HEMATOLOGY AND CLINICAL CHEMISTRY IN GOATS

Factors Affecting the Reference Values

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CONTENTS

PREFACE

The initial objective of this work was to gather as much information on the hematological and clinical chemical reference values for goats. It was desired to present the work in easily readable tables and figures to facilitate comparisons and reference assessments. To achieve this objective it was thought natural, first to review the literature on the factors that affect the reference values and secondly to investigate the trends under the influence of some of the factors. On the second part studies were conducted on growth, environment and metabolic influence on the reference values. This thesis is based on these investigations. Data reported in conventional units were transformed into the International System of Units (SI) to enable comparisons from different sources. Although the contents of the thesis are far from the desired perfection of the objective, it is a pleasure to state that some parts of the original purposes have been accomplished. Credit for this part of the work goes to the Central Laboratory, Department of Clinical Studies, Royal Veterinary and Agricultural University, Frederiksberg and the financial sponsor, the Danish International Development Agency.

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Gabriel Mbassa
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ABBREVIATIONS

ALAT	= Alanine aminotransferase	μkat/l
ALP	= Alkaline phosphatase	μkat/l
ASAT	= Aspartate aminotransferase	μkat/l
CK	= Creatine kinase	μkat/l
EDTA	= Ethylene diamine tetraacetate	
Hb	= Hemoglobin concentration	mmol/l
GDH	= Glucose dehydrogenase	μkat/l
MCH	= Mean corpuscular hemoglobin	fmol
MCHC	= Mean corpuscular hemoglobin concentration	mmol/l
MCV	= Mean corpuscular volume (femtoliters)	fl
PCV	= Packed cell volume (= Hematocrit)	l/l
RBC	= Red blood cells	$x10^{12}$/l
RPM	= Revolutions per minute	
TSP	= Total serum proteins	g/l
SCE	= Scandinavian committee on enzymes	
WBC	= White blood cells	$x10^{9}$/l
μkat	= Enzyme units of catalytic activity/second	

SUMMARY

The purpose of the present investigation was to determine the reference hematological and clinical chemical values in goats, in particular Danish landrace and Tanzanian breeds, and the factors affecting the ranges. The influences of age, breed, environment, pregnancy and lactation were studied and age specific reference values determined for Danish landrace goats. Parametric (means and one or two standard deviations) were compared with corresponding nonparametric statistics; median, 5^{th} to 95^{th} percentile interval values for each hematological and clinical chemical analyte. The results of the two statistical methods were close to each other and the data of most parameters followed a *Gaussian* distribution.

Comparative studies on cell counts revealed that hemocytometric erythrocyte counts are 5.63 % higher while the leukocyte counts 2.79 % lower than electronic counter results. Both analytical methods have therefore large influence on clinical reference ranges. The large differences reported in hematological and clinical chemical values are attributable at least in part to the analytical techniques.

Neonatal hematology on the morphology of erythrocytic cells in external jugular vein blood samples from newborn dwarf and Danish landrace kids showed three cell types after supravital staining with new methylene blue. These were large diffusely basophilic chromatophilic erythrocytes (macrocytes), punctate or aggregated reticulocytes and mature erythrocytes. Continued studies from one day to 12 months of age indicated that the diffusely basophilic polychromatophilic erythrocytes and punctate/aggregated reticulocytes diminished and were not observed in kids older than 1 to 2 months. Romanowsky stained blood smears showed marked anisocytosis and poikilocytosis in which polychromatophilic macrocytic erythrocytes were numerous. The erythrocytes decreased in sizes and increased in number with age.

The erythrocyte, total and differential leukocyte counts increased during growth from neonatal minimum values and were highest at 6-12 months of age. The hemoglobin concentration and hematocrit decreased from the neonatal values in two weeks of life, whereafter they increased. Mean corpuscular volume and mean corpuscular hemoglobin were higher in neonates and decreased with age but the mean corpuscular hemoglobin concentration fluctuated only very little. The number of lymphocytes and neutrophils were very low in new born kids but very high counts were observed within 8 months of age. Basophil, monocyte and eosinophil cells increased slightly with age.

Plasma calcium and phosphorus were higher while magnesium was lower in very young kids than in older goats. Sodium and potassium changed very little with age. Differences in mean electrolyte concentrations attributable to sex were not observed

in most age groups.

Alanine aminotransferase, aspartate aminotransferase and creatine kinase levels were low at birth and increased during growth, whereas for alkaline phosphatase it was vice versa. Creatinine, bilirubin, urea and total serum protein levels increased with age in dwarf and landrace goats. Glucose and cholesterol concentrations were high at birth and decreased with age. Differences between female and male landrace kids of the same ages were observed in plasma urea, creatinine, glucose and total serum proteins.

The investigation on profiles during pregnancy and lactation showed that the hematocrit, hemoglobin concentration, number of erythrocytes and leukocytes were higher in young (8-12 months old) nonpregnant and 1-2 years old adult pregnant than in adult pregnant and lactating goats of two years and above. Hematocrit, hemoglobin concentration and number of erythrocytes decreased in late pregnancy and early lactation. At the same time mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration increased. There were more changes in pluriparous than in primiparous goats. After parturition the former group of parameters increased and the latter decreased. There was an increase in the number of total leukocytes close to and after parturition and it was more in first lactation than in pluriparous goats. There were significant differences in many parameters between adult goats from different herds (within similar physiological states).

Calcium, phosphorus, alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase decreased in late gestation. Magnesium and creatine kinase decreased in early lactating goats but increased in subsequent lactation period. There were little changes in sodium and potassium during pregnancy and lactation. Calcium and magnesium changes during pregnancy and lactation were inversely, while phosphorus directly proportional to parity. The transferases and creatine kinase were higher in young (1-2 years old) than in old goats, while alkaline phosphatase was unpredictably high or low in individual goats.

Urea, creatinine and bilirubin were higher in young nonpregnant than in other goats. Urea decreased during early and mid lactation stages, directly proportional to parity. Creatinine increased in late lactation, more in goats of higher parity than in others. Bilirubin was higher in mid lactation stage, much more in goats of higher parity than in others. Glucose concentration decreased in pregnant goats and increased during lactation. The decrease during pregnancy was more in higher parity goats than in others. Plasma cholesterol and total serum proteins increased during lactation directly proportional to parity.

Comparisons were made for RBC counts, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte and differential leukocyte counts between on the one hand, blended, indigenous East African goats at 6-12 months of age, 15-45 day old

kids, 8 months old apparently healthy male and above 3 years old pregnant Norwegian dairy goats reared in Tanzania. On the other hand were 6-12 months old dwarf and Landrace goats, and adult pregnant Danish landrace goats reared in Denmark. Hemoglobin concentration, hematocrit, the number of RBC and white blood cells were lowest in Norwegian kids. Highest values were observed in 6-12 months old goats in all the breeds. The mean corpuscular volumes were highest in kids followed by adult pregnant goats, and lowest in 6-12 months old goats. East African and Blended goats had the smallest mean corpuscular volumes. The hemoglobin levels, erythrocyte and leukocyte counts were highest in indigenous East African, followed by young Norwegian and Blended goats. The mean corpuscular hemoglobin concentration was highest in Blended while the mean corpuscular hemoglobin higher in pregnant than in other goats.

Investigations after grouping all the Danish landrace goats according to age from birth to over five years of age confirmed the increasing trends for hemoglobin levels, packed cell volume, number of erythrocytes and leukocytes. These were lower in neonates and juveniles than in adult goats increasing to maximum values by 6-12 months of age. The mean corpuscular volume and mean corpuscular hemoglobin were highest in neonatal kids and decreased with age concomitantly with increases in erythrocyte counts, hemoglobin concentration and hematocrit. There were large significant age differences in hematological parameters except for mean corpuscular hemoglobin concentration. Calcium, inorganic phosphorus, alkaline phosphatase, urea, glucose and cholesterol were higher in young goats of less than 6 months of age than in adults. Sodium, magnesium, creatine kinase, bilirubin, creatinine, total serum proteins and alanine amino and aspartate amino transferases were moderately to significantly lower in juvenile than in adult goats. Potassium was not significantly different between goats of different ages. Sex differences were not significant in most parameters and were noted at some ages for urea, glucose, creatinine, bilirubin, cholesterol, aspartate aminotransferase and creatine kinase.

There were large significant differences in most hematological and clinical chemical values between breeds (in goats of similar age), age groups (within similar breeds) and herds (in goats of similar age within the breeds). Statistically significant differences between female and male kids of similar ages within the same breed were not observed in most parameters.

SAMMENDRAG

Formålet med den foreliggende undersøgelse var at fastsætte de hæmatologiske og klinisk-kemiske referenceværdier hos geder, specielt Dansk Landrace og Tanzanianske racer, saul undersøge faktorer der påvirker variationerne. Påvirkninger såsom alder, race, miljø, drægtighed og diegivning blev undersøgt, og specifikke aldersreferenceværdier blev herefter bestemt for geder af Dansk landrace.

Parametre (middel og en eller to standardafvigelser) blev sammenlignet med tilsvarende ikke-parametriske statistikværdier, middeltal, 5 og 95% gruppernes værdier for hver hæmatologiske og klinisk-kemiske analyse. Resultaterne for disse 2 statistiske metoder var meget tæt på hinanden og tallene fra de fleste parametre var normalfordelte.

Sammenlignende studier på celle beregninger afslørede at hæmocytometriske erytrocyttal er 5.63% højere, hvorimod leukocyttallene er 2.79% lavere end de elektroniske tællinger. Begge analyse metoder har derfor stor indflydelse på kliniske referenceområder. Den store afvigelse meddelt i hæmatologiske og klinisk-kemiske værdier kan derfor delvist tillægges den analytiske teknik.

Neonatal hæmatologi vedrørende morfologien af erytrocytoide celler fra nyfødte dværggeder og Danske Landrace kid viste 3 celletyper efter supravital farvning med methylenblå. Disse var diffust basofile erytrocytter (makrocytter), enkelte eller reticulocytter/aggregater og modne erytroctytter. Fortsatte studier fra fødsel til 12 måneders alderen tyder på de diffust basofile polycromatophile erytrocytter og enkelt reticulocyt/aggregater formindskedes og blev ikke observeret i kid der var ældre end 1-2 måneder. Romanowskyfarver blodudstrygningspræparat viste karakteristisk anisocytosis og poikilocytosis i hvilke polychromatophile markocytiske erytrocytter var talrige. Erytrocytterne mindskedes i størrelse og i antal med alderen.

Erytrocytterne, totalt og differentieret leukocyttal øgedes igennem væksten fra minimumsværdier hos nyfødte og maximumværdier ved 6-12 måneders alderen. Hæmoglobinkoncentationen og hæmatokrit formindskedes fra de nyfødtes værdi i løbet af de første 2 leveuger, hvorefter de øgedes. MCV og MCH var højere ved nyfødte og blev mindre med alderen, men MCH koncentrationen varierede kun lidt. Antallet af lymphocytter og neutrofile var meget lavt i nyfødte kid, medens meget høje tal blev observeret inden kiddene var 8 måneder gamle. Basofile, monocytter og eosinofile celler øgedes ubetydeligt med alderen.

Calcium og fosfor i plasma var højere medens magnesium var lavere i meget unge kid end i ældre geder. Natrium og kalium forandrede sig meget lidt med alderen. Køns-relaerene forskelle i middel elektrolytkoncentrationen sat i forhold til kønnet blev ikke observeret i de fleste aldersgrupper.

ALAT, ASAT og creatinkinase værdierne var lave ved fødslen, men steg med alderen hvorimod det for basisk fosfatase var omvendt. Creatinin, bilirubin, urinstof og totalt serumprotein værdierne steg med alderen i Dværggederne og i Landracegederne. Glukose og kolesterol koncentrationer var høje ved fødslen og faldt med alderen. Plasma urinstof, creatinin, glukose og total serumprotein forskelle mellem hundyr og handyr hos kiddene af Landrace i samme aldersgruppe blev observeret. Profilundersøgelser under drægtigheden og diegivning viste at hæmatokrit, hæmoglobin koncentrationen, antallet af erytrocytter og leukocytter var højere i unge (8-12 måneder) ikke drægtige og 1-2 år voksne drægtige geder, end i voksne drægtige og diegivende geder der var ældre end 2 år. Hæmatokrit, hæmaglobin koncentationen og antallet af erytrocytter faldt i slutningen af drægtigheden og i begyndelsen af diegivningen. Samtidig forøgedes MCV, MCH og MCHC værdierne. Der var flere forandringer i flergangsfødende geder, end i førstegangsfødende geder. De førstnævnte parametre forøgedes efter fødslen og de sidstnævnte parametre formindskedes. Der var en forøgelse af antallet af total leukocyt lige før og efter fødslen og der var flere i førstegangsfødende end der var i geder der havde født flere gange. Der var signifikant forskel i mange af parametrene imellem de voksne geder fra de forskellige flokke (indenfor ensartede fysiologiske tilstande).

Calcium, fosfor ALAT, basisk fosfatase og ASAT formindskedes i slutningen af drægtigheden. Magnesium og creatinin kinase formindskedes i den første del af diegivningen, men forøgedes senere hen i diegivningsperioden. Der var kun små forandringer i natrium og kalium i graviditetsperioden og diegivningsperioden. I løbet af diegivningsperioden var forandringerne for Calcium, Magnesium og Kalium omvendt, mens fosfor var direkte proportionelt til fødselsstatus. Transferaseværdierne og creatininkinaseværdierne var højere i unge (1-2 år gamle) geder end de var i de gamle geder, medens alkalisk phosphatase var uforudsigeligt højt eller lavt i de individuelle geder. Urinstof, creatinin og bilirubin var højere i unge ikke-drægtige geder end de var i andre geder.

Urinstoffet formindskedes tidligt - og under diegivning, direkte proportionelt med alder og laktationer. Creatinin forøgedes i den sidste periode af diegivningen mere hos flergangsfødende geder end hos de andre geder. Bilirubin var langt højere i midten af diegivningsperioden, hos flergangsfødende end i de andre geder. Glukosekoncentrationen faldt i drægtige geder og forøgedes under diegivningen. Falder under drægtigheden var højere hos flergangsfødende end hos andre. Kolesterol i plasma og total serumprotein forøgedes under diegivningen direkte proportionalt med antal fødsler. Sammenligning af værdierne for henholdsvis de røde blodlegemer, hæmatokrit, hæmoglobin, MCV, MCH, MCHC, total leukocyt og differentialtææling i gruppen for blandede indfødte Østafrikanske geder 6-12 måneder gamle, 15-45 dage gamle kid, 8 måneder gamle hanner og omkring 3 år gamle drægtige Norske malkegeder

opdrættet i Tanzania imod gruppen af 6-12 måneder gamle Dværggeder og Landracegeder og voksne drægtige Dansk Landracegeder opdrættet i Danmark. Hæmoglobinkoncentrationen, hæmatokrit, antallet af røde og hvide blodlegemer var lavest i de norske kid. De højeste værdier blev observeret i 6-12 måneder gamle geder i alle racerne. MCV var højest i kid, efterfulgt af voksne drægtige geder og lavest i 6-12 måneder gamle geder. Østafrikanske geder og de blandede racer havde de mindste MCV-værdier. Hæmoglobinværdier, erytrocyttallene og leukocyttalene var højest i indfødte Østafrikanske geder, efterfulgt af unge Norske geder og de blandede gederacer. MCH koncentrationen var højest hos de blandede gederacer medens MCH-værdierne var højere i de drægtige geder end værdierne var hos andre geder.

Undersøgelse, efter gruppering af alle de Danske Landracegeder i alder fra fødsel til 5 år gamle, bekræftede den forøgede tendens for hæmoglobinniveauerne, hæmatokrit, antallet af leukocytter og erytrocytter. Disse var lavere i nyfødte og unge kid end de var i voksne geder, hvor værdierne forøgedes til maximum ved 6-12 måneders alderen. Værdierne for MCV og MCH var højest i nyfødte kid og blev formindsket med alderen, med en forøgelse af erytrocyttal, hæmoglobinkoncentration og hæmatokrit. Der var stor signifikant aldersforskel i de hæmatologiske parametre bortset fra MCH koncentrationen.

Calcium, uorganisk fosfor, alkalisk phosphatase, urinstof, glukose og kolesterol var højere i geder yngre end 6 måneder, end tallene var for de voksne geder. Natrium, Magnesium, cretininkinase, bilirubin, creatinin, total serumprotein og ALAT og ASAT var moderat til signifikant lavere i unge end i de voksne geder. Der var ikke signifikant forskel for Kalium imellem geder i de forskellige aldre. Kønsforskellige var insifnigikante og værdier for urinstof, glukose, creatinin, bilirubinm kolesterol, ASAT og creatininkinase blev noteret hos enkelte aldersgrupper. Der var tydelig signifikant forskel i de fleste hæmatologiske og klinisk-kemiske værdier imellem racerne (i geder indenfor samme aldersgruppe og race). Statistisk signifikante forskelle imellem handyr og hundyr (kid) i aldersgruppe indenfor den samme race blev ikke observeret i de fleste parametre.

CONVENTIONAL AND SI UNITS

The introduction of the International System of units of measurements in various parameters has made the transformations of hematological and clinical chemical values published in conventional units to this system essential. This facilitates comparisons of the various data sources. The nomenclature and the conversion factors were adopted from Dybkaer and Jørgensen (1966) as follows.

	Unit
Calcium (mmol/l)	= calcium mg/dl x 0.2495
	= calcium mEq/l x 0.500
Inorganic phosphorus (mmol/l)	= inorganic phosphorus mg/dl x 0.3228
Magnesium (mmol/l)	= magnesium mg/dl x 0.4112
Potassium (mmol/l)	= potassium mg/dl x 0.2558
Sodium (mmol/l)	= sodium mg/dl x 0.4350
Bilirubin (μmol/l)	= mg/dl x 11.7
Cholesterol (mmol/l)	= mg/dl x 0.02586
Creatinine (μmol/l)	= mg/dl x 88.4
Glucose (mmol/l)	= mg/dl x 0.05551
Plasma urea (mmol/l)	= mg/dl x 0.1665
Total serum proteins (g/l)	= g/dl x 10
Alanine amino transferase (μkat/l)	= U/L x 1/60
Alkaline phosphatase (μkat/l)	= U/L x 1/60
Aspartate amino transferase (μkat/l)	= U/L x 1/60
Creatine kinase (μkat/l)	= U/L x 1/60
Erythrocyte count ($x10^{12}$/l)	= count x $10^6/\mu$l x 10^6
Hemoglobin concentration (mmol/l)	= g/dl x 0.6205
Reticulocyte counts ($x10^9$/l)	= count $x10^3/\mu$l x 10^6
Hematocrit (l/l)	= l/l
Mean corpuscular hemoglobin fmol	= $\mu\mu$g x 0.06205
Mean corpuscular hemoglobin concentration mmol/l	= g/dl x 0.6205
Mean corpuscular volume (fl)	= μ^3 x 10
Leukocyte count ($x10^9$/l)	= count $x10^3/\mu$l $x10^6$

CHAPTER 1

INTRODUCTION

Goats provide a significant supply of milk and meat and are important for experimental and research purposes because they are easy to handle. They are widely distributed in tropical and temperate countries reflecting their ability to adapt to a variety of environments. Among the known breeds in the world the Angora, Damascus, Maltese, Barbari, Beetal, Kashmir, Malabar, Marwari, Maradi (Red Sokoto), Nubian, Benadir, Kigezi, Somali, Fiji, East African, Congo dwarf, West African dwarf, Kamori, Galla, Boer, Alpine, Anglonubian, Toggenburg and Saanen can be mentioned (Devendra and McLeroy, 1982). Utility of goats depends on effective control of the diseases that affect them, provision of high quality and adequate nutrition and good management and breeding. This requires knowledge on the anatomy and physiology of the goat, the external factors and microorganisms which alone or by interactions cause diseases. The frequencies of diseases, distribution and causes must be determined for the purpose of their economic evaluation, treatment, control and eradication. Clinical diagnosis is therefore of fundamental importance.

Clinical diagnosis can be made after a series of procedures including examination of sick and even healthy animals and their surroundings. The tissue reactions to diseases can be examined in the laboratory by parasitological, hematological or clinical chemical methods in appropriate samples. Hematological and clinical chemical analysis have become major tools that reveal pathophysiological states in animals leading to identification of the pathogenesis and in turn the diseases (Wilson *et al.*, 1986; Bogin *et al.*, 1988; Boyd, 1988; Mbassa *et al.*, 1989). In addition, they enable assessment of the body's ability to fight infections and predict the probable outcome of a disease (prognosis).

In order to arrive at reasonably correct diagnosis and prognosis, knowledge of the reference level intervals of blood parameters in healthy goats is a primary requirement. The reference range is formed by determining the minimum and maximum levels of hematological and clinical chemical parameters in strictly healthy animals. The laboratory results of a clinically sick animal can then be compared to this range for better interpretation and subsequent diagnosis. Several expressions are used for reference ranges, the minimum to maximum observations in a set of data, the parametric (mean ± 1 or 2 standard deviations) and the nonparametric 2.5^{th} to 97.5^{th} or 5^{th} to 95^{th} percentile intervals (Solberg, 1983). The parametric estimation technique requires that data fit a specified distribution type (commonly *Gaussian*). If the data

requires that data fit a specified distribution type (commonly *Gaussian*). If the data is distributed in a *Gaussian* (normal) manner, the interval between 2 standard deviations below and above the mean includes about 95 % of all the observations. If the data is not normally distributed, parametric statistics do not properly describe the biological behavior of the blood parameter and the tests are not applicable (Reed *et al.*, 1971; Wu *et al.*, 1975). Such data must be approximated to the *Gaussian* distribution by transformations to logarithmic values (Flensburg and Willeberg, 1976). Alternatively nonparametric (or distribution free) statistical techniques are employed because these make no assumptions about the type of distribution.

There has been a number of earlier hematological and clinical chemical studies in tropical and temperate goats (Mukherjee and Battacharrya, 1952; Wilkins and Hodges, 1962). In the years that followed extensive investigations were conducted on other breeds and the factors that affect the blood picture (Holman and Dew, 1963; 1964; 1965a; 1965b; 1966a; 1966b; De Shaw *et al.*, 1969; Lewis, 1976; Nettleton and Beckett, 1976, Oduye, 1976; Castro *et al.*, 1977a; Mostaghni, 1979; Bhargava, 1980; Earl and Carranza, 1980; Payne *et al.*, 1982; Neto *et al.*, 1986; Mgasa and Mbassa, 1988; Wesonga and Nandokha, 1989). The hematological and clinical chemical variations between these sources are enormous because of the diversity of the goat breeds studied and climatic conditions under which the animals were reared. Application of generalized data for all goat breeds for the purpose of disease diagnosis is consequently difficult.

A careful interpretation is therefore essential because the level of hematological and clinical chemical parameters are altered by other physiological intrinsic processes or extrinsic factors in addition to the influence of diseases. The influences of the breeds (Pugliese *et al.*, 1982; Ginting, 1987), age (Nangia *et al.*, 1968; Edjetihadi, 1978; Neto *et al.*, 1986; Wojcik *et al.*, 1986; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988), nutritional adequacy and quality (Blackwell and Libby, 1982; Biswas *et al.*, 1986), herd (Masoni *et al.*, 1985; Biagi *et al.*, 1988), sex (Vaidya *et al.*, 1970; Chiericato *et al.*, 1986), lactation (Mohy *et al.*, 1985; Hassan *et al.*, 1986; Biagi *et al.*, 1988), pregnancy (Masoni *et al.*, 1985; Vihan and Rai, 1987), season (Vrzgula *et al.*, 1985; Pospisil *et al.*, 1987) and ambient temperatures (Bas *et al.*, 1980; Upadhyay and Rao, 1985; Oyewale and Olowookorun, 1986) have been reported. The consequence of these influences is that hematological and clinical chemical data derived from healthy goats from different places is not consistent and is often contradictory. Furthermore neonatal hematology and clinical chemistry in goats is not well understood and is frequently taken for granted to be identical to that of adults. This leads to uncertain interpretation of results and in some cases misleading diagnosis. As a result of these variations it was found necessary to evaluate the factors that affect the reference ranges for hematological and clinical chemical values in goats and their magnitudes, with a view

of specifying them to age, breed and probably other factors, hence the purpose for the present study.

The influence of age, breed and herd (environment) were investigated for erythrocyte, total and differential leukocyte counts, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine kinase, urea, bilirubin, glucose, cholesterol, creatinine, calcium, magnesium, potassium, sodium, inorganic phosphorus and total serum proteins in dwarf and Danish landrace goats reared in Denmark and some hematological parameters in Norwegian dairy, Blended and indigenous East African goats reared in Tanzania. The results are presented in five categories (1) techniques of blood cell counting, (2) hematological and clinical chemical profiles in growing kids (3) hematological and clinical chemical profiles during pregnancy and lactation (4) reference ranges for hematological and clinical chemical values in Danish landrace goats from the first day of life to above 5 years of age and (5) reference ranges for hematological values in Blended and East African indigenous goats compared with those of Danish landrace and Norwegian breeds.

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CHAPTER 2

STUDY DESIGNS

Animals and Blood samples

Seven herds were selected for blood sampling in Denmark, which belonged to the Department of Veterinary Microbiology and Hygiene, Royal Veterinary and Agricultural University (A), Knud Larsen, Lilleeje Gede farm Sækhusvej 21, 4640 Fakse (B), Greta Schmedes, Sorøvej 429, Ganges Bro, 4700 Næstved (C), Jytte Anderson, Egelimvej 7, Slimminge 4100 Ringsted (D), Pia Jensen, Ringstedvej 129 Forslev, 4690 Haslev (E), Ingrid Dam and Claus Mamros, Petersmindevej 15, 4250 Fugleberg (F) and Copenhagen Zoo (G). The goats were classified to two breeds, white and brown Danish landrace and dwarf. The dwarf goats belonged to the department of Veterinary Microbiology and Copenhagen Zoo. Those in the former herd were kept for experimental purposes. All the goats were apparently clinically healthy except the University experimental goats some of which were infected with parasites. Animals that appeared sick were categorized as infected and separated from the statistical analysis with healthy goats. The exact dates of birth of most goats were obtained. Age estimation was done only in a few goats where it was not possible to know the date of birth.

Goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in summer in all herds except those for experimental purposes. In farms F and G goats were reared in the field for the entire summer and autumn. The details for nutrition, age and physiological states are described in specific chapters dealing with kids or physiological profiles in adults.

A total of 578 blood samples were collected from the external jugular vein over a period of 15 months. Four vacuum tubes (Becton-Dickinson vacutainers) were used for each goat. The anticoagulant in the first tube was 0.12 ml, 0.34 M potassium ethylene diaminotetraacetate (K_3EDTA), the second contained sodium heparin (143 USP units), the third sodium fluoride with sodium heparin (NaF/Na heparin, 143 USP units) and the fourth a clot activator for serum separation. Sampling started in pregnant goats as well as any available males and were continued at two months intervals through parturition until late lactation stages. The number of adult male and female goats, their ages and physiological states are described in detail in chapter 3 - 12. Pool analysis of all goats for hematological and clinical chemical reference values are presented in chapters 13 -14.

are presented in chapters 13 -14.

Blood samples were taken also from kids. Of the 578 blood samples 62 were from healthy Danish landrace and 34 dwarf kids of one day old to 12 months of age. Samples in kids were collected every 3 weeks and after 8 weeks of age every 2 months. The details on sex, breed and ages are presented in chapters 4 - 9 dealing with kids.

Blood samples stabilized in K_3EDTA were used for hematological examination of erythrocytes, reticulocytes, total and differential leukocyte counts, hemoglobin and hematocrit. Plasma was obtained after centrifugation of heparinized blood and used for determination of calcium, inorganic phosphorus, magnesium, sodium, potassium, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine kinase, urea, creatinine, bilirubin and cholesterol. Plasma from blood samples stabilized in sodium fluoride sodium heparin were used for determination of glucose while serum was utilized in measurement of total proteins concentration.

Other investigations were conducted in Tanzania involving a total of 331 blood samples from three herds. Only the goats that appeared to be clinically healthy are included in this thesis and presented in chapter 15. Sick animals were analyzed separately. All analytes in this group of goats were done by manual methods.

Principles of Laboratory Analysis

Determination of hematological values

Red blood cell (RBC) counts were determined within 1 - 3 hours of sampling using a Coulter counter model ZF with an aperture diameter of 100 μm, adjusted for compensation of coincident cell passages. The threshold was set at 6 and attenuation 500. The background counts were kept at 200 maximum. A small amount of blood (100 μl) was stained supravitally with new methylene blue (0.5g new methylene blue, 1.6g potassium oxalate in 100 ml distilled water) by mixing in the ratio of 1:1. After interacting for 20 minutes at room temperature (25 ^{0}C), smears were prepared and examined. In these smears the percentage of punctate and aggregated reticulocytes were obtained after counting 1000 red blood cells. The number of reticulocytes was obtained by converting the percentages to absolute values from the total erythrocyte number determined from the coulter counter.

Hemoglobin (Hb) concentration was determined in model S560 Coulter counter (Coulter electronics, England). Packed cell volume (PCV) was determined in microhematocrit capillary tubes centrifuged at 12,000 G in a microhematocrit centrifuge. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by

standard formulae below. The RBC diameters were measured on photographs at 1440 x magnification.

$$\text{MCV (fl)} = \frac{\text{PCV x 1000}}{\text{RBC (x10}^2\text{/l)}}$$

$$\text{MCH (fmol)} = \frac{\text{Hb (mmol/l)}}{\text{RBC (x10}^{12}\text{/l)}}$$

$$\text{MCHC (mmol/l)} = \frac{\text{Hb (mmol/l)}}{\text{PCV (l/l)}}$$

The total number of white blood cells (WBC) were determined in a Coulter counter model S560 (Coulter electronics England) with aperture diameter and length of 100 and 75 μm respectively within 1 - 3 hours of sampling. Thin blood films were made on slides, air dried and immediately stained with a Romanowsky stain in an automatic stainer (Hema - Tek). Differential leukocyte counts were made after permanently mounting of smears with xylene. The number of basophils, eosinophils, band and segmented neutrophils, monocytes and lymphocytes, were determined from 200 total cell counts.

Principles of plasma electrolyte determinations

Plasma calcium and magnesium were determined on an atomic absorption spectrophotometer model 5000 (Perkin Elmer). A 200 μl sample of plasma was diluted with 10 ml solution containing 29.86 g lantanoxid, 325 g 5 M HCl, 250 mg triton x-100, 1.5 g strontium chloride in 5 liters of distilled water (Merck). The atomic absorption spectrophotometer was calibrated with the diluent and two standard stock solutions (I and II). Solution I for both calcium and magnesium contained 2.94 g $CaCl_2.2H_2O$ proanalyse, 1.62 g $MgCl_2$ pro analyse, 818 mg NaCl, 37 mg KCl in 100 ml distilled water. The equivalent concentration of the solution was 0.2M Ca and 0.08 M Mg. Standard solution II contained 4.41 g $CaCl_2.2H_2O$ proanalyse, 2.64 g $MgCl_2$ pro analyse, 818 mg NaCl and 37 mg KCl (pro analyse) in 100 ml distilled water for equivalent concentration of 0.30M Ca and 0.13M Mg. Both stock solutions were

diluted to 1:100 in distilled water, thereby to contain respectively 2 and 3 mmol/l calcium and 0.8 and 1.3 mmol/l magnesium. The concentration of calcium and magnesium in the plasma were then determined at wavelengths of 422.7 and 285.2 respectively.

The principle for determination of inorganic phosphorus was that it reacts with sodium molybdate to form phosphomolybdate which is converted to colloidal molybdenum blue by reduction with paramethyl aminophenol sulphate. The absorbance of molybdenum blue was determined spectrophotometrically at 340 nm wavelength after incubation for 120 seconds at 37 °C.

An electrolyte module of the Cobas Fara automated analyzer was used to measure the plasma sodium and potassium concentrations. This module has flow through ion selective electrodes with open liquid junctions. The potassium electrode consists of a polyvinyl chloride valinomycin membrane which is selective to K^+. The sodium electrode consists of a glass membrane selective to Na^+. Sodium and potassium are measured on the principle that each electrode has a membrane permeable principally to the ion it senses. The third electrode is reference. The reference solution flows through the reference electrode and thereafter merges with the sample, so as to close the electrical path between the ion selective and the reference electrode. The electrical potential measured between these electrodes is a function of the ion concentration. The electrodes respond to the activity of the ions in the solution and automatically give the results in concentration because the standard solution have the same ionic strength as the plasma sample.

Principles of determination of plasma enzyme activities

Alanine amino transferase (EC. 2.6.1.2, L- alanine: 2- oxoglutarate aminotransferase, ALAT) was determined on the principle of its reaction;

L-Alanine + 2-oxoglutarate ⇌ pyruvate + L-glutarate (catalyzed by ALAT)
Pyruvate + NADH + H^+ → L-lactate + NAD^+ (by lactic dehydrogenase)

The rate of NADH oxidation is proportional to ALAT catalytic activity and the change in color intensity was determined by measuring the decrease in absorbance at 340 nm incubated at 37 ^{0}C for 180 seconds.

Aspartate aminotransferase (EC. 2.6.1.1, L-aspartate: 2-oxoglutarate aminotransferase; ASAT) was determined by its reaction;

L- aspartate + 2- oxoglutarate ⇌ oxaloacetate + L-glutamate (ASAT)
Oxaloacetate + NADH + H^+ ⇌ L- Malate + NAD^+ (malic dehydrogenase)

The rate of NADH oxidation is proportional to ASAT catalytic activity and was determined by measuring the decrease in absorbance at 340 nm at 37 ^{0}C for 180 seconds.

Alkaline phosphatase (EC. 3.1.3.1, ortho phosphoric monoester phosphohydrolase, ALP) activity was determined by end point colorimetric method under its catalytic reaction principle with p-nitrophenylphosphate as a substrate;

p-Nitrophenylphosphate + H_2O → Phosphate + p-nitrophenol (ALP)

The absorbance of p-nitrophenol was determined at 405 nm after incubation for 120 seconds at 37 ^{0}C.

Creatine kinase (EC. 2.7.3.2, Cratine-N-phosphotransferase, CK) was determined kinetically under its principle of action;

Creatine phosphate + ADP ⇌ Creatine + ATP (CK)
ATP + D-glucose ⇌ ADP + D-glucose-6-phosphate (Glucose hexokinase)
D-glucose-6-phosphate + NADP ⇌ D-glucose-6-phosphate + NADP + H^+
The last reaction is catalyzed by glucose-6-phosphatase dehydrogenase.

The rate of increase in NADPH is directly proportional to the CK catalytic activity and was determined photometrically by measuring the increase in absorbance at 340 nm incubated at 37 ^{0}C for 120 seconds.

Principles of determination of other plasma and serum clinical chemical parameters

Plasma urea was determined by enzymatic ultra violet light test method with urease and glutamate dehydrogenase under this reaction principle;

(1) Urea + H_2O → 2 NH_4 + HCO_3^- (urease)
(2) NH_4 + 2-oxoglutarate + NADH → L- glutamate + NAD^+ + H_2O

The enzyme catalyzing reaction 2 is glutamic dehydrogenase. The decrease in NADH concentration was determined by monitoring the decrease in absorbance at 340 nm incubated at 37 ^{0}C for 120 seconds.

Plasma creatinine was determined by the principle that it reacts with picric acid in alkaline solution to form a yellow red compound. The color intensity of this is directly proportional to the creatinine concentration, the absorbance was measured in the range of 490 to 510 nm after incubation at 37 ^{0}C for 120 seconds.

Plasma glucose concentration was determined after deproteinization with perchloric acid under the principle that glucose dehydrogenase (B-D-glucose; NAD- oxidoreductase) catalyzes the oxidation of glucose to D- gluconolactone.

B-D glucose $\rightarrow$ D- gluconolactone + NADH + H^+ (glucose dehydrogenase)

The reaction is accelerated by aldose-1-epimerase (mutarotase). The quantity of NADH formed is proportional to the glucose concentration incubation temperature being 37 ^{0}C for 120 seconds and absorbance read at 340 nm.

The concentration of bilirubin in the plasma was determined under the principle that it reacts with 4-sulphobenzenediazonium chloride to form a red compound azobilirubin. The color intensity of azobilirubin is proportional to the bilirubin concentration and was determined by monitoring the absorbance at 550 nm incubated at 37 ^{0}C for 120 seconds.

The concentration of cholesterol in the plasma was determined by enzymatic colorimetric method with cholesterol esterase, cholesterol oxidase and 4-aminophenazone (PAP) on the principle of the reactions below.

cholesterol esters + H_2O $\rightarrow$ cholesterol + fatty acids (cholesterol esterase)

cholesterol + O_2 $\rightarrow$ 4-cholesten-3-on + H_2O_2 (cholesterol oxidase)

The hydrogen peroxide produced couples with phenol and 4- aminophenazone to a red color quinoneimine derivative in the presence of peroxidase. The concentration of quinoneimine which is directly proportional to the cholesterol concentration is determined by measuring its absorbance at 500 nm after incubation at 37 ^{0}C for 5 minutes.

Total serum proteins were determined by the Biuret method on the principle that compounds with at least two peptide bonds react with copper salts in alkaline solutions to give a violet color with absorption maximum at 546 nm.

CHAPTER 3

COMPARISON BETWEEN MODIFIED HEMOCYTOMETRIC AND ELECTRONIC COUNTING OF BLOOD CELLS

Summary

Dilutions of goat blood with Hayem-Jørgensen's fluid ranging from 1:200 to 1:1000 were used for hemocytometer counting of red blood cells (RBC) in 27 goats. The optimal dilutions were 1:400 - 1:500. Correlation studies between the results obtained by the hemocytometer and the Coulter counter red blood cells (RBC) and white blood cell (WBC) counts were performed in 551 goat blood samples. The hemocytometer RBC counts were 5.63 % higher and WBC counts 2.79 % lower than those of the electronic counter. The method of blood cell counting therefore influences the clinical hematological diagnoses and reference values in domestic animals. New cell counters specifically designed to measure cells of small volumes e. g. goat erythrocytes are needed.

Introduction

Evaluation of red blood cell (RBC), total and differential leukocyte counts, hemoglobin and hematocrit is a prerequisite for diagnosis of several diseases (Goldston *et al.*, 1980). Blood stabilized in ethylene diamino tetraacetate (K_3EDTA) is suitable for their determinations within 6 hours of collection, beyond which deleterious effects occur. Aging of blood samples in warm conditions causes cellular degeneration and clumping (Valli *et al.*, 1980). Lactic acid accumulates due to glycolysis (Manston *et al.*,1974) and lowers the pH (Poulsen and Surynek, 1977; Assal *et al.*, 1978; 1980) leading to leukocytic basophilia, vacuolation and pyknosis (Gosset and Carakostas, 1984) and renders the sample unsuitable for differential leukocyte counts (Weiss, 1984). Refrigeration and freezing causes hemolysis and cell degeneration (Valli *et al.*, 1980; Larkin, 1984). RBC and white blood cell (WBC) counts of old samples are lower than those of fresh blood irrespective of storage temperatures (Manston *et al.*, 1974; Grenn *et al.*, 1976; Fountaine *et al.*, 1987a;b).

Introduction of electronic particle counters has simplified blood cell counting (Brecher *et al.*, 1956; Mattern *et al.*, 1957; Richar and Breakell, 1959). RBC and WBC counts are found to be satisfactorily accurate (Weiser, 1981). In Veterinary medicine, delay in blood analysis for electronic counting due to big distances to laboratories may

cause some artifactual results. Some electronic counters do not accurately count RBC with mean volumes (MCV) less than 55 femtolitres (fl) (Weiser, 1983). Several domestic animals have MCV's of less than 60 fL (Weiser, 1985; table 1), therefore counters for human.blood cells require modifications for use to these species (Weiser, 1987), especially in sheep and goats.

Discrepancies between hemocytometric and electronic blood cell counts in goats were briefly reported (Mattern *et al.*, 1957; Wisecup and Crouch, 1963). The relationship between counts of the two techniques have not been further evaluated. Since all reported counts are based on the two methods the relationship between them is important in comparative and clinical haematology. The aims of this investigation were to develop suitable methods for hemocytometric counting of goat erythrocytes and leukocytes, and compare the results with those of an electronic counter.

Materials and Methods

Goat blood samples were taken from the external jugular vein in vacuum tubes (Becton - Dickinson vacutainer) containing K_3EDTA. Erythrocytes were counted in a hemocytometer after dilution with Hayem-Jørgensen's solution (0.5 g mercuric chloride, 5 g sodium sulphate, 1 g sodium chloride in 200 ml distilled water) in test tubes (table 2). After thorough mixing the hemocytometer chamber was filled with a microhematocrit tube and settled for 5 minutes before counting. Cells in 5 secondary squares were counted and RBC count per liter in the original blood sample calculated. The number of erythrocytes were parallel determined through duplicate counts in model ZF Coulter counter with an adjustable aperture diameter of 70 - 100 μm (Coulter electronics England) which is able to count particles smaller than 60 fl. The optimum threshold was determined on frequency curves after counting erythrocytes of 10 goats at 4, 5, 6 and 7 settings. The maximum counts were recorded at 6, therefore optimum threshold. The Coulter counter was corrected for coincident particle passages and the background counts kept at 200 maximum.

Red blood cell counts were performed on 27 samples using the hemocytometer and electronic cell counter. The results were compared and the differences statistically analyzed (table 2-4). Different dilutions were utilized for study of suitability in hemocytometer RBC counting. A dilution of 1:200 was used in 5 goats and in 22 goats dilutions of 1:401, 1:501, 1:665 and 1:996 were examined (table 2). The chamber was filled 10 times with different pipettes at each dilution and RBC counted. The mean of the 10 counts (table 3) was compared to the Coulter counter results of the same sample. In two of the samples, the erythrocytes were electronically counted in 20 dilutions for reproducibility control of Coulter counter results.

For optimum and comparative studies between the methods, parallel counting of

leukocytes in 22 goats were performed ten times each in the hemocytometer and in duplicate in model S 560 Coulter counter, the aperture diameter and length of which were 100 and 75 μm respectively (table 4). The dilution for hemocytometric leukocyte counting was 1:20 (50 μl blood with 950 μl of methyl violet acetic acid solution). Both sides of the hemocytometer were filled with thoroughly mixed suspension for counting and calculation in the standard manner.

Further correlation studies between hemocytometric and electronic counting were conducted in 551 venous blood samples from 49 kids one to 30 days, 44 kids one to three months, 45 kids three to six months, 23 goats six to 12 months, 138 goats one to 2 years old, 212 more than 2 years of age and 40 six to 8 months old goats infected with parasites. For hemocytometer counting 1:501 and 1:20 dilutions were used for RBC and WBC respectively (table 2, 5, 6) and results statistically analyzed with version 6 of the statistical analysis system (SAS, USA, 1988) for means, standard deviations and 't' tests.

Table 1: The minimum and maximum MCV of domestic animals electronically measured (Weiser, 1982) or calculated from microhematocrit and RBC count values (Jain, 1986).

	MCV (fl)						
	Dog	Cat	Horse	Ox	Pig	sheep	Goat
Coulter counter	62 - 87	38 - 64	34 - 53	31 - 59	-	-	-
Calculated	37 - 55	39 - 55	37 - 59	40 - 60	50 - 60	28 - 40	1 7 - 38

Table 2: Number of samples at different dilutions for erythrocyte counts.

Dilution factor	Number of samples	Blood to diluent volume
1 : 200	5	10 μl : 1990 μl
1 : 401	4	10 μl : 4 ml
1 : 401	4	25 μl : 10 ml
1 : 501	1	20 μl : 10 ml
1 : 665	5	3 μl : 1990 μl
1 : 996	8	2 μl : 1990 μl

Results

Red blood cell counts

The high number of goat RBC in the hemocytometer, 300 to 500 cells in each secondary square renders the dilution of 1:200 (table 2) impractical for counting purposes, therefore higher dilutions are required. In 1:401 and 1:501 dilutions, there were 60 - 90 cells in each secondary square and the RBC counts for each sample were close to those of the Coulter counter (table 3) thus being the most suitable for microscopic counting of goat erythrocytes. RBC hemocytometer counts were higher than those of the Coulter counter, but the results were, however, well correlated. The 1:665 and 1:996 dilutions (table 2, 3) resulted in large variations between counts.

Hemocytometer and the Coulter counter RBC counts were well correlated in the 551 RBC samples (table 5). The correlation coefficients were 0.82 to 0.96. The regression coefficients were 0.83-1.16, whereas the coefficient of determination (R^2) expressing the amount of variations explained by the regression model were 0.68 - 0.92. Hemocytometer RBC counts were higher than electronic counter results in most pairs. The mean of the differences between each pair of the chamber and electronic counter counts and their standard deviations varied between ages, but were significantly different ($P < 0.05$) in all groups except in parasitic infected goats (table 6). The mean hemocytometer count in excess of the electronic counter was 0.66 x 10^{12}/l or 5.63 %.

Leukocyte counts

The 1:20 dilution of blood to methyl violet acetic acid solution for counting of WBC was used. The variance of the results of Coulter counter examination for one sample in which the leukocytes were counted 20 times in duplicate was smaller than in the hemocytometer counts. Leukocytes were well stained and easily counted in the microscope at 10 x magnification but the numbers were lower than those of the Coulter counter (table 4 - 5) except in 1 - 3 and 3 - 6 months groups. High correlation and regression coefficients were observed between the two methods. The differences between each pair of the counts were statistically significant in the goats older than 2 years and in the sick animal group (table 6). The mean difference between each pair was 0.37 x 10^{9}/l for all goat groups or 2.79 % over the hemocytometer values.

Discussion

Erythrocyte counts

This investigation revealed significantly higher hemocytometer than electronic erythrocyte counts in goats (table 5). Similar observations have been reported in man (Mattern *et al.*, 1957), cattle (Strauss *et al.*, 1978; Weide *et al.*, 1962) pig and sheep

(Weide *et al.*, 1962). The higher hemocytometer counts are suggested to be due to pipette, chamber, field and personnel errors (Berkson *et al.*, 1940), speed of chamber filling, planar cell concentration in the chamber and tight securing of the coverslip on the hemocytometer pillars before loading (Brecher *et al.* 1956; Mattern *et al.*, 1957). Pipette and chamber errors are due to variations in volumes, whereas the number of cells settling in the squares vary with fields. The 20 μl of blood used and the dilution with 10 ml diluent in a test tube, thorough mixing and settling time were made to increase the accuracy of counts. This is reflected in the small standard deviation of the different values in goats (table 3, 5).

The lower erythrocyte count in the electronic counter could be due to coincidence loss. Although this was corrected for, coincidence loss due to doublets, triplets or more follow a Poisson distribution (Mattern *et al.*, 1957), so that Coulter counter results are very accurate for suspensions having few cells. Increasing number of cells increases coincidence losses. Since goats have tremendous number of erythrocytes coincidence losses in electronic counters are likely to be higher than in other species. The present results show that the fewer the cells an animal has the closer the two methods agree on the count. The accuracy of cell counts in electronic counters depend on appropriate aperture width and length and threshold settings. These are critical for reduction of coincidence losses. The threshold setting that determines the size of the apertures is an important factor for electronic counting of goat RBC.

The mean value in excess of the electronic counter numbers was consistent (0.66 x 10^{12}/l or 5.63 %). The difference in counts may thus be due to both microscopic and electronic errors. The primary advantage of electronic counters is time saving (Mammerickx *et al.*, 1978a).

Leukocyte counts

The lower hemocytometric WBC counts than those from electronic counters observed in this investigation is in agreement with findings reported in cats (Weiser, 1987) and cattle (Wisecup and Crouch, 1963; Strauss *et al.*, 1978; Halliday *et al.* 1979). The mean difference between each pair of counts by the two methods in this study was 0.37 x 10^{9}/l (2.79 %) lower in the hemocytometer than in the electronic counter. The clear visibility of WBC in the hemocytometer after hemolysis and stromatolysis with acetic acid and methyl violet staining indicate that the difference in cell numbers between the two techniques attributable to human errors in counting was negligible. Furthermore the large paired counts from the 551 samples show consistently lower figures for the microscopic method. The electronic counter elevation of leukocyte counts over the hemocytometer may be due to errors in dilution (Richar and Breakell, 1959; Strauss *et al.*, 1978).

Table 3: Results of RBC counts ($x10^{12}/l$) of 27 samples based on 10 hemocytometer chamber fillings for 5 different dilutions and duplicate determinations in the Coulter counter.

	Hemocytometer counts			Electronic counter
Dilution	Minimum	Maximum	$\bar{x} \pm s$ ($n = 10$)	Duplicate counts $n = 2$
1:200	•	•	•	-
1:401	9.04	10.69	9.77 ± 0.41	9.96
1:401	15.78	16.36	16.09 ± 0.19	15.67 ± 0.14[1]
1:401	12.29	14.20	13.32 ± 0.56	13.72
1:401	12.25	20.25	16.80 ± 2.79	13.91
1:401	8.88	9.76	9.38 ± 0.32	8.54
1:401	10.99	13.53	11.72 ± 0.80	11.23
1:401	12.17	12.85	12.47 ± 0.25	12.06
1:401	9.58	11.54	10.26 ± 0.75	9.22
1:501	15.33	16.75	15.83 ± 0.42	15.20 ± 0.75[1]
1:665	13.05	15.64	13.58 ± 0.92	13.24
1:665	13.19	14.78	13.74 ± 0.57	13.02
1:665	13.22	15.48	13.66 ± 0.66	13.85
1:665	13.25	14.91	13.89 ± 0.64	13.74
1:665	13.29	15.28	13.79 ± 0.64	13.29
1:996	10.31	17.14	12.65 ± 2.17	12.65
1:996	12.20	17.88	14.78 ± 1.76	12.14
1:996	11.65	17.94	13.72 ± 1.84	10.41
1:996	11.95	14.69	13.20 ± 0.81	12.15
1:996	11.80	15.76	14.01 ± 1.13	12.45
1:996	13.00	16.65	14.07 ± 1.32	11.95
1:996	12.10	18.58	14.54 ± 2.05	13.34
1:996	14.79	17.62	15.88 ± 0.86	13.43

•Measured 5 samples but counting impossible due to RBC overlaps in the chamber, s= standard deviation, [1]based on 20 duplicate counts.

Table 4: Results of WBC counts ($x10^9/l$) in 22 goat blood samples at 1:20 dilution, 10 duplicate chamber fillings and duplicate Coulter counter analysis (model S560).

Hemocytometer			Coulter counter
Minumum	Maximum	$\bar{x} \pm s$ (n=10)	$\bar{x} \pm s$ (n= 2)
8.45	12.85	10.59 ± 1.14	17.16 ± 1.07[1]
14.00	19.25	17.13 ± 1.60	21.00
11.45	16.60	12.93 ± 1.48	14.50
14.45	16.35	15.36 ± 0.66	17.40
10.80	14.10	12.79 ± 0.92	11.80
10.20	13.25	11.04 ± 0.84	13.60
12.20	15.90	14.19 ± 1.03	21.10
12.20	17.65	15.31 ± 1.73	21.20
10.35	12.55	11.35 ± 0.63	13.60
8.05	10.60	9.91 ± 0.74	10.60
11.45	14.75	12.53 ± 1.00	11.80
11.10	12.15	11.61 ± 0.35	11.40
16.80	19.10	17.72 ± 0.74	19.90
13.30	17.35	16.18 ± 1.22	20.10
10.45	14.30	12.71 ± 1.60	14.00
11.80	12.95	12.52 ± 0.32	12.30
10.10	14.05	12.83 ± 1.19	14.10
14.60	19.00	17.94 ± 1.35	19.50
16.60	17.40	16.99 ± 0.31	17.70
15.50	17.80	16.38 ± 0.70	16.50
14.60	21.60	18.00 ± 2.13	20.50
13.10	18.75	15.71 ± 1.86	19.80

[1]based on 20 duplicate counts, $\bar{x} \pm s$= mean ± standard deviation.

Table 5: Comparison between the results of hemocytometer and elctronic countimg og RBC ($x10^{12}/l$) and WBC ($x10^{9}/l$) counts in 551 blood samples.

Group	*n*	E counter	Hemocyto-meter	Corr	Regr	Interc	R^2
Erythrocyte counts							
1-30 day	49	8.31±1.33	9.01±1.49	0.88	0.98	0.84	0.77
1-3month	44	11.38±1.36	12.18±1.61	0.88	1.04	0.31	0.78
3-6month	45	13.22±1.28	13.83±1.71	0.84	1.12	-0.99	0.70
6-12month	23	15.08±3.03	15.90±3.67	0.96	1.16	-1.62	0.92
1-2 years	138	11.46±1.56	12.16±1.57	0.82	0.83	2.69	0.68
> 2 years	212	10.91±1.68	11.44±1.75	0.83	0.86	2.12	0.68
sick goats	40	11.64±2.36	12.04±2.72	0.85	0.98	0.63	0.72
Means	551	11.71	12.37				
Leukocyte counts							
1-30 day	49	7.71±2.43	7.62±2.58	0.94	1.00	-0.07	0.88
1-3 month	44	10.83±3.04	11.02±3.42	0.82	0.93	0.98	0.68
3-6 month	45	14.68±3.21	15.03±3.87	0.86	1.04	-0.21	0.74
6-12 month	23	16.22±3.54	15.85±4.23	0.89	1.06	-1.32	0.78
1-2 years	138	13.23±3.39	12.61±3.53	0.85	0.89	0.89	0.72
> 2 years	212	13.63±3.81	12.71±3.64	0.91	0.87	0.91	0.82
Sick goats	40	19.10±5.18	17.97±5.26	0.97	0.98	-0.81	0.94
Means	551	13.63	13.25				

E = Electronic, Corr= correlation coefficient, Regr= regression coefficient, Interc= intercept, R^2= coefficient of determination, *n*= number of goats.

Table 6: The minimum and maximum differences between the pairs and their means and standard deviations of hemocytometer and electronic counter RBC ($x10^{12}/l$) and WBC ($x10^{9}/l$) counts and the results of 't' test between them.

Group	Erythrocyte counts			Leukocyte counts		
	Hemocytometer minus Electronic			Electronic minus Hemocytometer		
	range	$\bar{x} \pm s$	t value	range	$\bar{x} \pm s$	t value
1-30 day	-1.01-2.12	0.70±0.71	6.87***	-2.55-1.65	0.09±0.91	0.71ns
1-3 month	-1.65-2.28	0.80±0.76	6.99***	-9.90-2.50	0.19±1.97	-0.63ns
3-6 month	-0.89-4.76	0.61±0.94	4.35***	-7.25-4.50	0.35±1.96	-1.20ns
6-12month	-1.40-3.34	0.83±1.16	3.40*	-4.16-3.40	0.36±1.98	0.88ns
12-24month	-1.20-2.51	0.70±0.93	3.07*	-3.70-3.90	0.62±1.89	1.36ns
> 24 month	-3.06-3.29	0.54±1.02	7.68***	-3.15-6.75	0.91±1.61	8.24***
sick goats	-3.83-3.33	0.41±1.43	1.80ns	-1.40-4.00	1.12±1.30	5.47***
Means	0.66			0.37		

Critical value of t = 1.99, t value for test *$p<0.05$, **$p<0.01$, ***$p<0.001$, ns= non significant.

Electronic WBC counts in cattle, are reported to vary from one counter to another. This brings some doubt on the reliability of the results (Strauss *et al.*, 1978). Hemocytometer counts may be more reliable (Halliday *et al.*, 1979) but electronic counters save time in blood analysis (Lorenz *et al.*, 1978; Mammerickx *et al.*, 1978b).

The number of erythrocytes and leukocytes in the various age groups of goats both counted electronically and microscopically were within the reported values in goats (Jain, 1986). In conclusion, goat erythrocyte hemocytometer counts are higher than those of electronic counters whereas the reverse is true for leukocytes. A dilution of 20 - 25 µl of blood to 10 ml diluent was optimum for hemocytometer erythrocyte counts and a 1:20 dilution is suitable for WBC counts in goats.

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CHAPTER 4

ERYTHROCYTE MATURATION IN NEONATAL KIDS

Summary

The morphology of erythrocytic cell series was investigated in external jugular vein blood samples from dwarf and Danish landrace goats aged from one day old to 12 months. Three erythrocytic cell types were observed in neonates after supravital staining with new methylene blue. The first were macrocytes which were stained uniformly dark to muddy blue. They formed the majority of erythrocytic cells at birth, and were categorized as diffusely basophilic chromatophilic erythrocytes. The second type were punctate and aggregated reticulocytes, and the third type were mature erythrocytes. The size ranges of the three erythrocytic cells were 4.2 - 5.6, 4.9 - 6.3 and 2.8 - 3.5 μm in diameter respectively in dwarf, and 5.6 - 9.7, 4.2 - 9.0 and 3.8 - 4.2 μm respectively in landrace kids during the first week. Romanowsky stained blood smears from neonatal kids were characterized by anisocytosis and poikilocytosis in which polychromatophilic macrocytes were numerous, but reticulocytes were not clearly identifiable. The range of erythrocyte diameters in Romanowsky stained neonatal blood films were 4.4 - 5.8 (5.2 $\pm$ 0.39) and 4.1 - 6.7 (5.1 $\pm$ 0.67) μm in dwarf and landrace kids respectively, decreasing to 3.0 $\pm$ 0.15 and 3.3 $\pm$ 0.13 μm in the two species respectively within 12 months of age. The diffusely basophilic polychromatophilic erythrocytes and punctate/aggregated reticulocytes diminished with age and were not observed in 1 - 2 months old kids.

Introduction

Studies in neonatal goats have revealed biphasic osmotic resistance fragiligrams of red blood cells (RBC), in that resistant RBC diminish and fragile RBC increase with age (Perk *et al.*, 1964a). The findings suggest that there is an early immature RBC population which is resistant to hemolysis and a late or mature osmotically fragile RBC group. The two RBC populations, however, have not been clearly distinguished on morphological examination of Romanowsky stained blood smears of newborn kids. Some erythropoietic cells are not identifiable by routine Romanowsky stains but only under special staining methods such as supravital (Jain, 1986) or cytochemical techniques (Hayhoe and Quaglino, 1989). Reticulocytes are the most immature circulating erythrocytic cells and are revealed after supravital staining with new methylene blue. They are not normally observed in the circulation of healthy animals

but appear in stimulated or accelerated erythropoiesis as in regenerative anaemia. Their appearance in the circulation constitute an important test for bone marrow response to anaemia, which can then be classified and identified for appropriate therapy. It is known that reticulocytosis due to anaemia occurs very rarely in adult goats (Dorr *et al.*, 1986; Dellmann and Brown, 1987). Evaluation of hemopoietic response to anaemia is consequently difficult, but examination of the morphology of neonatal RBC may provide a guide on erythropoiesis and enable assessment of the response to anaemia in adult goats. The aim of the present investigation was to identify morphological categories of early RBC in kids, and in particular their staining affinity for new methylene blue, on the basis of physiological properties (i. e. the reported osmotic resistance).

Materials and Methods

Blood samples were collected from the external jugular vein in vacuum tubes (Becton-Dickinson Vacutainer) containing 0.12ml of 0.34 mol/l tri-potassium ethylene diamine tetraacetate (K_3EDTA) from one to seven day old kids and thereafter every three weeks; after 8 weeks they were collected every 2 months. A total of 34 dwarf and 62 landrace kids were examined (table 1). A small amount of blood (100 μl) was stained supravitally with new methylene blue (0.5g new methylene blue, 1.6g potassium oxalate in 100 ml distilled water) within 1 - 2 hours of sampling by mixing in the ratio of 1:1. After interacting for 20 minutes at room temperature (25 ^{0}C), smears were prepared and examined. In these smears the percentage of punctate and aggregated reticulocytes were obtained after counting 1000 red blood cells. The absolute number was calculated from the RBC count (table 1).

The number of red blood cells in each sample was determined in model ZF coulter counter with an aperture diameter of 100 μm and corrected for compensation of coincident particle passages (Coulter electronics, England). The threshold was set at 6, attenuation 500 and the background counts kept at 200 maximum. The diameters of erythrocytes and other erythropoietic cells on supravitally stained blood smears were measured on photographs at 1440 x magnification. A minimum of 50 cells per smear of each animal were measured. Smears of whole blood stained with Leishman stain were also examined and the erythrocyte diameters measured. The median and mean reticulocyte counts and standard deviations were calculated, and t tests done for differences between the breeds (in same age kids) and ages (within the breeds).

Results

Three erythrocytic cell types were observed in the blood of kids, within the first 30

Table 1: The medians, mean ± standard deviation ($\bar{x} \pm s$) and ranges of reticulocyte counts (x10 ^{12}/L) in supravital stained, and $\bar{x} \pm s$ and ranges of RBC diameters (µm) in Romanowsky stained smears in kids.

Age	Dwarf			Landrace		
	female	male	both	female	male	both
0 - 7 days						
Reticulocytes						
median	0.85[a]	0.26[a]	0.40	1.49[b]	0.65[b]	0.75
$\bar{x} \pm s$	0.80 ± 0.40	0.5 ± 0.29	0.6 ± 0.36[*]	1.30 ± 0.69	0.90 ± 0.55	1.00 ± 0.56[*]
range	0.40 - 1.20	0.20 - 0.80	0.20 - 1.20	0.60 - 2.50	0.50 - 2.60	0.50 - 2.60
RBC diameters						
$\bar{x} \pm s$	5.20 ± 0.72	5.1 ± 0.06	5.20 ± 0.39	5.10 ± 0.88	5.10 ± 6.70	5.10 ± 0.67
range	4.40 - 5.80	5.0 - 5.10	4.40 - 5.80	4.10 - 5.80	4.10 - 6.70	4.10 - 6.70
n	3	6	9	5	13	18
7 - 30 days						
Reticulocytes						
median	0.20	0.12	0.18	0.29[c]	0.51[c]	0.40
$\bar{x} \pm s$	0.20±0.01[*]	0.1±0.05[*]	0.20± 0.05[**]	0.40 ± 0.33	0.40 ± 0.25	0.40 ± 0.24
range	0.20 - 0.30	0.10 - 0.20	0.1 - 0.30	0.20 - 0.80	0.00 - 0.90	0.00 - 1.00
RBC diameters						
$\bar{x} \pm s$	4.10 ± 0.23	3.7 ± 0.30	3.90 ± 0.33	4.00 ± 0.74	4.10 ± 0.69	3.90 ± 0.69
range	3.80 - 4.30	3.4 - 4.1	3.40 - 4.30	3.10 - 6.00	3.10 - 5.30	3.10 - 5.30
n	4	4	8	11	8	19
1 - 2 months						
Reticulocytes						
median	0	0	0	0.15[d]	0.27[d]	0.27
$\bar{x} \pm s$	0	0	0	0.30 ± 0.16	0.27 ± 0.13	0.26 ± 0.14
range	-	-	-	0.00 - 0.40	0.00 - 0.20	0.00 - 0.40
RBC diameters						
$\bar{x} \pm s$	3.60 ± 0.18	3.4 ± 0.11	3.50 ± 0.15	3.50 ± 0.36	3.70 ± 0.40	3.50 ± 0.36
range	3.40 - 3.80	3.30 - 3.50	3.30 - 3.80	3.10 - 4.10	3.40 - 4.10	3.10 - 4.10
n	4	5	9	6	10	16
Other ages	0 reticulocyte RBC diameter 3.00 ± 0.15 (n=34)				(n=62)	3.30 ± 0.13

Similar superscripts= unequal medians, n= number of kids, differences in means $^{*}p<0.05$, $^{**}p<0.01$.

days of life using supravital staining with new methylene blue. The first type were non nucleated young diffusely basophilic polychromatophilic erythrocytes, uniformly stained dark to muddy blue (fig. 1, cell 1). The cytoplasma of these cells were intermediate in characteristics between punctate/aggregated reticulocytes and the nucleated metarubricytes. They formed the majority of circulating cells in the first week. They were the largest in size (macrocytes), measuring 4.2 - 5.6 and 5.6 - 9.7 micrometers (μm) in diameter in dwarf and landrace kids respectively.

The second cell type had pale to normochromatic cytoplasm which contained deep blue stained dots, strings, clumps or aggregates and were 4.9 to 6.3 and 4.2 to 9.0 μm in diameters in dwarf and landrace kids respectively. The cells were categorized as punctate or aggregated (reticulated) reticulocytes (fig. 1, cells 2a and 2b). These cells, however, were very similar and a distinction between aggregated and punctate reticulocytes was not always possible. Punctate and aggregated reticulocytes were counted relative to mature erythrocytes and diffusely basophilic polychromatophilic erythrocytes, the means, standard deviations and medians are presented in table 1.

The third type of cells were pale and uniformly acidophilic mature erythrocytes, with less variability in size and shape which measured 2.8 to 3.5 and 3.8 to 4.2 μm in diameter in dwarf and landrace kids respectively. The hemoglobin in each cell was distributed evenly, leaving a central pale area (fig. 1, cell 3).

The number of punctate and aggregated reticulocytes was highest during the first week of life (table 1) particularly in the first 3 days. In both breeds there were no significant differences in mean reticulocyte counts between female and male kids in the first week of life. The median reticulocyte counts, however, were 0.85 x 10^{12}/l and 0.26x10^{12}/l in female and male dwarf kids respectively. The same trend was observed in landraces where the median reticulocyte count was 1.49 x 10^{12}/l in female compared with 0.65 x 10^{12}/l in male kids. The overall reticulocyte counts in female kids of both breeds were higher than in males (table 1). When the sexes were taken together the mean number of reticulocytes was lower in dwarf than in landrace kids ($p < 0.05$). In the second to fourth week of life the number of reticulocytes was higher in female than in male dwarf kids ($p < 0.05$) with no difference between means and medians. The median reticulocyte count was higher in male than in female landrace kids. The number of reticulocytes in dwarfs (both sexes) was lower than those of landrace kids ($p < 0.01$).

Both female and male dwarf kids were examined after the sixth week and reticulocytes were not observed. There were no significant differences between mean reticulocyte counts of female and male landrace kids of 1 - 2 months of age but the median count was higher in males. The median reticulocyte count for male kids was 0.27 x10^{12}/l compared with 0.15 x10^{12}/l in females kids.

Diffusely basophilic polychromatophilic erythrocytes and reticulocytes diminished

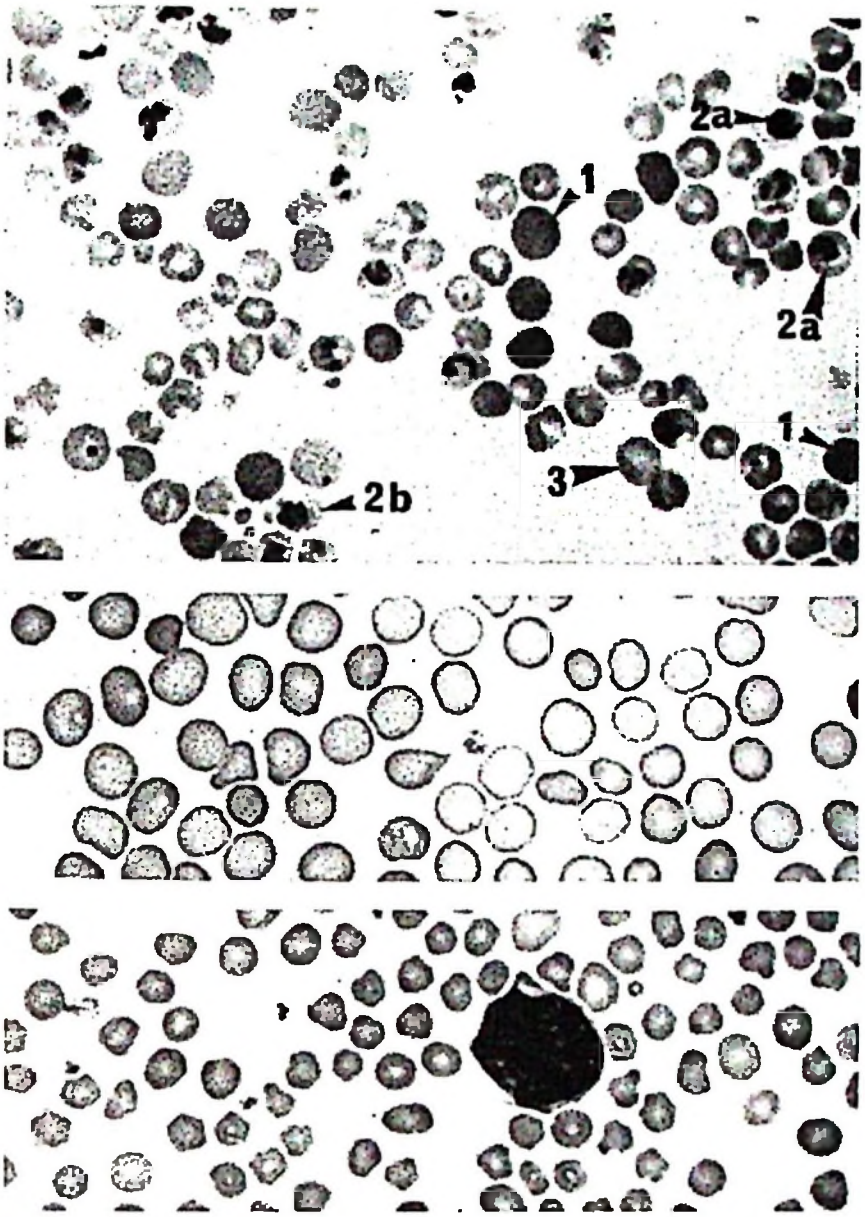

Figure 1: Smear of blood from 1 day old female dwarf kid supravitally stained with new methylene blue. Three types of erythrocytic cells are observed; diffusely basophilic polychromatophilic erythrocytes (1) which are the largest of the three; aggregated /punctate reticulocytes (2a/ 2b) and mature erythrocytes (3). Original magnification 1440 X.

Figure 2: Blood smear of a 3 day old male landrace kid showing the large erythrocytes at birth which decrease in size and increase in number with age. (Leishman stain, Original magnification 1440 X).

Figure 3: Blood smear of a 6 weeks old dwarf female kid showing the increased population of erythrocytes in a microscopic field and the reduced sizes. One large lymphocyte is present in the picture. (Leishman stain, original magnification 1440 X).

with age in and were not found in kids above 2 months oid (table 1). The intensity ofthe blue color of the cytoplasm in the latter decreased with age, eventually becoming identical to that of mature erythrocytes.

In blood smears from newborn kids stained with Romanowsky stains (figs. 2 and 3), punctate or aggregated reticulocytes could not be clearly distinguished, but polychromatophilic erythrocytes were identified as macrocytes, sometimes with fine intracytoplasmic granules circularly arranged in the cell periphery. Mature erythrocytes of normal size and macrocytes were observed.

Blood smears of newborn kids stained with Romanowsky stains therefore had marked polychromasia and anisocytosis. Mature erythrocytes were round and varied little in shape. RBC diameters in Romanowsky stained smears were 4.4 - 5.8 (5.2 ± 0.39) and 3.2 - 5.8 (4.7 ± 1.24) μm in neonatal dwarf and landrace kids respectively. Cells diminished in size with age to 2.7 - 3.1 (3.0 ± 0.15) and 3.2 - 3.4 (3.3 ± 0.13) μm in dwarf and landrace kids respectively (fig. 3), with non significant differences between them and between sexes.

In summary, three types of erythrocytic cells were observed in blood smears of newborn kids under supravital staining with new methylene blue; diffusely basophilic polychromatophilic erythrocytes which were macrocytes, punctate/aggregated reticulocytes and mature erythrocytes. The former two types of cells were observed from birth to 1 - 2 months of age. The erythroid patterns during development were similar in the two breeds of goats.

Discussion

The salient features of blood cells in newborn goats, are polychromasia, anisocytosis and reticulocytosis, most cells being macrocytes. Holman and Dew (1964) and Facello *et al.*, 1983) reported similar findings. Diffusely basophilic macrocytic polychromatophilic erythrocytes had the largest diameters followed by punctate and aggregated reticulocytes. The former were probably younger than the latter because in hematopoietic cell series young cells are larger than those in advanced stages of development (Jain, 1986; Dellmann and Brown, 1987). Furthermore, young cells have higher affinity for basic dyes than old ones, because they contain remains of nucleic acids (Laws, 1988; Weiss, 1988), thus stain more intensely with ordinary methylene and new methylene blue. The cytoplasm of diffusely basophilic polychromatophilic erythrocytes were stained intensely muddy blue, differing from the much less erythropoietically developed late polychromatic erythroblasts (metarubricytes) in that the former were non nucleated. Metarubricytes are the smallest of the nucleated erythrocytes, having small, highly condensed (pyknotic) nuclei which stain homogeneously deep purple with Romanowsky stains and is only found in active

hemopoietic tissues (Jain, 1986). These observations indicate that diffusely basophilic polychromatophilic erythrocytes were at a more advanced erythropoietic stage than metarubricytes. However, based on their size and cytoplasmic staining intensity they appeared to be less advanced than the punctate and aggregated reticulocytes. Judging from the morphological appearance it is probable that each type (diffusely basophilic polychromatophilic erythrocytes and punctate/aggregated reticulocytes) develops directly to a mature erythrocyte without changing to the form of the other (fig. 1).

The cytoplasm of punctate or aggregated reticulocytes was pale and contained remains of clumps and strings of reticulum (ribonucleoprotein) which stain deep blue with the supravital stain new methylene blue. These cells appear polychromatic in Romanowsky stained smears (Dellmann and Brown, 1987). They were probably at a more advanced erythropoietic stage than diffusely basophilic polychromatophilic erythrocytes because of their smaller in size and the normochromatic cytoplasm.

Vital stains cause aggregation of ribosomes and polyribosomes, thus forming a basis for identification of punctate/ aggregated reticulocytes (Jain, 1986). The homogeneous appearance of basophilic materials in the cytoplasm of diffuse basophilic polychromatophilic erythrocytes which changed with age to become normochromatic, with decrease in cell size may indicate that they matured to erythrocytes without passing through punctate or aggregated stages. In a regenerative anaemia, therefore, these cells may be dominant over punctate/aggregated reticulocytes. This appears to be the case for goats (see below). The distinction between punctate and aggregated reticulocytes, which is possible in dogs, is difficult or impossible in the goat (fig. 1).

Mature erythrocytes were small and as they do not contain any remains of ribonucleic acids, they were not stained with new methylene blue under supravital staining. The sizes of cells in female and male kids of both breeds were not significantly different.

The number of punctate and aggregated reticulocytes was highest at birth and gradually decreased in both dwarf and landrace kids. The reticulocyte counts given (table 1) are based on smears, that may be subject to errors. However, it appears that there are breed and sex influence on reticulocyte numbers in kids. An accurate method of reticulocyte counting by flow cytometry has been developed (Nobes and Carter, 1990) and might give further information.

In newborn kids, the diffusely basophilic polychromatophilic erythrocytes were more frequent than punctate and aggregated reticulocytes. The staining intensity and more frequent occurrence of diffusely basophilic cells in the circulation of kids suggest that they are probably more indicative of a regenerative response in goats than punctate or aggregated reticulocytes. The characteristic morphology of diffusely basophilic polychromatophilic erythrocytes appears to resemble that of cells observed by Dorr *et al.* (1986) in blood smears from adult goats recovering from severe

hemorrhage which were supravitally stained with new methylene blue. Punctate or aggregated reticulocytes were rarely observed in these smears.

The two erythrocytes types recognizable by osmotic fragility tests (Facello *et al.*, 1985) could probably correspond to the diffusely basophilic polychromatophilic erythrocytes and punctate or aggregated reticulocytes on one hand and mature erythrocytes on the other. The foetal and adult hemoglobins observed by Perk *et al.* (1964b) may possibly be components in these RBC types. Erythrocyte volumes vary with the type of hemoglobin they contain (Facello *et al.*, 1984). Furthermore, the reported decrease of hemoglobin, hematocrit, mean corpuscular hemoglobin and RBC count within two weeks of birth (Holman and Dew, 1964; Edjtehadi, 1978; Kanemaki *et al.*, 1986; Bialkowski *et al.*, 1988) is probably due to decrease of diffusely basophilic polychromatophilic erythrocytes and reticulocytes, which contain varying types and levels of hemoglobin.

In conclusion it was observed that polychromatophilic erythrocytes and punctate or aggregated reticulocytes are numerous in newborn kids. Both erythropoietic cell types circulate for a variable postnatal period, probably because of the demand for adequate oxygen transport until sufficient numbers of mature erythrocytes are present in the circulation. The erythroid pattern was identical in the two breeds of goats. These characteristics of blood cells of young goats may be useful comparative indices in erythropoiesis and of value in the evaluation of regenerative anaemia in adult goats where aggregated or punctate reticulocytes are normally not observed.

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CHAPTER 5

HEMATOLOGICAL PROFILE IN NEONATAL KIDS

Summary

The hematological profile in neonatal dwarf and Danish landrace kids was investigated in clinically healthy 62 Danish landrace and clinically healthy 34 dwarf kids from birth to 12 months of age in 7 herds. The objective was to determine the reference values in the breeds, and evaluate the influence of age and environment on hematological parameters. Parametric (means and standard deviations) and nonparametric (5^{th}, 95^{th} percentile, median) values were calculated for each analyte. Results of the two statistical methods were close to each other. The number of erythrocytes in both breeds increased with age from neonatal minimum values. Hemoglobin concentration and hematocrit decreased from the neonatal values in the following two weeks of life, whereafter they increased. Hemoglobin concentration and mean corpuscular volumes were significantly higher in neonatal dwarf than in landrace kids. Differences in erythrocyte counts and hematocrit were observed with subsequent growth. There were significant differences in erythrocyte counts, hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin, between breeds (in kids of similar age), age groups (within the breeds) and herds (in kids of similar age within the breeds). Statistically significant differences in hematological values between female and male kids of similar young ages within the same breed were not observed. Age, breed and environment influenced the level of hematological parameters in kids.

Introduction

Hematological and clinical chemical analysis of blood is an indispensable tool in the diagnosis of a variety of diseases (Lloyd, 1982; Sherman and Robinson, 1983). Hematological parameters are, however, influenced by breed, seasons and physiological states according to Vrzgula *et al.* (1985), Hassan *et al.* (1986), Wojcik *et al.* (1986) and Pospisil *et al.* (1987) and the different sources reveal tremendous disparity in relation to these factors. Furthermore, neonatal hematology in goats is not well understood because the reported values from the few investigations on kids are inconsistent and not in agreement with each other. Holman and Dew (1964) observed increasing erythrocyte counts, hemoglobin concentrations and packed cell volumes (hematocrit), but decreasing mean corpuscular volume, and thereby erythrocyte

diameters in growing goats with age. Mean corpuscular hemoglobin concentration fluctuated little during growth. However, Somvanshi *et al.* (1987) observed higher values in young kids than in old goats, similar to the findings by Nangia *et al.* (1968) and Wojcik *et al.* (1986) where the erythrocyte count, hemoglobin and hematocrit from birth to 6 months of age were higher than in old goats. These contradictive results complicate the evaluation of clinical chemical/hematological values for diagnostic purposes in different goat breeds. Studies on erythrocyte profiles for reference in kids from birth to one year of age specifying the breed and sex was therefore found necessary. It has been a common approach to express hematological data of clinically healthy animals in terms of mean ± standard deviation. In normally distributed data (Gaussian) two standard deviations above and below the mean includes about 95 % of the values. This approach has been criticized for the reason that the values of many blood parameters do not follow a Gaussian distribution (Reed *et al.*, 1971; Wu *et al.*, 1975). The present investigation was to determine hematological profile in young kids and the influence of environment, age, sex and breed in dwarf and landrace goats by using both parametric and nonparametric statistical methods.

Materials and Methods

Kids from seven Danish herds (A - G) located at The Royal Veterinary and Agricultural University (A), Fakse (B), Næstved (C), Ringsted (D), Haslev (E), Fugleberg (F) and Copenhagen Zoo (G) were used for this study. In all the farms kids naturally sucked milk from their mothers until weaning. The kids were gradually introduced to grass pellets and oats or natural grazing during the growth period. Goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms B to E in summer. In farms F and G goats were reared in the field for the entire summer and autumn.

Blood samples were collected from the external jugular vein in vacuum tubes (Becton-Dickinson Vacutainer) containing 0.12 ml of 0.34 mol/l tripotassium ethylene diamino tetraacetate (K_3EDTA) from 62 clinically healthy Danish landrace and 34 dwarf goats one day old to 12 months of age. Blood samples were collected every 3 weeks and after 8 weeks of age every 2 months (table 1) to make divisions of six age groups (0 - 7 days (1), 7 -30 days (2), 1 - 2 (3), 2 - 4 (4), 4 - 8 (5) and 8 - 12 (6) months old).

Red blood cell (RBC) counts were determined within 1 - 2 hours of sampling using a Coulter counter model ZF with an aperture diameter of 100 μm, set at threshold 6 and attenuation 500. The background counts were kept at 200 maximum and the instrument adjusted for compensation of coincident passages. Hemoglobin (Hb) concentration was determined in model S 560 Coulter counter (Coulter electronics,

England). Packed cell volume (PCV) was determined in microhematocrit capillary tubes centrifuged at 12,000 rpm for 5 minutes in a microhematocrit centrifuge (Clay-Adams). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by standard formulas. RBC diameters were measured on photographs at 1440 x magnification.

The parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentile, median) values were determined by the univariate procedure of a statistical analysis software (SAS, Carry USA, 1988). The coefficient of skewness, the degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte to the Gaussian distribution. The means of hematological values were tested for differences between kids in various age groups (within the breeds), breeds (in kids of similar age), herds (in kids of similar age, within the breeds) and sex (within the breeds) by using the general linear models procedure, a special method for analysis of variance for unbalanced data.

Results

The mean and median values of erythrocyte counts, hematocrit, hemoglobin concentration, MCV, MCH and MCHC in each age group and breed were close to each other (table 1 - 5). The coefficients of skewness and kurtosis were small. The Shapiro Wilk statistic (W) indicated that nearly all frequency distributions of hematological values followed a Gaussian distribution ($0.98 \leq W \leq 1$; table 1). Only in a few age groups were there any significant deviations from Gaussian distributions. The 5^{th} and 95^{th} percentile intervals were within mean ± 2 standard deviations in most age groups of both dwarf and landrace kids (tables 1-5).

The mean ± standard deviation of erythrocyte (RBC) counts in neonatal dwarf kids was 7.9 ± 0.9 x 10^{12}/l with a median count of 8.1x10^{12}/l (table 2). In landrace kids they were 8.8 ± 1.6 and 8.8 x10^{12}/l for mean ± standard deviation and median respectively (table 4) and were not significantly different from those of dwarf goats. RBC counts in dwarf kids decreased to significantly lower levels than of landrace kids during the second week (fig. 1, table 2 and 4). RBC counts increased thereafter with age to 16.3 ± 1.0 and 15.4 ± 2.7 x 10^{12}/l in the two breeds respectively at 8 - 12 months of age with significant differences at some ages. In dwarf goats RBC counts were slightly higher in males than in females but not significantly. There were significant differences between birth values and other ages in both breeds (fig. 1a-b)[1]

[1]Only significant differences between age group 1 and others are shown in figures, not among other groups.

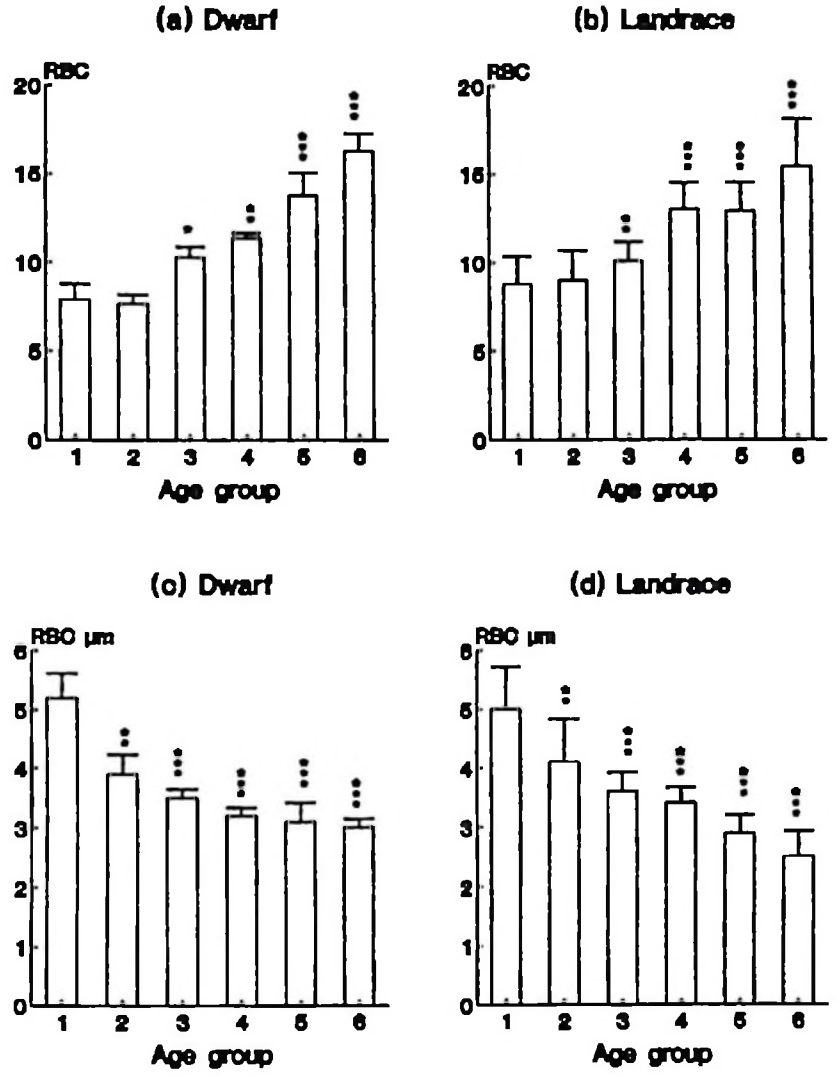

Figure 1: The mean number of RBC x10^{12}/l (a) and (b), erythrocyte diameters µm (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). Significant differences from values of group 1 kids $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

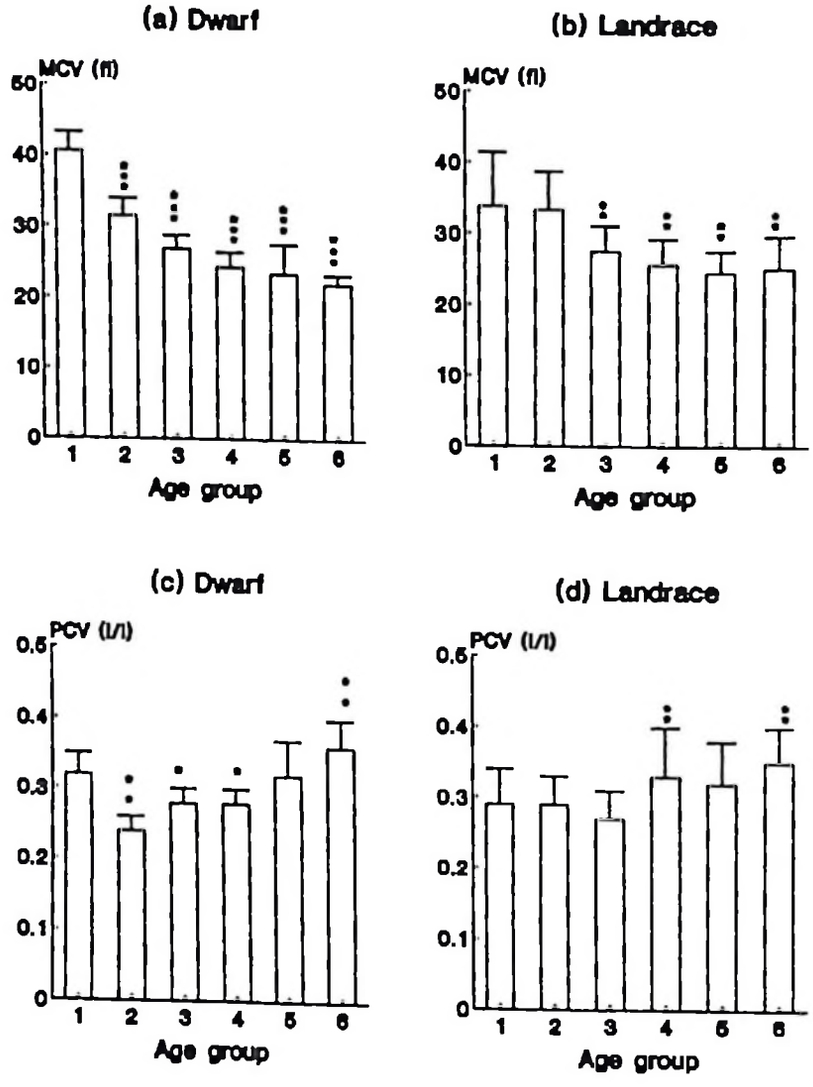

Figure 2: MCV fl (a) and (b), and hematocrit (PCV) (c) and (d) values with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). Significant differences from values of group 1 kids $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

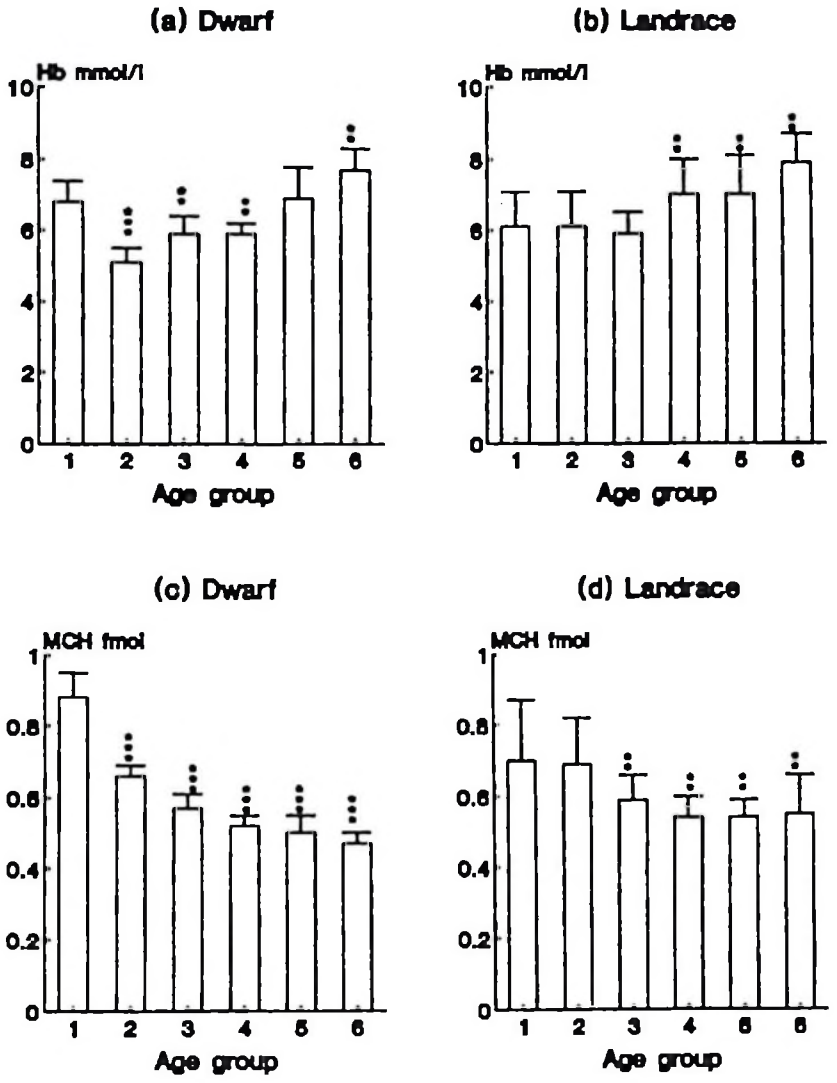

Figure 3: Blood concentration of hemoglobin mmol/l (a) and (b), and MCH fmol (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). Significant differences from values of group 1 kids $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

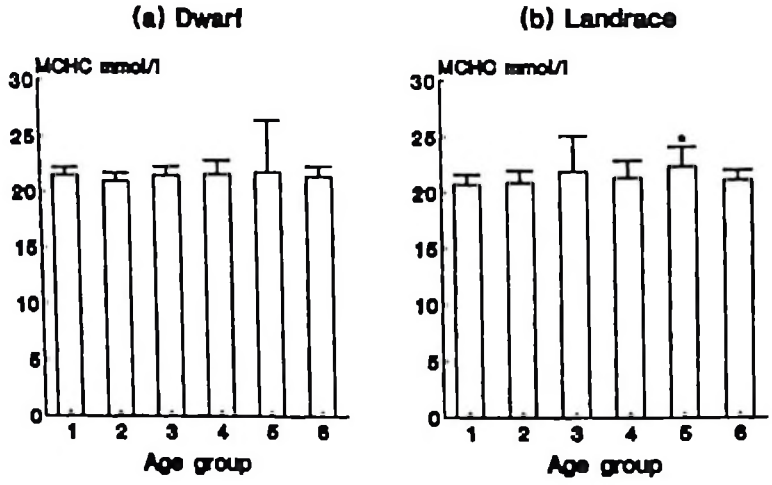

Figure 4: MCHC mmol/l values in growing dwarf (a) and landrace (b) with one standard deviation (bars) in dwarf and landrace kids, groups 1-6, ages as in figures 1-3. Significant differences from values of group 1 kids $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

and also between other age groups (2/4*[2], 2/5**, 2/6***, 3/4*, 3/5*, 3/6***, 4/5*, 4/6** and 5/6* for dwarf and 2/4*, 2/5*, 2/6**, 3/4*, 3/5*, 3/6**, 4/6* and 5/6* in landrace kids).

Erythrocytes in neonates were 4.4 - 5.8 (5.2 ± 0.4) and 4.1 - 6.7 (5.0 ± 0.7) µm in diameter in dwarf and landrace kids respectively and decreased to 2.7 - 3.1 (3.0 ± 0.15) and 2.1 - 3.4 (2.5 ± 0.43) µm respectively within 12 months without any significant difference between them. Erythrocyte diameters were inversely proportional to RBC counts but directly MCV (fig. 1-2). There were significant differences between birth values and other ages in both breeds (fig. 1c-d) and also between other age groups (2/3*, 2/4*, 2/5*, 2/6***, 3/5* and 3/6* in dwarf and 2/3*, 2/4*, 2/5*, 2/6***, 3/5*, 3/6*, 4/5* and 4/6* in landrace kids).

The MCV were highest at birth decreasing from mean neonatal values of 40.6 ± 2.7 and 33.9 ± 7.5 fl in dwarf and landrace kids respectively to 22.0 ± 1.4 and 25.0 ± 4.6 fl at 8 - 12 months of age in the two breeds respectively (fig. 2a-b, table 3 and 5).

[2] $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Significant differences were observed between the two breeds in the first, sixth and 12th week of age. There were significant differences between birth values and other ages in both breeds (fig. 2a-b) and also between other age groups (2/3*, 2/4*, 2/5*, 2/6**, 3/5* and 3/6* in dwarf and 2/3*, 2/4*, 2/5*, 2/6*** and 3/5* in landrace kids). There were no significant sex differences in MCV.

Hematocrit (PCV) was 0.32 ± 0.03 and 0.29 ± 0.05 l/l (table 2 and 4) in dwarf and landrace kids respectively at birth followed by a decrease in the first two weeks of life (fig. 2c-d). There was then an increase to 0.36 ± 0.04 and 0.35 ± 0.05 l/l (table 1 - 3) in dwarf and landrace breeds respectively at 8 - 12 months of age. Hematocrit was higher in dwarf than in landrace kids at birth, later significant differences were observed at some ages. There were significant differences between birth values and other ages in both breeds (fig. 2c-d) and also between other age groups (2/4*, 2/5*, 2/6***, 3/5*, 3/6*, 4/6* and 5/6* in dwarf and 2/4*, 2/6*, 3/4*, 3/5*, 3/6*, 4/6* and 5/6* in landrace kids).

Hemoglobin concentrations at birth were 6.8 ± 0.6 and 6.1 ± 1.0 mmol/l in dwarf and landrace kids respectively being significantly higher in the former than in the latter. Values decreased to 5.1 ± 0.4 and 5.9 ± 0.6 mmol/l in the second and fourth week of life in dwarf and landrace kids respectively (fig. 3a-b). Hemoglobin then increased to 7.7 ± 0.6 and 7.9 ± 0.8 mmol/l in dwarf and landrace kids respectively at 8 - 12 months of age, being significantly lower in dwarf than in landrace goats younger than 4 months of age (table 2 and 4). The differences between sexes were not significant. There were significant differences between birth values and other ages in both breeds (fig. 3a-b) and also between other age groups (2/4*, 2/5*, 2/6***, 3/5**, 3/6*, 4/5* and 5/6* in dwarf and 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 3/6**, 4/6* and 5/6* in landrace kids).

MCH was significantly higher in one day old dwarf kids at 0.88 ± 0.07, median = 0.89 fmol compared with 0.70 ± 0.17, median = 0.66 fmol in landrace kids (table 3 and 5). MCH decreased to 0.66 ± 0.28 and 0.69 ± 0.13 fmol within 2 weeks of life (fig. 3c-d) and further to 0.47 ± 0.03 and 0.53 ± 0.09 at 8 -12 months of age. The MCH was higher in neonatal dwarf than in landrace kids ($p < 0.001$) without further significant differences in subsequent ages, neither between female and male goats of similar ages within breeds. There were significant differences between birth values and other ages in both breeds (fig. 3c-d) and also between other age groups (2/3*, 2/4*, 2/5**, 2/6**, 3/4*, 3/5*, 3/6* and 4/6 in dwarf and 2/3*, 2/4*, 2/5*, 2/6**, 3/4* and 3/5* in landrace kids).

MCHC were 21.5 ± 0.7 and 20.7 ± 0.9 mmol/l (table 3 and 5) in dwarf and landrace kids respectively at birth significantly higher in the former than in the latter. The changes with age in both breeds were minor (fig. 4a-b). At 8 - 12 months of age the values were 21.4 ± 1.0 and 21.1 ± 0.9 mmol/l (table 3 and 5) in dwarf and

landrace kids respectively and breed differences were significant at some ages but not between sexes. Significant differences were observed between age groups 1 and 5 (fig. 4b), 2 and 5, and 4 and 5 in landrace kids.

The erythrocyte counts and diameters, PCV, hemoglobin concentration, MCV, MCH and MCHC of dwarf and landrace kids were found to be influenced by age, breeds and environment. Significant differences were observed between kids of different ages within breeds (fig. 1 - 4), between the breeds within similar ages (table 2 - 5) and between herds in similar ages within a breed (table 6).

In both breeds, sexes and all ages RBC counts were negatively correlated to MCH ($r=-0.694$) and MCV ($r=0.661$) but positively to hemoglobin ($r=0.655$) and hematocrit ($r=0.571$). The hemoglobin was correlated to the MCH ($r=0.922$) and hematocrit ($r=0.871$). The MCV was correlated to RBC diameter ($r=0.681$). Finally there was a negative correlation between MCHC and hematocrit ($r=-0.393$,) and MCV ($r=-0.371$).

Discussion

The mean, a parametric central measure of continuous numeric data is commonly used in hematological data. The median and mode are nonparametric, equal or unequal to the mean, depending on dispersion of observations. The mean ± standard deviation ($\bar{x} \pm s$) is used together with the range (interval from the minimum to the maximum) or the 2.5^{th} to 97.5^{th} or 5^{th} to 95^{th} percentile intervals (Zinkl *et al.*, 1990).

In the present investigation parametric and nonparametric ranges were not significantly different for RBC count, PCV, Hemoglobin concentration, MCV, MCH and MCHC in most age groups in both breeds. All $\bar{x} \pm s$ values in all age groups were within the 5^{th} to 95^{th} percentile interval (table 2 - 5). The 5^{th} to 95^{th} intervals were within the $\bar{x} \pm 2s$ range. The former including 90 % of the observations while the latter about 95 %. The data in most parameters followed a Gaussian distribution and the medians were very close to the means (table 2 - 5). Only a few significantly deviated from Gaussian (table 1). The conditions for the powerful parametric tests were therefore fulfilled (Siegel and Catellan, 1988) as earlier observed (Barakat and El-Guindi, 1967).

There were extensive changes in RBC counts, PCV and hemoglobin in kids during the first four weeks of life, showing increasing profile from minimum values in neonates (figs. 1-4, table 1-5). Erythrocyte diameters (fig. 1c-d), MCV (fig. 2a-b) and MCH (fig. 3c-d) showed a decreasing trend, while MCHC (fig. 4) changed little with growth. After three months, the changes were gradual. The blood picture in neonatal dwarf and landrace kids was characterized by low erythrocytes counts. The sizes of cells decreased with age, so that RBC counts were invesely related to cell volume.

Table 1; The 5th (P_5) to 95th (P_{95}) percentile interval of RBC counts, hemoglobin (Hb), PCV, MCV, MCH and MCHC (mmol/l) in growing Dwarf and Landrace kids.

Age	*n*	RBC x10^{12}/l	Hb mmol/l	PCV l/l	MCV fl	MCH fmol	MCHC
Dwarf							
0 - 7 day	9	6.02 - 8.94	5.40 - 7.60	0.26 - 0.35	34.7 - 43.2	0.72 - 0.99	20.6 - 22.6
7-30 day	8	6.93 - 8.37	4.60 - 5.60	0.22 - 0.29	29.6-37.5^{W}	0.63-0.72^{W}	19.3-21.6^{W}
1-2 month	9	9.06 - 11.0	5.20 - 6.60	0.25 - 0.31	25.1 - 30.9	0.52 - 0.63	20.4 - 22.7
2-4 month	10	10.8 - 11.7	5.50 - 6.40	0.23 - 0.30	19.6 - 27.2	0.47 - 0.57	20.0 - 23.9
4-8 month	17	10.9 - 16.3	5.10 - 8.40	0.22 - 0.38	15.7 - 34.9	0.41 - 0.56	13.4-38.2^{W}
8-12 month	7	15.3 - 18.0	6.50 - 8.20	0.30 - 0.40	19.4 - 23.8	0.42 - 0.50	20.5 - 23.0
Landrace							
0-7 day	18	5.56 - 11.6	4.30 - 8.00	0.20 - 0.39	25.1 - 47.8	0.50 - 1.00	17.9 - 21.8
7-30 day	20	5.92 - 12.4	4.40 - 7.90	0.22 - 0.37	23.3 - 42.1	0.43 - 0.92	18.8 - 22.9
1-2 month	16	8.38 - 12.1	4.70 - 6.80	0.18 - 0.38	19.6 - 33.8	0.46 - 0.70	20.0-33.3^{W}
2-4 month	62	10.5 - 15.1	5.40 - 8.50	0.25 - 0.43	20.8-33.3^{W}	0.46 - 0.65	17.6-23.1^{W}
4-8 month	44	10.2 - 15.4	5.30 - 8.90	0.23 - 0.41	20.1 - 29.5	0.47 - 0.62	19.7 - 24.6
8-12 month	13	10.5 - 20.6	7.00 - 9.30	0.32 - 0.45	16.5 - 33.9	0.38 - 0.72	19.6 - 23.4

W = Non *Gaussian* distributions ($p<0.05$), *n* = number of goats.

Table 2: Sex and age specific median and mean ± standard deviation ($\bar{x} \pm s$) values of RBC counts, hemoglobin (Hb) and PCV in Dwarf goats.

Age	*n*	RBC x10^{12}/l		Hb mmol/l		PCV l/l	
		$\bar{x} \pm s$	median	$\bar{x} \pm s$	median	$\bar{x} \pm s$	median
Both sex							
0-7 day	9	7.9 ± 0.90	8.10	6.80 ± 0.60*	7.00	0.32 ± 0.03	0.32
7-30 day	8	7.7 ± 0.50	7.80	5.10 ± 0.40*	5.10	0.24 ± 0.02	0.24
1-2 month	9	10.3 ± 0.60	10.3	5.90 ± 0.50	5.90	0.28 ± 0.02	0.27
2-4 month	10	11.4±0.30**	11.30	5.90± 0.30**	5.90	0.28 ± 0.02	0.28
4-8 month	17	13.8±1.30*	13.80	6.90 ± 0.90	6.70	0.32 ± 0.05	0.32
8-12 month	7	16.3 ± 1.00	16.0	7.70 ± 0.60	7.70	0.36 ± 0.04	0.37
female							
0-7 day	3	7.3 ± 1.10	7.70	6.60 ± 1.00	6.90	0.30 ± 0.04	0.31
7-30 day	4	7.6 ± 0.60	7.60	5.10 ± 0.50	5.10	0.25 ± 0.03	0.24
1-2 month	4	10.2 ± 0.70	10.40	6.10 ± 0.40	6.00	0.28 ± 0.02	0.28
2-4 month	5	11.3 ± 0.40	11.20	6.00 ± 0.30	6.00	0.28 ± 0.01	0.27
male							
0-7 day	6	8.2 ± 0.63	8.30	7.00 ± 0.40	7.10	0.33 ± 0.02	0.33
7-30 day	4	7.8 ± 0.58	8.00	5.10 ± 0.30	5.10	0.24 ± 0.01	0.24
1-2 month	5	10.3 ± 0.40	10.10	5.80 ± 0.50	5.80	0.27 ± 0.02	0.27
2-4 month	5	11.4 ± 0.30	11.40	5.80 ± 0.20	5.80	0.27 ± 0.03	0.28
4-8 month	17	13.8 ± 1.30	13.80	6.90 ± 0.90	6.70	0.32 ± 0.05	0.32
8-12 month	7	16.3 ± 1.00	16.00	7.70 ± 0.60	7.70	0.36 ± 0.04	0.37

n = number of goats, *$p<0.05$, **$p<0.01$, significant differences from same age landrace kids in table 4.

Table 3: Sex and age specific median and mean ± standard deviation ($\bar{x} \pm s$) values of MCV, MCH and MCHC in Dwarf goats.

Age	*n*	MCV fl		MCH fmol		MCHC	mmol/l
		$\bar{x} \pm s$	median	$\bar{x} \pm s$	median	$\bar{x} \pm s$	median
Both sex							
0-7 day	9	40.6 ± 2.70*	40.8	0.88 ± 0.07	0.89	21.5 ± 0.70	21.5
7-30 day	8	31.5 ± 2.50	30.9	0.66 ± 0.03	0.66	21.0 ± 0.70	21.1
1-2 month	9	26.9 ± 1.90	26.2	0.57 ± 0.04	0.57	21.5 ± 0.80	21.5
2-4 month	10	24.3 ± 2.10	24.5	0.52 ± 0.03	0.52	21.6 ± 1.30	21.1
4-8 month	17	23.5 ± 4.10	23.9	0.50 ± 0.05	0.49	21.8 ± 4.70	21.0
8-12 month	7	22.0 ± 1.40	21.6	0.47 ± 0.03	0.48	21.4 ± 0.90	21.0
female							
0-7 day	3	41.9 ± 1.60	42.4	0.93 ± 0.05	0.90	21.5 ± 0.90	21.2
7-30 day	4	32.5 ± 3.40	31.2	0.67 ± 0.03	0.65	20.7 ± 1.00	20.9
1-2 month	4	27.7 ± 2.50	27.3	0.60 ± 0.04	0.59	21.7 ± 1.00	21.9
2-4 month	5	24.6 ± 1.00	24.8	0.53 ± 0.04	0.54	21.7 ± 1.10	21.5
male							
0-7 day	6	39.9 ± 3.00	40.8	0.86 ± 0.07	0.88	21.4 ± 0.70	21.6
7-30 day	4	30.6 ± 0.90	30.6	0.65 ± 0.02	0.65	21.3 ± 0.30	21.3
1-2 month	5	26.3 ± 1.30	25.7	0.56 ± 0.04	0.54	21.2 ± 0.60	21.0
2-4 month	5	23.9 ± 2.90	24.2	0.51 ± 0.03	0.52	21.6 ± 1.60	20.7
4-8 month	17	23.5 ± 4.10	23.9	0.50 ± 0.05	0.49	21.8 ± 4.70	21.0
8-12 month	7	22.0 ± 1.40	21.6	0.47 ± 0.03	0.48	21.4 ± 0.90	21.0

n = number of goats, $^{*}p<0.05$, $^{**}p<0.01$, significant differences from same age landrace kids in table 5.

Table 4: Sex and age specific median and mean ± standard deviation ($\bar{x} \pm s$) values of RBC counts, hemoglobin (Hb) and PCV in Landrace goats.

Age	*n*	RBC x10^{12}/l		Hb mmol/l		PCV l/l	
		$\bar{x} \pm s$	median	$\bar{x} \pm s$	median	$\bar{x} \pm s$	median
Both sex							
0-7 day	18	8.80 ± 1.60	8.80	6.10 ± 1.00*	6.00	0.29 ± 0.05	0.29
7-30 day	20	9.00 ± 1.70*	8.80	6.10 ± 1.00*	6.10	0.29 ± 0.04***	0.29
1-2 month	16	10.1 ± 1.10	10.2	5.90 ± 0.60	5.90	0.27 ± 0.04*	0.28
2-4 month	62	13.0±1.50***	13.1	7.00± 1.00**	7.00	0.33 ± 0.06**	0.33
4-8 month	44	12.9±1.60*	12.8	7.00 ± 1.10	6.80	0.32 ± 0.06	0.31
8-12 month	13	15.4 ± 2.70	15.3	7.90 ± 0.80	7.90	0.35 ± 0.05	0.35
female							
0-7 day	5	9.50 ± 1.20	9.00	5.70 ± 0.30	5.80	0.29 ± 0.01	0.29
7-30 day	12	9.16 ± 1.80	8.80	6.20 ± 1.10	6.00	0.30 ± 0.04	0.30
1-2 month	6	9.80 ± 1.30	9.90	6.00 ± 0.80	6.10	0.29 ± 0.04	0.29
2-4 month	41	13.2 ± 1.50	13.3	7.20 ± 1.10	7.20	0.34 ± 0.06	0.34
4-8 month	38	12.8 ± 1.60	12.6	6.90 ± 1.10	6.80	0.31 ± 0.07	0.31
8-12 month	13	15.0 ± 2.40	15.2	7.90 ± 0.80	7.80	0.38 ± 0.05	0.37
male							
0-7 day	13	8.57 ± 1.60	8.20	6.20 ± 1.20	6.20	0.29 ± 0.06	0.29
7-30 day	8	8.61 ± 1.80	9.00	6.00 ± 1.00	6.00	0.28 ± 0.05	0.28
1-2 month	10	10.2 ± 1.00	10.2	5.90 ± 0.50	5.90	0.27 ± 0.04	0.28
2-4 month	21	12.7 ± 1.50	12.5	6.80 ± 0.90	6.70	0.32 ± 0.05	0.31
4-8 month	6	13.5 ± 1.00	13.5	7.50 ± 0.90	7.30	0.36 ± 0.04	0.36

n = number of goats, *$p<0.05$, **$p<0.01$, significant differences from same age Dwarf kids in table 2.

Table 5: Sex and age specific median and mean ± standard deviation ($\bar{x} \pm s$) values of MCV, MCH and MCHC in Landrace goats.

Age	*n*	MCV fl		MCH fmol		MCHC	mmol/l
		$\bar{x} \pm s$	median	$\bar{x} \pm s$	median	$\bar{x} \pm s$	median
Both sex							
0-7 day	18	33.9 ± 7.50**	32.3	0.70 ± 0.17	0.66	20.7 ± 0.90*	21.0
7-30 day	20	33.3 ± 5.40	33.3	0.69 ± 0.13	0.69	20.9 ± 1.10	21.0
1-2 month	16	27.4 ± 3.70	28.0	0.59 ± 0.07	0.60	21.9 ± 3.20	20.9
2-4 month	62	25.6 ± 3.70	25.2	0.54 ± 0.06	0.54	21.3 ± 1.60	21.6
4-8 month	44	24.4 ± 3.10	24.6	0.54 ± 0.05	0.54	22.3 ± 1.80	22.1
8-12 month	13	25.0 ± 4.60	26.0	0.55 ± 0.11	0.56	21.1 ± 0.90	21.1
female							
0-7 day	5	30.4 ± 3.40	32.0	0.61 ± 0.08	0.62	20.1 ± 1.50	20.0
7-30 day	12	33.3 ± 4.10	34.3	0.68 ± 0.10	0.68	20.6 ± 1.00	20.9
1-2 month	6	29.2 ± 3.50	29.4	0.62 ± 0.06	0.62	21.1 ± 0.90	20.9
2-4 month	41	25.9 ± 3.60	26.0	0.54 ± 0.05	0.55	21.2 ± 1.80	21.7
4-8 month	38	24.1 ± 3.20	23.7	0.54 ± 0.05	0.53	22.4 ± 1.90	22.7
8-12 month	13	25.7 ± 4.00	26.8	0.55 ± 0.11	0.56	20.9 ± 0.70	21.0
male							
0-7 day	13	35.2 ± 8.30	33.1	0.74 ± 0.18	0.69	21.0 ± 0.50	21.1
7-30 day	8	33.8 ± 7.10	33.5	0.71 ± 0.17	0.70	21.1 ± 1.30	20.9
1-2 month	10	26.2 ± 3.50	27.4	0.58 ± 0.07	0.60	22.3 ± 4.00	21.0
2-4 month	21	25.0 ± 4.00	23.3	0.54 ± 0.07	0.50	21.5 ± 1.30	21.5
4-8 month	6	26.3 ± 1.50	26.2	0.56 ± 0.04	0.57	21.0 ± 1.30	21.6

n = number of goats, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, significant differences from same age Dwarf kids in table 3.

Table 6: Environmental influence on RBC, PCV, MCV, MCH, MCHC and hemoglobin concentration in landrace kids (means ± standard deviations).

Age	*Hn*	RBC x10^{12}/l	Hb mmol/l	PCV l/l	MCV fl	MCH fmol	MCHC[1]
0-7 day	B14	9.40 ± 1.10*	5.80 ± 0.90	0.28 ± 0.04	30.3±3.10**	0.62± 0.07*	20.6±1.00
	C4	6.90 ± 1.40*	6.80 ± 1.20	0.32 ± 0.06	46.5±1.40**	0.98±0.03**	21.2±0.50
	G9	7.90 ± 0.90	6.80 ± 0.60	0.32 ± 0.03	40.6± 2.70	0.88±0.07	21.5±0.70
7-30 day	B13	9.50 ± 1.70^{a*}	6.20 ± 1.10	0.29 ± 0.04	31.4±4.30	0.66±0.1^{ab*}	21.0±0.80
	C2	5.90 ± 0.30^{ab*}	5.50 ± 0.30	0.25 ± 0.03	42.2±2.90	0.9±0.01^{abc*}	22.1±1.40*
	F5	9.00 ± 0.70^{b*}	6.20 ± 1.00	0.31 ± 0.04	34.6±5.20	0.79±0.14^{c*}	20.1±1.30*
	G9	7.70 ± 0.50	5.10 ± 0.40	0.24 ± 0.02	31.5±2.50	0.66±0.03	21.0±0.70
1-2 month	B16	10.1 ± 1.10	5.90 ± 0.60	0.27 ± 0.04	27.4±3.70	0.59±0.07	21.9±3.20
	G9	10.3 ± 0.60	5.90 ± 0.50	0.28 ± 0.02	26.9±1.90	0.57±0.04	21.5±0.80
2-4 month	B42	13.2 ± 1.30	7.00 ± 1.10	0.32± 0.05*	24.2±2.3^{ab*}	0.53±0.05^{a*}	21.8±0.8^{abc*}
	C1	13.9	7.80	0.35	25.1	0.56	22.3
	D7	12.3 ± 2.40	7.50 ± 0.90	0.34 ± 0.06	28.4±3.90^{b*}	0.62±0.1^{ab*}	22.0±1.6^{b*}
	E2	12.2 ± 0.40	7.20 ± 0.40	0.38 ± 0.06	31.2±3.60^{b*}	0.58±0.01	19.0±1.9^{bc*}
	F10	12.8 ± 1.60	6.80 ± 0.90	0.36±0.08*	28.6±5.10^{a*}	0.54±0.04^{b*}	19.1±2.3^{ab*}
	G10	11.4 ± 0.30	5.90 ± 0.30	0.28 ± 0.02	24.3±2.10	0.52±0.03	21.6±1.30
4-8 month	A17	13.8 ± 1.30	6.90 ± 0.90	0.32 ± 0.05	23.5±4.10	0.50±0.05	21.8±4.70
	B27	13.3 ± 1.50	7.30 ± 1.10*	0.33±0.06**	24.9±2.60	0.54±0.1^{abc*}	22.0±1.70
	E3	12.4 ± 1.90	7.10 ± 0.70	0.35 ± 0.04	28.4±2.50	0.58±0.1^{abc*}	20.4±0.7**
	F14	12.3 ± 1.40	6.40 ± 1.00*	0.28±0.06**	22.7±3.20	0.52±0.05^{b*}	23.1±1.9**
8-12 month	A7	16.3 ± 1.00	7.70 ± 0.60	0.36 ± 0.04	22.0±1.40	0.47±0.03	21.4±0.90
	E3	19.5 ± 1.00*	7.40 ± 0.40	0.34 ± 0.01	17.7±1.10**	0.38±0.00**	21.6±1.50
	F10	14.2 ± 1.70*	8.10 ± 0.80	0.39 ± 0.05	27.2±2.30**	0.57±0.05**	21.0±0.70

H= herd, *n* = number of goats, Herds A and G= dwarf, B-F= landrace. Where comparisons involve more than 2 herds, mean values with similar superscripts (a b c or d) in columns within age groups differ significantly. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

The increase in hematocrit and hemoglobin concentration with age has been observed in other breeds of goats (Holman and Dew, 1966; DeShaw *et al.*, 1969; Edjetihadi, 1978; Löhle *et al.*, 1990). Somvanshi *et al.* (1987) and Nangia *et al.* (1968), however, had observed a reverse profile of higher RBC counts, Hb, and PCV in goats younger than 6 months of age and lower MCV and RBC diameters, similarly, Bialkowski *et al.* (1988). Erythrocyte counts and PCV in Polish goats decreased from 16.7 x 10^{12}/l and 0.32 at one week of age to 14.6 x 10^{12}/l and 0.29 respectively seven months later (Bialkowski *et al.*, 1988), findings that are contrary to the present results. Hemoglobin and MCHC were also higher at birth (8.4 and 25.4 mmol/l) compared with the present values of 6.1 and 20.7 mmol/l respectively in landrace goats.

Values of many parameters were higher in Polish than in the present dwarf and landrace goats, probably due to breed and environmental differences. The tendency for breed influence was found in the present dwarf and landrace kids within similar ages (table 2 - 5). Furthermore, tremendous breed variations in hematologic values are observed in adult goats (Castro *et al.*, 1977; Pospisil *et al.*, 1987; Ginting, 1988). Goats of different breeds might acquire similar levels of hematological parameters if reared under the same environment and fed on identical nutrition. Toggenburg, Faun, Saanen and Camoun goat breeds kept under similar environment and nutrition have similarities in hematological values (Neto *et al.*, 1986). Minor differences were also observed between Tibetan and Tibetan/Maltese cross breed goats (Domina *et al.*, 1982). This, however, is not likely to be the general profile because of genetic control of breed characteristics including those of hematological parameters.

The lack of consistent differences in all parameters between male and female kids in both breeds studied indicate that sex has little influence in blood parameter levels in young goats. Wilkins and Hodges (1962), Oduye (1976) and Somvanshi *et al.* (1987) reported similar observations. Although Vaidya *et al.* (1970) had noted sex differences in RBC counts, PCV and MCV at all ages, this appears not to be a general principle except in old female animals where pregnancy and lactation might have influence, to be reflected as sex differences when compared with male goats.

Hematological values are influenced by seasonal changes according to Ginting (1987). Assessment of seasonal effect demands multiseasonal investigations and cannot be evaluated in growing goats but only in adult goats and in a long term study that enables breeding and study of animals at any time of the year. Our studies could not evaluate this factor. There were, however, significant differences in many parameters in kids from different herds within similar ages and breeds indicating the effect of the environment. Feeding influences hematological parameters. All the kids in this investigation were maintained on milk until weaning. Since milk composition is breed

specific and nutritionally dependent, this might indirectly affect neonatal hematology in kids. Our studies could not determine the nutritional influence on these blood parameters.

The negative correlation between RBC counts and MCV and cell diameters shows the inverse relationship between cell size and number. The negative correlation between RBC and MCH indicate that when there are few erythrocytes the hemoglobin content of each increased. Similarly the larger the erythrocyte population the higher the hemoglobin concentration and hematocrit.

In conclusion, erythrocyte counts, hemoglobin concentration and hematocrit show an increasing profile, while MCV, MCH and RBC diameters decrease with age in growing kids until adult values are acquired, breed and environment have large influences on these parameters. Age and breed are therefore necessary for specification in reference values without which hematological analysis may be difficult to interpret for diagnostic purposes. The hematological patterns during development are the same in the two breeds of goats studied.

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CHAPTER 6

LEUKOCYTE PROFILE IN NEONATAL KIDS

Summary

Total and differential leukocyte count profiles were studied in 62 clinically healthy Danish landrace kids and 34 clinically healthy dwarf kids from birth to 12 months of age in seven Danish herds. The objective was to determine the reference values in the two breeds and the influence of age, sex and environment and whether there are any breed differences. Parametric (mean ± 2 standard deviations) and the nonparametric (5^{th} to 95^{th} percentile interval) values for each leukocyte type were closely related. The medians were very close to the means. The number of leukocytes was low in neonates, 7.1 ± 1.5 and 7.0 ± 2.1 $x10^9/l$ in dwarf and landrace kids respectively. Values increased with age to 18.7 ± 2.1 and 13.4 ± 3.1 $x10^9/l$ in the two breeds respectively at 8 - 12 months of age. There were significant differences in leukocyte counts between the breeds at 1 - 2 and 8 - 12 months of age and at 2 - 4 months between herds within the breeds. The number of lymphocyte and neutrophil cells were 2.6 ± 0.8 and 3.9 ± 1.1 x $10^9/l$ respectively in neonatal dwarf and 4.1 ± 1.5 and 2.3 ± 1.1 x $10^9/l$ respectively in landrace kids. Very high numbers of these cells were observed in dwarf and landrace kids respectively within 8 months, 10.7 ± 1.1 and 8.2 ± 3.3 for lymphocytes and 6.8 ± 1.7 and 4.4 ± 2.6 $x10^9/l$ neutrophils for the two breeds. Significant differences between the ages (within the breeds), breeds (within similar ages) and herds (within breeds in kids of similar ages) were observed. Basophil, monocyte and eosinophil cells increased slightly with age. Sex variations were observed in some ages but were not significant.

Introduction

Examination of leukocytes is vital for diagnosis of many diseases in animals. Each type is produced in response to specific intrinsic or extrinsic factors. Reference leukocyte counts in very young animals are not available, and are therefore often taken for granted to be identical to those of adults. Interpretation of results under such conditions is difficult, thus the diagnosis laid upon is less reliable. Leukocyte counts were reported to be higher in growing kids than in old goats (Wilkins and Hodges, 1962; Nangia *et al.*, 1968; Vaidya *et al.*, 1970; Oduye, 1976; Earl and Carranza, 1980). Other reports, however revealed the opposite (DeShaw *et al.*, 1969; Edjetihadi, 1978; Neto *et al.*, 1986; Somvanshi *et al.*, 1987). These differences are

either due to the diversity of ages under which the animals were grouped for statistical analysis, especially because growing animals have a greater turnover of cells within a short time, or the influence of intrinsic and extrinsic factors which follow after birth. Accurate diagnostic information requires determination of breed, age and sex specific reference leukocyte counts under defined conditions. Knowledge of the leukocyte profile in healthy growing goats grouped within narrow age limits provides insights on the general reference pattern. Hematological data from clinically healthy animals are expressed in terms of mean ± standard deviation, but this approach has been criticized because the values of many blood parameters do not have a Gaussian distribution (Reed *et al.*, 1971; Wu *et al.*, 1975). The present study was addressed to determination of age, breed and sex specific absolute total and differential leukocyte counts for growing dwarf and Danish landrace goats using both parametric and nonparametric statistical analysis.

Materials and Methods

The animals in this were 62 Danish landrace and 34 dwarf kids from seven Danish herds (A - G) located at The Royal Veterinary and Agricultural University (A), Fakse (B), Næstved (C), Ringsted (D), Haslev (E), Fugleberg (F) and Copenhagen Zoo (G). In all the herds, kids naturally suckled milk from their mothers until weaning and were introduced to grass pellets and oats or natural grazing during the growth period. Goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms B to E in summer. In farms F and G goats were reared in the field for the entire summer and autumn.

Blood samples were collected from the external jugular vein in vacuum tubes (Becton-Dickinson Vacutainer) containing 0.12 ml of 0.34 mol/l potassium ethylene diaminotetraacetate (K_3EDTA) from one day to 12 months of age. Samples were collected every 3 weeks and after 8 weeks of age every 2 months to make divisions of six age groups 1 - 6 [(0 - 7 days (1), 7 - 30 days (2), 1 - 2 (3), 2 - 4 (4), 4 - 8 (5) and 8 - 12 (6) months old)].

The total number of white blood cells (WBC) were determined in a Coulter counter model S 560 (Coulter electronics England) within 1 - 3 hours of sampling. Thin blood films were made on slides, air dried and immediately stained with Leishman stain in an automatic stainer (Hema - Tek) for differential leukocyte counts after a permanent mounting under coverslip with xylene. The number of basophils, eosinophils, band and segmented neutrophils, monocytes and lymphocytes, were determined from 200 total cell counts.

The parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentile, median) values were determined by univariate procedure of a statistical

analysis software (SAS, Carry NC, USA 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each leukocyte type to the Gaussian distribution. The means of leukocyte counts were tested for differences between kids in various age groups (within breeds), breeds (in similar age kids), herds (in similar age kids, within the breeds) and sex (within breeds) by using the general linear models procedure.

Results

The range of total leukocyte counts were 4.9 - 12.3 $x10^9/l$ in landrace and 5.8 - 9.7 $x\ 10^9/l$ in dwarf goats at birth (Table 1) and increased to 8.3 - 21.4 and 15.7 - 21.9 $x\ 10^9/l$ respectively at 8 - 12 months of age. These values were within the 5^{th} to 95^{th} percentile interval. The 5^{th} to 95^{th} percentile intervals were included within the mean $\pm$ 2 standard deviations ($\bar{x} \pm 2s$) range in most age groups of both dwarf and landrace kids (Table 1).

The mean and median values of total leukocyte, absolute lymphocyte and segmented neutrophil counts in each age group and breed were close to each other (Table 1 - 4). The coefficients of skewness and kurtosis were small in most age groups (Table 5). The Shapiro Wilk statistic (W) showed that nearly all frequency distributions of the leukocytes were Gaussian ($0.98 \leq W \leq 1$, Table 5). Only in a few age groups were there any significant deviations from Gaussian. Leukocyte counts increased with age in both breeds, being significantly lower in landrace than in dwarf kids at 2 and 8 months of age (Table 2).

The majority of leukocytes were lymphocytes at all ages. Lymphocyte counts were also low at birth in both breeds and increased with age. The difference in lymphocyte counts between the two breeds were significant at some ages (Table 2). Segmented neutrophil counts were significantly higher in dwarf than in landrace goats for most of the 12 months study period. There were significant differences in total leukocyte counts between the birth values and other age groups in both breeds (Fig. 1a-c)[1], and also among other age groups ($2/4^{*}$[2], $2/5^{*}$, $2/6^{***}$, $3/6^{*}$, $4/5^{*}$, $4/6^{*}$ and $5/6^{*}$ in dwarf, and $2/3^{*}$, $2/4^{*}$, $2/6^{**}$, $3/4^{*}$, $3/5^{*}$ and $3/6^{*}$ in landrace kids). There were significant differences in lymphocyte counts between birth values and other age groups in both breeds (Fig. 2a-b), and also among other age groups ($2/5^{***}$, $2/6^{***}$, $3/5^{*}$, $3/6^{*}$, $4/6^{*}$ and 5/6 in dwarf, and $2/4^{**}$, $2/5^{**}$, $2/6^{**}$, $2/6^{*}$, $3/4^{*}$, $3/5^{*}$ and $3/6^{*}$ in landrace kids).

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[1]Only significant differences between group 1 and others appear in figures, not among other groups

[2]$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

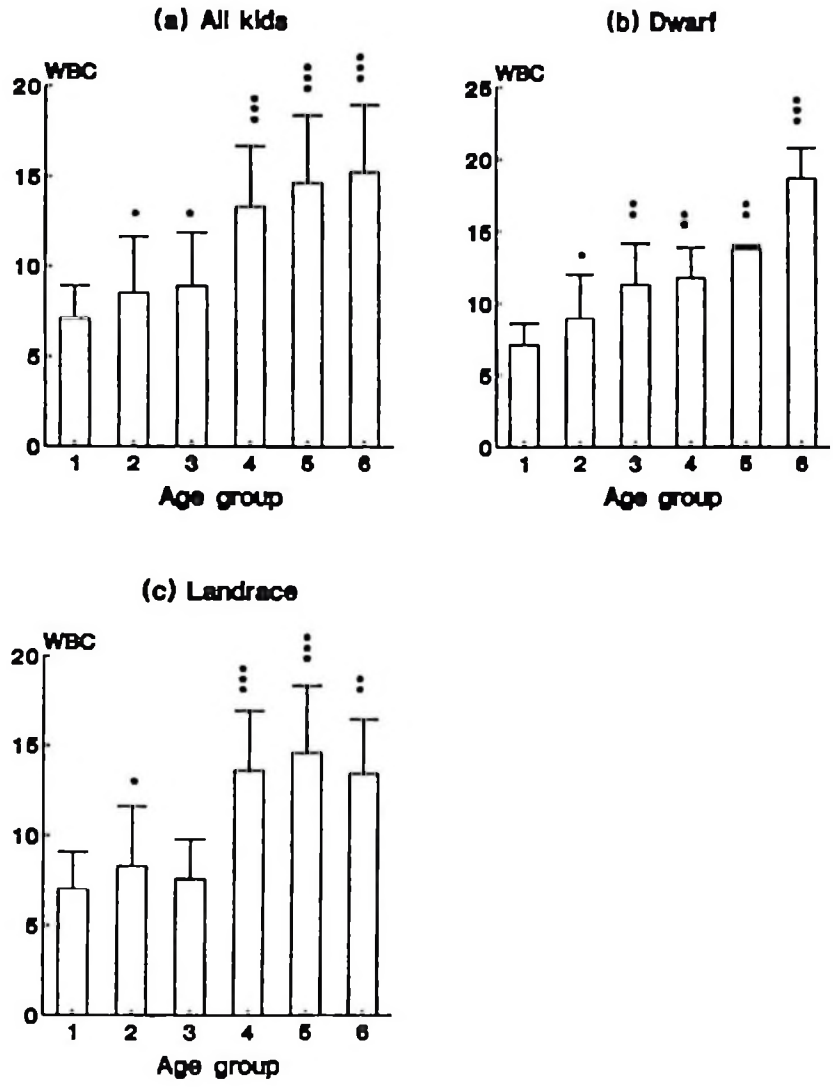

Figure 1a: The mean number of total leukocytes (WBC $x10^9/l$) with one standard deviation (bar) in both growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). For significant differences between values of group 1 and others; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Figure 1 (b) and (c): The mean number of leukocytes ($x10^9/l$) with one standard deviation (bar) in growing dwarf (b) and landrace (c) kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). For significant differences between values of group 1 and others; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

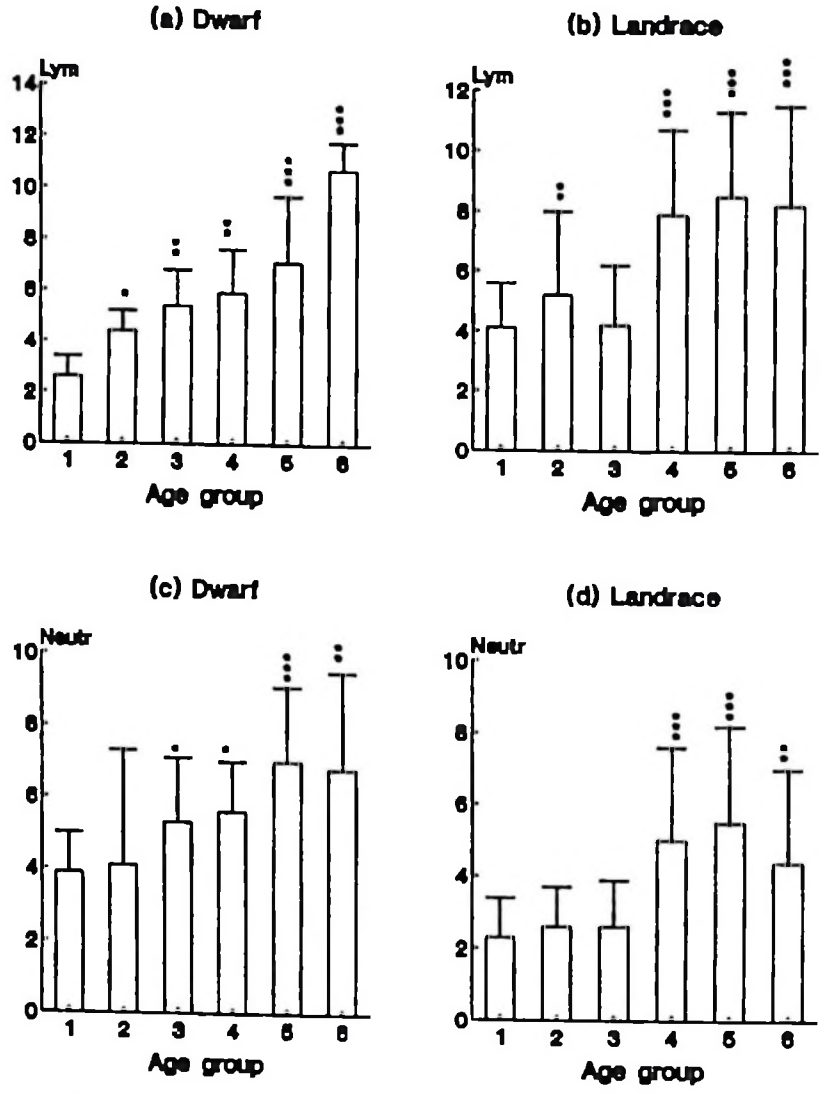

Figure 2: The mean number of lymphocytes (x10^9/l) in growing dwarf (a) and landrace (b) kids, and the mean number of segmented neutrophils (x10^9/l) with one standard deviation (bars) in growing dwarf (c) and landrace (d) kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). For significant differences between values of group 1 and others; $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Table 1: Comparisons between leukocyte count range, 5th (P_5) to 95th (P_{95}) percentile and $\bar{x} \pm 2s$ intervals, and the median counts (Q_2) in growing kids (all $x10^9/l$)

		WBC $x10^9/l$				Lymphocytes $x10^9/l$				Segmented neutrophils $x10^9/l$			
Age	*n*	Range	P_5-P_{95}	$\bar{x}$-2*s* to $\bar{x}$+2*s*	Q_2	Range	P_5-P_{95}	$\bar{x}$-2 to $\bar{x}$+2*s*	Q_2	Range	P_5-P_{95}	$\bar{x}$-2*s* to $\bar{x}$+2*s*	Q_2
Dwarf													
0-7 day	9	5.8-9.7	6.9-8.9	4.1-10.1	6.4	1.6-4.1	1.6-4.1	1.0-4.2**	2.5	2.4-5.1	2.4-5.1	1.7-6.1**	4.1
7-30 day	8	5.8-15.7	5.8-15.7	2.7-15.1	8.6	3.4-5.7	3.4-5.7	2.8-6.00	4.5	1.8-11.5	1.8-11.5	0.0-10.3*W	3.2
1-2 month	9	6.6-15.0	6.6-15.0	5.5-17.1***	11.5	3.2-7.5	3.2-7.5	2.6-8.20	5.0	3.2-8.2	3.2-8.2	1.7-8.9***	4.7
2-4 month	10	8.8-15.6	8.8-15.6	7.6-16.0	11.7	3.6-9.7	3.6-9.7	2.5-9.3***	5.4	3.6-8.4	3.6-8.4	2.8-8.40	5.6
4-8month ♂	17	7.7-20.4	7.7-20.4	11.2-16.4	13.8	3.4-11.0	3.4-11.3	1.9-12.3*	6.7	2.6-10.5	2.6-10.5	2.8-11.2*	6.8
8-12month ♂	7	15.7-21.9	15.7-21.9	14.5-22.9***	18.4	9.0-12.0	9.0-12.0	8.5-12.9*	10.7	4.1-8.8	4.1-8.8	3.4-10.2*	6.5
Landrace													
0-7 day	18	4.9 12.3	4.9-12.3	2.8-11.2	6.8	2.1-7.0	2.1-7.9	1.1-7.1**	3.7	0.6-4.9	0.6-4.9	0.1-4.5**	2.2
7-30 day	20	4.6-16.9	4.6-15.8	1.7-14.9	7.5	1.8-13.0	1.8-11.3	0.0-10.8	4.4	1.1-5.0	1.1-4.5	0.4-4.8*	2.5
1-2 month	16	4.0-11.9	4.0-11.9	3.2-12.0***	7.4	2.0-9.1	2.0-9.1	0.2-8.20W	4.4	0.4-4.3	0.4-4.3	0.0-5.2**	2.4
2-4 month	62	4.4-20.7	8.7-19.1	6.8-20.4	13.4	3.0-14.6	4.0-13.7	2.3-13.5***	7.6	0.2-13.3	1.6-9.2	0.0-10.2	4.7
4-8 month	44	5.6-23.6	9.4-21.2	7.0-22.2	14.6	2.6-15.0	4.0-12.6	2.9-14.1*	8.7	1.1-18.2	2.6-8.4	0.1-10.9*W	5.2
8-12month ♀	13	8.3-21.4	8.3-21.4	7.2-19.6***	12.5	2.3-14.0	2.3-14.1	1.6-14.8*	8.1	0.8-12.2	0.8-12.2	0.0-9.6*	4.6

n = number of goats, ♂ = males only, ♀ = females only.

Differences between breeds *$p<0.05$, **$p<0.01$, ***$p<0.001$. WBC = white blood cells.

Table 2: The mean ± one standard deviation of total leukocyte, lymphocyte and segmented neutrophil counts and ranges for rare leukocyte types ($x10^9/l$) in growing kids.

Age	*n*	Total WBC	Lymphocyte	[s]Neutrophil	[b]Neutrophil	Basophil	Monocyte	Eosino-phil
Dwarf								
0-7 day	9	7.10±1.50	2.6±0.80**	3.9±1.10**	0.12 - 0.23	0 - 0.23	0 - 0.64	0 - 0.28
7-30 day	8	8.90±3.10	4.4±0.80	4.1±3.20*W	0 - 0.31	0 - 0 16	0 - 0.36	0 - 0.53
1-2 month	9	11.3±2.90***	5.5±1.40	5.3±1.80***	0 - 0.15	0 - 0.18	0.13 - 0.77	0 - 0.19
2-4 month	10	11.8±2.10	5.9±1.70***	5.6±1.40	0	0 - 0.16	0.10 - 0.41	0 - 0.28
4-8month ♂	17	13.8±1.30	7.1±2.60*	7.0±2.10*	0 - 0.67	0 - 0.40	0 - 0.96	0 - 1.44
8-12month ♂	7	18.7±2.10***	10.8±1.10*	6.8±1.70*	0 - 0.55	0 - 0.44	0 - 0.1.27	0 - 0.44
Landrace								
0-7 day	18	7.0±2.10	4.1±1.50**	2.3±1.10**	0 - 0.70	0 - 0.30	0.1 - 0.80	0 - 0.50
7-30 day	20	8.3±3.30	5.2±2.80	2.6±1.10*	0 - 0.20	0 - 0.60	0 - 1.80	0 - 0.40
1-2 month	16	7.6±2.20***	4.2±2.00W	2.6±1.30***	0 - 0.50	0 - 0.60	0 - 0.90	0 - 0.10
2-4 month	62	13.6±3.40	7.9±2.00***	5.0±2.60	0 - 0.50	0 - 0.40	0.04 - 1.00	0 - 0.70
4-8 month	44	14.6±3.80	8.5±2.80*	5.5±2.70*W	0 - 0.30	0 - 0.50	0 - 0.80	0 - 1.40
8-12month ♀	13	13.4±3.10***	8.2±3.30*	4.4±2.60*	0 - 0.50	0 - 0.60	0 - 1.70	0 - 0.90

n = number of goats, ♂ = males only, ♀ = females only.

[b] = Band neutrophils, s = segmented neutrophils, W = non *Gaussian* distribution ($p<0.05$)

Table 3: The median (Q_2) and mean ± one standard deviation of leukocyte counts $x10^9/l$ in dwarf kids according to breed, sex and age.

		WBC			Lymphocyte			Neutrophil		
Age	*n*	range	Q_2	$\bar{x} \pm s$	range	Q_2	$\bar{x} \pm s$	range	Q_2	$\bar{x} \pm s$
females										
0-7 day	3	5.8-7.8	7.00	6.9 ± 1.00	2.1 - 2.5	2.1	2.2 ± 0.2	2.40-5.10	4.10	3.90±1.30
7-30 day	4	5.8-15.7	9.00	9.9 ± 4.20	3.4 - 5.0	4.2	4.2 ± 0.8	2.0-11.5	3.90	5.30±4.30
1-2 month	4	6.6 - 12.9	10.5	10.1 ± 2.7	3.2 - 6.1	4.8	4.7 ± 1.2	3.20-7.50	4.30	4.80±1.90
2-4 month	5	8.8-13.5	9.90	10.9 ± 2.1	3.6 - 7.3	4.8	5.2 ± 1.4	3.90-6.20	5.70	5.30±0.90
males										
0-7 day	6	5.9-9.7	6.30	7.2 ± 1.70	1.6 - 4.1	2.9	2.8 ± 0.9	2.60-5.10	3.90	3.90±1.10
7-30 day	4	5.9-9.1	8.40	8.0 ± 1.50	3.7 - 5.7	4.5	4.6 ± 0.8	1.80-4.30	2.70	2.90±1.20
1-2 month	5	8.9-15.0	13.1	12.2 ± 2.9	4.4 - 7.5	6.1	5.9 ± 1.4	3.80-8.20	5.40	5.70±1.80
2-4 month	5	10.6-15.6	11.8	12.7 ± 2.0	5.0 - 9.7	5.7	6.5 ± 1.9	3.60-8.40	5.70	5.80±1.80
4-8 month	17	7.7-20.4	15.1	15.0 ± 3.6	3.4 - 11.3	6.7	7.1 ± 2.6	2.60-10.5	6.80	7.00±2.10
8-12 month	7	15.7-21.9	18.4	18.7 ± 2.1	9.6 - 12.0	10.7	10.7 ± 1.1	4.10-8.80	6.50	6.80±1.70

n = number of goats

WBC = white blood cells

Differences between female and male kid, $*p<0.05$, $**p<0.01$, $***p<0.001$

Table 4: The median (Q_2) and mean ± one standard deviation of leukocyte counts x10^9/l in landrace kids according to breed, sex and age.

		WBC			Lymphocyte			Neutrophil		
Age	*n*	range	Q_2	$\bar{x} \pm s$	range	Q_2	$\bar{x} \pm s$	range	Q_2	$\bar{x} \pm s$
females										
0-7 day	5	5.8-7.6	6.90	6.8 ± 0.6*	2.4 - 6.00	5.0	4.5 ± 1.40	0.6-2.90	1.80	1.80±0.90
7-30 day	12	4.7-16.9	7.50	8.4 ± 3.50	2.8 - 13.2	4.3	5.4 ± 3.20	1.1-4.00	2.40	2.40±0.90
1-2 month	6	4.0-11.7	6.90	7.2 ± 2.60	2.0 - 9.10	3.0	3.8 ± 2.70	0.90-4.00	2.80	2.60±1.20
2-4 month	41	4.00-20.7	13.4	13.6 ± 3.40	3.1 - 14.6	7.6	8.1 ± 2.90	0.2-13.30	4.70	4.90±2.50
4-8 month	38	5.60-23.6	14.5	14.5 ± 3.90	2.6 - 15.0	8.5	8.4 ± 2.90	1.10-18.2	5.30	5.50±2.80
8-12 month	13	9.70-19.8	12.6	13.7 ± 3.00	2.3 - 14.1	8.7	8.6 ± 3.10	0.90-9.00	3.80	4.20±2.70
males										
0-7 day	13	4.90-12.3	8.20	8.6 ± 1.6'	2.1 - 7.00	3.3	4.0 ± 1.60	1.30-4.90	2.40	2.50±1.10
7-30 day	8	4.60-14.6	7.20	8.0 ± 3.30	0.8 - 9.50	4.6	4.9 ± 2.60	1.10-4.00	2.40	2.40±1.10
1-2 month	10	5.30-11.9	7.70	7.8 ± 2.00	2.9 - 7.40	4.4	4.4 ± 1.50	0.40-4.30	2.40	2.60±1.30
2-4 month	21	7.40-20.0	12.7	13.5 ± 3.60	4.0 - 14.4	7.4	7.5 ± 2.70	1.60-12.5	4.70	5.20±2.70
4-8 month	6	10.9-20.0	14.7	14.9 ± 3.40	5.0 - 11.1	9.9	9.3 ± 2.40	1.80-8.10	5.20	5.00±2.40

WBC= whilte blood cells, *n* = number of goats, Differences between female and male kid, $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table 5: Tests for closeness of fit to *Gaussian* distribution; skewness (g1), kurtosis (g2) and Shapiro Wilk statistic (W).

Age	WBC			Lymphocyte			Neutrophil		
	g1	g2	W	g1	g2	W	g1	g2	W
Dwarf									
0-7 day	1.02	-0.46	0.84	0.69	0.15	0.96	-0.15	-1.67	0.91
7-30 day	1.60	3.55	0.84	0.28	-0.91	0.95	2.21	5.36	0.72*
1-2 month	-1.18	-1.11	0.95	0.12	-0.90	0.95	0.64	-0.98	0.92
2-4 month	-0.32	-0.65	0.97	1.24	2.12	0.91	0.58	1.32	0.94
4-8 month	-0.10	-0.41	0.97	0.26	-1.43	0.92	-0.15	-0.14	0.98
8-12 month	0.36	-0.31	0.94	-0.39	-0.77	0.96	-0.43	-0.99	0.94
Landrace									
0-7 day	1.63	2.32	0.80**	0.55	-0.97	0.92	0.91	0.91	0.93
7-30 day	1.22	1.29	0.89*	1.32	2.17	0.89	0.43	-0.70	0.94
1-2 month	0.62	0.18	0.95	1.37	1.47	0.86*	-0.13	-1.31	0.94
2-4 month	-0.08	-0.11	0.98	0.62	-0.17	0.94	0.91	1.38	0.95
4-8 month	0.14	-0.02	0.99	0.04	-0.41	0.98	2.37	10.4	0.84***
8-12 month	0.74	-0.06	0.94	0.13	-0.18	0.98	0.87	0.59	0.94

Zero g1= symmetrical, + g1= skewed left, - g1= skewed right, zero g2= Gaussian distribution, + g2= kurtosis, -g2= flat topped, W must be > 0 and ≤ 1, small values indicate non Gaussian distribution. Where W is significantly small *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 7: Comparison of mean reference values of leukocyte counts $x10^9/l$ according to age by different authors.

Age days	Source	WBC	Lymph-ocyte	Neutro-phil (s)	Neutrop-hil (b)	Eosino-phil	Mono-cyte	Baso-phil
0-7	1	8.1	3.04	4.88	0.01	0.06	0.16	-
0-30	1	12.3	8.12	3.74	0.01	0.16	0.38	-
30-60	2	12.2	10.84	-	-	-	-	-
0-30	3	15.8	9.55	6.52	-	0.16	0.24	0
0-30	4	9-14	0.5-8.2	3.3-5.8	0.2-0.6	0.0-0.14	0.02-0.22	-
0-30	5	6.7	3.70	2.50	-	0.15	0.29	-
30-60	6	12.3	9.23	2.71	0.16	0.02	-	-
0-7[1]	7	7.10	2.60	3.90	0.27	0.28	0.64	0.23
0-7[2]	7	7.00	4.10	2.30	0.70	0.50	0.80	0.30
7-30[1]	7	8.00	4.40	4.10	0.31	0.53	0.36	0.16
7-30[2]	7	8.30	5.20	2.60	0.20	0.40	1.80	0.60

WBC= white blood cells, neutrophils (s)= segmented and (b)= bands.
[1]Dwarf and [2]Landrace kids
Source; 1 Holman and Dew (1966), 2 DeShaw *et al.* (1969), 3 Edjetihadi (1978), 4 Neto *et al.* (1986), 5 Somvanshi *et al.* (1987), 6 Bialkowski *et al.* (1988) and 7 present studies.

Significant differences were observed too in segmented neutrophils counts between neonatal values and those of other ages (Fig. 2c-d) and among other age groups ($2/5^{*}$, $2/6^{***}$ and $3/5^{*}$ in dwarf, and $2/4^{**}$, $2/5^{**}$, $2/6^{*}$, $3/4^{*}$, $3/5^{*}$ and $3/6^{*}$ in landrace kids).

Total leukocyte and lymphocyte counts were slightly higher in male than female dwarf kids but not statistically significant (Table 3). In one day old landrace kids the total leukocyte count was higher in males than in females (Table 4). Total leukocyte counts were significantly higher in 2 - 4 month old landrace kids in farm B than those in farm D (Table 6). There were differences in the number of lymphocytes and neutrophils in goats from different herds within some similar ages above 2 months old (Table 6). The band neutrophil population was generally high in newborn goats of both breeds, then decreased within the first week of life, whereafter they increased only slightly with age (Table 2).

Basophil counts were 0.23 and 0.3 x 10^9/l in dwarf and landrace kids at birth, but 0.44 and 0.6 x 10^9/l at 8 - 12 months of age (Table 2). Monocyte and eosinophil counts fluctuated little with age. There were no significant differences between female and male kids in the number of band neutrophils, basophils, monocytes and eosinophils in both breeds.

Discussion

Hematological data can be expressed as mean ($\bar{x}$), a parametric central measure of continuous numeric data or the nonparametric statistics, median and mode which may or may not be equal to the mean, depending on dispersion of observations. The range (interval from minimum to maximum observation) or the 2.5th to 97.5th or the 5th to 95th percentile intervals are used in blood parameters together with mean ± 2 standard deviations ($\bar{x} \pm 2s$) (Zinkl *et al.*, 1990).

In the present investigation all the $\bar{x} \pm s$ values of WBC count, lymphocytes and neutrophils in all age groups were within the 5th to 95th percentile interval (Table 1 - 4). The 5th to 95th percentile interval was within the range of $\bar{x} \pm 2s$ which was close to the observed range (Table 1). This agreed with the fact that in Gaussian distributions $\bar{x} \pm 2s$ includes about 95 % of the observations, compared with 90 in the 5th to 95th interval. The parametric and nonparametric ranges therefore did not have significant differences for these parameters. Most of the distributions were Gaussian ($0.98 \leq W \leq 1$, Table 5) except only a few distributions. The medians were very close to the means in most age groups of both breeds (Table 1 - 4). The conditions for the powerful parametric tests were therefore satisfied (Siegel and Castellan, 1988), as earlier noted (Barakat and El-Guindi, 1967).

Leukocyte counts in goats are influenced by age and breed (Figs. 1 and 2, Tables 1 - 3). There are great variations among sources on the profile from birth to maturity. The opinion that leukocyte counts are higher in young than in old animals (Wilkins and Hodges, 1962; Nangia *et al.*, 1968; Vaidya *et al.*, 1970; Nettleton and Beckett, 1976; Kumaresan and Ndzingu Awa, 1984) is based on observations after grouping of goats less than 12 months old as young, and those above as mature. Grouping of goats within the first 12 months, however, reveals minimum counts at birth and increasing values to maximum counts at 6 - 12 months. This was observed by Holman and Dew (1966), DeShaw *et al.* (1969), Castro *et al.* (1977), Kanemaki *et al.* (1986), Somvanshi *et al.* (1987) and confirmed in the present investigation. Contrary to this Earl and Carranza (1980) found highest WBC counts at birth which decreased with age. Similarly, Bialkowski *et al.* (1988) observed decreases of leukocyte counts from 12.30

x 10^9/l in the first month of life to 5.90 x 10^9/l six months later, whereas Neto *et al.* (1986) revealed no differences between breeds and no particular profile with age. These disagreements might be due to differences in breed and specific conditions. The present dwarf and landrace goats show a firm increasing profile for leukocytes of all types (Figs. 1 and 2, Tables 1 - 4).

Breed, nutrition in different herds and management factors seem to be important in altering the pattern. If leukocyte counts of kids less than 30 days of age from different sources are compared, large variations are noted (Table 7). The variations result from breed, immunodynamics, nutrition and management of the doe, and environment. The environment was observed in this study to play a role, because differences were observed between herds within breeds in dwarf and landrace kids of similar ages (Table 6). The reference ranges for the leukocyte counts are therefore dependent on age, breed and environment in addition to pathological influences of exposures to various microrganisms, parasites and toxicological agents. There are also extreme individual variations in the number of leukocytes, thus the standard deviations at some ages were large.

Lymphocyte and neutrophil counts are low in number at birth and increase to maximal values by 6 - 12 months of age. In some breeds of goats there appears to be more neutrophils than lymphocytes at birth (Table 7), but the reverse is true in mature goats (Table 2). In the latter 50 - 70 % of leukocytes are lymphocytes and 30 - 50 % are neutrophils according to Holman and Dew (1966) and Payne *et al.* (1982). Other sources, however, indicate reversed profiles (Nangia *et al.*, 1968; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988). The present results in dwarf and landrace goats show that most leukocytes are lymphocytes at most ages. Band neutrophils, eosinophil, basophil and monocyte counts are very low in all ages, therefore only the ranges have been shown (Table 2).

In conclusion, the leukocyte population is lowest at birth in the examined breeds of goats and increases with age to maximum 6 - 12 months later. The total and differential leukocyte types are age, breed and environment specific which must be specified for reference values.

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CHAPTER 7

PROFILES OF PLASMA ELECTROLYTES IN GROWING KIDS

Summary

The concentration of calcium, magnesium, sodium, potassium and inorganic phosphorus in the plasma were determined in 62 Danish landrace and 34 dwarf healthy kids from birth to 12 months of age. The objective was to obtain their reference values in the two breeds specified to age and sex. Comparisons between parametric (mean ± standard (s) deviation and the corresponding nonparametric (5^{th} and 95^{th} percentiles, median) values calculated for each electrolyte indicated no apparent differences. The data were found to be distributed in a Gaussian manner. The mean ±s levels in dwarf neonates were 3.00 ± 0.10, 0.92 ± 0.11, 146.2 ± 1.92, 4.42 ± 0.24 and 2.83 ± 0.27 mmol/l respectively. The respective values for landrace kids were 2.91 ± 0.18, 0.87 ± 0.10, 146.6 ± 1.50, 4.48 ± 0.41 and 3.43 ± 0.53 mmol/l. Plasma concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and inorganic phosphorus in dwarf kids of 8 - 12 months of age were 2.65 ± 0.05, 0.86 ± 0.05, 148.6 ± 1.20, 4.90 ± 0.38 and 2.81 ± 0.58 mmol/l respectively. The respective values for landrace kids of the same age were 2.46 ± 0.15, 0.93 ± 0.10, 144.1 ± 6.42, 4.12 ± 0.56 and 2.32 ± 0.80. There were significant differences in mean concentrations of ions between kids of different ages (within the breed), breeds (in kids of the same age) and herds (within the breeds in kids of the same age). Differences attributable to sex were not observed in most age groups in both breeds.

Introduction

Electrolytes are vital for cellular metabolism. Calcium is required in osteogenesis, capillary and cell membrane permeability, nerve impulse transmission, muscle contraction and blood clotting. Phosphorus is integral in osteogenesis, absorption and high energy phosphate bonds. Magnesium is an activator of phosphatase and enzymes that involve ATP and thiamine pyrophosphate. Sodium and potassium play roles in intra- and extracellular ion equilibrium and maintain the body's fluid balance through renal osmotic regulations. A stable fluid and acid-base balance is required for optimal activity of enzymes, coenzymes and neurotransmitter compounds. Plasma concentration of these ions are useful indices in diagnosis and treatment of many diseases and pathophysiological conditions (Feldman and Thomason, 1989). Calcium is bound to albumin (Capen and Rosol, 1989) and since the latter is lost in hemorrhagic parasitic

infections (Saad *et al.*, 1984), plasma calcium level has been observed to be a useful index of certain parasitic infections (Mbassa *et al.*, 1989). Furthermore a type of hemolytic anaemia in cattle has been attributed to hypophosphatemia (Ogawa *et al.*, 1989). Plasma/serum electrolyte levels are thus important complement tests in clinical diagnosis of diseases.

Investigations in various goat breeds under different environments (Castro *et al.*, 1977; Bogin *et al.*, 1981; Catarsini *et al.*, 1982; Wojcik *et al.*, 1986) indicate large variations between them and according to many factors. The detection of the magnitude of factors such as breed, age and environmental influence on electrolytes is a prerequisite for interpretation of laboratory results and simplify the diagnostic decision making. Studies on electrolytes in newborn kids are few (Braun *et al.*, 1983) and are based on small sample sizes, missing particularly in dwarf and landrace goats. Clinical chemical data from clinically healthy animals are commonly presented as mean ± standard deviation ($\bar{x} \pm s$). In normally distributed data (Gaussian) $\bar{x} \pm 2s$ includes about 95 % of the values. This approach has been criticized because the values of many blood parameters do not have a Gaussian distribution (Reed *et al.*, 1971; Wu *et al.*, 1975). The present study was to determine the plasma concentrations of electrolytes which are regularly used in diagnosis of diseases, in growing dwarf and landrace kids.

Materials and Methods

In this study 62 Danish landrace and 34 dwarf healthy kids of one day old to 12 months of age from 7 Danish herds (A - G) located at the Royal Veterinary and Agricultural University (A), Fakse (B), Næstved (C), Ringsted (D), Haslev (E), Fugleberg (F) and Copenhagen Zoo (G) were used. In all the herds, kids naturally suckled milk from their mothers until weaning and introduced to grass pellets or natural grazing, and oats or barley grains during the growth period. Goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms B to E in summer. In farms F and G goats were reared in the field for the entire summer and autumn.

Blood samples were collected from the external jugular vein in sodium heparin vacuum tubes (Becton-Dickinson vacutainers) and the plasma separated by centrifugation at 3500 rpm for 5 minutes. Samples were collected every 3 weeks and after 8 weeks of age every 2 months. The goats were categorized in age groups of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6).

Plasma calcium and magnesium concentrations were determined by atomic absorption spectrophotometer (Perkin Elmer), sodium and potassium by ion selective

electrode module and inorganic phosphorus spectrophotometrically in an auto-analyzer Cobas Fara (ROCHE). All determinations were made within 3 - 24 hours of sampling with plasma stored at 4 ^{0}C when necessary.

Parametric (means, standard deviation) and nonparametric (5th, 95th percentile, median) values were determined by univariate procedure of a statistical analysis system software (SAS, Carry NC, USA, 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated to determine the closeness of fit for the data of each electrolyte to the Gaussian distribution. The means of plasma electrolytes were tested for differences between kids in various age groups (within the breeds), breeds (in same age kids), herds (in same age kids within the breeds) and sex (within the breeds) by the general linear models procedure.

Results

The mean and median values of calcium, magnesium, inorganic phosphorus, sodium and potassium in each age group and breed were close to each other (table 1-4). The coefficients of skewness and kurtosis were small and W between 0.98 and 1.00, therefore the data in most age groups of both breeds for all the electrolytes except a few were distributed in a Gaussian manner (table 1 - 2). The 5th and 95th percentile intervals were included in the range of $\bar{x} \pm 2s$ in most age groups of both dwarf and landrace kids.

Plasma calcium was 3.0 ± 0.10 and 2.91 ± 0.18 mmol/l in neonatal dwarf and landrace kids respectively. The levels decreased to 2.65 ± 0.05 and 2.46 ± 0.15 mmol/l in the two breeds respectively at 8 - 12 months of age (fig. 1a and b). Calcium levels were significantly higher in dwarf than in landrace kids of similar age (table 1), except in the first and 6th weeks of age. Plasma calcium in neonatal dwarf kids was significantly higher than those of 4 - 8 and 8 - 12 months of age (fig. 1a)[1]. There were significant differences in several other groups (2/3[*2], 2/5*, 2/6*, 3/5*, 3/6*, 4/5*, and 4/6*). Calcium was higher in landrace neonates than in kids of 2 - 4, 4 - 8 and 8 - 12 months of age. (fig. 1b), and several differences among other age groups were observed (2/5*, 2/6*, 3/5*, 3/6*, 4/5*, and 4/6*).

Magnesium concentrations were 0.92 ± 0.11 and 0.87 ± 0.10 mmol/l in neonatal dwarf and landrace kids respectively (fig. 1c and d, table 1). After a slight increase up to the 12 th week, levels decreased to 0.86 ± 0.05 and 0.93 ± 0.10 mmol/l in the two breeds respectively at 8 - 12 months of age. Magnesium was significantly lower in

[1]Only significant differences between group 1 and others are shown in figures, not among other groups.

[2]*$p<0.05$, **$p<0.01$, ***$p<0.001$.

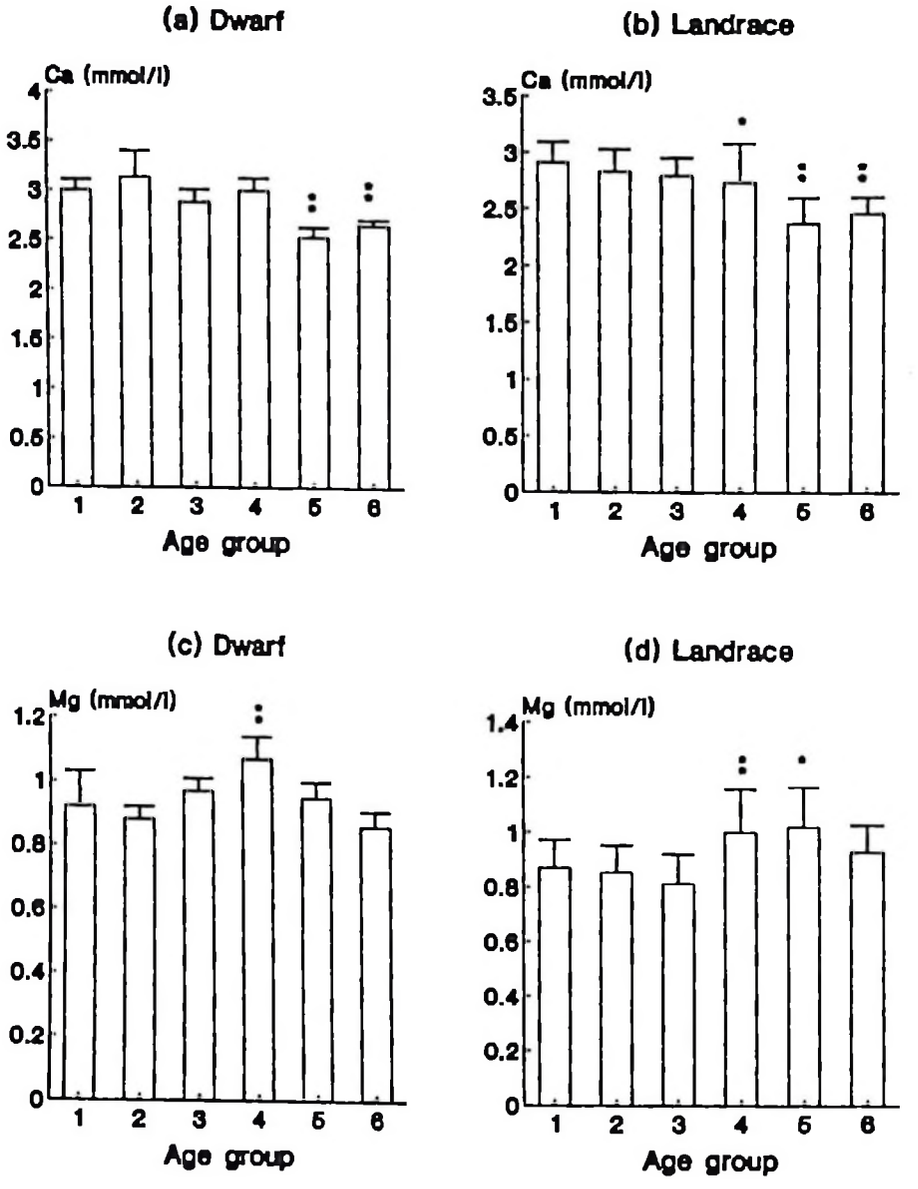

Figure 1: The mean plasma concentration of calcium (a) and (b) and magnesium (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Means significantly different from neonatal values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

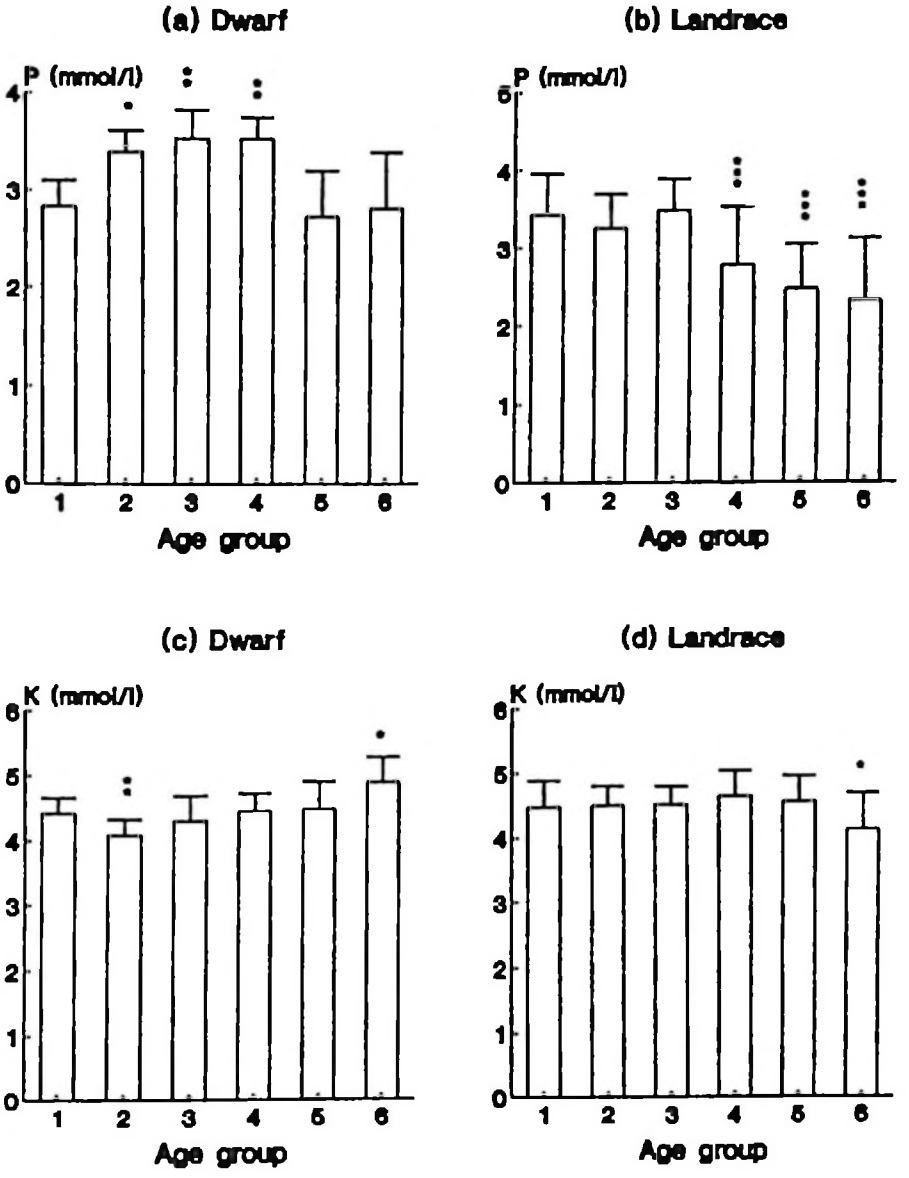

Figure 2: The mean plasma inorganic phosphorus (a) and (b) and potassium (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Means significantly different from neonatal values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

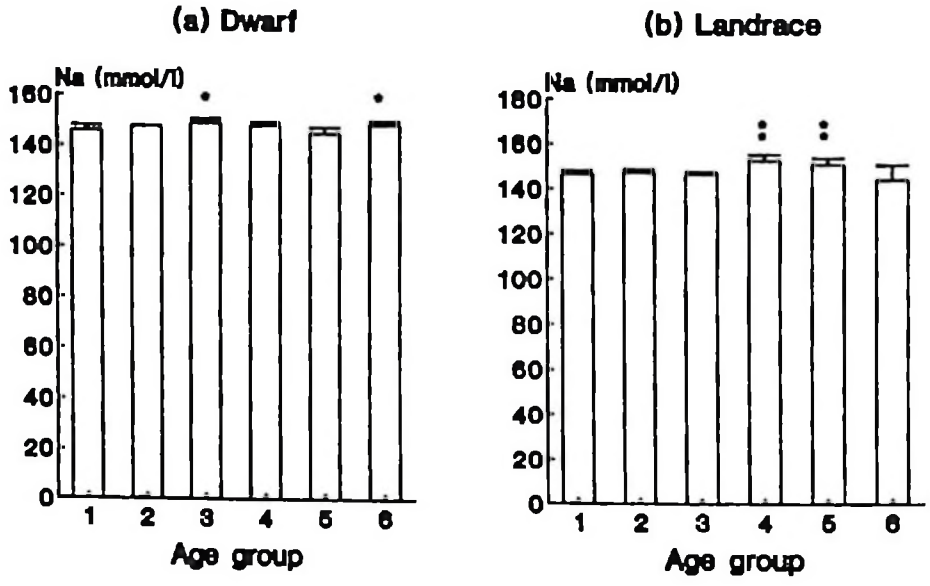

Figure 3: The mean plasma concentration of sodium with one standard deviation (bar) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Means significantly different from neonatal values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

neonatal than in 2 - 4 months old dwarf kids (fig. 1c) and differed between other age groups (2/3*, 2/4*, 2/5*, 3/4*, 3/6*, 4/5*, 4/6* and 5/6*). The concentration was significantly lower in neonatal than in 2 - 4 and 4 - 8 landrace kids (fig. 1d) with differences in other groups (2/4*, 2/5*, 3/4*, 3/5* an 3/6*).

Inorganic phosphorus in neonatal dwarf and landrace kids were 2.83 ± 0.27 and 3.43 ± 0.53 mmol/l respectively, significantly lower in the former than in the latter (fig. 2, table 1). There was a slight increase up to the 12 th week whereafter levels

Table 1: The 5th (P_5) to 95th (P_{95}) percentile interval, medians (Q_2) and $\bar{x} \pm s$ of plasma calcium, magnesium and inorganic phosphorus levels in growing dwarf and landrace kids.

			Calcium mmol/l		Magnesium mmol/l			Phosphorus mmol/l		
Age	*n*	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$
Dwarf										
0-7d	9	2.83-3.10	3.02	3.00±0.10	0.78-1.10	0.91	0.92±0.11	2.51-3.17	2.77	2.83±0.27**
7-30d	8	2.64-3.61	3.10	3.13±0.27**	0.82-0.94	0.87	0.88±0.04	2.99-3.61	3.45	3.39±0.22
1-2m	9	2.72-3.08	2.85	2.88±0.13	0.91-1.03	0.98	0.97±0.04	2.92-3.90	3.52	3.53±0.30
2-4m	10	2.86-3.21	2.97	3.00±0.12**	0.99-1.17	1.06	1.07±0.07	3.16-3.97	3.53	3.53±0.22**
4-8m	17	2.35-2.69	2.53	2.53±0.10**	0.83-1.03	0.94	0.95±0.05*	2.01-3.57	2.68	2.73±0.47
8-12m♂	7	2.57-2.71	2.65	2.65±0.05**	0.81-0.94	0.84	0.86±0.05W	2.00-3.84	2.75	2.81±0.58
Landrace										
0-7d	18	2.55-3.15	2.95	2.91±0.18W	0.71-1.05	0.89	0.87±0.10	2.07-3.95	3.58	3.43±0.53**W
7-30d	20	2.41-3.08	2.84	2.83±0.20**	0.71-1.06	0.85	0.85±0.10	2.66-3.90	3.22	3.25±0.45
1-2m	16	2.52-3.07	2.76	2.79±0.16	0.61-1.03	0.82	0.81±0.11	2.46-3.97	3.62	3.48±0.41
2-4m	62	2.35-3.20	2.66	2.73±0.35**W	0.81-1.33	0.97	1.00±0.16W	1.77-3.94	2.75	2.78±0.75**
4-8m	44	2.66-2.41	2.41	2.37±0.23**W	0.83-1.22	1.01	1.02±0.15*	1.45-3.49	2.50	2.47±0.58
8-12m♀	13	2.15-2.73	2.52	2.46±0.15**	0.81-1.16	0.90	0.93±0.01	0.61-3.80	2.52	2.32±0.80

n = number of goats, d = days, m = months, ♂ = males only, ♀ = females only, W = non Gaussian distributions ($p<0.05$)
For differences in means between the breeds in similar age groups *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 2: The 5th (P_5) to 95th (P_{95}) percentile interval, medians (Q_2) and $\bar{x} \pm s$ of plasma calcium, magnesium and inorganic phosphorus levels in growing dwarf and landrace kids.

			Sodium (mmol/l)		Potassium mmol/l		
Age	*n*	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$
Dwarf							
0-7 day	9	142.6-148.6	146.2	146.2±1.92	4.12-4.73	4.36	4.42±0.24
7-30 day	8	146.8-148.2	147.8	147.6±0.51**	3.53-4.37	4.09	4.08±0.25**
1-2 month	9	147.0-152.6	148.7	149.0±1.71***	3.90-5.04	4.20	4.30±0.39
2-4 month	10	146.4-150.5	147.2	147.8±1.39***	4.02-4.99	4.45	4.45±0.28
4-8 month ♂	17	141.9-148.6	144.3	144.9±02.30***	3.82-5.24	4.40	4.48±0.43
8-12 month ♂	7	146.4-149.9	148.8	148.6±1.20*	4.55-5.67	4.76	4.90±0.38**
Landrace							
0-7 day	18	144.1-150.2	146.3	146.6±1.50	4.05-5.63	4.36	4.48±0.41W
7-30 day	20	145.3-149.4	147.4	147.3±1.29	4.13-5.12	4.48	4.50±0.30**
1-2 month	16	142.2-148.7	146.4	146.4±1.17***	4.01-5.04	4.55	4.51±0.28
2-4 month	62	147.1-156.1	152.4	152.5±2.81***	4.08-5.24	4.64	4.64±0.40
4-8 month	44	146.8-154.8	150.6	150.9±2.78***	3.69-5.13	4.63	4.55±0.40
8-12month ♀	13	137.6-154.5	140.7	144.1±6.42***	2.80-4.95	4.11	4.12±0.57**

n = number of goats
♂ = males only, ♀ = females only
W = non Gaussian distributions ($p<0.05$)
For differences in means between the breeds in similar age groups *$p<0.05$, **$p<0.01$, ***$p<0.001$.

decreased (fig. 2). In neonatal dwarf kids phosphorus was significantly lower than those at 7 - 30 days, 1 - 2 and 2 - 4 months old (fig. 2b). Significant differences between other age groups were observed (2/5*, 2/6*, 3/5*, 3/6*, 4/5* and 4/6*). In landrace kids phosphorus was higher in neonates than in 2 - 4, 4 - 8 and 8 - 12 months of age (fig. 2a). There were other age group differences (2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 3/6*, 4/5* and 4/6*).

Potassium levels in neonatal dwarf and landrace kids were 4.42 ± 0.24 and 4.48 ± 0.41 mmol/l respectively and fluctuated slightly during growth (fig. 2, table 2 and 5). At some ages significant differences were observed between dwarf and landrace kids (table 2). In neonatal dwarf kids potassium levels were significantly higher than in 7-30 days but lower than in 4-8 months of age (fig. 2c). There were differences in other age

Table 3: The 5th (P_5) to 95th (P_{95}) percentile interval, medians (Q_2) and $\bar{x} \pm s$ of plasma electrolytes in growing dwarf and landrace kids.

			Calcium mmol/l			Magnesium mmol/l			Phosphorus mmol/l		
Breed and sex	Age	*n*	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$
Dwarf females	0-7 day	3	2.83-3.07	2.98	2.96±0.12	0.79-1.10	0.90	0.93±0.16	2.51-2.98	2.72	2.74±0.23
	7-30 day	4	3.04-3.61	3.07	3.20±0.27	0.84-0.92	0.88	0.88±0.04	2.99-3.59	3.40	3.34±0.27
	1-2 month	4	2.72-3.08	2.89	2.89±0.16	0.91-0.98	0.94	0.94±0.03*	3.35-3.90	3.47	3.55±0.24
	2-4 month	5	2.86-3.21	2.93	2.97±0.14	0.99-1.15	1.05	1.06±0.06	3.16-3.71	3.54	3.50±0.20
Males	0-7 day	6	2.85-3.10	3.04	3.02±0.09	0.78-1.05	0.93	0.92±0.09	2.52-3.17	2.92	2.88±0.30
	7-30 day	4	2.64-3.28	3.14	3.05±0.28	0.82-0.94	0.86	0.87±0.05	3.18-3.61	3.48	3.44±0.19
	1-2 month	5	2.76-3.08	2.85	2.87±0.12	0.95-1.03	1.00	1.00±0.03	2.92-3.83	3.53	3.52±0.36
	2-4 month	5	2.87-3.18	3.05	3.03±0.11	1.00-1.17	1.07	1.08±0.07	3.30-3.97	3.53	3.57±0.45
Landrace females	0-7 day	5	2.91-3.15	3.02	3.03±0.11	0.87-0.99	0.94	0.93±0.05	3.48-3.95	3.61	3.69±0.20**
	7-30 day	12	2.67-3.11	2.91	2.90±0.14	0.68-1.13	0.85	0.85±0.11	2.68-3.79	3.26	3.26±0.38
	1-2 month	6	2.57-2.92	2.75	2.75±0.15	0.65-1.03	0.81	0.82±0.15	3.38-3.97	3.66	3.68±0.23
	2-4 month	41	2.45-3.45	2.70	2.79±0.39*	0.80-1.39	1.00	1.02±0.18*	1.77-3.84	2.65	2.68±0.74
	4-8 month	38	1.85-2.70	2.43	2.37±0.24	0.82-1.44	1.02	1.04±0.15*	1.45-3.54	2.54	2.56±0.56
Males	0-7 day	13	2.55-3.05	2.94	2.86±0.18	0.71-1.05	0.89	0.85±0.11	2.07-3.95	3.56	3.34±0.59
	7-30 day	8	2.41-3.06	2.76	2.71±0.23	0.75-1.00	0.85	0.86±0.09	2.65-3.99	3.06	3.23±0.56
	1-2 month	10	2.52-3.07	2.80	2.81±0.17	0.61-0.91	0.84	0.80±0.10	2.46-3.83	3.55	3.35±0.45
	2-4 month	21	2.34-3.03	2.57	2.63±0.24*	0.85-1.10	0.95	0.97±0.11*	1.91-3.94	2.87	2.96±0.76
	4-8 month	6	2.24-2.65	2.40	2.42±0.13	0.83-0.97	0.88	0.89±0.05*	1.39-2.35	1.85	1.91±0.39

n = number of goats, For differences in means between sexes within the breeds in similar age groups $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table 4: The 5th (P_5) to 95th (P_{95}) percentile interval, medians (Q_2) and $\bar{x} \pm s$ of plasma sodium and potassium levels in growing dwarf and landrace kids.

			Sodium mmol/l			Potassium mmol/l		
Breed and sex	Age	*n*	P_5-P_{95}	Q_2	$\bar{x} \pm s$	P_5-P_{95}	Q_2	$\bar{x} \pm s$
Dwarf females	0-7 day	3	143.9-146.6	146.1	145.5±1.44	4.18-4.73	4.47	4.46±0.27
	7-30 day	4	146.8-147.9	147.5	147.4±0.56	4.08-4.37	4.22	4.22±0.13
	1-2 month	4	147.0-152.6	149.7	149.8±2.44	3.93-4.84	4.27	4.34±0.36
	2-4 month	5	146.4-148.4	147.4	147.4±0.83	4.02-4.72	4.46	4.43±0.25
Males	0-7 day	6	142.6-148.6	146.9	146.5±2.16	4.12-4.73	4.33	4.41±0.25
	7-30 day	4	147.0-148.2	147.8	147.7±0.50	3.53-4.09	4.06	3.93±0.27
	1-2 month	5	147.8-149.2	148.2	148.5±0.67	3.90-5.04	4.11	4.27±0.44
	2-4 month	5	146.7-150.5	147.0	148.2±1.84	4.17-4.99	4.30	4.47±1.17
Landrace females	0-7 day	5	144.9-147.3	146.3	146.3±1.01	4.09-4.66	4.34	4.39±0.23
	7-30 day	12	144.8-149.5	147.5	147.4±1.53	4.12-4.73	4.42	4.43±0.20
	1-2 month	6	146.0-148.7	146.5	146.8±1.01	4.35-4.85	4.59	4.58±0.21
	2-4 month	41	149.6-156.1	153.0	153.4±2.55	3.89-5.24	4.63	4.62±0.44
	4-8 month	38	146.6-158.0	150.5	150.8±2.89	3.65-4.99	4.63	4.54±0.36*
Males	0-7 day	13	144.1-150.2	146.4	146.7±1.67	4.05-5.63	4.39	4.51±0.47
	7-30 day	8	146.1-148.6	147.0	147.1±0.88	4.08-5.25	4.63	4.66±0.38
	1-2 month	10	144.2-148.3	146.3	146.3±1.27	4.01-5.04	4.53	4.47±0.32
	2-4 month	21	146.8-153.3	151.5	150.7±2.49	4.30-5.08	4.70	4.67±0.31
	4-8 month	6	149.6-154.0	152.0	151.8±1.90	3.69-5.53	4.58	4.63±0.65*

n = number of goats, For differences in means between sexes within the breeds in similar age groups $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table 5: Environmental influence on plasma calcium, magnesium, inorganic phosphorus, sodium and potassium levels (mmol/l) in dwarf and landrace kids (means ± standard deviations).

Age	Herd	*n*	Calcium	Magnesium	Phosphorus	Sodium	Potassium
0-7d	B	14	2.98 ± 0.12	0.91 ± 0.07	3.67 ± 0.07	146.6 ± 1.13	4.41 ± 0.32
	C	4	2.65 ± 0.08	0.73 ± 0.04	2.59 ± 0.47	146.4 ± 2.67	4.70 ± 0.65
	G	9	3.00 ± 0.10	0.92 ± 0.11	2.83 ± 0.27	146.2 ± 1.92	4.42 ± 0.24
7-30d	B	13	2.86 ± 0.16	0.87 ± 0.12	3.41 ± 0.45	147.1 ± 1.44	4.52 ± 0.15
	C	2	2.41 ± 0	0.75 ± 0.00	2.73 ± 0.09	146.5 ± 0.42	5.12 ± 0.18
	F	5	2.89 ± 0.15	0.85 ± 0.01	3.03 ± 0.23	148.2 ± 0.51	4.29 ± 0.33
	G	8	3.13 ± 0.27	0.88 ± 0.04	3.39 ± 0.22	147.6 ± 0.51	4.08 ± 0.25
1-2m	B	16	2.79 ± 0.16	0.81 ± 0.11	3.48 ± 0.41	146.4 ± 1.17	4.51 ± 0.28
	G	9	2.88 ± 0.13	0.97 ± 0.04	3.53 ± 0.30	149.0 ± 1.71	4.30 ± 0.39
2-4m	B	42	2.65 ± 0.22	0.98 ± 0.13	2.70 ± 0.58	153.4 ± 2.32	4.66 ± 0.39
	C	1	2.63	0.85	3.94	153.1	4.30
	D	7	2.70 ± 0.17	0.90 ± 0.06	3.88 ± 0.52	147.3 ± 0.95	4.72 ± 0.27
	E	2	2.54 ± 0.03	0.95 ± 0.01	3.13 ± 0.37	150.7 ± 0.64	4.90 ± 0.25
	F	10	3.19 ± 0.58	1.18 ± 0.21	2.15 ± 0.73	152.4 ± 1.54	4.45 ± 0.51
	G	10	3.00 ± 0.12	1.07 ± 0.07	3.53 ± 0.22	147.8 ± 1.39	4.5 ± 0.28
4-8m	A	17	2.53 ± 0.10	0.95 ± 0.05	2.73 ± 0.47	144.9 ± 2.30	4.48 ± 0.43
	B	27	2.34 ± 0.25	1.03 ± 0.17	2.62 ± 0.48	149.9 ± 2.40	4.55 ± 0.34
	E	3	2.23 ± 0.30	1.00 ± 0.17	2.29 ± 0.88	150.3 ± 4.41	4.19 ± 0.64
	F	14	2.46 ± 0.17	1.00 ± 0.09	2.21 ± 0.64	153.2 ± 1.79	4.64 ± 0.44
8-12m	A	7	2.65 ± 0.05	0.86 ± 0.05	2.81 ± 0.58	148.6 ± 1.20	4.90 ± 0.38
	E	3	2.48 ± 0.11	0.99 ± 0.09	1.99 ± 0.53	152.0 ± 2.23	4.61 ± 0.34
	F	10	2.46 ± 0.17	0.91 ± 0.11	2.42 ± 0.87	141.7 ± 5.15	3.97 ± 0.55

n = number of kids, d = days, m = month, Where comparisons involve more than 2 herds, mean values with similar superscripts (a b c d e) differ significantly. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

groups (2/4*, 2/5*, 2/6*, 3/6*, 4/6* and 5/6*). In landrace kids potassium levels were significantly lower at 8 - 12 months of old than in all other age groups (fig. 2d).

Sodium concentrations were 146.2 ± 1.92 and 146.6 ± 1.50 mmol/l in neonatal dwarf and landrace kids respectively with significant differences at some ages within the growth period (fig. 3, table 2). Sodium was significantly different between age groups in dwarf kids (1/3 and 1/6 (fig. 3a), 2/5*, 3/5*, 4/5* and 5/6*). There were differences in landrace kids among groups 1/4 and 1/5 (fig. 3b) and 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 4/5* and 4/6*. The calcium to phosphorus s were 1.06, 0.92, 0.82, 0.93 and 0.94 in dwarf and 0.85, 0.87, 0.80, 0.98, 0.96 and 1.06 in landrace kids of group 1 to 6 respectively. These were constant throughout with only minor fluctuations. The concentrations of these plasma electrolytes were not significantly different between female and male kids of both breeds in most age groups (table 3-4). There were highly significant differences in mean electrolyte concentrations between kids of the same breed from different herds (table 5).

Discussion

The influence of age on plasma electrolytes for the Danish Landrace and Dwarf goats of one day to one year of age have been elucidated in this investigation. The parametric and nonparametric statistics did not differ significantly for plasma electrolytes. The mean is the parametric central measure of numeric data, while the median is a corresponding nonparametric statistic. The range (minimum to maximum observation interval) or the 2.5^{th} to 97.5^{th} or the 5^{th} to 95^{th} percentile intervals are used in blood parameters together with $\bar{x} \pm s$ (Zinkl *et al.*, 1990). All the $\bar{x} \pm s$ values of calcium, magnesium, inorganic phosphorus, sodium and potassium in all age groups of both breeds were within the 5^{th} to 95^{th} percentile interval which was in turn within $\bar{x} \pm 2s$. The medians were very close to the means in most age groups of both breeds for all the ions (table 1 - 4). Since the distributions were Gaussian and sampling was random the data satisfied the conditions for use of parametric tests, taking advantage of their power (Siegel and Castellan, 1988).

Plasma calcium, magnesium, inorganic phosphorus, sodium and potassium appeared to be influenced by breed as there were significant differences between dwarf and landrace kids of similar ages within most of the growth period (table 1 - 2). This indicates some possible breed influence on their concentrations. Similar differences were observed by Catarsini *et al.* (1982). There were also highly significant differences in all the electrolytes between kids of similar ages from different herds within the breeds (table 5), indicating major environmental influence. Herd variations probably account for most differences observed, thus explaining the disagreements between different investigations (Davies and Sims, 1983).

The influence of age on plasma electrolytes was enormous (figs. 1 - 3). The effect of age on ion concentrations in very young animals is described to be due to variations in basal metabolic rates (Bogin *et al.*, 1981) and nutrition (Vrzgula *et al.*, 1985). Electrolyte levels are reported to be high in young animals (Bogin *et al.*, 1981; Kumaresan and Ndzingu Awa, 1984; Bhattacharyya and Duttagupta, 1987; Gray *et al.*, 1988) due to high metabolic rates, milk source and fast osteogenesis, particularly for calcium and phosphorus. The present studies agree with this trend (fig. 1-2, table 1 - 4). The differences in these electrolytes between ages within the breeds were also extensive (fig. 1 - 2). The rise in Ca, Mg, and P within one week of birth could be due to colostral intake (Braun *et al.*, 1983). Sodium concentrations follow similar patterns but the changes are slight, whereas potassium levels increase slightly with age.

Differences in plasma calcium, magnesium, phosphorus, sodium and potassium due to sex were not observed in kids younger than 2 months, which is in agreement with the results of Youssef (1985) and Chiericato *et al.* (1986). This profile indicates that interpretation of laboratory results for diagnosis require careful use of generalized data for all goat categories (Lloyd, 1982; Sherman and Robinson, 1983; Gray *et al.*, 1988).

It is apparent from these results that the calcium - phosphorus is constant irrespective of age, being slightly higher in younger than in older kids. It is known that an imbalance of these electrolytes produces rickets due to reduced disposition of calcium in growing bone and osteomalacia from fully developed bones in adult animals.

In conclusion, the profiles and age specific values for plasma calcium, magnesium, inorganic phosphorus, sodium and potassium concentrations in Danish landrace and dwarf goats from birth to 12 months of age have been determined. The concentrations are kept within very narrow limits and apart from the changes in the first three months the concentrations are very constant. It is concluded unequivocally that age is a determinant factor in the levels of plasma electrolytes in young goats. Because of the narrow limits, electrolytes form an important test for identification of pathophysiological states when the reference intervals are known in strictly healthy goats. The levels are higher in newborn kids than in adult animals. Differences between female and male kids were not significant.

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CHAPTER 8

PROFILES OF SOME PLASMA ENZYME ACTIVITIES IN KIDS

Summary

Plasma alanine aminotransferase, aspartate aminotransferase, creatine kinase and alkaline phosphatase activities were studied in 62 Danish landrace and 34 dwarf kids in seven herds from birth to 12 months of age. The purpose was to evaluate the influence of age, breed and herd on reference values. The mean ($\bar{x}$) enzyme levels ± standard deviation (s) in neonatal dwarf kids were 0.09 ± 0.04, 1.23 ± 0.24, 2.79 ± 1.50 and 18.3 ± 11.0 μkat/l respectively. The respective values in landrace kids were 0.13 ± 0.06, 1.06 ± 0.22, 2.44 ± 1.60 and 37.6 ± 23.6 μkat/l. In 8 - 12 months old dwarf kids they were 0.30 ± 0.11, 1.49 ± 0.13, 3.28 ± 0.44 and 11.1 ± 2.4 μkat/l respectively and 0.23 ± 0.05, 1.12 ± 0.34, 3.68 ± 1.63 and 14.1 ± 8.40 μkat/l respectively in same age landrace kids. The 5th to 95th percentile intervals of the enzyme activities for most age groups in both breeds were within the $\bar{x} \pm 2s$ range except alkaline phosphatase. The means and medians were close to each other for the values of alanine aminotransferase, aspartate aminotransferase and CK but not for alkaline phosphatase. Alanine aminotransferase, aspartate aminotransferase and creatine kinase levels were low at birth and increased with age, whereas for alkaline phosphatase it was vice versa. Significant differences were observed in mean enzyme activities between; kids of different ages (within breeds), breeds (in same age kids) and herds (within same breed and age kids). Sex variations (within the breeds) were not observed. It was concluded that plasma enzyme activities are dependent on age, breed and environment.

Introduction

Quantitative analysis of plasma/serum enzyme activities yield important clues to differential diagnosis of affected organs in diseases, metabolic disorders, nutritional deficiencies and parasitism (Kassuku *et al.*, 1986; Bogin *et al.*, 1988; Boyd, 1988). In order to obtain reliable conclusions based on the enzyme pattern, plasma or serum enzyme activities in clinically healthy animals must be known because apart from the influences due to diseases, other factors bring about variations in enzyme activities. Fluctuations associated with breed, developmental processes and also individual animal characters have been reported (Bialkowski *et al.*, 1988). Furthermore, values reported in goats, particularly growing kids, are inconsistent and vary from place to

place (Chiofalo *et al.*, 1982; Bialkowski *et al.*, 1988). These observations lead to the possible concept of an existence of specific plasma/serum enzyme activity levels for each age, breed and herd. In addition to these factors, sex, season, nutrition and physiological states may account for a part of the reported variations. The magnitude of non disease factor influence in healthy animals must therefore be known in order to obtain the desired information of reference values. Knowledge is available in some goat breeds (Edjetihadi, 1978; Bogin *et al.*, 1981; Chiofalo *et al.*, 1982), but there is scarce information on plasma enzyme activities in dwarf goats and the dairy Danish landrace goats. The present investigations were therefore conducted to study the plasma enzyme activity profiles in newborn kids of two breeds and in the subsequent growth period.

Materials and Methods

Blood samples were collected from the external jugular vein in heparinized vacuum tubes (Becton - Dickinson Vacutainers) in healthy 62 Danish landrace and 34 dwarf kids from the first day of life to 12 months of age. Blood samples were collected every 3 weeks and after 8 weeks of age every 2 months to make divisions of six age groups [(0 - 7 days (1), 7 -30 days (2), 1 - 2 (3), 2 - 4 (4), 4 - 8 (5) and 8 - 12 (6) months old)].

The kids were from seven Danish herds (A - G) located at The Royal Veterinary and Agricultural University (A), Fakse (B), Næstved (C), Ringsted (D), Haslev (E), Fugleberg (F) and Copenhagen Zoo (G). In all the farms kids naturally suckled milk from their mothers until weaning. The kids were gradually introduced to grass pellets or natural grazing, and oats or barley grains during the growth period. The goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms B to E in summer. In farms F and G goats were reared in the field for the entire summer and autumn.

The activities of alanine aminotransferase (ALAT, EC 2.6.1.2), aspartate aminotransferase (ASAT, EC 2.6.1.1) and creatine kinase (CK, EC 2.7.3.2) were determined kinetically at 37 ^{0}C, according to the recommendations of the Scandinavian Committee on Enzymes in an auto- analyzer Cobas Fara (ROCHE). Alkaline phosphatase (ALP, EC 3.1.3.1) activity was measured by end point colorimetric method in the same analyzer. Determinations were made immediately or within 24 hours of sampling and plasma separation.

The parametric (mean, standard deviation) and nonparametric (5^{th}, 95^{th} percentile, median) values were determined by normal univariate procedure of a statistical analysis software (SAS, Carry, USA). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte to the Gaussian distribution. The mean plasma

enzyme activities were tested for differences between kids in ages (within the breeds), breeds (in same age kids), herds (in same age and breed kids) and sex (within breeds) by the general linear models procedure.

Results

The enzyme activity distributions showed mild skewness and kurtosis but most of them did not significantly deviate from Gaussian distributions in most age groups in both breeds ($0.98 \leq W \leq 1$, table 1-2). In a few age groups significant deviations from Gaussian distributions were noted (table 2).

The activity of ALP was 18.3 ± 11.0 (mean ± standard deviation) and 37.6 ± 23.6 μkat/l in neonatal dwarf and landrace kids respectively (table 2). The activity was higher in 0 - 7 day old dwarf kids (age group 1) than in 4 - 8 and 8 - 12 months of age (fig. 1a). In landrace kids ALP activity was higher in the first week than in 2 - 4, 4 - 8 and 8 - 12 months old kids (fig. 1b)[1]. Differences in ALP activity among dwarf kids of other age groups were observed (2/5*[2], 2/6*, 3/5*, 4/5** and 5/6**). In landrace kids significant differences were also observed between the age groups (2/4**, 2/5*, 2/6*, 3/5* and 3/6*). ALP levels decreased to 11.1 ± 2.4 and 14.1 ± 8.4 μkat/l in dwarf and landrace kids at 8 - 12 months of age respectively. The activity of ALP was significantly higher in landrace than in dwarf kids at various ages during the growth period (table 2).

Plasma ALAT activity in the first week of life was 09 ± 0.04 and 0.13 ± 0.06 μkat/l in dwarf and landrace kids respectively (table 2) and increased within 12 weeks of age. ALAT activity was lowest in neonates than in the other age groups (fig. 1c-d). Significant differences were also observed among kids of the other age groups (2/4*, 2/5*, 2/6*, 3/4*, 3/5* and 3/6* in dwarfs and 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 3/6* and 4/5* in landraces).

Plasma ASAT activity was 1.23 ± 0.24 and 1.06 ± 0.22 μkat/l in the first week of life in dwarf and landrace kids respectively, then the values gradually increased to reach levels of 1.49 ± 0.13 and 1.12 ± 0.34 μkat/l in the two breeds respectively (table 2) whereafter they decreased slightly. ASAT activity in 0 - 7 day kids was significantly lower than at 2 - 4 and 4 - 8 months of age (fig. 2a-b). The activity in 7 - 30 day old dwarf kids was also significantly lower than in 2 - 4 months old kids while many other significant differences were observed between various age groups in landrace kids (2/4*, 2/5*, 3/4*, 3/5*, 4/6* and 5/6*).

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[1]Only significant differences between group 1 and others are shown in figures, not among other age groups.

[2]*$p<0.05$, **$p<0.01$, ***$p<0.001$.

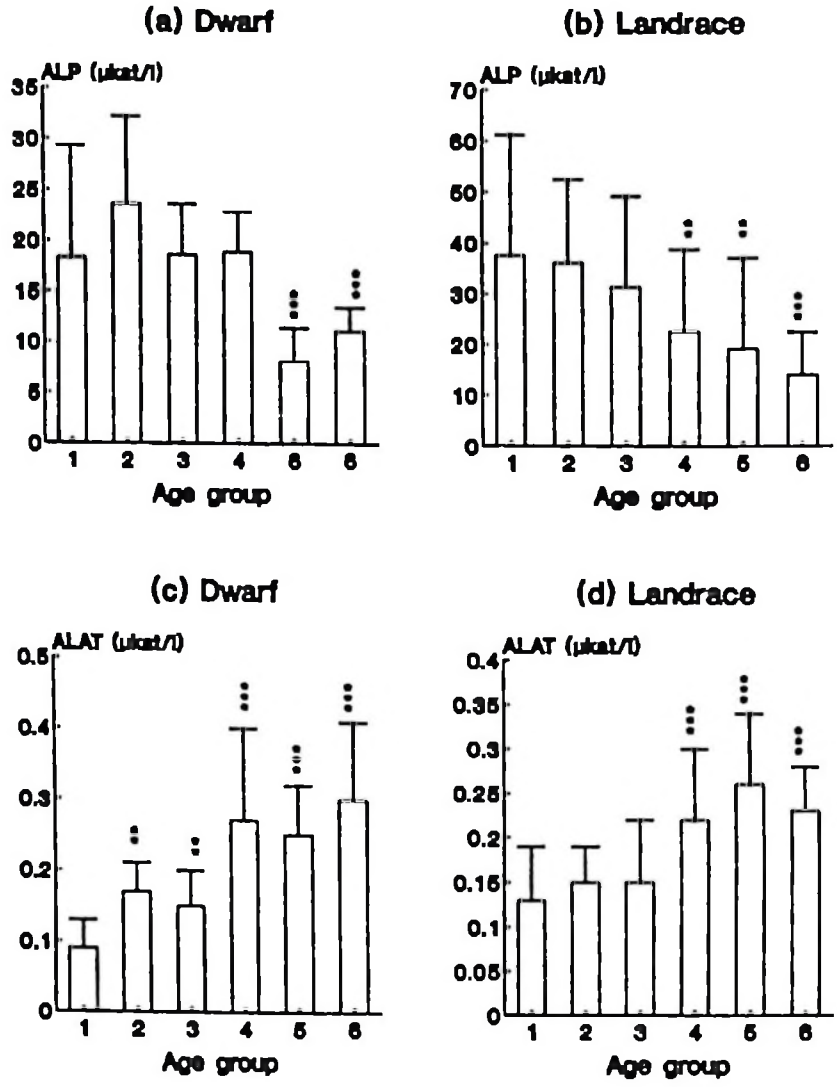

Figure 1: Plasma ALP (a) and (b), and ALAT (c) and (d) activities with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). For means differing significantly from neonatal values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

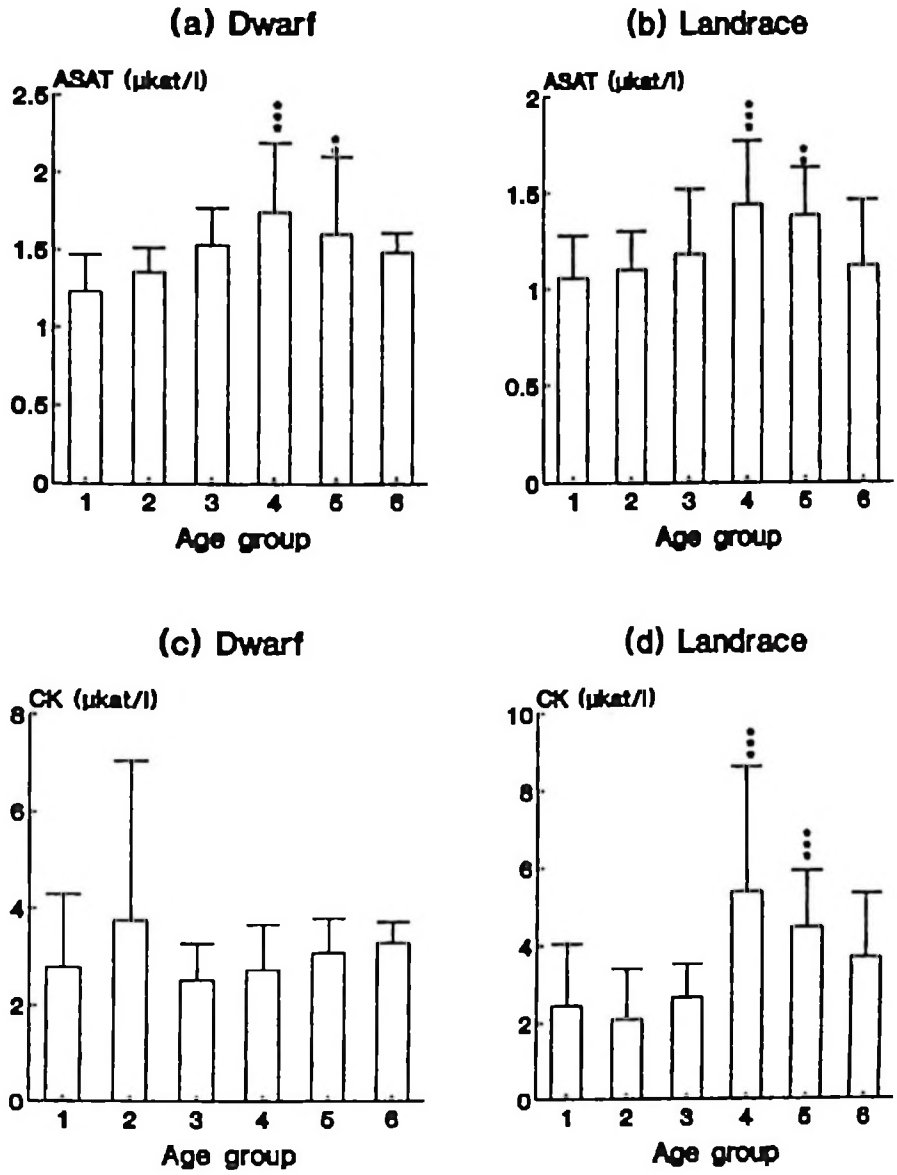

Figure 2: Plasma ASAT (a) and (b), and CK (c) and (d) activities with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months old (group 6). For means differing significantly from neonatal values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Table 1: Comparison between the 5th (P_5) to 95th (P_{95}) percentile intervals and the mean ± standard deviation $\bar{x} \pm s$, the median (Q_2) and the mean values of plasma enzymes in growing kids in relation to sex

	ALAT µkat/l			ASAT µkat/l			CK µkat/l			ALP µkat/l		
Age (n)	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
Dwarf females												
0-7d (3)	0.08-0.15	0.13	0.12±0.04	1.07-1.59	1.48	1.38±0.27	1.62-5.00	2.26	2.96±1.79	9.83-36.2	13.1	19.7±14.4
7-30d (4)	0.17-0.22	0.19	0.19±0.02	1.22-1.44	1.40	1.37±0.10	2.15-11.5	3.25	5.04±4.35	11.4-37.5	23.2	23.8±10.8
1-2m (4)	0.12-0.24	0.21	0.19±0.05	1.21-1.79	1.58	1.50±0.26	1.53-3.62	2.82	2.70±1.07	14.0-23.9	22.1	20.5±4.41
2-4m (5)	0.22-0.58	0.27	0.34±0.15	1.42-2.68	1.53	1.84±0.54	1.67-4.25	3.65	3.25±1.02	13.7-22.8	21.5	18.9±4.30
Dwarf males												
0-7d (6)	0.02-0.11	0.08	0.07±0.04	0.90-1.51	1.13	1.15±0.21	1.29-4.70	2.24	2.71±1.50	8.96-36.0	13.3	17.6±10.5
7-30d (4)	0.09-0.19	0.16	0.15±0.04	1.16-1.61	1.31	1.35±0.22	1.29-4.19	2.20	2.47±1.43	17.3-33.7	21.2	23.3±7.45
1-2m (5)	0.08-0.14	0.13	0.12±0.02	1.24-1.81	1.50	1.54±0.26	1.78-2.99	2.34	2.37±0.49	10.2-25.4	16.8	17.1±5.44
2-4m (5)	0.14-0.31	0.17	0.20±0.07	1.27-2.28	1.60	1.67±0.38	1.32-2.78	2.16	2.17±0.54	13.3-25.0	18.3	19.0±4.28
Landrace females												
0-7d (5)	0.13-0.20	0.15	0.16±0.03	0.97-1.28	1.11	1.12±0.11	1.37-3.41	1.84	2.14±0.79	24.9-50.4	44.0	40.6±9.75
7-30d (12)	0.09-0.22	0.15	0.15±0.04	0.82-1.66	1.04	1.14±0.24	0.55-6.84	1.68	2.13±1.58	16.3-64.8	42.0	38.1±14.4
1-2m (6)	0.12-0.22	0.15	0.16±0.04	1.02-1.40	1.15	1.19±0.17	1.17-3.26	2.54	2.49±0.75	18.5-66.7	36.0	40.8±21.6
2-4m (41)	0.11-0.13	0.21	0.21±0.05	1.11-1.75	1.38	1.40±0.23	2.89-9.21	4.63	5.57±3.50	4.20-47.0	18.1	21.4±14.0
4-8m (38)	0.11-0.42	0.26	0.26±0.08	0.97-1.91	1.35	1.41±0.25	2.19-7.62	4.40	4.59±1.50	1.21-60.2	13.2	18.1±17.0
Landrace males												
0-7d (13)	0.03-0.27	0.11	0.12±0.07	0.56-1.57	1.03	1.03±0.25	1.11-6.77	1.83	2.56±1.84	3.22-86.5	24.9	36.5±27.5
7-30d (8)	0.09-0.23	0.15	0.16±0.05	0.86-1.28	1.02	1.06±0.13	1.32-3.55	1.71	1.99±0.78	2.44-61.7	26.0	33.2±19.4
1-2m (10)	0.04-0.37	0.11	0.14±0.09	0.80-2.25	1.06	1.17±0.41	1.68-4.97	2.43	2.73±0.98	15.0-51.7	20.2	25.7±13.2
2-4m (21)	0.14-0.53	0.20	0.23±0.12	0.97-2.25	1.42	1.51±0.47	2.05-8.87	4.34	4.99±2.70	7.20-62.8	20.3	25.0±19.9
4-8m (6)	0.15-0.27	0.24	0.23±0.05	1.01-1.27	1.16	1.15±0.11	2.15-4.31	3.67	3.52±0.65	0.92-57.0	16.5	26.1±23.2

n = number of goats, d = days, m = months, For differences in means between sex within the breeds $^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p<0.001$.

Table 2: Comparison between the 5th (P_5) to 95th (P_{95}) percentile intervals and the mean ± standard deviation ($\bar{x} \pm s$), the median (Q_2) and the mean values of plasma enzyme activity levels in growing dwarf and landrace kids

Age (n)	ALAT µkat/l P_5-P_{95}	Q_2	$\bar{x} \pm s$	ASAT µkat/l P_5 - P_{95}	Q_2	$\bar{x} \pm s$	CK µkat/l P_5-P_{95}	Q_2	$\bar{x} \pm s$	ALP µkat/l P_5 - P_{95}	Q_2	$\bar{x} \pm s$
Dwarf												
0-7d (9)	0.02-0.15	0.09	0.09±0.04*	0.90-1.59	1.21	1.23±0.24	1.29-5.00	2.26	2.79±1.50	9.00-36.2	13.1	18.3±11.0W
7-30d (8)	0.09-0.22	0.18	0.17±0.04	1.16-1.61	1.40	1.36±0.16**	1.29-11.5	3.08	3.76±3.29	11.4-37.5	22.6	23.6±8.60*
1-2m (9)	0.08-0.24	0.13	0.15±0.05	1.21-1.81	1.50	1.54±0.24**	1.53-3.62	2.34	2.51±0.76	10.2-25.4	17.6	18.6±5.03*
2-4m (10)	0.14-0.58	0.24	0.27±0.13	1.27-2.68	1.56	1.75±0.45*	1.32-4.25	2.62	2.71±0.96*	13.3-25.0	19.5	18.9±4.00
4-8m (17)♂	0.16-0.40	0.24	0.25±0.07	1.24-3.48	1.50	1.61±0.50W	1.84-4.97	3.22	3.08±0.71***	2.94-14.7	9.00	8.09±3.33***
8-12m (7)♂	0.15-0.46	0.29	0.30±0.11	1.29-1.63	1.47	1.49±0.13**	2.48-3.84	3.43	3.28±0.44	8.33-14.6	10.9	11.1±2.39
Landrace												
0-7d (18)	0.03-0.27	0.15	0.13±0.06*	0.56-1.57	1.08	1.06±0.22	1.11-6.77	1.83	2.44±1.60W	3.22-86.5	33.4	37.6±23.6**
7-30d (20)	0.09-0.23	0.15	0.15±0.04	0.84-1.54	1.02	1.10±0.20**	0.88-5.19	1.68	2.09±1.30W	9.38-63.2	39.6	36.1±16.3*
1-2m (16)	0.04-0.37	0.13	0.15±0.07W	0.80-2.25	1.08	1.18±0.34*W	1.17-4.97	2.48	2.64±0.89	15.0-66.7	22.3	31.4±17.8W
2-4m (62)	0.13-0.32	0.20	0.22±0.08W	1.04-1.93	1.39	1.44±0.33*W	2.26-9.21	4.59	5.37±3.24*W	4.80-59.3	18.6	22.6±16.2W
4-8m (41)	0.13-0.39	0.26	0.26±0.08	1.01-1.83	1.30	1.38±0.25	2.19-6.56	4.26	4.45±1.46***	1.21-57.0	13.2	19.2±17.9*W
8-12m (13)♀	0.13-0.33	0.22	0.23±0.05	0.73-2.01	1.14	1.12±0.34**	1.17-6.07	3.59	3.68±1.63	1.05-25.2	12.9	14.1±8.40

n = number of goats, d = days, m = months, ♂ = males only, ♀ = females only
W = non Gaussian distributions ($p<0.05$).
For differences between means of the two breeds within same ages in columns *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 3: Environmental influence on plasma enzymes in growing dwarf kids (herd A and G) and landrace kids in the rest of the herds (means ± standard deviations).

Age	H (n)	ALAT µkat/l	ASAT µkat/l	CK µkat/l	ALP µkat/l
0-7d	B (14)	0.15 ± 0.05^{ab*}	1.11 ± 0.16^{a*}	2.30 ± 1.43	43.4 ± 23.7^{ab**}
	C (4)	0.05 ± 0.02^{a*}	0.85 ± 0.32^{ab**}	2.95 ± 2.31	17.7 ± 7.5^{a**}
	G (9)	0.09 ± 0.04^{b*}	1.23 ± 0.24^{b**}	2.79 ± 1.50	18.3 ± 11.0^{b**}
7-30d	B (13)	0.15 ± 0.04	1.15 ± 0.21^{a*}	2.41 ± 1.46	35.8 ± 18.9
	C (2)	0.10 ± 0.01^{ab**}	1.08 ± 0.09^{a*}	2.01 ± 0.96	25.4 ± 0.93
	F (5)	0.18 ± 0.03^{a*}	0.99 ± 0.18^{b*}	1.20 ± 0.41*	41.2 ± 9.75*
	G (8)	0.17 ± 0.04	1.36 ± 0.16^{ab*}	3.76 ± 3.30*	23.6 ± 8.70*
1-2	B (16)	0.15 ± 0.07	1.18 ± 0.34*	2.64 ± 0.89	31.4 ± 17.8*
	G (9)	0.15 ± 0.05	1.54 ± 0.24*	2.52 ± 0.76	18.6 ± 5.0*
2-4m	B (42)	0.22 ± 0.09	1.50 ± 0.34^{a*}	5.41 ± 1.77^{a**}	19.0 ± 12.9^{a***}
	C (1)	0.30	1.31	4.57	12.6^{a*}
	D (7)	0.20 ± 0.02	1.35 ± 0.31^{b*}	7.10 ± 2.50^{b**}	46.5 ± 19.3^{abcde**}
	E (2)	0.14 ± 0.01	1.04 ± 0.09^{c*}	3.65 ± 0.59	21.5 ± 19.0^{d*}
	F (10)	0.25 ± 0.05	1.31 ± 0.24^{d*}	4.43 ± 1.60	22.5 ± 14.6^{c*}
	G (10)	0.27 ± 0.13	1.75 ± 0.45^{abcd*}	2.71 ± 0.96^{ab**}	18.9 ± 4.0^{d*}
4-8m	A (17)	0.25 ± 0.07^{ad*}	1.61 ± 0.05^{ab*}	3.08 ± 0.71^{ab*}	8.1 ± 3.33^{a*}
	B (27)	0.24 ± 0.06^{be*}	1.43 ± 0.24	4.65 ± 1.51^{a*}	23.6 ± 19.0^{ab**}
	E (3)	0.15 ± 0.04^{cde*}	1.11 ± 0.21^{b*}	4.86 ± 1.54^{b*}	13.1 ± 12.9
	F (14)	0.31 ± 0.08^{abc*}	1.34 ± 0.24^{a*}	3.97 ± 1.34	12.1 ± 14.4^{b**}
8-12m	A (7)	0.30 ± 0.07	1.49 ± 0.13^{a*}	3.28 ± 0.44^{a*}	11.1 ± 2.4
	E (3)	0.25 ± 0.07	1.46 ± 0.48^{b*}	5.07 ± 1.14^{ab*}	16.9 ± 6.9
	F (10)	0.23 ± 0.05	1.02 ± 0.24^{ab*}	3.26 ± 1.55^{b*}	13.3 ± 9.0

H= Herd, *n*= number of kids, d= days, m= month.
Where comparisons involve more than 2 herds, means with similar superscripts (a b c d e) in columns within the age groups differ significantly, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Herd A and G= dwarf, B-F= landrace.

Plasma CK activity in neonatal dwarf and landrace kids were 2.79 ± 1.50 and 2.44 ± 1.60 µkat/l respectively. There was an increase to 3.28 ± 0.44 and 3.68 ± 1.63 µkat/l in the two breeds respectively at 8 - 12 months of age. There were no significant differences among different age groups in dwarf kids (fig. 2c). Plasma CK activity was lower in 0 - 7 day than in 2 - 4 and 4 - 8 month old landrace kids (fig. 2d). There were significant differences in mean CK activity between several age

groups (2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 4/5* and 4/6*).

Significant differences were observed in the mean plasma activities of ASAT and ALP between dwarf and landrace kids of the same age (table 2). There were non significant differences in plasma enzyme activities between female and male kids of the same age within the same breed (table 1).

Highly significant differences were observed in the enzyme activities between kids of the same age and breed, but from different herds (table 3) indicating an environmental influence.

Discussion

The mean ± standard deviation (s) is used in expressing blood parameters together with the range (interval from the minimum to maximum observation) or the nonparametric statistics 2.5th to 97.5th or 5th to 95th percentile intervals (Zinkl *et al.*, 1990). The parametric ($\bar{x}$ ± 2*s*) and nonparametric ranges were not significantly different for ALAT, ASAT and CK. All the mean ± s values in all age groups were within the 5th to 95th percentile intervals which in turn were within the $\bar{x}$ ± 2*s* ranges. The means and medians were very close to each other in most age groups of both breeds (table 1-2). Parametric tests were used for plasma enzyme levels because the data satisfied the conditions (randomness, Gaussian distribution and/or equal population variances). Parametric tests are most powerful when all the statistical model assumptions are fulfilled (Siegel and Castellan, 1988). Use of parametric tests for data that are not proved to be normally distributed may lead to misinterpretation of results (Reed *et al.*, 1971; Wu *et al.*, 1975).

Parametric and nonparametric ranges for alkaline phosphatase were significantly different at some ages in both breeds. Values in the present landrace kids ranged widely (0.92 to 86.46 μkat/l) indicating a large individual animal variations. This is probably due to the occurrence of many multiple molecular isoforms (Milne, 1985; Tolling, 1988), in the microvilli of the secretory and absorptive epithelia (bile duct canaliculi, intestinal tract, renal tubules and placenta), liver cells, adrenal gland and osteoblasts. Application of mean ± 2s would result in negative values for the lower reference limit, therefore the 5th to 95th percentile interval is the important clinical range.

The results of the present investigation show higher ALP activities in newborn dwarf and landrace kids and a decrease with age (fig. 1a, b, table 2). Plasma alkaline phosphatase activity is reported to be higher in young than in adult animals because of bone formation (Bogin *et al.*, 1981; Bhattacharyya and Duttagupta, 1987; Bialkowski *et al.*, 1988; Kramer, 1989) and in pregnant animals due to fetal demands (Kumaresan and Ndzingu Awa, 1984). ALP activity varies with age and environment

(table 1 and 3) and differs between breeds but not sexes (table 1 and 2), hence the lack of agreement in clinical chemical values between various sources.

ALAT is present in small quantities in ruminants (Kramer,1989). It was observed to be low at birth, then increased with age (fig. 1), probably due to increasing body mass and metabolic activities. Bogin *et al.* (1981) had observed no difference in plasma ALAT activity in young and old animals but Braun *et al.* (1983) and Raviart *et al.* (1987) noted a slight increase after birth. It was noted in the present dwarf and landrace kids that breed differences occurred within the first week, above which ALAT activity levels were not significantly different (fig. 1). There were, however, herd differences in same age kids within the breeds (table 3).

ASAT occurs in larger amounts than ALAT and in two isoenzymes, anionic cytosol and cationic mitochondrial in cells of many tissues and is thereby a good indicator of soft tissue damage (Boyd, 1988, Kramer, 1989). ASAT activity was observed to increase gradually up to two months of age whereafter the levels became constant (fig. 2). These findings are similar to those reported in Polish goats (Bialkowski *et al.*, 1988). A wide age grouping of goats was probably responsible for the lack of differences between young kids and old animals reported by Bogin *et al.* (1981). The activity levels of this enzyme were also different in kids of the same age and breed, from different herds (table 3) indicating an environmental influence.

CK is located mainly in skeletal and cardiac muscles and brain. Plasma activities were observed to increase gradually from neonatal values to constant levels within 8 - 12 months of age. CK levels probably depend on the muscle mass, thus increase only gradually. The individual goat variations in CK activity were very high as revealed by the large standard deviations (table 1 - 2).

The results of this study on the influence of sex agree with that of Chiericato *et al.* (1986) that there were no sex differences in enzyme activities in young goats. The differences between herds were highly significant (table 3) indicating strong environmental influences, probably a reflection of the different feeds.

In conclusion, plasma ALAT, ASAT and CK were observed to be low in neonates and increase with age only slightly. This appears to be due to cell increases in body tissues. ALP activity levels are high in neonates and decrease with age. There were no sex differences in plasma enzymes in both breeds. All the four enzymes were found to be age, breed and environment dependent. Parametric and nonparametric statistical analysis were in close agreement for ALAT, ASAT and CK, but there were differences for ALP. Biological differences in ALP could be better tested by nonparameteric methods.

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CHAPTER 9

PROFILES OF CLINICAL CHEMICAL PARAMETERS IN KIDS

Summary

Plasma creatinine, urea, bilirubin, total serum proteins, glucose and cholesterol were analyzed in dwarf and landrace kids from birth to 12 months of age. The purpose was to determine the reference range and factors that affect the profiles. The mean ± standard deviation ($\bar{x} \pm s$) in neonatal dwarf kids were 80.0 ± 29.0 μmol/l, 8.0 ± 2.8 mmol/l, 19.3 ± 7.0 μmol/l, 61.3 ± 5.9 g/l, 4.8 ± 0.8 mmol/l and 2.5 ± 1.1 mmol/l respectively. The respective values for neonatal landrace kids were 50.3 ± 10.7 μmol/l, 5.1 ± 2.5 mmol/l, 9.6 ± 4.4 μmol/l, 54.0 ± 6.4 g/l, 5.4 ± 1.4 mmol/l and 2.9 ± 0.7 mmol/l. The mean and median values for analytes were closely related. The $\bar{x} \pm s$ for most parameters were in the 5th to 95th percentile intervals which in turn was within the $\bar{x} \pm 2s$ range. These parameters were very much dependent on age. Creatinine, urea and total serum protein levels increased gradually with age in both breeds. Glucose and cholesterol levels were high at birth and then decreased with age. Significant differences in these parameters were observed between kids of different ages (within the breeds), breeds (within similar age) and herds (within the same age and breed). Differences between female and male landrace kids of the same ages were observed in plasma urea, creatinine, glucose and total serum proteins. It is concluded that age has a major influence on the clinical chemical reference values in young goats, followed by herd and breed, but the influence of sex is small and negligible in clinical diagnosis.

Introduction

Analyses of concentration of clinical chemical parameters in plasma or serum are important for diagnosis and differential diagnosis of several diseases, metabolic disorders and nutritional deficiencies (Boyd, 1984; Wilson *et al.*, 1986; Feldman and Thomason, 1989). The reported values of many clinical chemical parameters in goats, particularly growing kids are inconsistent and vary from place to place, proving difficult to compare (Davies and Sims, 1983). Variations in plasma or serum levels due to age, breed, nutrition, season and individual characters are reported (Bialkowski *et al.*, 1988). The existence of numerous goat breeds therefore require specification of the conditions under which the reference values of clinical chemical parameters are determined. If these are not specified interpretation of laboratory results of clinical

cases for diagnostic purposes will be uncertain and in some cases may mislead. Serum or plasma levels of many parameters in young animals are different from those of adults, particularly in neonates because of abrupt changes for adaptation to terrestrial life (Braun *et al.*, 1983). The extensive metabolic changes that occur during growth are obviously accompanied by parallel changes in the whole blood, plasma or serum levels of many parameters. To be able to distinguish changes due to age from those brought about by disease, knowledge of the profiles in growing healthy goats is of fundamental importance. Studies in clinical chemical reference values in dwarf and Danish landrace goats are scarce. Some studies have been performed in the former breed (Castro *et al.*, 1977a; 1977b), but there is no information on the latter. Investigations were therefore initiated to study the profiles of clinical chemical parameters that are commonly used in disease diagnosis namely plasma urea, creatinine, bilirubin, total serum proteins, glucose and cholesterol in neonatal dwarf and landrace kids and at subsequent growth periods. The Danish landrace breed goats are large dairy, weighing 30 to 40 Kg, commonly white but brown and black colors occur. They form the commonest breed in Denmark. There has been criticisms on the inferences based on parametric tests whose models assume a Gaussian distribution (Reed *et al.*, 1971; Wu *et al.*, 1975). Since these are the most frequently used statistical tests, it was thus felt appropriate to use both parametric and nonparametric statistical methods and compare the results in order to eliminate any statistical method influences.

Materials and Methods

Blood samples were collected from the external jugular vein in vacuum tubes (Becton-Dickinson vacutainers) containing 143 USP units of sodium heparin and sodium fluoride with sodium heparin (NaF/Naheparin) and clot activator for serum separation, from 62 clinically healthy Danish landrace and 34 dwarf kids of one day to 12 months of age. Blood samples were collected every 3 weeks and after 8 weeks of age every 2 months. These were categorized in age groups of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 (group 6) months old.

The kids were from 7 Danish herds (A - G) located at The Royal Veterinary and Agricultural University (A), Fakse (B), Næstved (C), Ringsted (D), Haslev (E), Fugleberg (F) and Copenhagen Zoo (G). In all the farms kids naturally suckled milk from their mothers until weaning. The kids were gradually introduced to grass pellets or natural grazing, and oats or barley grains during the growth period. Goats were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms B to E in summer. In farms F and G goats were reared in the field for the entire summer and autumn.

Plasma urea, creatinine, bilirubin, cholesterol and glucose were determined in an automated analyzer COBAS FARA (ROCHE) by enzymatic spectrophotometric methods. Total serum proteins were determined by the Biuret method in the same analyzer.

The parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentile, medians) were determined by the univariate procedure of a statistical analysis software (SAS, Cary USA, 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte to the Gaussian distribution,. The means of analytes were tested for differences between kids of different ages (within the breeds), breeds (in kids of similar age), herds (in kids of same age and breed) and sex (within the breeds) by using the general linear models procedure.

Results

The mean and median values of plasma urea, creatinine, bilirubin, cholesterol, glucose and total serum proteins in most age groups in both breeds were close to each other (table 1-4). The coefficients of skewness and kurtosis were also small in most age groups in both breeds. The Shapiro Wilk (W) statistic testing for Gaussian distribution proved that the frequency distributions of all the clinical chemical parameters in most age groups were Gaussian ($0.98 \leq W \leq 1$). Only in a few age groups were there significant deviations from Gaussian (table 1). The 5^{th} and 95^{th} percentile intervals were included in the mean ± 2 standard deviation range in most age groups of both dwarf and landrace kids (table 1-4).

Plasma urea concentration was 8.0 ± 2.8 and 5.1 ± 2.5 mmol/l (mean ± standard deviation) in dwarf and landrace kids respectively in the first week of life (fig. 1a and b, table 1). Values decreased in the second week in both breeds, whereafter a gradual increase was found. Urea varied more in dwarf than in landrace kids during growth and the concentration was significantly higher in the former than in the latter except during the second week of age (table 1). There were significant differences in urea between neonates and age groups 2, 3, 4 and 6 in dwarf (fig. 1a)[1] and 2, 3 and 6 in landrace kids (fig. 1b). There were significant differences among other age groups (2/5*[2], 2/6*, 3/4*, 3/5*, 3/6*, 4/5*, 4/6* and 5/6* in dwarf and 2/4*, 2/5*, 2/6*, 3/4*, 3/6*, 4/6* and 5/6* in landrace kids).

Plasma creatinine levels were 80.0 ± 29.7 and 50.3 ± 10.7 μmol/l in dwarf and

[1]Only significant differences between group 1 and others is indicated in figures, not among other groups.

[2]*$p<0.05$, **$p<0.01$, ***$p<0.001$.

landrace kids respectively in the first week of life (fig. 1c-d, table 1). The levels increased with age to 102.8 ± 12.8 and 76.4 ± 22.4 μmol/l in dwarf and landrace kids respectively at 8 - 12 months of age. Creatinine was significantly higher in dwarf than in landrace kids during the period of study. Significant differences were observed between neonatal kids and those in age groups 4 and 6 in dwarf (fig. 1c) and 4, 5 and 6 in landrace kids (fig. 1d). There were also significant differences among other age groups (2/6*, 3/5*, 3/6*, 4/5*, 5/6* in dwarf and 2/6*, 3/5*, 3/6* and 4/6* in landrace kids).

Bilirubin values in neonatal dwarf and landrace kids were 19.3 ± 7.0 and 9.6 ± 4.4 μmol/l respectively, being significantly higher in the former than in latter kids in the first 4 months of age (fig. 2a and b, table 1). After 4 months of age the concentrations did not differ significantly between the two breeds. Levels decreased with age to 3.6 ± 0.9 and 3.9 ± 1.6 μmol/l in dwarf and landrace kids respectively at 8-12 months of age. There were significant differences between neonates and kids in age groups 2, 3, 4, 5 and 6 in dwarf (fig. 2a) and 4, 5 and 6 in landrace (fig. 2b) kids. Significant differences were observed among other age groups (2/5*, 2/6*, 3/5*, 3/6, 4/5* and 4/6* in dwarf and 2/4*, 2/5*, 2/6*, 3/4*, 3/5* and 3/6 in landrace kids).

Plasma glucose levels were 4.8 ± 0.8 and 5.4 ± 1.4 mmol/l in neonatal dwarf and landrace kids respectively, decreasing to 3.9 ± 0.9 and 2.9 ± 0.3 mmol/l, in the two breeds respectively at 8-12 months of age (fig. 2c, table 2). The levels were significantly higher in landrace than in dwarf kids at 6 weeks but higher in the latter than in the former at 8-12 months of age. Significant differences were observed between neonates and age groups 2, 5 and 6 in dwarf (fig. 2c) and 4, 5 and 6 in landrace (fig. 2d) kids. There were also significant differences among other age groups (2/3*, 2/4*, 2/5*, 2/6*, 3/5* and 4/5* in dwarf and 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 3/6*, 4/5* and 4/6* in landrace kids).

Total serum proteins in dwarf and landrace kids of 0 - 7 days of age were 61.3 ± 5.9 and 54.0±6.4 g/l respectively (fig. 3a and b, table 2). After very slight drop within 2-4 weeks, serum proteins increased with age with significant differences between the breeds at some ages. Protein levels were 68.6 ± 4.1 and 69.4 ± 5.7 g/l in dwarf and landrace kids respectively at 8-12 months of age. There were significant differences between birth values and age groups 2, 4, 5, 6 in dwarf (fig. 3a) and 4, 5, 6 in landrace kids (fig. 3b). Similarly significant differences were observed among other age groups (2/5*, 2/6*, 3/5*, 3/6*, 4/5* and 4/6* in dwarf and 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 3/6* and 4/6* in landrace kids).

Plasma cholesterol levels were 2.5 ± 1.1 and 2.9 ± 0.7 mmol/l in dwarf and landrace kids respectively in the first week of life (fig. 2b, table 2) increasing dramatically in dwarfs during the following 6 weeks, whereafter it decreased.

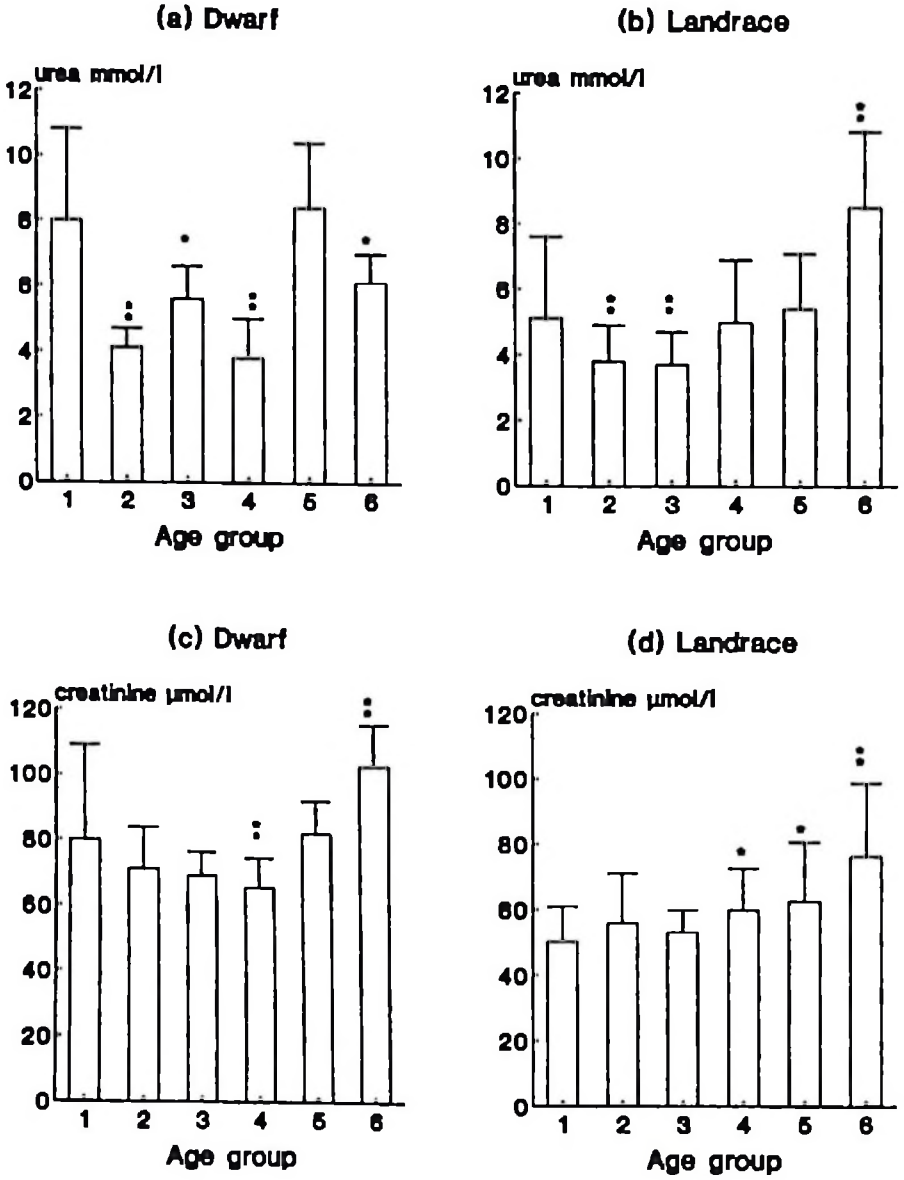
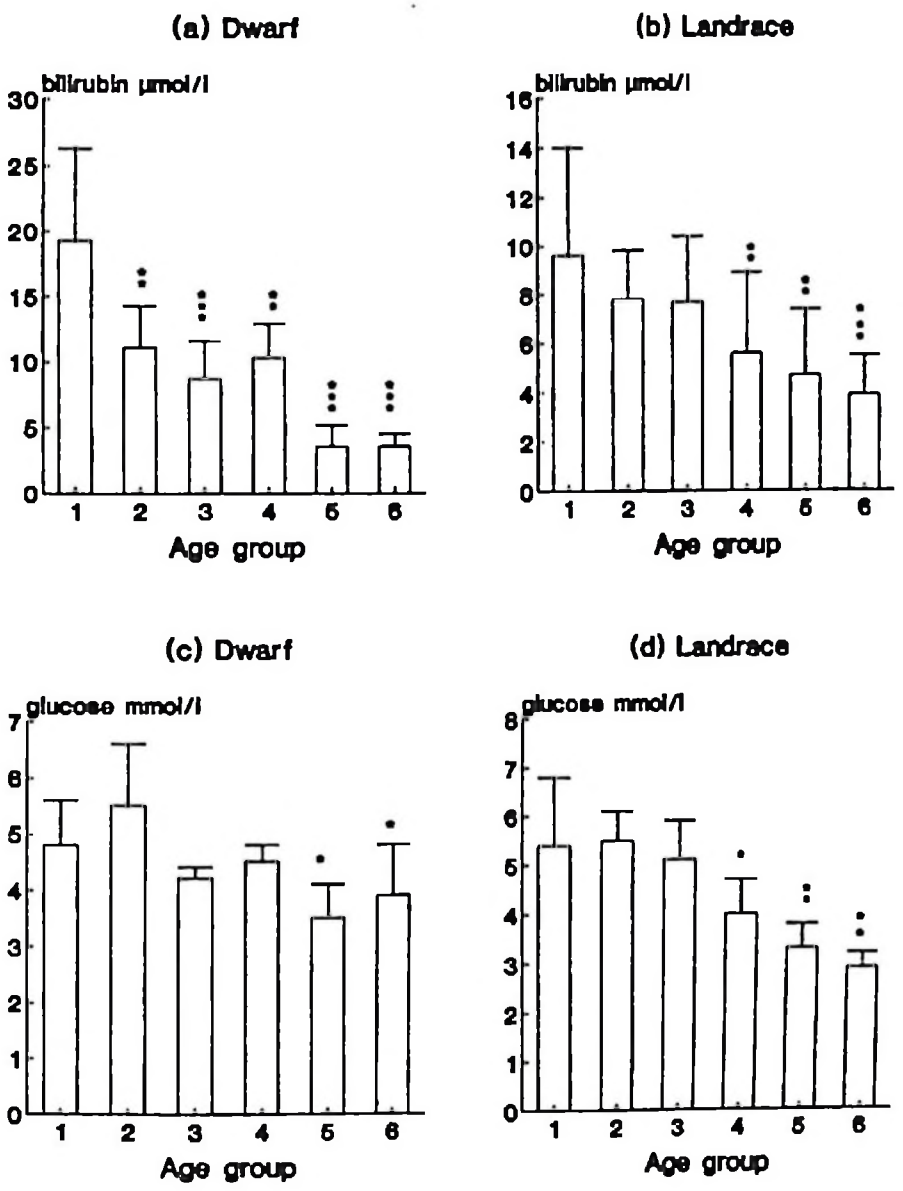


Figure 1: The mean plasma urea (a) and (b), creatinine (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Significant differences from group 1 values $^{*}p<0.05$, $^{**}p<0.01$. $^{***}p<0.001$.



Figures 2: The mean plasma bilirubin (a) and (b) and glucose (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Significant differences from group 1 values $^{*}p<0.05$, $^{**}p<0.01$. $^{***}p<0.001$.

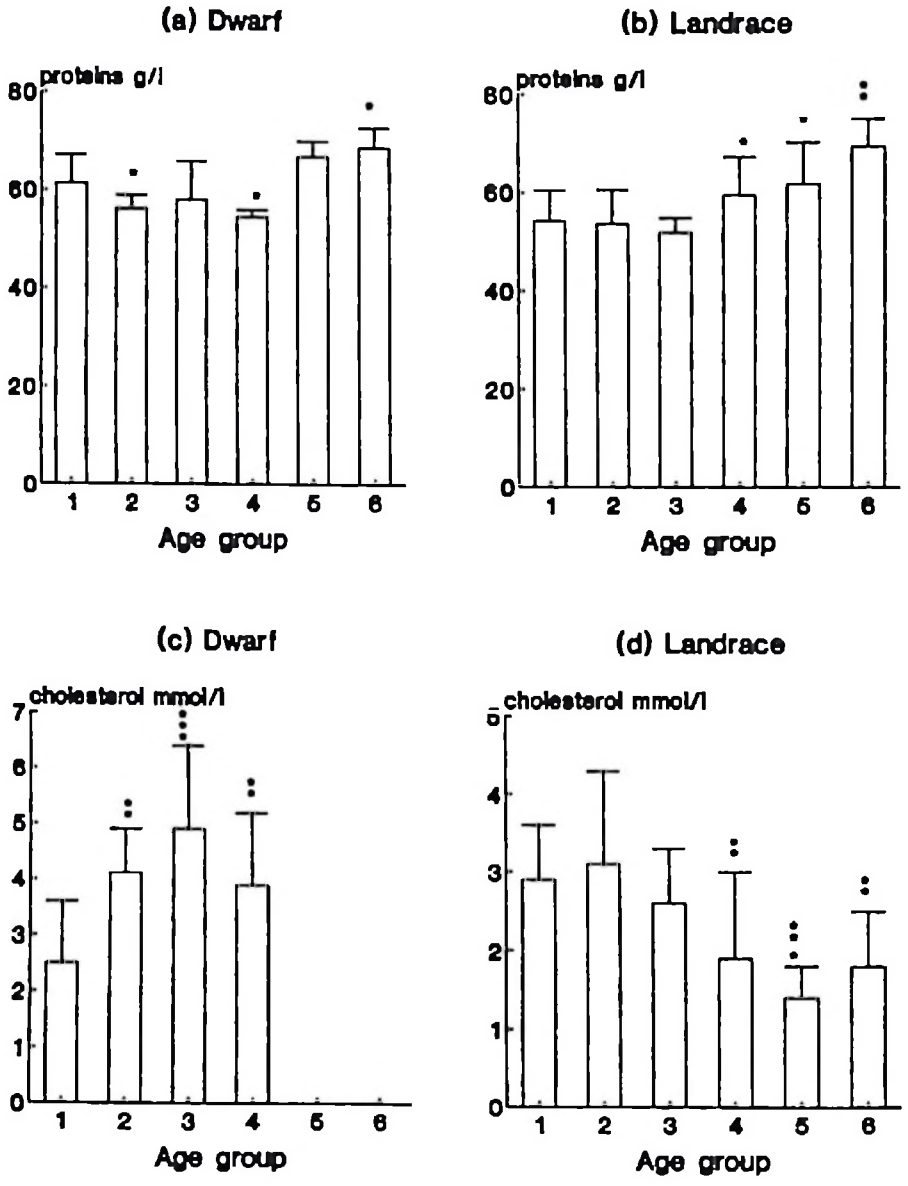


Figure 3: The mean total serum proteins (a) and (b), plasma cholesterol (c) and (d) with one standard deviation (bars) in growing dwarf and landrace kids of 0 - 7 days (group 1), 7 - 30 days (group 2), 1 - 2 months (group 3), 2 - 4 months (group 4), 4 - 8 months (group 5) and 8 - 12 months (group 6). Significant differences from group 1 values $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Table 1: Comparisons between the 5th (P_5) to 95th (P_{95}) percentile intervals and mean ± standard deviation ($\bar{x} \pm s$), and mean and median values (Q_2) and of plasma urea, creatinine and bilirubin levels in growing dwarf and landrace kids.

		Urea mmol/l			Creatinine µmol/l			Bilirubin µmol/l		
Age	(n)	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	(Q_2)	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
Dwarf										
0-7d	(9)	4.5-14.7	7.6	8.0±2.8**	39.0-140.0	81.0	80.0± 29**	12.0-29.7	18.1	19.3±7.0***
7-30d	(8)	3.3-5.0	(4.1)	4.1±0.6	53.0-89.0	70.5	71.0±12.8**	7.1-17.1	10.2	11.1±3.2*
1-2m	(9)	4.6-8.0	(5.2)	5.6±1.0*	61.0-81.0	66.0	69.0±7.3**	5.6-14.8	8.1	8.7±2.9
2-4m	(10)	2.3-6.9	(3.7)	3.8±1.2W	52.0-85.0	65.5	65.5±9.0	7.1-14.1	9.9	10.3±2.6***
4-8m♂	(17)	6.0-11.5	(8.0)	8.4±2.0**	68.0-109.0	80.0	82.0±10.1**	1.7-7.3	3.5	3.6±1.6
8-12m♂	(7)	5.0-7.0	(6.0)	6.1±0.9*	87.0-118.0	105.0	102.8±12.8***	2.7-5.5	3.5	3.6±0.9
Landrace										
0-7d	(18)	3.2-9.1	4.2	5.1±2.5*W	39.0-62.0	46.5	50.3±10.7*W	4.9 - 15.7	8.5	9.6±4.4***
7-30d	(20)	1.8-5.6	3.8	3.8±1.1	39.5-77.0	53.0	56.1±10.9**	4.9 - 11.3	8.1	7.8±2.0**
1-2m	(16)	2.6-6.1	3.4	3.7±1.0*	40.0-64.0	52.5	53.2±6.8*	4.5 - 13.3	6.9	7.7±2.7
2-4m	(62)	2.2-7.8	4.8	5.0±1.9	44.0-79.0	58.5	59.9±12.7	2.1 - 11.1	5.0	5.6±3.3***W
4-8m	(44)	2.9-7.8	5.2	5.4±1.7**	38.0-90.0	65.0	62.7±18.0**	1.9 - 10.0	3.5	4.7±2.7
8-12m♀	(13)	2.5-11.9	8.9	8.5±2.3**	54.0-140.0	68.0	76.4±22.4**W	0.7 - 7.5	4.0	3.9±1.6W

n= number of goats, d= days, m= months, ♂= males only, ♀= females only,
W= distributions that deviated from Gaussian ($P<0.05$).
For differences of means between the two breeds at same ages *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 2: Comparison between the 5th (P_5) to 95th (P_{95}) percentile intervals and the mean ± standard deviation ($\bar{x} \pm s$) and the means and median (Q_2) values of total serum proteins, plasma glucose and cholesterol levels in growing dwarf and landrace kids.

	Serum proteins g/l			Glucose mmol/l			Cholesterol§ mmol/l		
Age	P_5 - P_{95}	(Q_2)	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
Dwarf									
0-7d	53.0-71.0	61.0	61.3±5.9^{*W}	3.0-5.7	4.9	4.8±0.8	0.9-4.3	2.8	2.5±1.1
7-30d	52.0-61.0	56.0	56.1±2.8	4.6-7.9	5.1	5.5±1.1^{W}	2.7-5.1	4.3	4.1±0.8*
1-2m	51.0-78.0	56.0	58.0±7.9*	3.8-4.5	4.2	4.2±0.2*	3.1-8.0	4.6	4.9±1.5**
2-4m	52.0-56.5	54.5	54.5±1.4*	4.0-5.1	4.5	4.5±0.3	2.3-7.0	3.7	3.9±1.3**
4-8m♂	62.0-73.0	66.0	66.8±3.1*	2.9-5.4	3.5	3.5±0.6^{W}	-	-	-
8-12m♂	62.0-74.0	70.0	68.6±4.1	2.2-5.0	4.0	3.9±0.9*	-	-	-
Landrace									
0-7d	40.0-63.0	53.0	54.0±6.4**	2.8-5.8	5.5	5.4±1.4^{W}	1.9-3.9	3.0	2.9±0.7
7-30d	44.0-66.5	52.0	53.6±7.0	4.5-6.7	5.5	5.5±0.6	1.6-5.9	2.8	3.1±1.2*
1-2m	47.0-58.0	51.5	51.8±3.1*	3.4-6.5	5.0	5.1±0.8*	1.6-3.4	2.7	2.6±0.7**
2-4m	52.0-77.0	58.0	59.6±7.7^{*W}	3.0-5.3	4.0	4.0±0.7	0.9-4.3	1.5	1.9±1.1^{*W}
4-8m	44.0-73.0	63.5	61.8±8.5*	2.4-4.1	3.3	3.3±0.5	0.9-1.9	1.4	1.4±0.4
8-12m♀	61.0-79.0	68.0	69.4±5.7	2.3-3.4	2.8	2.9±0.3*	1.1-3.2	1.9	1.8±0.7

§= Not determined in dwarf goats after 4 months of age, d= days, m= months
W= as in table 1
Test for means between breeds at same ages $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$
Number of goats see table 1, ♂= male goats only, ♀= females only.

Table 3: The values of the 5th (P_5) to 95th (P_{95}) percentile intervals, medians (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) of plasma urea, creatinine and bilirubin levels in growing dwarf and landrace kids according to sex and age.

Age	(n)	Urea mmol/l P_5 - P_{95}	Q_2	$\bar{x} \pm s$	Creatinine µmol/l P_5 - P_{95}	Q_2	$\bar{x} \pm s$	Bilirubin µmol/l P_5 - P_{95}	(Q_2)	$\bar{x} \pm s$
Dwarf females										
0-7d	(3)	4.5-7.8	6.2	6.2±1.6	39.0-92.0	81.0	70.7±28.0	12.3-29.7	16.2	19.4±9.1
7-30d	(4)	3.8-5.0	4.1	4.2±0.5	53.0-85.0	76.5	72.7±13.8	7.1-13.7	11.1	10.8±3.0
1-2m	(4)	4.9-5.9	5.3	5.3±0.4	61.0-76.0	65.5	67.0±7.0	5.6-11.0	8.4	8.4±2.2
2-4m	(5)	3.5-6.9	4.0	4.4±1.4	58.0-74.0	62.0	64.0±6.1	7.1-14.0	8.5	9.6±2.9
Dwarf males										
0-7d	(6)	6.2-14.7	8.2	9.0±3.0	59.0-140	75.0	84.8±30.9	12.0-28.6	18.7	19.2±6.7
7-30d	(4)	3.3-4.5	4.0	3.9±0.6	59.0-89.0	64.5	69.2±13.5	8.4-17.1	10.2	11.5±3.9
1-2m	(5)	4.6-8.0	5.2	5.8±1.3	65.0-81.0	66.0	71.0±7.8	5.9-14.8	8.1	9.0±3.5
2-4m	(5)	2.3-3.9	3.6	3.2±0.7	52.0-85.0	66.0	67.2±11.7	8.4-14.1	10.6	11.1±2.4
Landrace female										
0-7d	(5)	3.3-5.7	4.4	4.4±0.9	42.0-51.0	44.0	44.8±3.7	4.9-10.1	6.5	7.2±2.0
7-30d	(12)	3.3-4.8	3.8	4.0±0.5	39.0-73.0	51.0	51.3±8.4**	4.7-9.4	8.1	7.5±1.7
1-2m	(6)	2.8-6.1	4.5	4.4±1.2*	46.0-58.0	51.0	52.0±4.6	5.0-9.4	6.7	6.8±1.6
2-4m	(41)	2.5-7.8	5.3	5.4±1.8**	46.0-83.0	60.0	62.2±13.6*	2.0-11.1	4.5	5.1±3.2*
4-8m	(38)	2.9-7.8	5.2	5.3±1.7	39.0-92.0	65.0	64.1±18.1	1.9-10.0	4.0	5.1±2.8*
Landrace males										
0-7d	(13)	3.2-12.5	4.0	5.4±2.9	39.0-82.0	51.0	52.5±11.8	4.5-21.2	9.0	10.5±4.8
7-30d	(8)	1.6-5.9	3.2	3.6±1.6	49.0-82.0	63.5	63.4±10.4**	5.1-12.5	8.0	8.3±2.6
1-2m	(10)	2.6-4.3	3.2	3.3±0.6*	40.0-66.0	54.0	53.9±8.0	4.5-13.3	7.0	8.3±3.2
2-4m	(21)	1.8-7.3	4.3	4.2±1.8**	44.0-69.0	55.0	55.5±9.5*	2.8-11.1	5.1	6.6±3.4*
4-8m	(6)	4.0-8.4	5.3	5.5±1.5	37.0-74.0	53.5	53.7±16.4	1.5-2.8	2.4	2.3±0.5*

§ = Not determined in dwarf goats after 4 months of age, d = days, m = months. For differences in means between sex within the breeds $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table 4: The 5th (P_5) to 95th (P_{95}) percentiles, medians (Q_2), mean ± standard deviations of total serum proteins, plasma glucose and cholesterol levels in growing dwarf and landrace kids according to sex and age.

	Serum proteins g/l			Glucose mmol/l			Cholesterol§ mmol/l		
Age	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
Dwarf female									
0-7d	54.0-66.0	59.0	59.7±6.0	4.6-5.7	4.8	5.0±0.6	0.9-3.2	2.9	2.3±1.2
7-30d	52.0-61.0	56.0	56.2±4.0	4.9-6.5	5.1	5.4±0.7	3.6-5.1	4.6	4.4±0.7
1-2m	51.0-59.0	54.0	54.5±3.3	4.0-4.3	4.1	4.2±0.1	3.7-8.0	4.3	5.1±2.0
2-4m	52.0-57.0	54.0	54.2±1.9	4.2-5.1	4.7	4.6±0.4	2.9-7.0	4.2	4.4±1.6
Dwarf males									
0-7d	53.0-71.0	63.0	62.2±6.3	3.0-5.6	4.9	4.6±0.9	1.2-4.3	2.6	2.5±1.2
7-30d	54.0-58.0	56.0	56.0±1.6	4.6-7.9	5.2	5.7±1.5	2.7-4.8	3.9	3.8±0.9
1-2m	55.0-78.0	57.0	60.8±9.7	3.8-4.5	4.3	4.2±0.2	3.1-5.9	5.1	4.7±1.1
2-4m	54.0-56.0	55.0	54.8±0.8	4.0-4.8	4.4	4.4±0.3	2.3-4.4	3.2	3.4±0.8
Landrace female									
0-7d	48.0-59.0	53.0	53.6±4.4	5.6-5.8	5.7	5.7±0.1	3.0-3.6	3.5	3.4±0.3
7-30d	43.0-63.0	50.0	51.1±5.9*	4.6-6.8	5.4	5.5±0.6	1.2-4.8	3.3	3.1±1.0
1-2m	49.0-57.0	52.5	52.3±2.8	4.7-6.5	5.5	5.5±0.6	1.8-3.6	2.9	2.8±0.7
2-4m	51.0-81.0	59.0	60.6±8.9	2.8-5.3	3.8	3.9±0.7*	1.9-4.4	1.6	2.0±1.3
4-8m	51.0-73.0	64.0	63.0±7.0**	2.4-4.7	3.3	3.4±0.5*	0.8-1.9	1.4	1.4±0.4
Landrace males									
0-7d	40.0-69.0	53.0	54.2±7.1	2.8-9.7	5.4	5.2±1.7	1.9-4.0	2.4	2.7±0.7
7-30d	51.0-70.0	55.5	57.4±0.7*	4.4-6.6	5.5	5.5±0.7	2.1-7.0	2.8	3.2±1.6
1-2m	47.0-58.0	50.5	51.5±3.3	3.4-6.8	4.7	4.9±0.9	1.6-3.4	2.5	2.5±0.6
2-4m	53.0-66.0	56.0	57.6±4.4	3.5-5.2	4.2	4.3±0.7*	0.9-3.3	1.5	1.8±0.8
4-8m	44.0-75.0	48.0	54.3±13.3**	2.3-3.3	3.0	2.9±0.4*	0.5-1.8	1.3	1.2±0.5

§ = Not determined in dwarf goats after 4 months of age, d = days, m = months, Number of goats as in table 3. Test of means between sex within the breeds *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 5: Environmental influence on urea (mmol/l), creatinine (µmol/l), bilirubin (µmol/l), proteins (g/l), glucose (mmol/l) and cholesterol (mmol/l) levels in landrace kids (means ± standard deviations).

Age	Herd (n)	Urea	Creatinine	Bilirubin	Proteins	Glucose	Cholesterol
0-7d	B (14)	4.0 ± 0.7*	46.4 ± 6.2*	9.1 ± 3.7	53.4 ± 3.8	5.3 ± 0.8	3.1 ± 0.7*
	C (4)	9.1 ± 2.6*	64.2 ± 12.1*	11.3 ± 6.7	56.2 ± 12.7	5.5 ± 3.0	2.2 ± 0.3*
	G (9)	8.0 ± 2.8	80.0 ± 29.0	19.3 ± 7.0	61.3 ± 5.9	4.8 ± 0.8	2.5 ± 1.1
7-30d	B (13)	3.8 ± 0.7^{a**}	52.8 ± 0.5*	7.7 ± 1.9	49.5 ± 3.6^{ab*}	5.5 ± 0.5	2.6 ± 0.7*
	C (2)	4.8 ± 0.3^{ab**}	70.5 ± 16.3*	6.2 ± 0.1	66.5 ± 4.9^{bc*}	6.1 ± 0.7	2.8 ± 0.03
	F (5)	4.6 ± 1.0^{b**}	59.2 ± 13.8	8.7 ± 2.7	59.0 ± 3.4^{bc*}	5.4 ± 0.9	4.5 ± 1.6*
	G (9)	4.1 ± 0.6	71.0 ± 12.8	11.1 ± 3.3	56.1 ± 2.8	5.5 ± 1.1	4.1 ± 0.8
1-2m	G (9)	5.6 ± 1.0	69.0 ± 7.3	8.7 ± 2.9	58.0 ± 7.9	4.2 ± 0.2	4.9 ± 1.5
2-4m	B (42)	4.5 ± 2.0^{ab*}	61.4 ± 12.9	5.4 ± 3.5	57.6 ± 4.5^{ac*}	4.1 ± 0.7^{ab*}	1.7 ± 0.9^{a*}
	C (1)	4.3	58.7	4.9 ±	63.3^{b*}	4.5	2.3
	D (7)	5.5 ± 1.3	51.7 ± 6.0	9.6 ± 1.8	53.0 ± 2.6^{abc*}	4.8 ± 0.6^{ac*}	3.3 ± 1.3^{abc*}
	E (2)	7.6 ± 0.3^{a*}	47.3 ± 14.6	4.7 ± 0.7	64.0 ± 2.8^{d*}	4.0 ± 0.05	1.4 ± 0.05^{c*}
	F (10)	6.0 ± 1.1^{b*}	61.7 ± 13.3	3.9 ± 1.3	71.7 ± 9.4^{abc*}	3.2 ± 0.5^{bc*}	2.0 ± 1.2^{a*}
	G (10)	3.8 ± 1.2	65.5 ± 9.0	10.3 ± 2.6	54.5 ± 1.4	4.5 ± 0.3	3.9 ± 1.3
4-8m	A (17)	8.4 ± 2.0	82.0 ± 10.1	3.6 ± 1.6	66.8 ± 3.1	3.5 ± 0.6	-
	B (2)	5.0 ± 1.4^{a*}	71.1 ± 16.3^{a*}	6.0 ± 2.8^{a**}	66.8 ± 4.7^{ab*}	3.6 ± 0.5^{ab*}	1.3 ± 0.3
	E (3)	8.6 ± 2.2^{ab*}	65.7 ± 3.8^{b*}	3.6 ± 1.6	58.0 ± 12.5^{a*}	2.7 ± 0.5^{b}	1.6 ± 0.9
	F (14)	5.4 ± 1.4^{b*}	49.2 ± 12.9^{ab*}	2.6 ± 0.4^{a**}	54.5 ± 6.7^{b*}	3.2 ± 0.6^{a}	1.4 ± 0.3
8-12m	A (7)	6.1 ± 0.9	102.8 ± 12.8	3.6 ± 0.9	68.6 ± 4.1	3.9 ± 0.9	-
	E (3)	9.6 ± 1.2	90.7 ± 22.8	2.3 ± 1.5*	64.3 ± 4.2	2.7 ± 1.5	1.5 ± 0.6
	F (10)	8.1 ± 2.4	72.9 ± 12.9	4.5 ± 1.3*	70.8 ± 5.0	2.9 ± 0.3	2.1 ± 0.8

n= number of kids, d= days, m= month.
Where there are more than 2 herds, similar superscripts (a b c d e) indicate significant differences between those the means in columns within the age groups (*$p<0.05$, **$p<0.01$, ***$p<0.001$).
Where n=0 at any age the herd is not indicated.
Herd A and G= dwarf and B-F= Landrace goats.

In the landrace a gradual decrease was found after 4 weeks of age and levels were 1.8 ± 0.7 mmol/l at 8 - 12 months of age. Significant differences were observed among different age groups (1/2, 1/3 and 1/4 (fig. 3c) for dwarf and 1/4, 1/5 and 1/6 (fig. 3d), 2/4*, 2/5*, 2/6*, 3/4*, 3/5*, 4/5* and 5/6* for landrace kids.

Plasma urea, creatinine, bilirubin and glucose and total serum protein levels at some ages were significantly different between female and male landrace kids but not in dwarfs (table 3-4). There were significant differences in several parameters between same age landrace kids from different herds (table 5). The age differences were highly significant for all parameters.

Discussion

The statistical comparisons have shown that parametric and nonparametric ranges were apparently not significantly different for the values of urea, creatinine, glucose, total serum proteins, bilirubin and cholesterol levels. The data followed a Gaussian distribution except a few (table 1 and 2). The critical determinants of these differences appear to be sample sizes and accuracy of laboratory analysis. The inferences based on parametric tests are valid and they are the most powerful when their models are fulfilled (Siegel and Castellan, 1988).

There are wide range intervals of clinical chemical values in goats (Lloyd, 1982; Sherman and Robinson, 1983; Okorie and Anugwa, 1986). This makes diagnostic decisions by laboratory analysis difficult because the magnitudes of variations due to intrinsic and extrinsic factors in healthy goats are known. This investigation has shown the magnitudes of the influence of age on the plasma profiles of urea, creatinine, total bilirubin, glucose, cholesterol and total serum proteins plasma profiles. It is unequivocally shown that age has a profound influence on the levels, probably more than any other factor (figs. 1 - 3, tables 1 - 4).

There is a decrease in urea within the first week of life, but later, levels increase with age in both dwarf and landrace kids. This occurs in other goat breeds (Braun *et al.* (1983). Creatinine concentrations increased slightly with age (fig. 1b and d), consistent with observations that plasma and serum creatinine values are constant in healthy animals. Plasma values depend on total body content of creatine, the sole precursor of creatinine. Creatine concentration depends on the diet, rate of synthesis from renal, intestinal and pancreatic arginine and glycine and on muscle mass (Finco, 1989). The changes in neonates and young animal might be influenced by the change in intra- and extracellular masses. Creatinine levels in dwarf were higher than in landrace kids.

It is shown that in dwarf and landrace goats there are higher plasma bilirubin levels at birth than at other ages and decrease steadily with age to nearly constant concentrations, contrary to the transient decreases observed in neonatal kids of other breeds (Braun *et al.*, 1983). This discrepancy is probably due to breed differences.

Glucose and cholesterol concentrations in the present dwarf and landrace breeds were higher in younger than in older kids. After a further slight increase values decreased to adult levels as has been observed in other goats (Castro *et al.*, 1975; Bogin *et al.*, 1981; Pereka and Riis, 1987; Bialkowski *et al.*, 1988). The higher glucose levels in young than in adult goats is probably due to high metabolic rates in the former. Both glucose and cholesterol levels in adult goats are highest in the first week following parturition (Vihan and Rai, 1987; Biagi *et al.*, 1988). It is likely that goat colostrum has high content of cholesterol. The higher levels in young kids are

therefore due to dietary sources, and the initial increase is brought about by the first colostral intake (Raviart *et al*, 1987).

Total serum protein concentrations were low at birth in dwarf and landrace kids, then increased with age (fig. 3a and b, table 3). An immediate increase is reported to occur following colostral ingestion due to gamma globulins (Braun *et al.*, 1983; Kanemaki *et al.*, 1986; Raviart *et al.*, 1987). Slight decrease in total serum proteins were noted in the first to 6th week of life, in dwarf and landrace kids (fig. 3a and b), and Polish goats (Bialkowski *et al.*, 1988). The decreases may be due to disappearance of colostral proteins (Braun *et al.*, 1983). After this decrease total serum proteins increase with age. This finding is in agreement with the reported trends in Israel and Indian goats (Bogin *et al.*, 1981; Bhattacharrya and Duttagupta, 1987), and is most probably because of gradual build up of immunoglobulins.

Differences were observed in urea, creatinine, bilirubin, glucose and serum protein concentrations between dwarf and landrace kids of the same age (table 1 and 2). The differences may be attributed to the genotypic influence of breed. Such differences of genotypic nature were observed in Tibetan and Maltese/Tibetan cross bred goats for proteins, albumin, lipids, glucose, cholesterol and urea (Pugliese *et al.*, 1982). Many other goat breeds have been found to differ in various parameters (Davies and Sims, 1983).

Significant differences were observed too among the landrace kids of different herds (table 5), indicating environmental, and probably nutritional influences among herds. Sex influence was also observed in landrace kids (table 3-4) but not in dwarfs. Sex influence is reported in some goat breeds (Castro *et al.*, 1977a; Chiericato *et al.*, 1986) the differences are not consistent in young goats and can be regarded as of less importance for clinical diagnosis.

In conclusion the reference ranges and age profiles of the commonly used clinical chemical parameters in diagnosis of diseases have been determined in growing dwarf and landrace goats. It is revealed that plasma creatinine and total serum protein levels are low at birth in dwarf and landrace kids and increase with age. Plasma glucose, cholesterol and bilirubin levels are high at birth and decrease with age. Cholesterol levels showed sharp postnatal increase then decreased with age. There are enormous differences between kids from different herds within the breeds. Age and environment are therefore the major factors influencing the level of these parameters in serum and plasma of growing animals.

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CHAPTER 10

THE INFLUENCE OF PREGNANCY, LACTATION AND HERD ON HEMATOLOGICAL VALUES

Summary

Hematological analysis was performed in young and adult non pregnant non lactating, early and late pregnant, early mid and late lactating Danish landrace goats from five herds. The purpose was to determine whether their levels are significantly different in these states and the effect of age and parity on the changes. The hematocrit, hemoglobin concentration, number of erythrocytes and leukocytes were higher in 8 - 12 months old nonpregnant and 1 - 2 years old pregnant than in adult pregnant and lactating goats of over two years of age. Hematocrit, hemoglobin concentration and number of erythrocytes decreased in late pregnancy and early lactation. At the same time mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration slightly increased. The changes were directly proportional to parity, more in pluriparous than in primiparous goats. After parturition the former group of parameters increased and the latter decreased. There was an increase in the number of total leukocytes close to and after parturition due to neutrophils and lymphocytes and it was more pronounced in first lactation than in pluriparous goats. There were significant differences in many parameters between goats from different herds (within similar physiological states).

Introduction

Sustained alterations in the level of hematological parameters due to the influence of pregnancy and lactation are described in cattle (Payne *et al.*, 1970; Hewett, 1974; Rowlands *et al.*, 1975; Rowlands, 1984; Ghergariu *et al.*, 1984). These form the basis of metabolic profile tests which help to predict the occurrence, thereby prevention of production diseases such as milk fever (hypocalcaemia), hypomagnesemia, toxaemia and ketosis occurring in cattle due to feeding and production imbalances (Payne *et al.*, 1970; Sansom, 1973). Sheep and goats are affected by these syndromes (King, 1984; Farnigham, 1988). The introduction of intensive methods of husbandry in goats for higher milk yields from minimum feeds is likely to increase the incidence rates.
The hematocrit, erythrocyte, total leukocyte, neutrophil, lymphocyte, basophil, monocyte and eosinophil counts have been shown to decrease in pregnant and early lactating ewes and cows (Hewett, 1974; Manston *et al.*, 1975; Rowlands *et al.*, 1975).

The number of leukocytes increases after parturition (Fortagne and Schafer, 1989). The hematological profile in pregnant goats follows that of dairy cows (Mohy *et al.*, 1985).

Metabolic profile tests have been applied in Saanen goats (Biagi *et al.*, 1988). It is impossible, however, to formulate a universal test because of the variations between herds (Masoni *et al.*, 1985), nutritional quality and level (Biswas *et al.*, 1986), age, season and ambient temperatures (Vrzgula *et al.*, 1985; Upadhyay and Rao, 1985; Oyewale and Olowookorun, 1986; Pospisil *et al.*, 1987), stage of gestation and lactation (Mohy *et al.*, 1985) and breed (Domina *et al.*, 1982; Unanian, 1986; Ginting, 1987). Knowledge in non lactating pregnant, nonpregnant lactating and non pregnant non lactating goats is not very elucidative on the influence of parity, age and environment. The aim of this investigation was to study the alterations in hematological parameter levels in different stages of pregnancy and lactation and the dependency of the changes on age and parity in Danish landrace goats.

Materials and Methods

Danish landrace goats from 5 Danish herds (A - E) located at Fakse (A), Næstved (B), Ringsted (C), Haslev (D) and Fugleberg (E) were sampled four times during a year in pregnancy and lactation cycle. All were apparently clinically healthy 13 non pregnant 8 - 12 months old (group 1), 22 more than 13 months old in the first month of first pregnancy (group 2), 76 in advanced pregnancy of 120 to 130 days (group 3), 40 (group 4), 73 (group 5) and 61 (group 6) at 20, 60 and above 90 days in lactation respectively, and 11 adult nonpregnant nonlactating goats (group 7). The pregnant group 3 goats were non lactating 41 of them were at third, 28 at 4th and 7 above 5 pregnancies respectively. In group 4, 21 goats were at their third, 9 at 4th and 10 above 5 lactations respectively. Group five was composed of 41 goats at third, 17 at fourth and 15 above 5 lactations. In group 6, 31 goats were at third, 22 at fourth and 8 above 5 lactations. Four of group 7 goats were at fourth and the rest above 5 lactations.

Goats were bred once per year during mid October to mid November for kidding in mid March to mid April. and were kept indoors throughout the year. Some degree of outdoor rearing was allowed in farms A to D in summer. Nutrition in these herds was composed of green grass pellets and about 0.5 kg of oats or barley concentrates in addition to dry hay straw. In farms E, goats were reared in the field for the entire summer and autumn without any concentrate supplementation.

Blood samples were collected from the external jugular vein in vacuum tubes (Becton-Dickinson vacutainers) containing 0.12 ml of 0.34 mol/l tripotassium ethylene diamine tetraacetate (EDTA). The samples were from the same goats beginning with

advanced pregnancy (group 3), then after parturition and in the subsequent lactation period and in the second year the from 8-12 months and 1-2 years old off-springs (groups 1-2).

The erythrocyte counts (RBC) were determined in model ZF coulter counter of 100 μm aperture diameter, adjusted for compensation of coincident passages (Coulter electronics, England). The threshold was set at 6 and attenuation 500 and a maximum of 200 background counts was allowed. Hemoglobin concentration (Hb) and total leukocyte counts (WBC) were determined in model S560 coulter counter and hematocrit (packed cell volume, PCV) in microhematocrit capillary tubes in a microhematocrit centrifuge (CLAY ADAM). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae. The number of lymphocytes, band and segmented neutrophils, monocytes, eosinophils and basophils were determined from a count of 200 leukocytes on thin Romanowsky stained blood smears.

The parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles, median) values and coefficient of variations were determined by univariate procedure of statistical analysis system software (SAS, Carry NC, USA, 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk (W) were determined in order to determine closeness of fit for the data of each analyte in each group to the Gaussian distribution. The means ($\bar{x}$) of hematological analytes were tested for differences between non pregnant, pregnant and lactating goats between herds (in similar groups), parity (among pregnant and lactating goats in herds with many animals) and groups (in goats of all herds combined) by using the general linear models procedure. Wilcoxon Mann Whitney tests were performed for parameters whose distributions significantly deviated from Gaussian manner. Values were ranked ascendingly and the average ranks tested for differences. In order to study the changes occurring during pregnancy and lactation, group 4 goats (14 -20 days postparturient) were selected as reference. The mean of each hematological parameter in this group ($\bar{x}_r$) was subtracted from the corresponding parameter mean ($\bar{x}_g$) in each parity group ($\bar{x}_g$-$\bar{x}_r$) and plotted.

Results

The mean and median value of RBC, PCV, MCV, MCH, MCHC, Hb, WBC, lymphocyte and neutrophil counts in each goat group were close to each other (table 1). The coefficients of variations (CV) were also relatively small except for leukocyte counts (table 2). The coefficient of skewness and degree of kurtosis were small and non significant, therefore the data in all parameters in most groups followed a

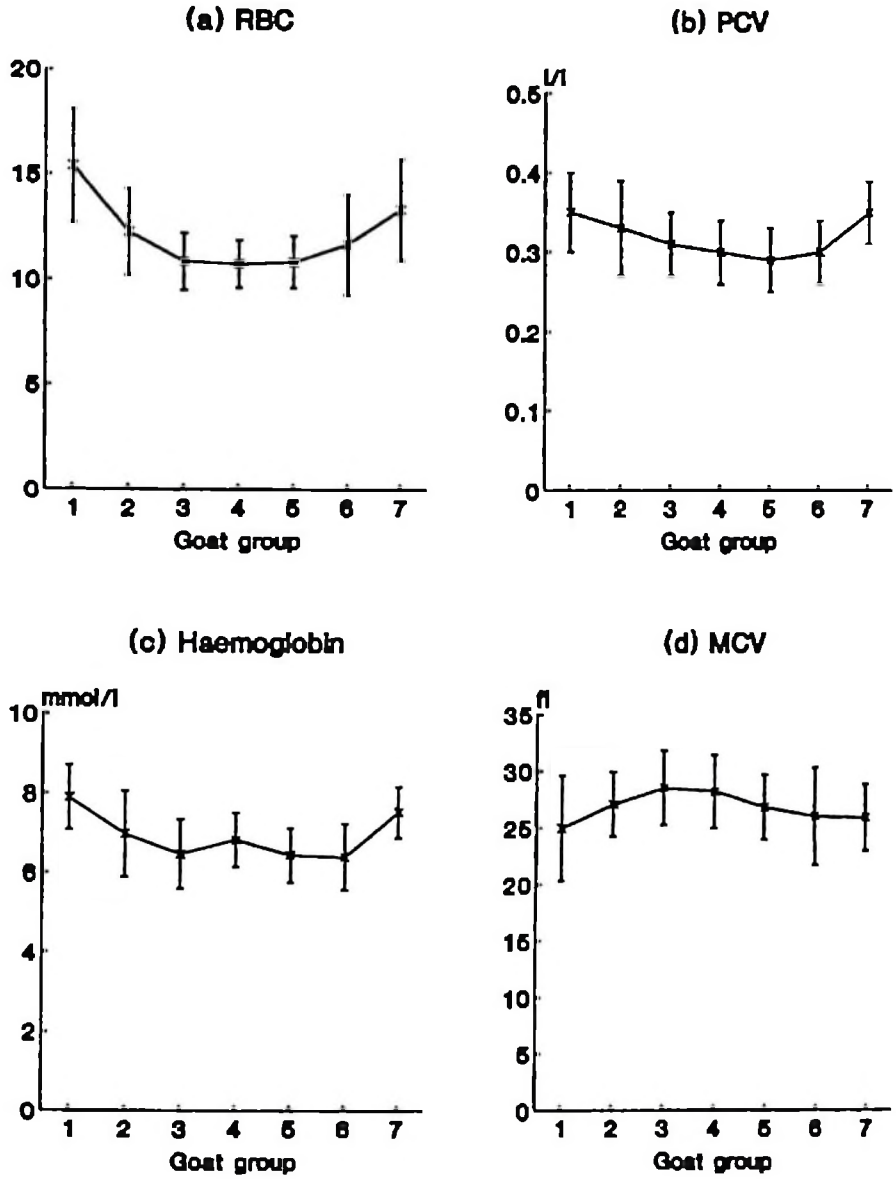

Figure 1: The mean red blood cell count (RBC $x10^{12}/l$) (a), packed cell volume (PCV) (b), hemoglobin concentration (c) and MCV (d) with one standard deviation above and below (vertical lines with bars) in young nonpregnant (group 1), young pregnant (group 2), advanced pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) lactations and dry adult (group 7) goats from all herds combined.

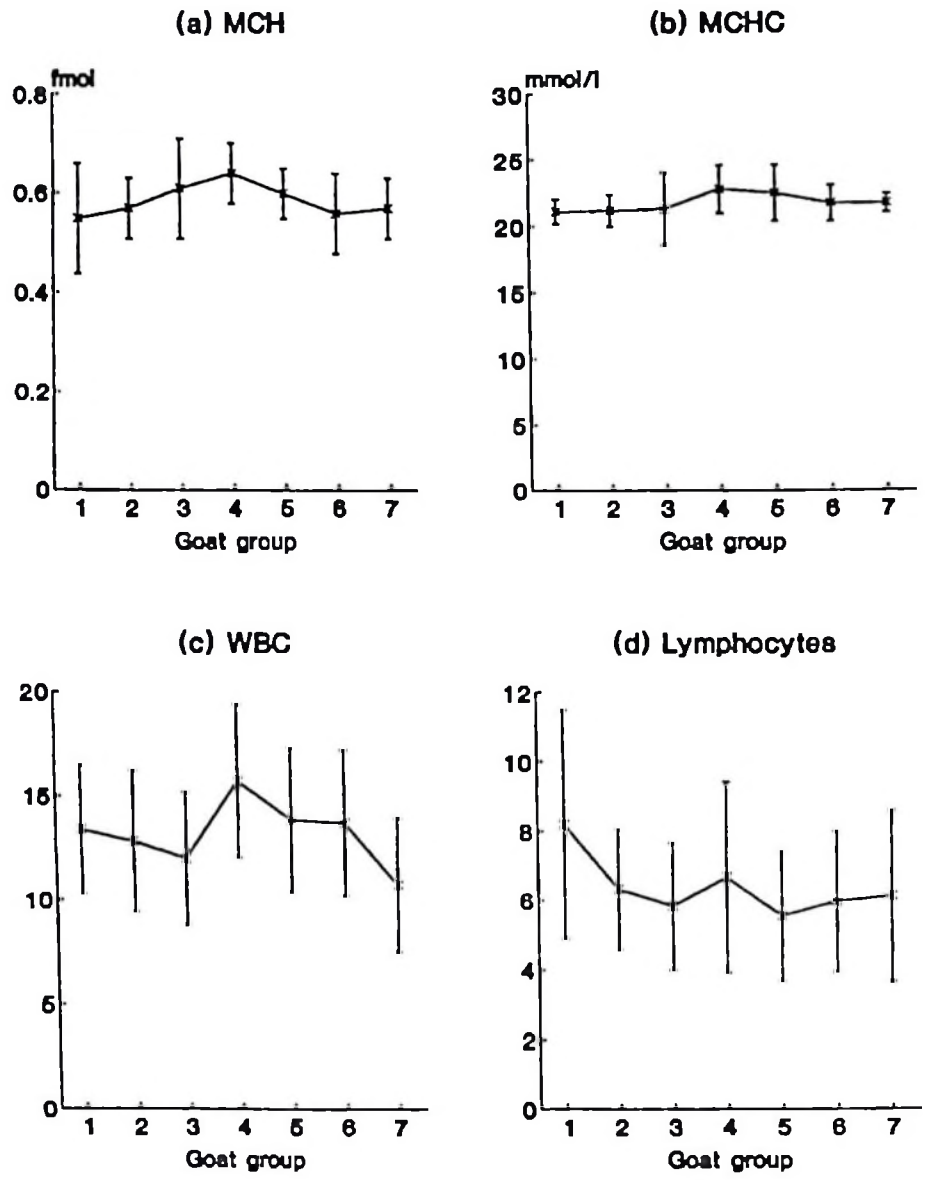

Figure 2: The mean MCH (a), MCHC (b), WBC x10^9/l (c) and lymphocytes x10^9/l (d) with one standard deviation above and below (vertical lines with bars) in young nonpregnant (group 1), young pregnant (group 2), advanced pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) lactations and dry adult (group 7) goats from all herds combined.

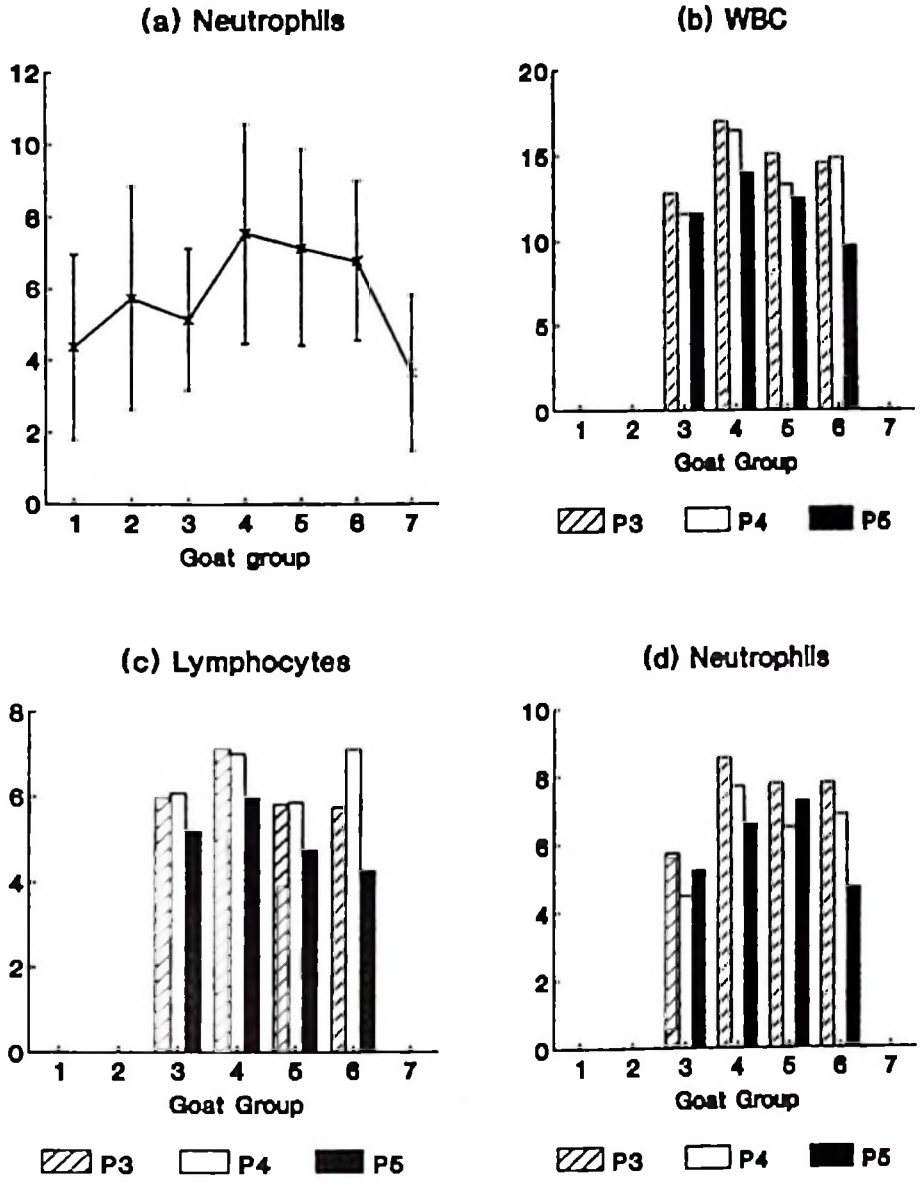

Figure 3a: The mean number of segmented neutrophils with one standard deviation above and below (vertical lines with bars) in goats of all herds combined, figure 3b-d; The mean total WBC count x10^9/l (b), lymphocyte count x10^9/l (c) and segmented neutrophil count x10^9/l in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state groups as in figures 1 and 2.

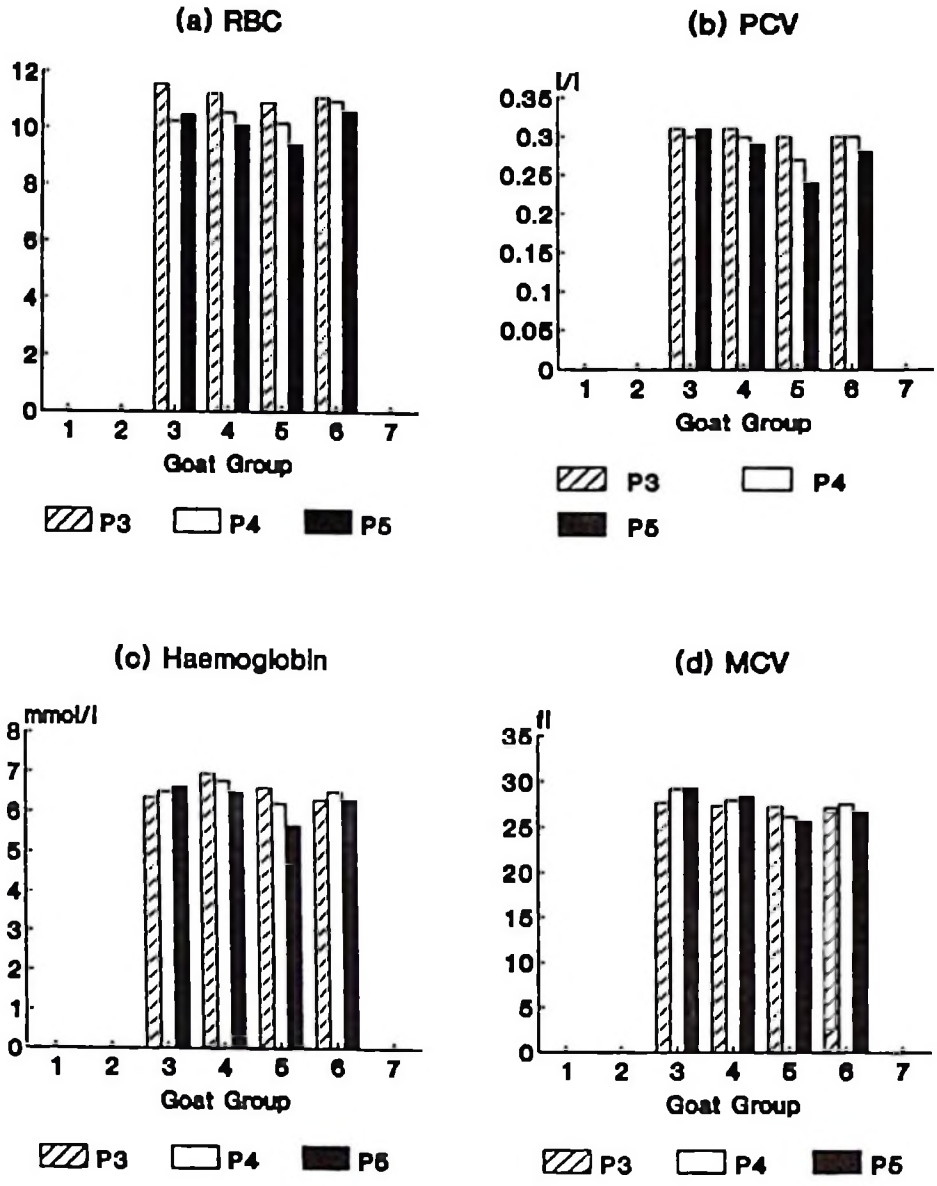

Figure 4: The mean RBC count $x10^{12}/l$ (a), PCV (b), hemoglobin concentration (c) and MCV (d) in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state groups as in figures 1 and 2.

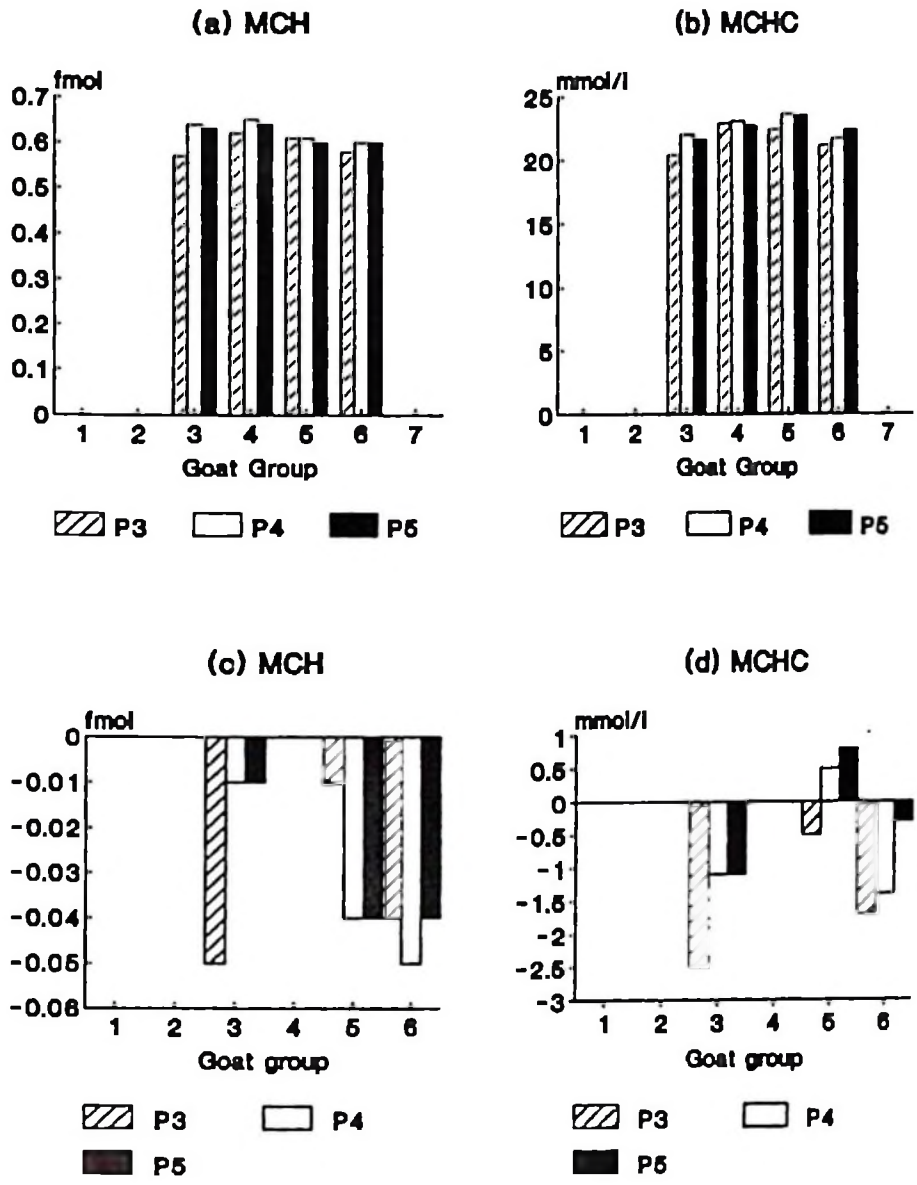

Figure 5a-b: The mean MCH (a) and MCHC (b) in herd A goats of parity 3 (p3), 4(P4) and 5 and above (P5) and the same physiological state group as in figures 1 and 2.
Figure 5c-d: The differences and the direction of the changes from group 4 herd A goats mean values (reference group) for MCH (c) and MCHC (d), parity 3 (P3), 4 (P4) and 5 and above (P5).

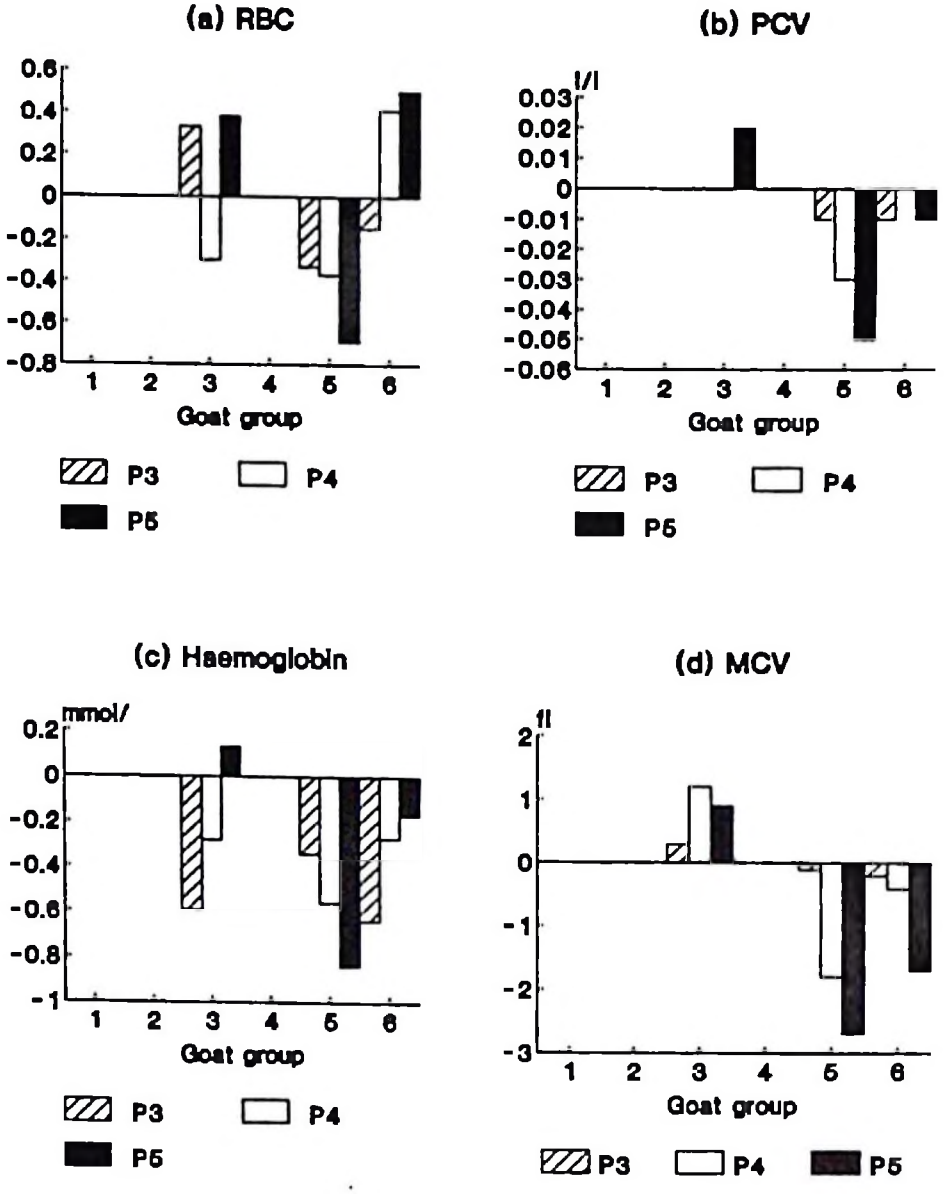

Figure 6: The differences and the direction of the changes from reference group 4 herd A goats mean values for RBC count $x10^{12}/l$ (a), PCV (b), hemoglobin concentration (c) and MCV (d), parity 3 (P3), 4 (P4) and 5 and above (P5).

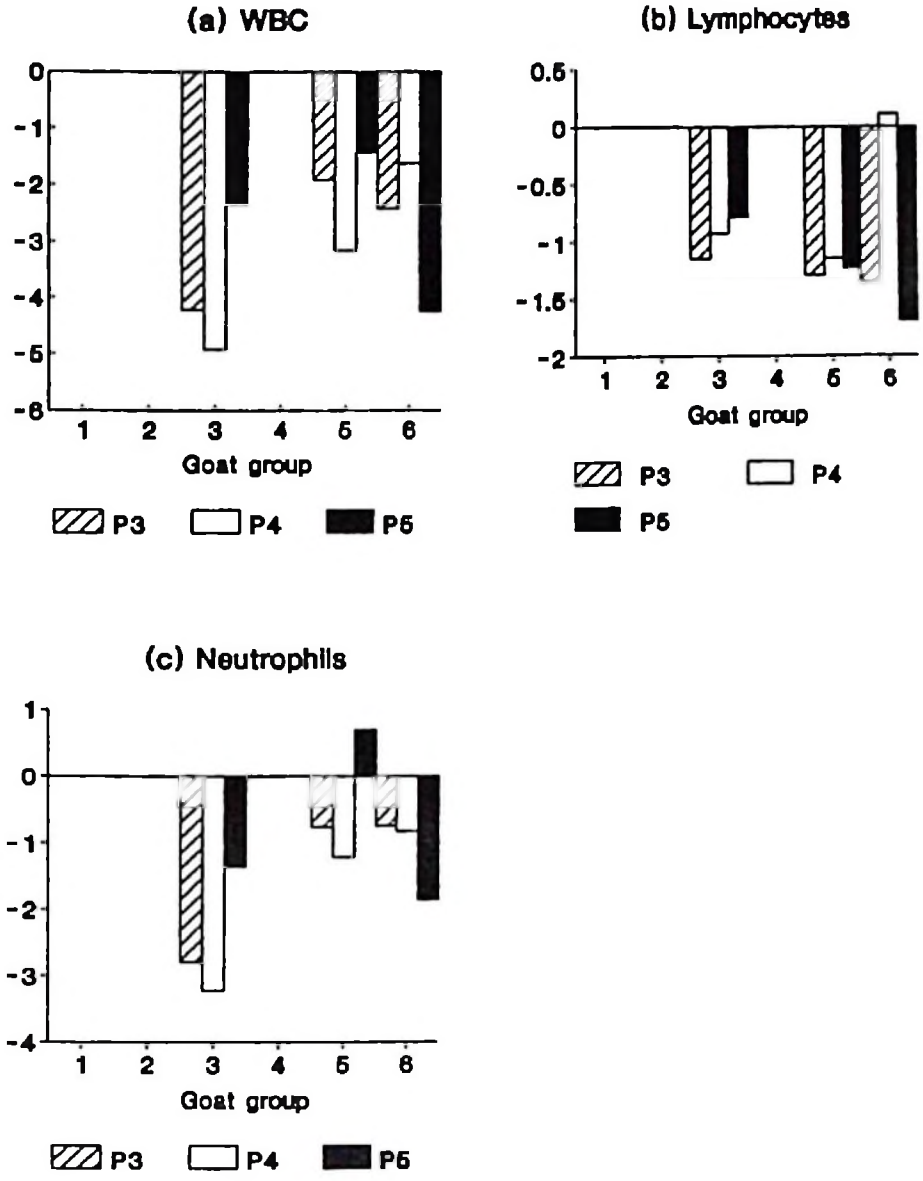

Figure 7: The differences and the direction of the changes from reference group 4 herd A goats mean values for total WBC $x10^{12}/l$ (a), lymphocyte (b) and segmented neutrophil counts (c), parity 3 (P3), 4 (P4) and 5 and above (P5).

Table 1: The median (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) of hematological values in pregnant, lactating and dry goats in all herds.

Parameter	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$
RBC $x10^{12}/l$	15.3	15.4 ± 2.70	11.8	12.26 ± 2.05	10.69	10.8 ± 1.35	10.5	10.7 ± 1.14	10.8	10.8 ± 1.23^{a}	11.0	11.7 ± 2.39^{a}	13.3	13.3 ± 2.43^{W}
PCV l/l	0.35	0.35 ± 0.05	0.34	0.33 ± 0.06	0.30	0.31 ± 0.04^{a}	0.30	0.30 ± 0.04^{b}	0.29	$0.29 \pm 0.04)^{abc}$	0.29	0.30 ± 0.04^{c}	0.35	0.35 ± 0.04
Hb mmol/l	7.90	7.90 ± 0.80	7.20	6.99 ± 1.07	6.5	6.49 ± 0.88^{ad}	6.90	6.80 ± 0.68^{abcde}	6.50	6.46 ± 0.69^{e}	6.30	6.42 ± 0.83^{be}	7.50	7.54 ± 0.64
MCV fl	26.0	25.0 ± 4.60	27.0	27.1 ± 2.83	27.9	28.5 ± 3.24^{aW}	27.8	28.2 ± 3.20	26.8	26.8 ± 2.83^{a}	26.1	26.0 ± 4.27	25.6	25.9 ± 2.91
MCH fmol	0.56	0.55 ± 0.11	0.56	0.57 ± 0.06	0.61	0.61 ± 0.10^{aW}	0.63	0.64 ± 0.06^{ab}	0.59	0.60 ± 0.05	0.57	0.56 ± 0.06^{b}	0.56	0.57 ± 0.06
MCHC mmol/l	21.1	21.1 ± 0.90	21.1	21.2 ± 1.18	22.0	21.3 ± 2.71^{acW}	22.7	22.8 ± 1.79^{bcW}	22.4	22.5 ± 2.09^{adW}	21.8	21.7 ± 1.36^{bd}	21.6	21.7 ± 0.69
WBC $x10^{9}/l$	12.5	13.4 ± 3.10	13.0	12.83 ± 3.39	12.2	12.0 ± 3.2^{acde}	16.5	15.7 ± 3.7^{abc}	13.2	13.8 ± 3.48^{be}	13.9	13.7 ± 3.5^{ad}	10.0	10.7 ± 3.22
Lymph. $x10^{9}/l$	8.10	8.20 ± 3.30	6.11	6.31 ± 1.74	5.60	5.81 ± 1.84^{W}	6.15	6.66 ± 2.75	5.25	5.53 ± 1.87^{W}	5.72	5.95 ± 2.03	6.00	6.12 ± 2.47
SN $x10^{9}/l$	4.60	4.40 ± 2.60	5.84	5.75 ± 3.11	5.04	5.15 ± 1.99	6.64	7.55 ± 3.06	6.98	7.15 ± 2.72	6.98	6.79 ± 2.22	3.61	3.63 ± 2.17
Eosinophil %	3	0 - 15	2	0 - 6	6	0 - 19	5	0 - 14	4.8	0 - 26	3	0 - 13	3	0 - 18
Basophil %	1	0 - 4	1	0 - 4	0.7	0 - 4	1.2	0 - 4	0.9	0 - 5	1	0 - 4	1	0 - 4
Monocytes %	3	0 - 6	2.5	0 - 6	4.2	0 - 14	5.2	0 - 16	2.6	0 - 11	2	0 - 4.5	4	0 - 6.5
B. neutrophil %	1	0 - 3	1	0 - 3	2	0 - 10	0.9	0 - 4	0.3	0-2.5	1	0 - 5.5	2	0 - 5
Number of goats	13		22		76		40		73		61		11	

Similar superscripts = significantly different group means ($p<0.05$). For rare leukocytes mean % and ranges are given

SN = segmented neutrophils. W = non Gaussian distributions. Groups; 1 = nonpregnant 8-12 months old, 2 = pregnant 1-2 years old, 3 = nonlactating pregnant above two years old, 4 = nonpregnant 14-20 days in lactation above 2 years old, 5 = nonpregnant 60 days in lactation, 6 = nonpregnant more than 90 days in lactation above 2 years old, 7 = nonpregnant nonlactating above 2 years old.

Table 2: The 5th (P_5) to 95th (P_{95}) percentile interval and coefficient (CV %) of hematological analytes in dry, pregnant and lactating goats from all farms combined.

Analyte	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV
RBC $x10^{12}$/l	10.52- 0.63	20.3	9.1-15.85	16.7	8.98-13.11	12.5	9.20-12.63	10.6	8.86-12.78	11.4	9.42-17.10	20.5	10.89-19.4	18.2
PCV l/l	0.32-0.45	12.1	0.22-0.41	17.2	0.25-0.37	13.0	0.24-0.36	12.6	0.22-0.36	14.9	0.23-0.37	14.6	0.28-0.42	10.3
Hb mmol/l	7.00-9.30	9.19	4.60-8.30	15.3	4.80-8.20	13.5	5.80-7.90	9.9	5.30-7.60	10.6	5.10-8.00	12.9	6.30-8.80	8.5
MCV fl	16.5-33.90	19.3	22.0-31.1	10.4	24.9-35.3	11.4	23.2-32.2	11.3	22.4-31.6	10.6	18.5-31.9	16.4	20.8-29.6	11.2
MCH fmol	0.38-0.72	19.6	0.49-0.66	11.0	0.40-0.72	16.9	0.56-0.74	8.9	0.52-0.67	8.4	0.40-0.68	14.0	0.45-0.65	11.0
MCHC mmol/l	19.6-23.40	4.85	19.8-22.7	5.5	13.90-23.8	12.1	20.4-26.5	7.8	19.2-25.6	9.3	19.4-23.4	6.3	20.9-23.1	3.2
WBC $x10^9$/l	8.30-21.40	28.0	6.70-17.10	26.4	6.00-17.20	26.6	8.75-20.9	23.3	7.80-20.4	26.7	8.30-18.7	25.6	6.50-15.4	30.0
Lym. $x10^9$/l	2.33-14.12	41.6	4.09-9.58	27.6	2.92-9.13	31.6	2.15-11.7	41.3	2.77-9.30	33.9	3.02-9.15	34.2	2.79-9.65	40.4
Neu. $x10^9$/l	0.86-12.20	64.9	1.39-9.90	54.1	2.18-8.77	38.6	2.58-11.7	40.5	2.84-12.71	38.0	3.62-9.98	32.8	1.03-9.24	59.8
n	13		22		76		40		73		61		11	

Lym. = Lymphocytes, Neu. = Neutrophils, N = number of goats.

Table 3: Hematological values in pregnant, lactating and dry goats from different herds.

Group	H(n)	RBC $x10^{12}/l$	PCV l/l	Hb mmol/l	MCV fl	MCH fmol	MCHC mmol/l	WBC $x10^9/l$	Lymphocytes[G]	Neutrophil[G]
1	D(3)	19.50 ±1.00*	0.34 ± 0.01	7.40 ± 0.40	17.7 ± 1.10**	0.38 ± 0.00**	21.6 ± 1.50	12.47 ± 2.85	6.06 ± 2.30	5.60 ± 1.01
	E(10)	14.20 ±1.70*	0.39 ± 0.05	8.10 ± 0.80	27.2 ± 2.30**	0.57 ± 0.05**	21.0 ± 0.70	14.40 ± 3.86	8.68 ± 3.24	4.73 ± 3.65
2	C(2)	11.91 ± 1.00	0.34 ± 0.02	7.40 ±0.14	28.9 ± 0.64	0.62 ± 0.04	21.5 ± 0.91	9.55 ± 4.88	5.28 ± 1.17	3.84 ± 3.58
	D(5)	13.47 ± 1.84	0.37 ± 0.03	7.66 ± 0.63	27.5 ± 2.02	0.57 ± 0.06	20.8 ± 0.84	10.62 ± 3.18	5.73 ± 1.56	4.18 ± 2.49
	E(15)	11.90 ± 2.14	0.32 ± 0.06	6.71 ± 1.15	26.7 ± 3.17	0.57 ± 0.07	21.3 ± 1.32	14.01 ± 2.81	6.64 ± 1.84	6.53 ± 3.13
3	A(66)	10.90 ± 1.33	0.31 ± 0.04	6.43 ± 0.88	28.3 ± 3.1	0.60 ± 0.01*	21.1 ± 2.67	12.29 ± 3.12	5.98 ± 1.79*	5.24 ± 2.04
	C(5)	9.87 ± 1.59	0.30 ± 0.04	7.00 ± 0.84	30.9 ± 5.49	0.72 ± 0.11*	23.4 ± 0.96	9.60 ± 3.68	4.23 ± 1.65*	4.62 ± 2.03
	E(5)	11.21 ± 1.32	0.32 ± 0.05	6.78 ± 0.87	28.4 ± 2.35	0.61 ±0.03	22.9 ± 1.21	10.78 ± 3.06	5.20 ±2.05	4.50 ± 1.06
4	A(30)	10.86 ± 1.21	0.30 ± 0.04	6.84 ± 0.67	27.7 ± 2.8	0.63 ± 0.05	22.9 ± 1.45	16.40 ± 3.50***	6.90 ± 2.70	8.00 ± 3.00
	B(4)	10.47±0.96	0.31 ± 0.03	7.22 ± 0.67	29.4 ± 1.10	0.69 ± 0.07	23.7 ± 3.30	11.00±3.70**	4.30 ± 2.20***	5.10 ± 3.50
	C(3)	9.84 ± 1.00	0.30 ± 0.07	6.53 ± 0.84	30.9 ± 7.20	0.66 ± 0.07	21.9 ± 2.77	14.37 ± 3.20*	5.27 ± 2.30	8.78 ± 3.30
	E(3)	10.74 ± 0.76	0.31 ± 0.05	6.70 ± 0.95	28.8 ± 3.50	0.62 ± 0.05	21.7 ± 1.60	16.80 ± 0.85	8.95 ± 1.75***	5.93 ± 0.23
5	A(57)	10.49 ±1.03abc*	0.28 ± 0.04ab*	6.40 ± 0.68a*	26.8 ± 2.60	0.61 ± 0.04a*	22.90 ± 2.00ab*	14.28 ± 3.50a*	5.70 ± 1.90	7.40 ± 2.60a*
	C(5)	11.84 ± 1.27c*	0.33 ± 0.02a*	7.34 ± 0.46ab*	27.8 ± 3.60	0.62 ± 0.09b*	22.50 ± 0.42	9.44 ± 2.31ab*	4.80 ± 1.37	4.27 ± 1.60ab*
	D(3)	11.73 ± 1.18a*	0.32 ± 0.05	6.60 ± 0.95	27.5 ± 0.37	0.56 ± 0.03ac*	20.4 ± 1.15a*	13.07 ± 3.72b*	5.10 ± 0.86	5.73 ± 1.78
	E(8)	12.41 ± 1.00b*	0.32 ± 0.04b*	6.52 ± 0.40b*	25.8 ± 4.08	0.53 ± 0.04ab*	20.7 ± 2.14b*	13.80 ± 4.57	5.20 ± 1.95	7.89 ± 3.27b*
6	A(48)	11.01±1.37ac**	0.30 ± 0.04a*	6.40 ± 0.83	27.3 ± 3.60ab**	0.58 ± 0.06ab*	21.5 ± 1.30a*	14.40 ± 3.20a**	6.20 ± 2.10a**	7.20 ± 2.00a**
	C(5)	15.88 ± 2.48cd*	0.30 ± 0.02	6.98 ± 0.55*	19.5 ± 2.49ac**	0.45 ± 0.08ac*	23.0 ± 1.71ab*	7.42 ± 1.99abc**	3.79 ± 1.10ab**	2.94 ± 1.15abc**
	D(3)	17.39±2.48ab**	0.33 ± 0.01b*	6.87 ± 0.21	19.4 ± 2.45bd**	0.40 ± 0.04bd*	20.6 ± 0.29bc*	14.33 ± 0.75b**	7.16 ± 1.26b**	5.95 ± 1.45c*
	E(5)	10.30 ± 0.52bd*	0.25 ± 0.04ab*	5.76 ± 0.82*	24.6 ± 2.37cd*	0.56 ± 0.06cd*	22.7 ± 0.46c*	12.9 ± 2.18c*	5.25 ± 1.48	6.86 ± 2.01b*
7	C(5)	14.12 ± 3.50*	0.34 ± 0.05	7.58 ± 0.89	23.7 ± 2.33**	0.53 ± 0.07	22.0 ± 0.83	9.18 ± 3.08*	4.89 ± 2.54*	3.62 ± 0.62**
	D(3)	11.94 ± 0.79*	0.34 ± 0.01	7.37 ± 0.15	28.8 ± 1.13**	0.62 ± 0.03	21.5 ± 0.34	13.87 ± 1.33*	7.37 ± 1.27*	5.76 ± 3.01**
	E(3)	13.44 ± 0.14	0.36 ± 0.03	7.63 ± 0.61	26.5 ± 2.24	0.57 ± 0.05	21.4 ± 0.60	10.20 ± 3.12	6.92 ± 2.99	1.53 ± 0.53**

H = Herd, (n)= number of goats. Where there are more than 2 herds, means with similar superscripts (a b c) differ significantly. *p<0.05, **p<0.01, ***p<0.001. G= $x10^9/l$.

Table 4: Hematological values in pregnant, lactating and dry goats of different parities in herd A.

Group	P[1]	N	RBC $x10^{12}$/l	PCV l/l	Hb mmol/l	MCV fl	MCH fmol	MCHC mmol/l	WBC $x10^9$/l	Lymphocyte[G]	Neutrophil[G]
1	≤1	0	-	-	-	-	-	-	-	-	-
2	1-2	0	-	-	-	-	-	-			
3	2-3	38	11.56 ± 1.15**	0.31 ± 0.04*	6.37 ± 0.96	27.7 ± 2.40*	0.57 ± 0.09**	20.4 ± 3.09*	12.80 ± 3.10	5.97 ± 1.57	5.74 ± 2.12*
	3-4	25	10.26 ± 1.40**	0.30 ± 0.04*	6.51 ± 0.80	29.2 ± 3.86*	0.64 ± 0.10**	22.0 ± 1.73*	11.59 ± 3.20	6.09 ± 2.11	4.47 ± 1.81*
	> 5	3	10.48 ± 0.17	0.31 ± 0.01	6.63 ± 0.15	29.3 ± 1.19	0.63 ± 0.02	21.6 ± 0.38	11.63 ± 2.02	5.19 ± 2.08	5.22 ± 0.21
4	2-3	16	11.23 ± 1.31	0.31 ± 0.04	6.96 ± 0.68	27.4 ± 3.01	0.62 ± 0.06	22.9 ± 1.72	17.02 ± 3.10	7.11 ± 3.09	8.53 ± 3.07
	3-4	8	10.56 ± 0.94	0.30 ± 0.03	6.79 ± 0.54	28.0 ± 2.45	0.65 ± 0.05	23.1 ± 1.07	16.51 ± 3.32	7.01 ± 2.19	7.69 ± 2.70
	> 5	5	10.10 ± 0.90	0.29 ± 0.05	6.50 ± 0.80	28.4 ± 3.18	0.64 ± 0.05	22.7 ± 1.16	13.98 ± 4.47	5.97 ± 2.63	6.58 ± 3.53
5	2-3	34	10.90 ± 1.01^{ab*}	0.30 ± 0.04^{ab*}	6.62 ± 0.58^{ab*}	27.3 ± 2.41	0.61 ± 0.04	22.4 ± 1.67*	15.11 ± 3.22*	5.82 ± 1.88	7.77 ± 2.49
	3-4	14	10.19 ± 0.66^{a*}	0.27 ± 0.04^{a*}	6.23 ± 0.66^{ac*}	26.2 ± 3.27	0.61 ± 0.04	23.6 ± 2.81*	13.35 ± 3.61	5.87 ± 1.60	6.48 ± 2.68
	> 5	9	9.41 ± 0.57^{bc*}	0.24 ± 0.02^{b*}	5.66 ± 0.49^{bc*}	25.7 ± 1.96	0.60 ± 0.06	23.5 ± 1.58	12.56 ± 3.58*	4.74 ± 2.53	7.27 ± 2.97
6	2-3	27	11.08 ± 1.55	0.30 ± 0.05	6.32 ± 0.84	27.2 ± 3.62	0.58 ± 0.06	21.2 ± 1.11	14.61 ± 3.21^{a*}	5.75 ± 1.97^{a**}	7.79 ± 1.93*
	3-4	18	10.97 ± 1.19	0.30 ± 0.04	6.52 ± 0.86	27.6 ± 3.73	0.60 ± 0.06	21.7 ± 1.56	14.89 ± 2.83^{b*}	7.12 ± 1.95^{ab**}	6.87 ± 1.82
	> 5	3	10.60 ± 0.82	0.28 ± 0.04	6.33 ± 0.78	26.7 ± 2.83	0.60 ± 0.05	22.4 ± 0.52	9.73 ± 1.36^{ab*}	4.27 ± 0.94^{b**}	4.72 ± 0.94*
7	1-2	0	-	-	-	-	-	-	-	-	-
	> 5	0	-	-	-	-	-	-	-	-	-

[1]P Parity, N = number of goats. In >2 age groups, same superscript = significantly different means $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. [G] = $x10^9$/l

Gaussian distribution, only a few for MCV, MCH, and MCHC in group 3 significantly deviated (W in table 1). The 5th and 95th percentile interval appeared to be within mean ± 2 standard deviations ($\bar{x} \pm 2$) in most groups of goats.

The RBC, PCV, Hb, WBC and lymphocytes were higher in young goats 8 - 12 months and 1 - 2 years of age (fig. 1a, b, c, 2c, d) but vice versa for neutrophils (fig. 3a), while the MCV, MCH and MCHC were lower than in adult goats of above 2 years of age (fig. 1d, 2a, b, table 1). The RBC count, PCV, and Hb declined in pregnant goats and increased after parturition (fig. 1a-c). The increasing trend in these parameters continued during lactation to reach much higher levels in dry goats. The MCV, MCH and MCHC (fig. 1d, 2a-b) increased during pregnancy and decreased after parturition and lactation. The WBC, lymphocyte and neutrophil counts were low during pregnancy, increased near parturition period and decreased during lactation (fig. 2c, d, 3a). The eosinophil number was higher in early lactation and decreased thereafter. The number of monocytes and basophils fluctuated little in pregnant and lactating animals.

There were no significant differences in parameters (except RBC, MCV and MCH) in young goats (group 1 and 2) of different herds (table 3). The MCH was significantly higher and the number of lymphocytes lower in advanced pregnant group 3 goats of herd C than others (table 4). Other significant differences were observed in many parameters during subsequent stages of lactation in goats from different herds.

Parity influence was examined in one herd (A) in which many animals were sampled (table 4). Significant differences were observed between goats of different parity in RBC, MCH, MCHC and neutrophils in pregnant goats. There were significant differences in many parameters with advancing lactation. Animals with many lactations had lower values for RBC counts, PCV, Hb (fig. 4a-c) and WBC, lymphocyte and neutrophil counts (fig. 3b, c, d) but higher values for MCV, MCH and MCHC (fig. 4d, 5a, b) than young goats with low number of lactations. The decrease ($\bar{x}_g - \bar{x}_r$) in RBC and Hb during pregnancy was more in group 3 goats at their 4th pregnancy (fig. 4a, b, c, 6a, c).

The RBC, PCV and Hb values were higher at the period around parturition (reference group) than most other groups (negative $\bar{x}_g - \bar{x}_r$) (fig. 6a-c). Hb was relatively high in 2 weeks of birth and showed declining trends in both pregnancy and lactation. RBC, PCV and Hb clearly declined in pregnancy until mid lactation (fig. 6a-c).
MCV was higher in pregnancy but declined in lactation (fig. 6d), more in goats of lower parity. MCH, MCHC, WBC, lymphocyte and neutrophils were maximum around birth and lower in both pregnancy and lactation (fig. 5c-d, 7a-c). The influence of pregnancy for Hb, MCH and MCHC was greater in 3rd lactation than in goats of

higher parity (fig. 5c-d, 6c). The influence of pregnancy and lactation on hematological parameters were dependent on age and parity of the goat. However, it was of the same magnitude in goats of all parity for total and differential leukocytes (fig. 7a-c).

Discussion

The results of the tests for closeness of fit to a Gaussian distribution in the present study (coefficient of skewness, degree of kurtosis, Shapiro Wilk statistic (W)) indicate that data of most parameters were distributed normally (table 1). As the samples were random, the conditions for parametric tests were fulfilled (Reed *et al.*, 1971; Wu *et al.*, 1975; Siegel and Castellan, 1988). The goats from all herds were combined for testing differences between groups (table 1) because of small samples in some and similar observed changes. Tests were also made for differences between herds (table 3).

Young goats of groups 1 and 2 (8-12 months and 1-2 years of age) had higher values for RBC, PCV and Hb (fig. 1, table 1) than adult animals, as reported by Nangia *et al.* (1968) and Somvanshi *et al.* (1987). The number of lymphocytes were also higher but that of neutrophils lower in younger than in adult goats (fig. 2d, 3a).

The decrease in RBC counts, Hb and PCV during pregnancy (fig. 1a-c), agree with findings by Mohy *et al.* (1985), Biagi *et al.* (1988) and Löhle *et al.* (1990) in Baladi, Saanen and Dwarf goats respectively, in cattle (Rowlands *et al.*, 1975; Junid and Krad, 1987) and canines (Allard *et al.*, 1989). The MCV, MCH and MCHC remain constant in pregnancy and increase slightly in early lactation (fig. 1d, 2a-b). The low number of leukocytes during pregnancy and the increase at parturition and early lactation is probably a response to uterine involution. The results agree with others in goats (Mohy *et al.*, 1985; Biagi *et al.*, 1988) and cattle (Fortagne and Schafer, 1989; Löhle *et al.*, 1990).

Some studies have observed opposite trends to the mentioned above (increases in RBC and WBC counts, MCV in gestation) (Perreira *et al.*, 1987), with higher MCV and MCH, but lower RBC and WBC counts in older than in younger animals. Others did not observe any statistically significant difference in these parameters between pregnant and non pregnant goats (Pospisil *et al.*, 1987). With these exceptions the frequently reported influences of pregnancy are reduction of Hb, PCV and RBC counts, increases in cell size and hemoglobin contents with WBC increasing at parturition and early lactation (see above). The differences between investigations are probably due to nutrition, because Hb and PCV are correlated with dietary crude protein intake and stage of lactation in cows (Pelletier *et al.*, 1985). The decreases are proportional to milk yield (Payne *et al.*, 1975; Hassan *et al.*, 1986).

The degree of changes in blood parameters due to pregnancy and lactation appear to be more with increasing parity. The MCH and MCHC were higher in group 4 (14-20 days post parturition) and low in lactation and pregnancy, the magnitude varying in each parity group. Pregnancy had larger influence on third pregnancy goats whereas lactation affected 4th lactation goats most (fig. 5c-d). More or less the same trend was observed for Hb (fig. 6c). The influence of lactation on RBC, PCV and MCV was more pronounced in goats of greater than 5 lactations (fig. 6a, b, d). The MCV, MCH and MCHC values were larger in pluriparous than in primiparous goats because of the indirect relationship of cell size and number and hemoglobin content (fig. 4d, 5a-b, 5c-d). The increase in WBC, lymphocyte and neutrophil counts following parturition was distinct. The values in the reference group were higher than those of any other group (fig. 3b-d, 7a-c), as reported by Vihan and Rai (1987). The decrease during pregnancy was more pronounced in 4th pregnancy goats whereas lactation influenced more the > 5th lactation goats. However, the decline in leukocytes in pregnancy and lactation might be masked by the higher initial values in young goats (Nangia *et al.*, 1968). The number of neutrophil was much lower in pluriparous than in primiparous goats, as in cattle (Baglioni *et al.*, 1987). The number of all types of leukocytes were higher in the reference than in other groups (negative $\bar{x}_g - \bar{x}_r$) (fig. 7a-c).

The influence of herds on hematological parameters is described in goats (Masoni *et al.*, 1985) and cattle (Hewett, 1974; Rowlands *et al.*, 1975). The present investigation have confirmed them in Danish landrace dairy goats. This is probably because of nutritional adequacy and quality differences between herds.

In conclusion, sustained alterations on the level blood parameters occur as a result of pregnancy and lactation. The magnitudes of the changes depend on age, parity and herd.

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CHAPTER 11

THE INFLUENCE OF PREGNANCY, LACTATION AND HERD ON ELECTROLYTES AND ENZYMES

Summary

Plasma calcium, magnesium, inorganic phosphorus, sodium and potassium concentrations, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and creatine kinase activities were determined in young and adult nonpregnant nonlactating, early and late nonlactating pregnant and early, mid and late nonpregnant lactating Danish landrace goats in five herds. The purpose was to determine the influence of pregnancy and lactation on the levels of these parameters and the effect of age and parity on the changes. Calcium, phosphorus, alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase decreased in late gestation. Magnesium and creatine kinase decreased in early lactating goats but increased in subsequent lactation period. Sodium and potassium fluctuated little during pregnancy and lactation. Calcium, magnesium and potassium profiles were inversely, while phosphorus directly proportional to parity. There were significant differences in most ions and enzymes between goats of different herds (within the same physiological state). The transferases and creatine kinase were higher in young than in old goats, while alkaline phosphatase was unpredictably high or low in individual goats. Alterations in the level of plasma electrolytes and enzyme activities occur due to pregnancy and lactation and the degree depends on age and parity, influenced also by environment.

Introduction

Variations in the concentration of plasma or serum electrolytes and enzyme activities occur in goats in relation to physiology, nutrition and disease (Vrzgula *et al.*, 1985; Chiericato *et al.*, 1986; Mbassa *et al.*, 1989). Some studies have focused on hematological and clinical chemical changes during pregnancy and lactation (Vihan and Rai, 1987; Cissik *et al.*, 1987; Biagi *et al.*, 1988). During gestation maternal mineral homeostasis and plasma activities of certain enzymes such as alkaline phosphatase are strained due to foetal requirements for bone formation (Kumaresan and Ndzingu Awa, 1984). In some species the mother adjusts to mineral requirements of the foetus with alteration of metabolism of vitamin D and intestinal mineral absorption (Paulson and Langman, 1990). In lactating and pregnant ewes and cows,

calcium and phosphorus decrease, magnesium increases while sodium and potassium fluctuate little (Rowlands *et al.*, 1975; Manston *et al.*, 1975; Sigurdsson, 1988), erythrocyte potassium increases while erythrocyte sodium decreases (Mulei and Daniel., 1990). The effects are proportional to the milk yield (Rowlands *et al.*, 1975) and age, particularly for calcium which decreases shortly after calving especially in older cows with many lactations (McAdam and O'Dell, 1982), hence the high incidence rates of postparturient hypocalcaemia with increasing parity.

The influence of pregnancy and lactation on plasma electrolytes and enzyme activities in goats appear to resemble that observed in cows (Vihan and Rai, 1987). However, the effects of age and parity have not been adequately investigated in goats. This study was undertaken to determine if plasma electrolyte concentrations and enzyme activities are significantly altered in pregnancy and lactation and the effects of age and parity on the changes.

Materials and Methods

The goats for this study were from 5 Danish herds (A - E) located at Fakse (A), Næstved (B), Ringsted (C), Haslev (D) and Fugleberg (E), all of Danish landrace breed and were sampled regularly in one and a half years. They were apparently clinically healthy, 13 non pregnant 8 - 12 months old (group 1), 22 of 13 - 24 months of age in the first month of first pregnancy (group 2), 76 adults in advanced pregnancy of 120 to 130 days (group 3), 40 (group 4), 73 (group 5) and 61 (group 6) at 20, 60 and above 90 days in lactation respectively, and 11 adult nonpregnant nonlactating (group 7). The pregnant group 3 goats were non lactating 41 at third, 28 at 4th and 7 above 5 pregnancies respectively. In group 4, 21 goats were at third, 9 at 4th and 10 above 5 lactations respectively. Group five was composed of 41 goats at third, 17 at fourth and 15 above 5 lactations. In group 6, 31 goats were at third, 22 at fourth and 8 above 5 lactations. Four of group 7 goats were at fourth and the rest above 5 lactations. Goats were bred once per year during mid October to mid November for kidding in mid March to mid April.

Goats were kept indoors with degree of outdoor rearing in farms A to D in summer. Nutrition in all was composed of green grass pellets and about 0.5 kg of oats or barley concentrates in addition to dry hay straw. In farms E, goats were reared in the field for the entire summer and autumn without any concentrates.

Blood samples were collected from the external jugular vein in sodium heparin containing vacuum tubes (Becton-Dickinson vacutainers) between 09.00 and 10.00 am and centrifuged at 3500 rpm for 5 minutes within 3 hours for plasma separation. Sampling began during pregnancy (group 3) and continued in the same goats after parturition and subsequent lactation. In the second year blood samples were taken also from the 8 - 12 months (group 1) and 1 - 2 years (group 2) old off-springs.

Plasma enzyme analysis was done immediately or within 24 hours, during which it was stored at 4 ^{0}C. Plasma calcium (Ca) and magnesium (Mg) concentrations were determined on model 5000 atomic absorption spectrophotometer (Perkin Elmer), inorganic phosphorus (P) by phosphomolybdate reaction in end point mode on the Cobas Fara autoanalyzer (Roche). Sodium (Na) and potassium (K) concentrations were determined with electrolyte module of the Cobas Fara analyzer in diluted samples using flow through ion selective and reference electrodes with open liquid junctions.

Aspartate aminotransferase (ASAT, EC 2.6.1.1), alanine aminotransferase (ALAT, EC 2.6.1.2) and creatine kinase (CK, creatine-N-phosphotransferase, EC 2.7.3.2) were determined by kinetic reactions on the Cobas Fara at 37 ^{0}C according to the recommendations of the Scandinavian committee on enzymes. Alkaline phosphatase (ALP, EC 3.1.3.1) activity was determined by end point colorimetric method on the Cobas Fara analyzer by hydrolysis of p-nitrophenylphosphate to phosphate and p-nitrophenol at 37 ^{0}C.

Parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles, median) and coefficient of variations were determined by univariate procedure of a statistical analysis system software (SAS, Carry North Carolina, 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated to determine the closeness of fit for the data of each analyte in each group to the Gaussian distribution. The means of plasma analytes were tested for differences between non pregnant non lactating, pregnant and lactating goats between; herds (in similar physiological groups), parity (in pregnant and lactating goats in a herd with many animals) and physiological groups (in goats of all herds combined) by the general linear models procedure. A Wilcoxon Mann Whitney test was performed for electrolytes and enzymes whose distributions significantly deviated from Gaussian manner.

To study the effect of parity, herd A where many goat samples were collected was used. Goats were grouped according to age and parity (parity was proportional to age, the first pregnancy in 1-2 years old goats was taken to be parity number 1). In order to study the trend of influence of pregnancy or lactation, the group which was closest to the period of parturition (group 4 at 14 - 20 days post parturient) were chosen as reference group. The mean of each parameter in the reference group ($\bar{x}_r$) was subtracted from the corresponding parameter group mean ($\bar{x}_g$) and the differences ($\bar{x}_g$-$\bar{x}_r$) plotted.

Results

The mean and median value of calcium, magnesium, inorganic phosphorus, sodium, potassium, ALAT, ASAT and CK were close to each other (table 1). The biological

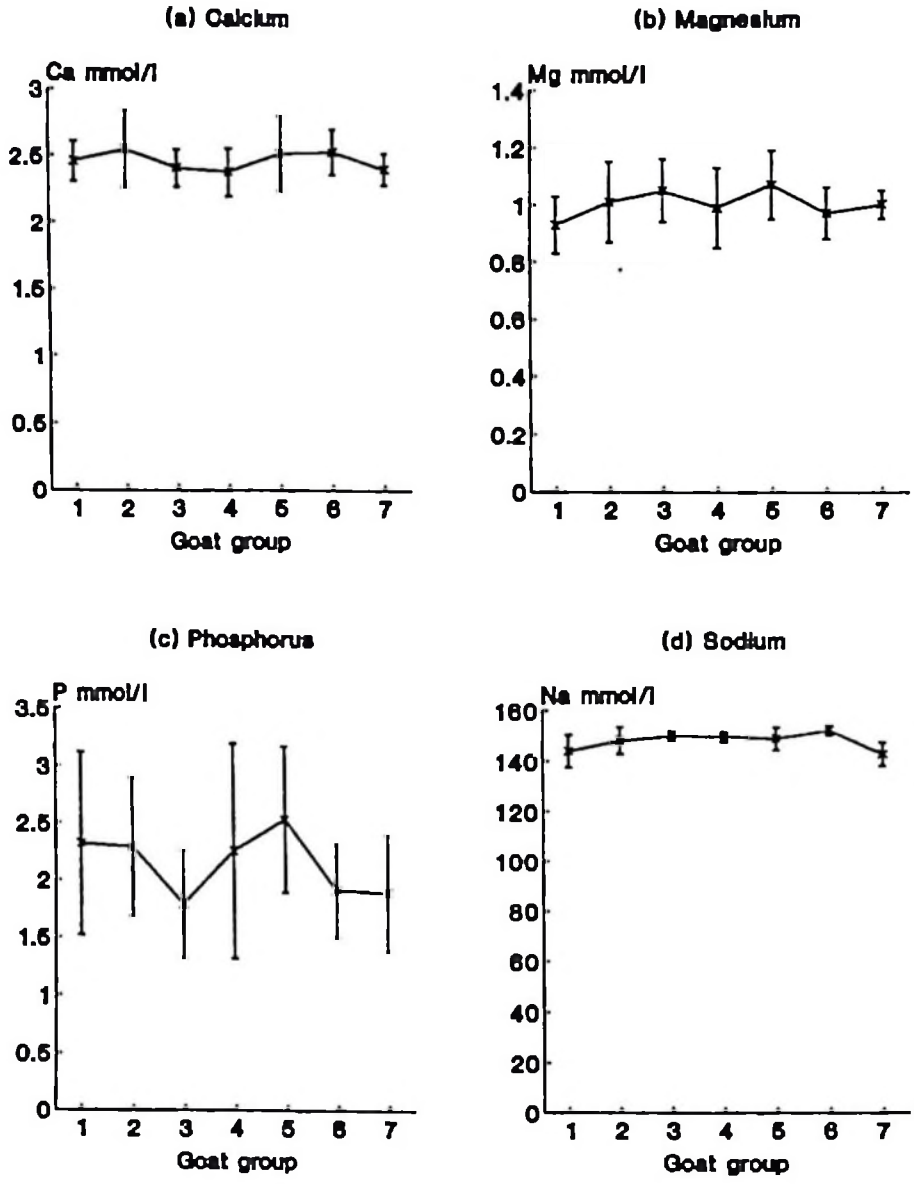

Figure. 1: The mean calcium (a), magnesium) (b), inorganic phosphorus (c) and sodium (d) levels with one standard deviation above and below (vertical lines with bars) in young non pregnant (group 1), young pregnant (group 2), non lactating pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) nonpregnant lactating and nonpregnant non lactating adult (7) goats from all herds combined.

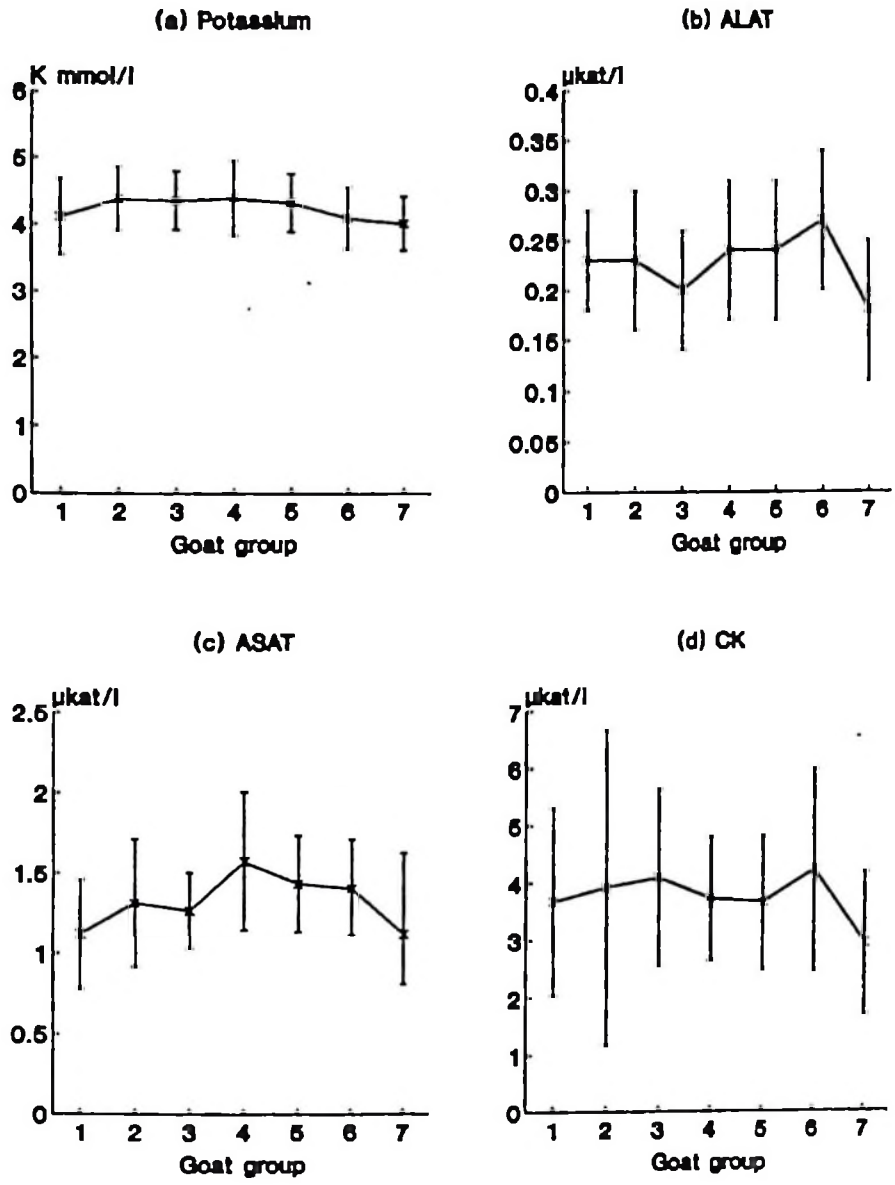

Figure 2: The mean potassium concentration (a), ALAT (b), ASAT (c) and CK (d) activities with one standard deviation above and below (vertical lines with bars) in young nonpregnant (group 1), young pregnant (group 2), non lactating pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) nonpregnant lactating and nonpregnant non lactating adult (group 7) goats from all herds combined.

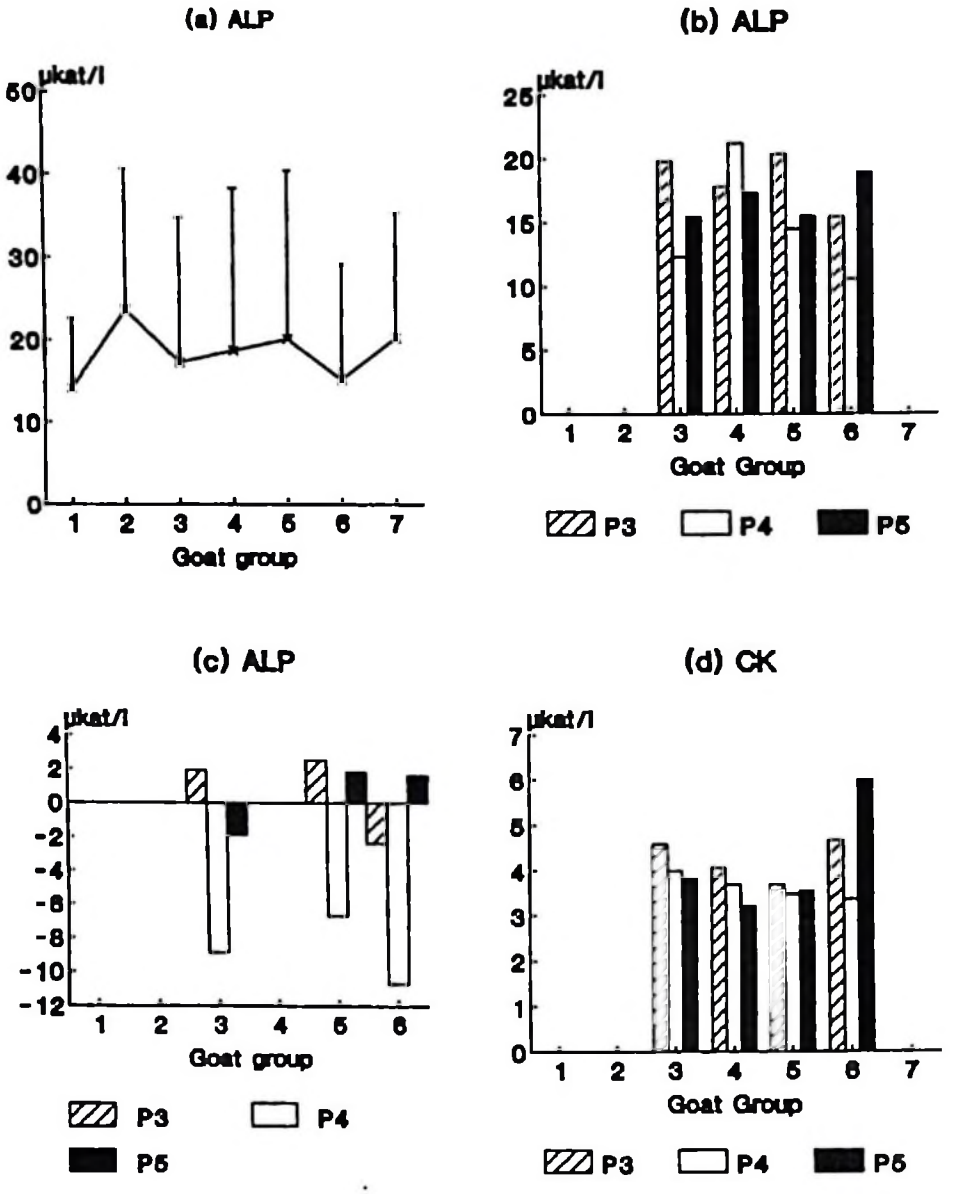

Figure 3a: The mean ALP activity with one standard deviation above (vertical lines with bars) in goats of all herds combined, Figure 3b-d; The mean ALP activity in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) in b. The change in ALP activity on either side of parturition (c) in herd A goats of parity 3 (P3), 4 (P4) and 5 (P5). The mean CK activity in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) in d.

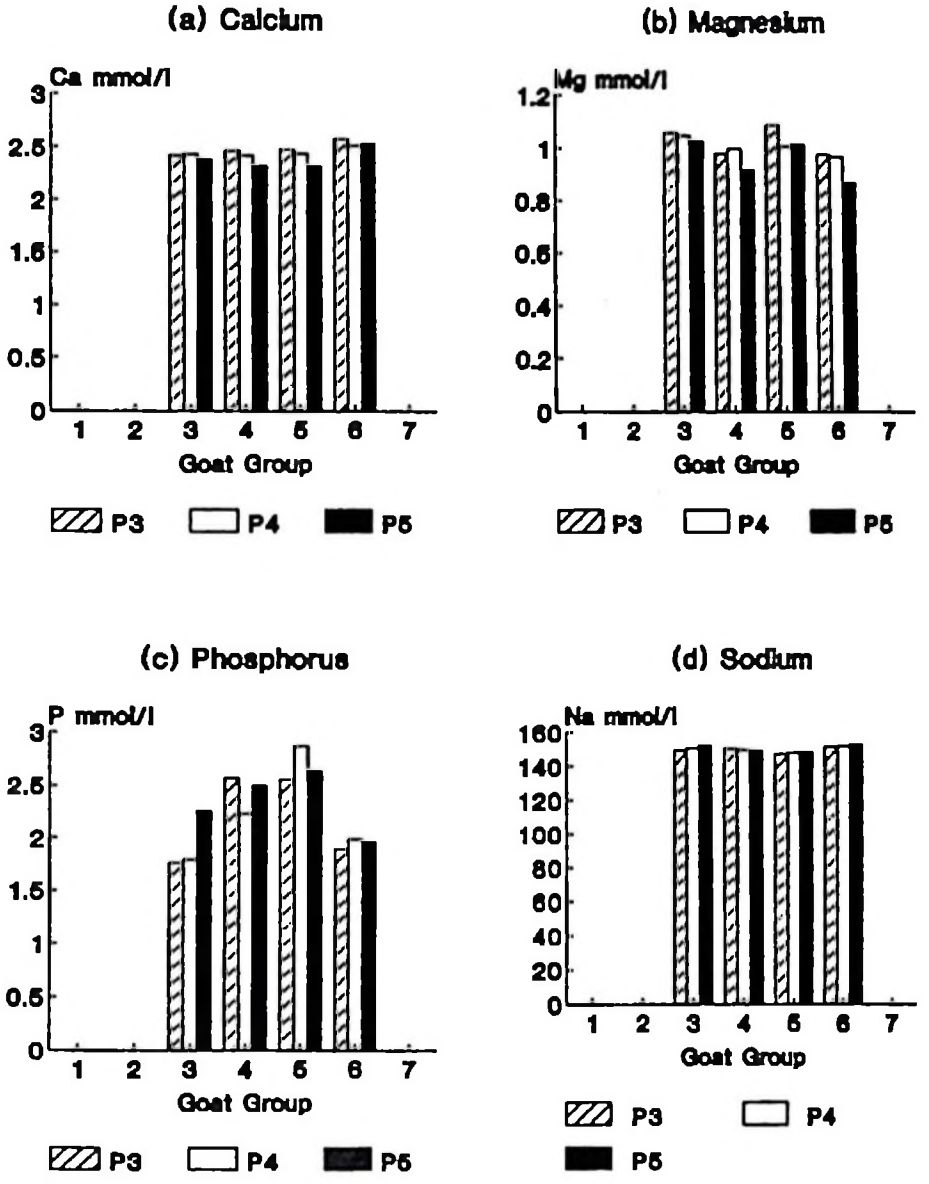

Figure 4: The mean concentration of calcium (a), magnesium (b), phosphorus (c) and sodium (d) in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state group as in figure in 1.

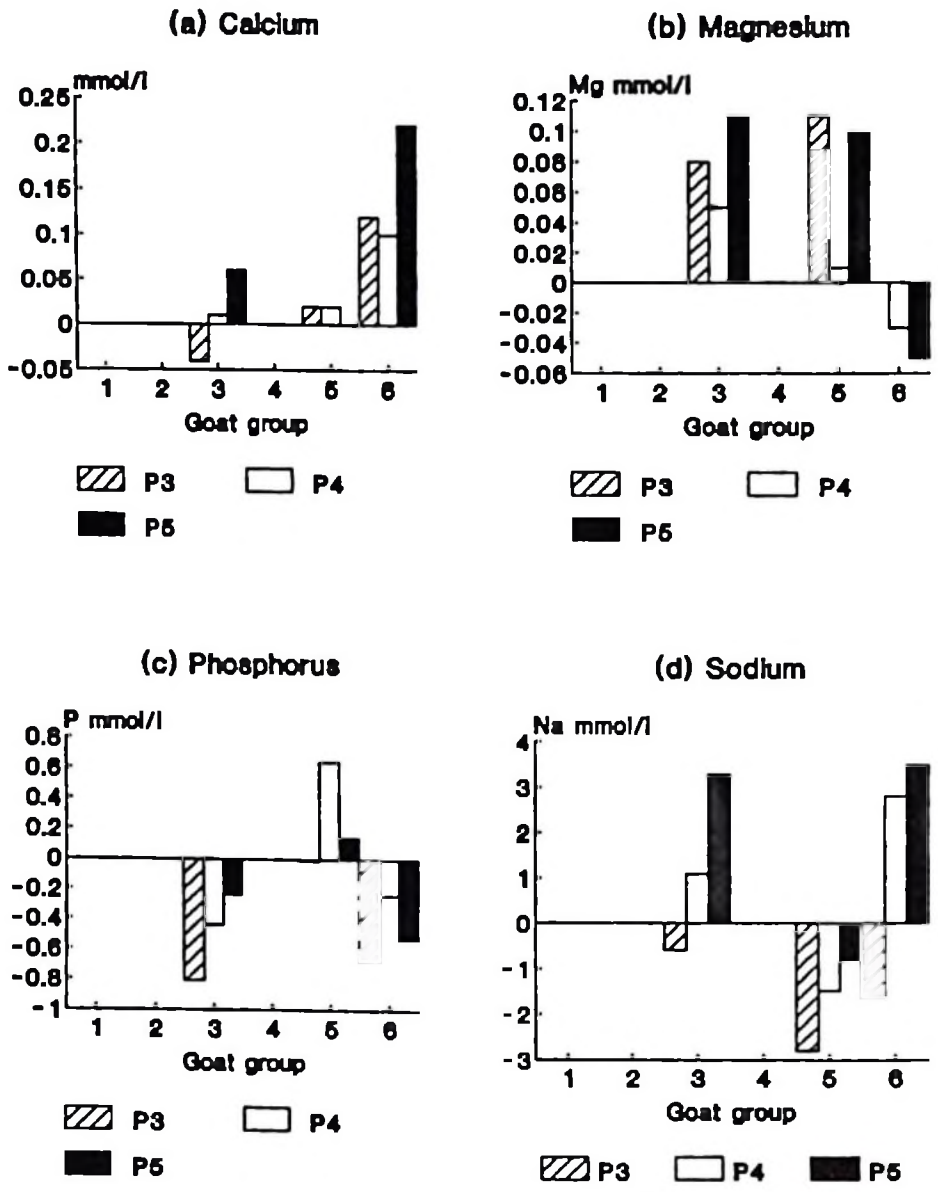

Figure 5: The differences ($\bar{x}_s$ - $\bar{x}_r$) and the direction of the changes in mean values between group 4 goats (reference group) close to parturition and those of other groups in herd A, parity 3 (P3), 4 (P4) and 5 (P5) goats for Ca (a), Mg (b), P (c) and Na (d) concentration.

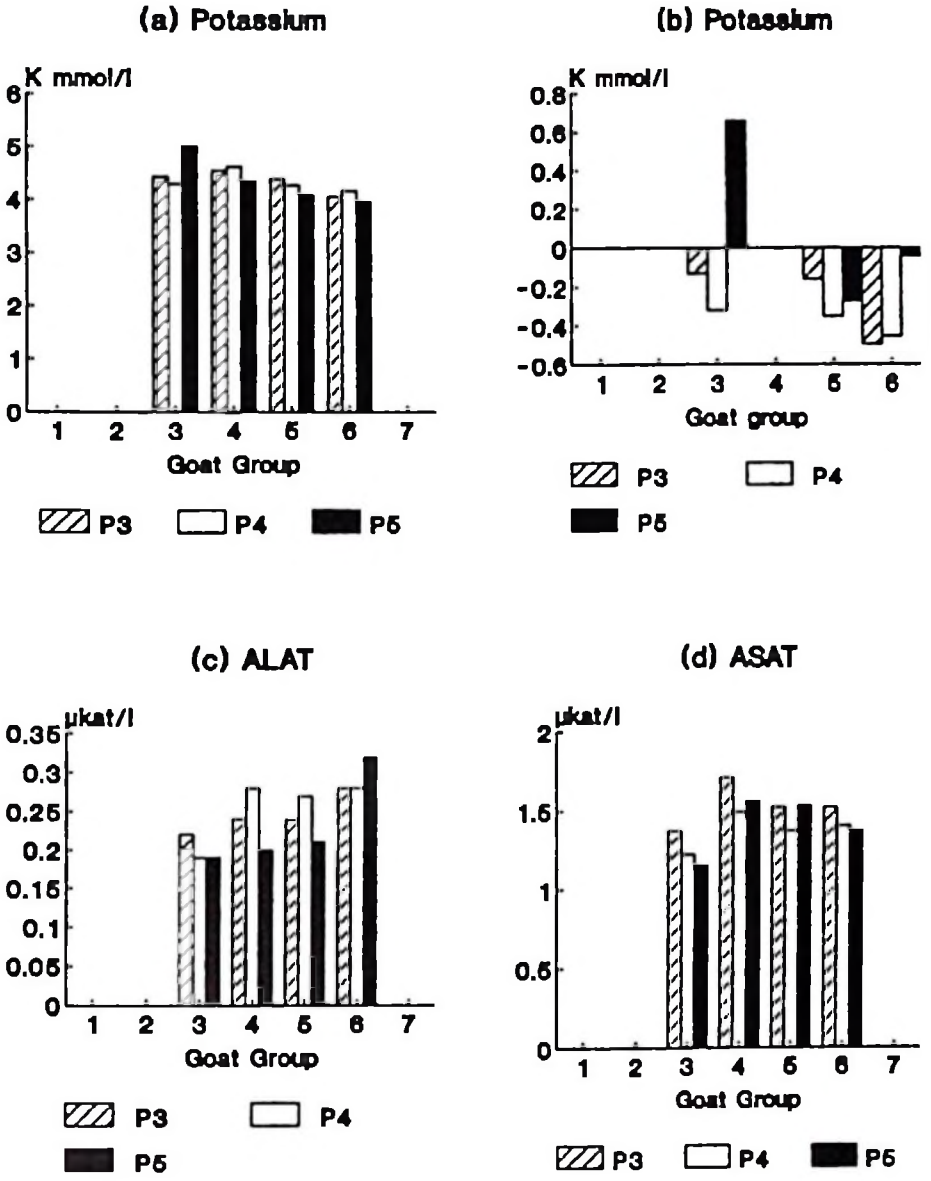

Figure 6a-b: The mean potassium concentration in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state groups as in figures 1 and 2 (a). The changes in mean potassium values from reference group (b). Figure 6c-d: The mean ALAT (c) and ASAT (d) activity in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state groups as in figure 1 and 2.

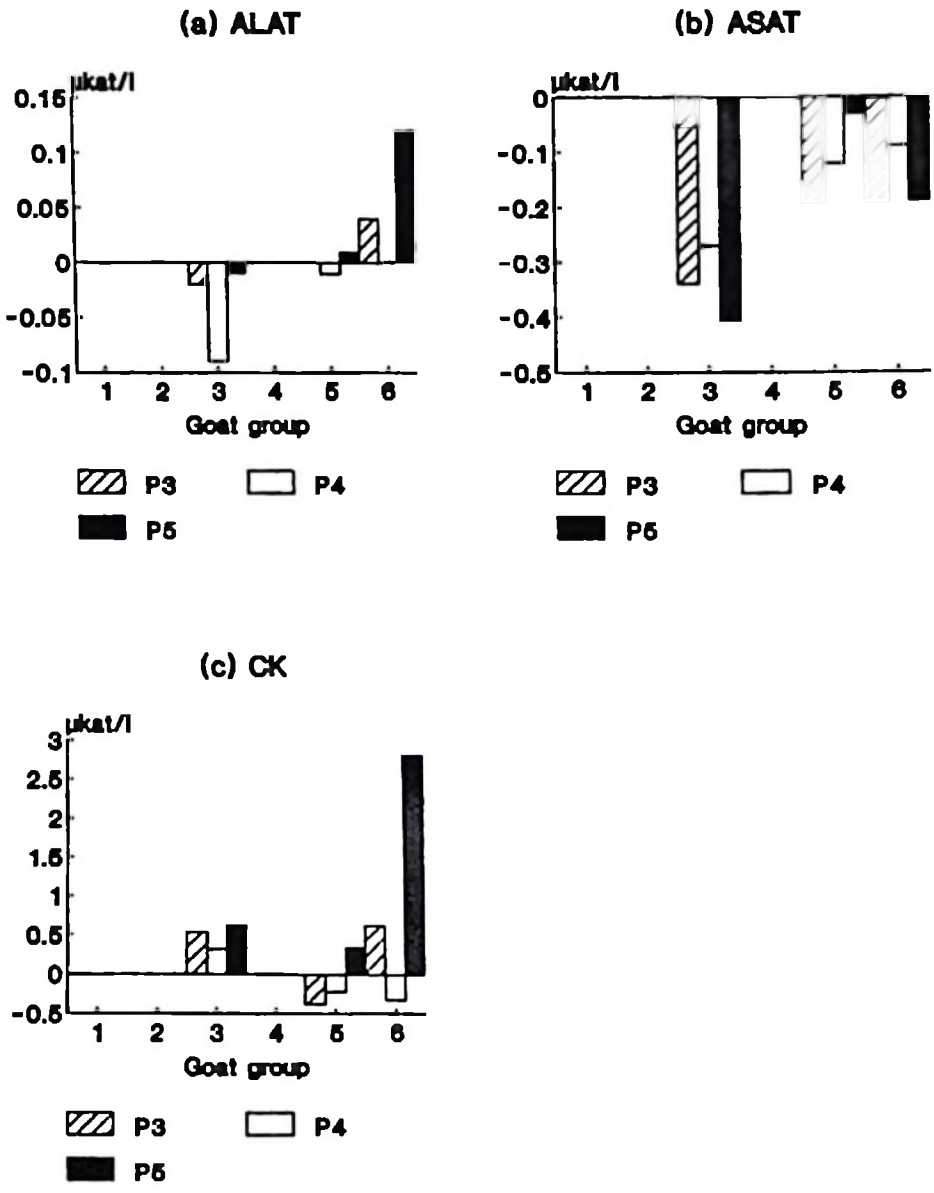

Figure 7: The differences ($\bar{x}_4 - \bar{x}_i$) and the direction of the changes in mean values between group 4 goats (reference group) close to parturition and those of other groups in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) for ALAT (a), ASAT (b) and CK (c).

Table 1: The median (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) of plasma electrolytes (mmol/l) and enzyme activities (μkat/l) in pregnant, lactating and dry goats in all herds.

Analyte	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$
Ca	2.52	2.46 ± 0.15	2.53	$2.55 \pm 0.29^{ab*}$	2.43	$2.41 \pm 0.14^{bce*}$	2.40	$2.38 \pm 0.18^{bdf*}$	2.49	$2.52 \pm 0.28^{ef*}$	2.52	$2.53 \pm 0.17^{ad*}$	2.44	2.40 ± 0.12
Mg	0.90	$0.93 \pm 0.10^{abcde*}$	1.00	$1.01 \pm 0.14^{c*}$	1.07	$1.05 \pm 0.11^{cd*}$	1.02	$0.99 \pm 0.14^{ac*}$	1.04	$1.07 \pm 0.12^{ab*}$	0.96	$0.97 \pm 0.09^{bd*}$	1.00	1.00 ± 0.05
P	2.52	$2.32 \pm 0.80^{dfh*}$	2.32	$2.29 \pm 0.61^{ef*}$	1.82	$1.79 \pm 0.47^{adh*}$	2.19	$2.26 \pm 0.94^{agh*}$	2.55	$2.5 \pm 0.64^{abcd*}$	1.84	$1.92 \pm 0.41^{beg*}$	1.95	$1.90 \pm 0.51^{e*}$
Na	140.7	$144.1 \pm 6.4^{abcd*}$	149.7	$148.3 \pm 5.3^{df*}$	149.7	$150.0 \pm 1.8^{adg*}$	149.5	$149.6 \pm 2.1^{bh*}$	148.6	$149.0 \pm 4.4^{ei*}$	152.2	$152.2 \pm 2.0^{abcdf*}$	141.3	$142.9 \pm 4.7^{abgj*}$
K	4.11	$4.12 \pm 0.57^{dghi*}$	4.24	$4.39 \pm 0.48^{cdej*}$	4.39	$4.36 \pm 0.44^{fg*}$	4.30	$4.40 \pm 0.56^{abk*}$	4.33	$4.33 \pm 0.43^{hij*}$	3.97	$4.10 \pm 0.46^{acfk*}$	4.08	$4.02 \pm 0.40^{beg*}$
ALAT	0.22	$0.23 \pm 0.05^{j*}$	0.23	$0.23 \pm 0.07^{e*}$	0.20	$0.20 \pm 0.06^{dfghi*}$	0.24	$0.24 \pm 0.07^{bk*}$	0.23	$0.24 \pm 0.07^{abcf*}$	0.28	$0.27 \pm 0.07^{abcdej*}$	0.15	$0.18 \pm 0.07^{gjk*}$
ASAT	1.14	$1.12 \pm 0.34^{bk*}$	1.28	$1.32 \pm 0.40^{e*}$	1.25	1.27 ± 0.24	1.50	$1.58 \pm 0.43^{abcdei*}$	1.39	$1.44 \pm 0.30^{afj*}$	1.44	$1.42 \pm 0.30^{bhl*}$	1.12	$1.22 \pm 0.41^{fg*}$
ALP	12.9	14.1 ± 8.42	19.7	23.6 ± 17.0	11.3	17.3 ± 17.5	13.5	18.7 ± 19.6	16.5	20.1 ± 20.3	12.8	15.2 ± 14.0	16.9	20.2 ± 15.1
CK	3.59	$3.68 \pm 1.63^{de*}$	2.91	3.92 ± 2.75	3.84	$4.09 \pm 1.54^{ce*}$	3.61	3.72 ± 1.08	3.70	$3.65 \pm 1.17^{e*}$	3.79	$4.22 \pm 1.76^{abd*}$	3.30	$2.93 \pm 1.24^{bc*}$
n	13		22		76		40		73			61		11

n = number of goats. Similar superscripts = significantly different group means within rows, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Groups; 1 = nonpregnant 8-12 months old, 2 = pregnant 1-2 years old, 3 = nonlactating pregnant above two years old, 4 = nonpregnant 14-20 days in lactation above 2 years old, 5 = nonpregnant 60 days in lactation, 6 = nonpregnant more than 90 days in lactation above 2 years old, 7 = nonpregnant nonlactating above 2 years old.

Table 2: The 5th (P_5) to 95th (P_{95}) percentiles and coefficient (CV %) of plasma electrolytes (mmol/l) and enzyme activities (µkat/l) in dry, pregnant and lactating goats from all farms combined.

Analyte	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV	P_5 - P_{95}	CV
Ca	2.15-2.73	6.2	2.15-3.15	11.3	2.15-2.60	5.7[W]	2.12-2.63	7.4	2.11-3.05	11.0[W]	2.30-2.81	6.8	2.13-2.53	5.2
Mg	0.81-1.16	11.2	0.87-1.27	14.1	0.85-1.21	10.6	0.74-1.18	14.4	0.90-1.32	11.5	0.82-1.12	9.7	0.93-1.13	5.5
P	0.61-3.80	34.6	1.63-3.05	26.8	1.01-2.58	26.5	0.84-3.54	41.4	1.61-3.52	25.2	1.32-2.49	21.6	1.10-2.72	26.9
Na	137.6-154.5	4.45[W]	138.6-153.4	3.6[W]	147.4-153.3	1[W]	146.3-154.6	1.4[W]	144.1-155.8	2.9[W]	149.1-155.3	1.3	137.4-152.0	3.3
K	2.80-4.95	13.9	3.82-5.23	11.0	3.59-5.21	10.2	3.58-5.35	12.6	3.67-4.97	9.9	3.38-4.76	11.3	3.19-4.60	9.9
ALAT	0.13-0.33	23.7	0.12-0.37	32.3	0.11-0.29	28.7	0.11-0.37	29.2	0.15-0.37	27.9	0.16-0.38	26.3	0.12-0.34	36.8
ASAT	0.73-2.01	30.7	0.75-1.93	30.4	0.92-1.68	18.9	1.07-2.30	27.2[W]	1.03-1.95	21.1[W]	1.02-1.88	21.3	0.82-1.96	33.8
ALP	1.05-25.2	59.7	5.94-45.7	72.0[W]	1.71-53.5	101.4[W]	0.95-58.5	104.8[W]	2.15-56.1	101.0[W]	1.64-37.9	92.3[W]	1.40-43.9	75.0
CK	1.17-6.07	44.2	1.59-7.19	70.2[W]	1.86-7.51	37.7[W]	2.29-5.70	29.0	2.15-5.93	31.9	2.52-6.29	41.6[W]	1.51-5.07	42.2
n		13		22		76		40		73		61		11

n= number of goats, W= non Gaussian distributions.

Table 3: The mean plasma electrolytes and enzyme activities in pregnant, lactating and dry goats from different herds.

Group	Herd	N	Ca mmol/l	Mg mmol/l	P mmol/l	Na mmol/l	K mmol/l	ALAT µkat/l	ASAT µkat/l	ALP µkat/l	CK µkat/l
1	D	3	2.48 ± 0.11	0.99 ± 0.09	1.99 ± 0.53	152.0 ± 2.23	4.61 ± 0.34	0.25 ± 0.07	1.46 ± 0.48^{a}	16.9 ± 6.90	5.07 ± 1.14
	E	10	2.46 ± 0.17	0.91 ± 0.11	2.42 ± 0.87	141.7 ± 5.15	3.97 ± 0.55	0.23 ± 0.05	1.02 ± 0.24^{a}	13.3 ± 9.00	3.26 ± 1.55
2	C	2	2.67 ± 0.20	0.98 ± 0.06	2.92 ± 0.18	150.6 ± 3.89	4.76 ± 1.18	0.19 ± 0.02	1.78 ± 0.91	27.7 ± 25.3	2.02 ± 0.45^{a}
	D	5	2.35 ± 0.15	1.04 ± 0.06	2.30 ± 1.05	147.9 ± 4.49	4.30 ± 0.34	0.18 ± 0.06	1.36 ± 0.36	24.9 ± 6.15	5.90 ± 4.65^{a}
	E	15	2.60 ± 0.31	1.01 ± 0.17	2.20 ± 0.42	148.2 ± 5.91	4.37 ± 0.43	0.26 ± 0.07	1.24 ± 0.32	22.7 ± 19.3	3.52 ± 1.76
3	A	66	2.42 ± 0.14	1.06 ± 0.11^{a}	1.79 ± 0.46	150.0 ± 1.89	4.40 ± 0.44	0.20 ± 0.06	1.31 ± 0.23^{ab}	16.7 ± 17.5^{a}	4.34 ± 1.85^{ab}
	C	5	2.34 ± 0.16	1.03 ± 0.08	2.10 ± 0.58^{a}	149.8 ± 0.82	4.22 ± 0.38	0.17 ± 0.06	1.09 ± 0.25^{a}	33.5 ± 15.7^{ab}	2.19 ± 0.81^{b}
	E	5	2.36 ± 0.09	0.94 ± 0.07^{a}	1.42 ± 0.28^{a}	150.2 ± 2.22	4.04 ± 0.45	0.19 ± 0.05	1.05 ± 0.15^{a}	8.96 ± 9.20^{b}	2.66 ± 1.52^{a}
4	A	30	2.42 ± 0.14	0.97 ± 0.14	2.47 ± 0.75^{ac}	149.8 ± 2.22	4.53 ± 0.52^{a}	0.24 ± 0.07	1.62 ± 0.43	18.6 ± 20.51	3.83 ± 1.92
	B	4	2.18 ± 0.30	1.07 ± 0.17	1.00 ± 0.43^{bc}	149.5 ± 1.17	4.34 ± 0.53	0.25 ± 0.12	1.39 ± 0.22	15.9 ± 12.86	3.44 ± 0.80
	C	3	2.33 ± 0.12	1.04 ± 0.09	2.72 ± 1.88^{b}	148.5 ± 3.53	3.63 ± 0.17^{a}	0.19 ± 0.02	1.55 ± 0.74	31.0 ± 26.94	2.84 ± 0.40
	E	3	2.37 ± 0.14	1.01 ± 0.04	1.45 ± 0.31^{a}	149.3 ± 0.71	3.94 ± 0.28	0.23 ± 0.02	1.37 ± 0.19	10.6 ± 7.79	3.93 ± 1.80
5	A	57	2.45 ± 0.18^{ac}	1.06 ± 0.11^{a}	2.65 ± 0.61^{a}	147.7 ±4.03^{abc}	4.31 ± 0.43^{a}	0.24 ± 0.06	1.48 ± 0.32	18.10 ± 17.4^{a}	3.63 ± 2.18
	C	5	2.40 ± 0.14^{a}	0.97 ± 0.07^{a}	2.56 ± 0.59^{a}	154.3 ± 0.58^{a}	4.72 ± 0.19^{ab}	0.20 ± 0.03	1.21 ± 0.25	45.40 ± 39.7^{ab}	2.97 ± 1.18
	D	3	2.76 ± 0.62^{ab}	0.99 ± 0.04^{a}	2.21 ± 0.20	154.5 ± 2.17^{a}	4.45 ± 0.41	0.29 ± 0.13	1.42 ± 0.25	8.44 ± 9.93^{b}	4.44 ± 0.94
	E	5	2.99 ± 0.26	1.21 ± 0.10^{abc}	1.84 ± 0.55^{ab}	153.1 ± 1.48	4.17 ± 0.42^{b}	0.25 ± 0.06	1.27 ± 0.11	22.97 ± 17.9	3.99 ± 1.04
6	A	46	2.50 ± 0.17^{a}	0.97 ± 0.09	1.93 ± 0.39	152.0 ±2.05^{a}	4.09 ± 0.45	0.28 ± 0.07	1.47 ± 0.29^{a}	13.77 ±14.09	4.25 ± 1.92
	C	5	2.36 ± 0.18^{a}	1.02 ± 0.09	1.79 ± 0.41	153.2 ±0.68	4.32 ± 0.43^{a}	0.22 ± 0.04	1.13 ± 0.22^{a}	24.2 ± 15.82	4.46 ± 1.23
	D	2	2.45 ± 0.05	0.94 ± 0.06	1.87 ± 0.60	154.5 ±1.61^{a}	3.61 ± 0.27^{ab}	0.20 ± 0.06	1.42 ± 0.48	19.15 ± 15.10	4.65 ± 1.75
	E	5	2.56 ± 0.24	0.92 ± 0.10	1.91 ± 0.66	151.9 ± 1.99	4.28 ± 0.59^{a}	0.29 ± 0.08	1.30 ± 0.24	17.6 ± 10.67	3.19 ± 0.48
	A	7	2.56 ± 0.35	0.99 ± 0.06	2.01 ± 0.60	143.3 ± 4.88	4.00 ± 0.39	0.22 ± 0.08	1.30 ± 0.42	32.1 ± 13.67^{ab}	3.23 ± 1.54
	C	3	2.65 ± 0.06	1.05 ± 0.07	1.78 ± 0.37	146.8 ± 2.91^{a}	3.83 ± 0.59	0.15 ± 0.04	0.94 ± 0.16	10.8 ± 8.53^{a}	2.88 ± 1.17
	E	3	2.47 ± 0.35	0.98 ± 0.04	1.75 ± 0.58	138.5 ± 0.97^{a}	4.25 ± 0.09	0.15 ± 0.02	1.36 ± 0.55	9.72 ± 6.38^{a}	2.50 ± 1.02

N = number of goats. Similar superscripts = significantly different herd means, *p<0.05, **p<0.01, ***p<0.001.

Table 4: Plasma electrolytes and enzyme activities in pregnant, lactating and dry goats of different parities in herd A.

Group	P[1]	N	Ca mmol/l	Mg mmol/l	P mmol/l	Na mmol/l	K mmol/l	ALAT µkat/l	ASAT µkat/l	ALP µkat/l	CK µkat/l
1	≤1	0	-	-	-	-	-	-	-	-	-
2	1-2	0	-	-	-	-	-	-			
3	2-3	38	2.42 ± 0.15	1.06 ± 0.12	1.76 ± 0.46	149.6 ± 1.90*	4.42 ± 0.40a*	0.22 ± 0.05	1.37 ± 0.24ab*	19.7 ± 20.3	4.60 ± 1.70
	3-4	25	2.43 ± 0.12	1.05 ± 0.11	1.79 ± 0.45	150.5 ± 1.66	4.30 ± 0.44b*	0.19 ± 0.06	1.22 ± 0.19a*	12.3 ± 11.8	4.01 ± 0.92
	> 5	3	2.38 ± 0.21	1.03 ± 0.06	2.26 ± 0.51	152.5 ± 1.04*	5.01 ± 0.57ab*	0.19 ± 0.04	1.15 ± 0.07b*	15.4 ± 18.6	3.83 ± 0.57
4	2-3	16	2.46 ± 0.14	0.98 ± 0.15	2.57 ± 0.79	150.2 ± 2.78	4.55 ± 0.62	0.24 ± 0.07	1.71 ± 0.52a*	17.8 ± 17.9	4.07 ± 1.06
	3-4	8	2.42 ± 0.15	1.00 ± 0.14	2.23 ± 0.78	149.4 ± 1.24	4.62 ± 0.44	0.28 ± 0.07	1.49 ± 0.26ab*	21.2 ± 27.0	3.70 ± 1.08
	> 5	5	2.32 ± 0.14	0.92 ± 0.16	2.50 ± 0.59	149.2 ± 0.88	4.35 ± 0.28	0.20 ± 0.05	1.56 ± 0.32b*	17.3 ± 21.8	3.21 ± 1.05
5	2-3	34	2.48± 0.14	1.09 ± 0.12*	2.56 ± 0.58	147.4 ± 4.71	4.39 ± 0.46	0.24 ± 0.06	1.52 ± 0.30	20.3 ± 18.4	3.70 ± 1.40
	3-4	14	2.44 ± 0.22*	1.01 ± 0.07*	2.87 ± 0.60	147.9 ± 3.41	4.27 ± 0.39	0.27 ± 0.09*	1.37 ± 0.23	14.5 ± 16.5	3.48 ± 0.64
	> 5	9	2.32 ± 0.23*	1.02 ± 0.13	2.64 ± 0.73	148.4 ± 1.61	4.08 ± 0.31	0.21 ± 0.05*	1.53 ± 0.46	15.5 ± 15.1	3.55 ± 1.00
6	2-3	27	2.58 ± 0.19	0.98 ± 0.09	1.90 ± 0.38	151.8 ± 1.94	4.06 ± 0.48	0.28 ± 0.05	1.52 ± 0.31	15.4 ± 15.3	4.69 ± 1.70*
	3-4	18	2.52 ± 0.13	0.97 ± 0.09	1.99 ± 0.42	152.2 ± 2.32	4.17 ± 0.45	0.28 ± 0.10	1.40 ± 0.27	10.5 ± 12.0	3.38 ± 0.98a*
	> 5	3	2.54 ± 0.11	0.87 ± 0.08	1.96 ± 0.31	152.7 ± 1.61	3.95 ± 0.06	0.32 ± 0.04	1.37 ± 0.23	18.9 ± 15.5	6.02 ± 4.83b*
7	1-2	0	-	-	-	-	-	-	-	-	-
	> 5	0	-	-	-	-	-	-	-	-	-

[1]P = parity and is proportional to age, n = number of goats. Same superscript = significantly different parity means within the groups, *p<0.05, **p<0.01, ***p<0.001.

coefficients of variations ranged from small to high values especially for P and enzymes (table 2). The coefficient of skewness and degree of kurtosis were small and non significant in most parameters. The Shapiro Wilk statistic (W) testing for normal distribution indicated that nearly all frequency distributions of the electrolyte and enzyme values were Gaussian (table 2), except a few for ALP and CK. The 5^{th} to 95^{th} interval appeared to be within the $\bar{x}\pm2$ standard deviation in most groups of goats. The medians and means for plasma ALP in different groups were different but there were no significant differences between groups under Wilcoxon tests.

Plasam calcium and inorganic phosphorus concentrations decreased in advanced pregnancy and shortly following parturition (fig. 1a, c). Plasma magnesium, sodium and potassium were relatively constant in both pregnancy and lactation (fig. 1b, d, 2a). Phosphorus was, however, higher in young pregnant (group 2) than in adult pregnant group 3 goats. The concentration of the various electrolytes and enzymes that significantly differed between physiological groups are shown in table 1. There was a slight increase of magnesium in mid lactation in group 5 goats (fig. 1b).

Calcium tended to be lower in goats with more than parity 5 (groups 3-6) from pregnancy to mid lactation (fig. 4a, 5a). The level was inversely proportional to parity. Calcium levels increased at the end of lactation, more so in > 5 lactations goat group (fig. 5a). Magnesium and potassium also showed tendencies of decreasing with increasing parity during pregnancy and lactation (fig. 4b, 6a). The magnitude of changes during the two physiological periods varied with parity (fig. 5b, 6b). Plasma phosphorus was higher in pregnant goats of higher than of lower parity. Levels increased in gestation and lactation but decreased at the end of the latter state (fig. 4c, 5c). Plasma sodium was not much influenced by parity (fig. 4d) but showed an increasing trend during pregnancy, decreased in mid lactation and increased again at the end of lactation period (fig. 5d).

Plasma ALAT activity was higher in young than in old goats (fig. 2b), the levels decreased in pregnancy but increased following parturition and during lactation to decrease again in dry animals. Plasma ASAT activity was lower in both young and adult non lactating non pregnant goats (fig. 2c), slightly decreased in advanced pregnancy (group 3) and increased close to parturition. ASAT levels were highest in early lactation (2 weeks postpartum) and decreased with lactation. Plasma CK activity varied in individual goats but were significantly lower in old nonpregnant nonlactating goats (fig. 2d). Levels appeared not to be altered in pregnancy but decreased after parturition. Plasma ALP highly varied between individuals (fig. 3a, table 2) and there was no significant differences among the age and physiological groups.

ALAT, ASAT and CK activities in pregnancy and lactation were higher in young goats with low parity except at the end of lactation when they were vice versa (fig. 3d, 6c-d). The three enzymes were highest around parturition (in reference group) except

ALAT and CK which increased at the end of lactation period (fig. 7a-c), with small differences between parity groups.

There were significant differences in plasma electrolytes and enzymes between goats of different herds within similar physiological groups (table 3).

Discussion

The data of all ions and enzymes in this study for each group were tested for closeness of fit to the Gaussian distribution by the coefficient of skewness, degree of kurtosis and Shapiro Wilk statistic (W) and the results indicate, that nearly all were distributed normally (except W in table 2). Since the samples were taken randomly, the conditions for parametric tests were satisfied (Reed *et al.*, 1971; Wu *et al.*, 1975; Siegel and Castellan, 1988), but in those which deviated (for ALP), nonparametric tests were used. In the tests for influence of lactation and pregnancy (table 4) goats from all herds were combined because the goats were of the same breed and the same changes were observed. Tests were also made for differences between herds for all parameters (table 3). The results show decreases of calcium, magnesium and phosphorus towards the end of parturition. Sodium and potassium levels change very little with pregnancy and lactation. There is therefore an initial low level of electrolytes at the onset of lactation. The concentrations increase up to the end of lactation and drop. These results agree with a study in cattle where calcium, phosphorus and sodium were found to decrease towards parturition and then increased with lactation while magnesium and potassium decreased (Akinsoyinu, 1982). Vihan and Rai (1987) observed no differences in calcium and magnesium between pregnant and non pregnant goats, similar to comparisons of Cissik *et al.* (1987), while phosphorus was high at parturition and postparturient periods. Biagi *et al.* (1988) observed slight increases of calcium, magnesium and potassium in lactating goats.

The lack of agreement between these sources are probably due to nutrition, breed, environment, seasons, age of the goats, ambient temperatures and analytical methods (Bas *et al.*, 1980; Ridoux *et al.*, 1981; Vrzgula *et al.*, 1985). Plasma Ca, P, and Mg are higher in young than in old cows during the lactation cycle (McAdam and O'Dell, 1982) and most electrolytes are higher in young than in old goats.

The influence of pregnancy and lactation on electrolytes appears to be higher with increasing parity. During pregnancy much lower calcium, magnesium and potassium but higher phosphorus were observed in goats of higher than of lower parity (fig. 4a-c). Sodium fluctuated little with parity. This trend resembles that seen in cows (McAdam and O'Dell, 1982). In cattle the serum electrolyte profile matches the clinical picture so that adult cows with many lactations are more prone to post parturient hypocalcaemia than young ones.

ALAT and ASAT were found to decrease in the last stages of pregnancy in the present investigation and increase following parturition, as observed by Vihan and Rai (1987) in other goats, by Felbinger (1987) in horses and Karadjole *et al.* (1985) in sheep. In sheep Transferases were constant but ALP increased with lactation to a peak in the 5th month (Karadjole *et al.*, 1985). During pregnancy and early lactation ALAT, ASAT, ALP and CK are lower in goats of higher than those of lower parity (fig. 3b-d, 6c-d, 7a-c).

Plasma CK activity slightly declined initially in the present goats after parturition then increased with lactation. The rise with lactation was earlier observed (Garnier *et al.*, 1984).

ALP activity varied so greatly that it was impossible to confirm any trend. The Wilcoxon Mann Whitney tests did no show any significant differences between pregnant and lactating goats. Biagi *et al.* (1988) observed a slight ALP rise during lactation but Kramer and Carthew (1985) noted wide variations that made conclusions impossible on the metabolic pattern of this enzyme. The unpredictable ALP level makes it of little clinical use in goats. Kumaresan and Ndzingu Awa (1984) observed high ALP in young and adult pregnant goats, an evidence of age influence. In the former category of goats (under one year of age) the same trend was observed in this study. ALP decreases in lactating cows (Timet *et al.*, 1985), especially at maximum milk yield, contrary to the findings in sheep (Karadjole *et al.*, 1985). The high bone ALP isoform in young animals diminishes on maturity. The increase or decrease of ALP activity during pregnancy or lactation may be associated with fetal demands and secretion into the milk.

Parity and age influence ALP level, but unknown other factors of probably renal, accessory sex glands or nutritional nature have effects on this enzyme.

The present study have revealed large differences in electrolytes and enzymes in Danish landrace dairy goats from different herds, which have been describe also by Masoni *et al.* (1985), and in cattle by Hewett (1974) and Rowlands *et al.* (1975). They probably result from nutritional quality and quantity in different herds. Metabolic profile tests are therefore herd specific (Biagi *et al.* (1988).

In conclusion, this study have revealed significant sustained alterations in plasma electrolytes and enzyme activities due to pregnancy and lactation. The changes are most pronounced in old animals with greater number of lactations or pregnancies and depend on the herd, probably as a result of nutritional adequacy and quality.

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CHAPTER 12

INFLUENCE OF PREGNANCY, LACTATION AND HERD ON PLASMA UREA, CREATININE, BILIRUBIN, CHOLESTEROL, GLUCOSE AND TOTAL SERUM PROTEINS

Summary

Plasma urea, creatinine, bilirubin, glucose, cholesterol and total serum proteins were determined in Danish landrace goats from five herds in early and late gestation, during lactation and in dry goats. The purpose was to determine if there are sustained alterations in the levels of these parameters due to pregnancy and lactation and whether the changes are dependent on age, parity and environment. Urea, creatinine and bilirubin were higher in young nonpregnant than in other goats. Urea decreased in goats at early and mid lactation directly proportional to parity so that the higher the parity the more the decrease. Creatinine was higher in young and adult nonpregnant than in other goats. There was an increase in late lactation that was more in goats of higher parity than in others. Bilirubin was higher in mid lactation stage, much more in goats of higher parity than in others. Glucose concentration was lower in pregnant than in lactating goats and increased during lactation. The decrease during pregnancy was more in higher parity goats than in others. Plasma cholesterol and total serum proteins increased during lactation directly proportional to parity. There were significant differences in biochemical parameters between goats from different herds (within similar physiological states). Sustained alterations of these biochemical parameters occur during pregnancy and lactation in goats, the magnitude of changes depends on age and parity, and vary between herds.

Introduction

Several investigators have studied the variations in the concentration of clinical chemical parameters in goats in relation to physiology, nutrition and disease (Pugliese *et al.*, 1982; Vrzgula *et al.*, 1985; Okorie and Anugwa, 1986; Mbassa *et al.*, 1989). There has been some studies on hematological and clinical chemical changes in pregnancy and lactation (Vihan and Rai, 1987; Cissik *et al.*, 1987; Biagi *et al.*, 1988a). During pregnancy and lactation maternal metabolic activities are strained due to foetal and off-spring growth requirements which pose extra demands for water and nutrients (Maltz and Shkolnik, 1984). In some species the mother adjusts to nutritional

requirements of the foetus with alteration of metabolism of vitamin D and intestinal nutrient absorption (Paulson and Langman, 1990). Thyroid hormone (T_3 and T_4) levels are increased in lactating goats and follow a linear regression with respect to the number of lactations (Biagi *et al.*, 1988b). The adjustments and effects of pregnancy and lactation on plasma and serum biochemical parameters in cows and sheep have been described (Rowlands *et al.*, 1975; Manston *et al.*, 1975; Vihan and Rai, 1987, Siggurdson, 1988). The influence of pregnancy and lactation on clinical chemical parameters in relation to age and parity in goats, however, have not been adequately investigated. The effect of parity is important in interpretation of metabolic profile tests. This study was undertaken to determine if the reference biochemical values are subject to sustained alterations due to pregnancy, lactation and the influence of parity.

Materials and Methods

The goats for this study were of Danish landrace breed from 5 herds (A - E) located at Fakse (A), Næstved (B), Ringsted (C), Haslev (D) and Fugleberg (E) which were sampled regularly for one and a half years. All were apparently clinically healthy 13 non pregnant 8 - 12 months old (group 1), 22 of 13 - 24 months of age in the first month of first pregnancy (group 2), 76 in advanced pregnancy of 120 to 130 days (group 3), 40 (group 4), 73 (group 5) and 61 (group 6) at 20, 60 and above 90 days in lactation respectively, and 11 adult dry goats (group 7). The pregnant group 3 goats were non lactating 41 of them were at third, 28 at 4th and 7 above 5 pregnancies respectively. In group 4, 21 goats were at their third, 9 at 4th and 10 above 5 lactations respectively. Group five was composed of 41 goats at third, 17 at fourth and 15 above 5 lactations. In group 6, 31 goats were at third, 22 at fourth and 8 above 5 lactations. Four of group 7 goats were at fourth and the rest above 5 lactations.

Goats were bred once per year during mid October to mid November for kidding in mid March to mid April. They were kept indoors throughout the year but some degree of outdoor rearing was allowed in farms A to D in summer. Nutrition in these herds was composed of green grass pellets and about 0.5 kg of oats or barley concentrates in addition to dry hay straw. In herd E, goats were reared in the field for the entire summer and autumn any concentrate supplementation.

Blood samples were collected from the external jugular vein in sodium heparin and clot activator containing vacuum tubes (Becton-Dickinson vacutainers) between 9 and 10 am and centrifuged for plasma and serum separation at 3500 rpm for 5 minutes within 3 hours. Analyses were performed immediately or within 24 hours during which plasma and serum were stored at 4 ^{0}C.

Plasma urea was determined by enzymatic ultra-violet test with urease and glutamate dehydrogenase, creatinine by the kinetic picrate reaction without

deproteinization, glucose by oxidation to D-gluconolatone under glucose dehydrogenase after deproteinization with perchloric acid and bilirubin by sulphobenzenediazonium chloride method in an automated analyzer Cobas Fara (Roche). Total serum proteins were determined by the biuret method in the same analyzer.

Parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles, median) values were determined by univariate procedure of statistical analysis system software (SAS, 1988). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte in each group to the Gaussian distribution. The means of plasma and serum analytes were tested for differences between nonpregnant nonlactating, nonlactating pregnant and nonpregnant lactating goats between herds (in similar groups), parity (in pregnant, lactating and nonpregnant nonlactating goats in a herd with many animals) and groups (in goats from all herds) by using the general linear models procedure and Wilcoxon -Mann- Whitney tests for biochemical parameters whose data significantly deviated from Gaussian manner. Parameter values were ranked in ascending order and the average ranks tested for differences between physiological groups and herds (in similar physiological states) by the rank and general linear models procedures.

To study the effect of parity, the herd where sufficient goat samples were collected (A) was used. Goats were grouped according to age and parity (parity was proportional to age, the first pregnancy in 1 - 2 year old goats was taken to be parity number 1). In order to study the trend and magnitude of influence of pregnancy or lactation, the group which was closest to the period of parturition (group 4 at 14 - 20 days post parturient) were chosen as standard reference group. The mean of each parameter in the reference group ($\bar{x}_r$) was subtracted from the corresponding parameter group mean ($\bar{x}_g$) and the differences ($\bar{x}_g - \bar{x}_r$) plotted.

Results

The mean and median values for urea, creatinine, glucose, bilirubin, cholesterol and total serum proteins were close to each other (table 1). The 5^{th} and 95^{th} percentile intervals in most parameters were within the mean ± 2 standard deviations interval in most groups of goats (table 1 and 2). The coefficient of skewness and the degree of kurtosis were small and non significant in most parameters. The Shapiro Wilk statistic (W) testing for normal distributions indicated that the data of most analytes were Gaussian distributions (except W in table 2).

Plasma urea and creatinine concentrations were higher in young nonpregnant, and for the latter also in adult dry goats (fig. 1a, b, 2a, b, table 1 - 2). Plasma urea

concentration decreased in early and mid but increased in late lactating goats (fig. 1a, 2a). Urea increase during pregnancy was directly proportional to the number of lactations. The higher the parity the more the increase (fig. 2a, 4a, table 3) and this appeared to be similar during late lactation.

Creatinine concentration did not differ much between pregnant and lactating goats (fig. 1b). However, creatinine increased in late lactation in herd A (fig. 2b). The increase was directly proportional to parity so that the higher the parity the greater the increase (fig. 2b, 4b). Plasma bilirubin was higher in young non pregnant and mid stage lactating goats (fig. 1c, 2c, table 1 - 2). Bilirubin concentration increased during pregnancy, directly proportional to parity (fig. 2c, 4c, table 3). The decrease after parturition was directly proportional to parity (fig. 2c). Bilirubin levels increased in mid lactation especially more in goats of low parity and decreased in late lactation stages (fig. 2c, 4c).

Plasma glucose concentration was not affected by age as it was the same in young and adult nonpregnant non lactating goats but increased during lactation (fig. 1d, 2d, table 1 - 2). Glucose levels were lower in pregnancy than in lactation (fig. 2d) and the decrease was more in goats of high than those of low parity (fig. 2d, 4d).

Total serum protein concentrations were higher during lactation than in pregnancy (fig. 3a) and more so in goats of higher than of lower parity (fig. 3c, table 1-2). The magnitude of changes in pregnancy and lactation, however, varied between groups (fig. 4e).

Cholesterol levels were lower in young nonpregnant, non lactating and adult pregnant than in lactating and adult dry goats (fig. 3b). The decrease in cholesterol levels in pregnant goats was much more in those with the highest number of lactations. Cholesterol levels were highest during early lactation, an increase that was directly proportional to parity (fig. 3d). The magnitude of change during pregnancy and lactation was more in goats with larger number of lactations than in younger animals (fig. 4f).

Significant differences were observed in many parameters between goats from different herds (table 4).

Discussion

The data of all parameters for each group were tested for closeness of fit to a Gaussian distribution. The results indicate showed data of nearly all parameters for all groups were distributed in a Gaussian manner. Since the samples were random and the goats of one breed, all conditions for parametric tests were satisfied (Reed *et al.*, 1971; Wu *et al.*, 1975; Siegel and Castellan, 1988) and Wilcoxon Mann Whitney test was used for non Gaussian distributions. It was possible to combine goats from all

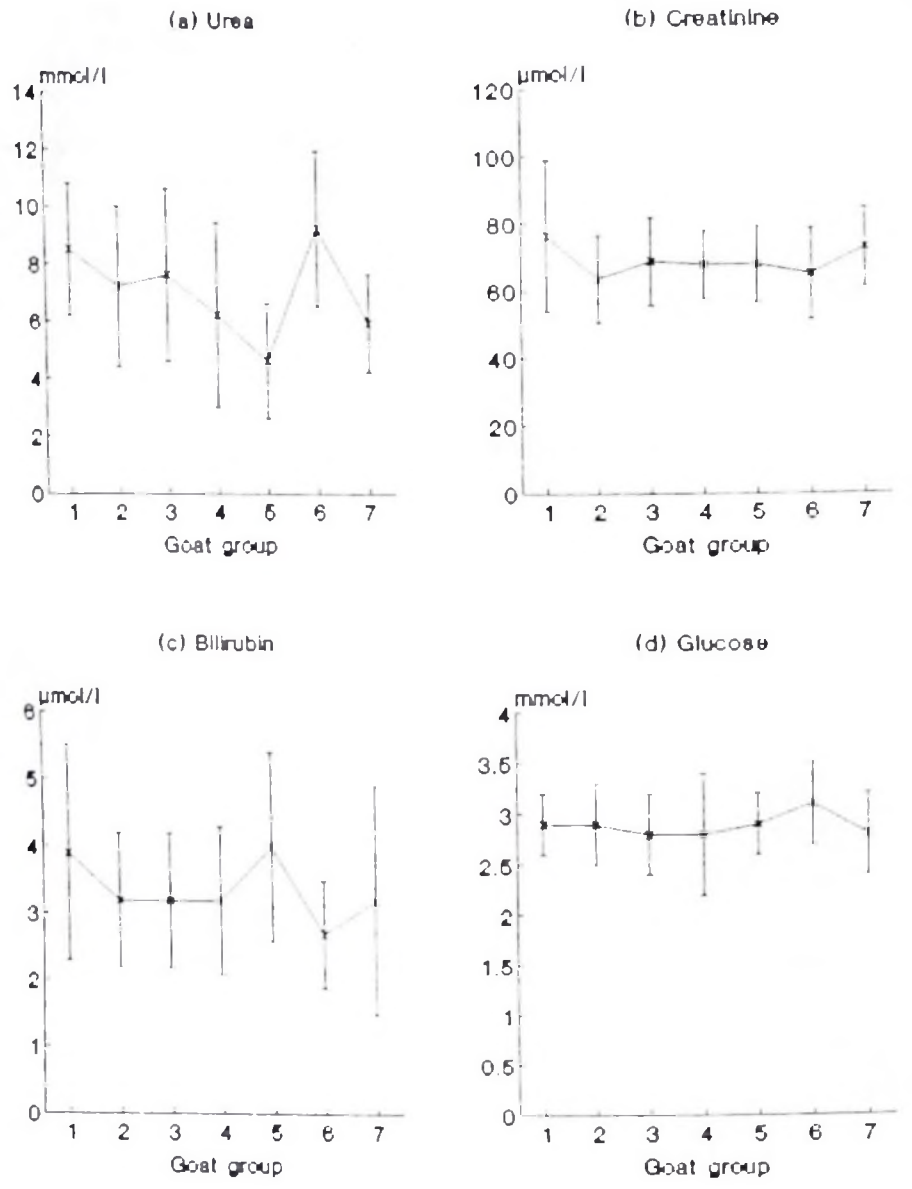

Figure 1: The mean urea (a), Creatinine (b), Bilirubin (c) and Glucose (d) plasma levels with one standard deviation above and below (vertical lines with bars) in young non pregnant (group 1), young pregnant (group 2), advanced non lactating pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) nonpregnant lactating and non lactating nonpregnant adult (group 7) goats from all herds combined.

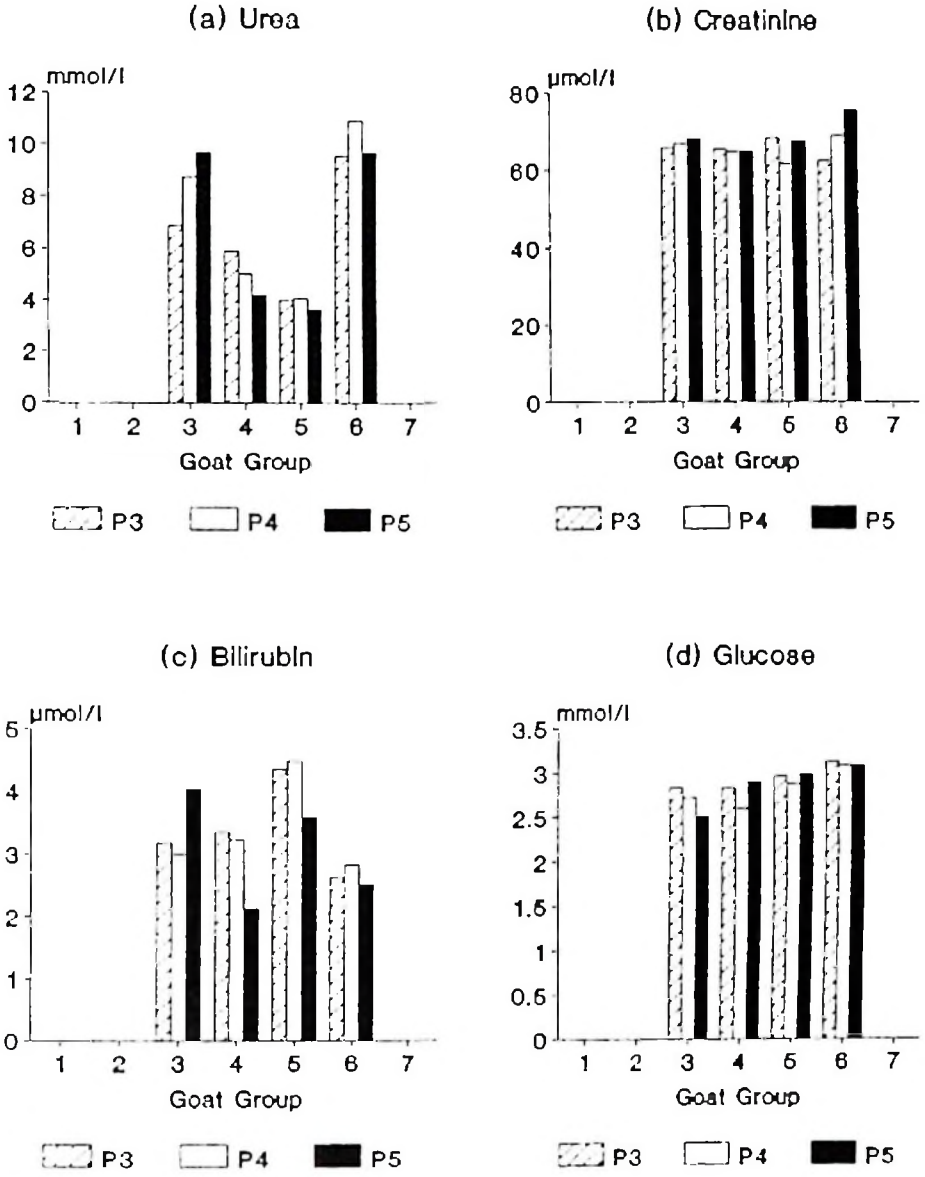

Figure 2: The mean urea (a), creatinine (b), bilirubin (c) and glucose (d) plasma concentration in herd A goats of parity 3 (P3), 4 (P4) and 5 (P5) and the same physiological state group as in figure 1.

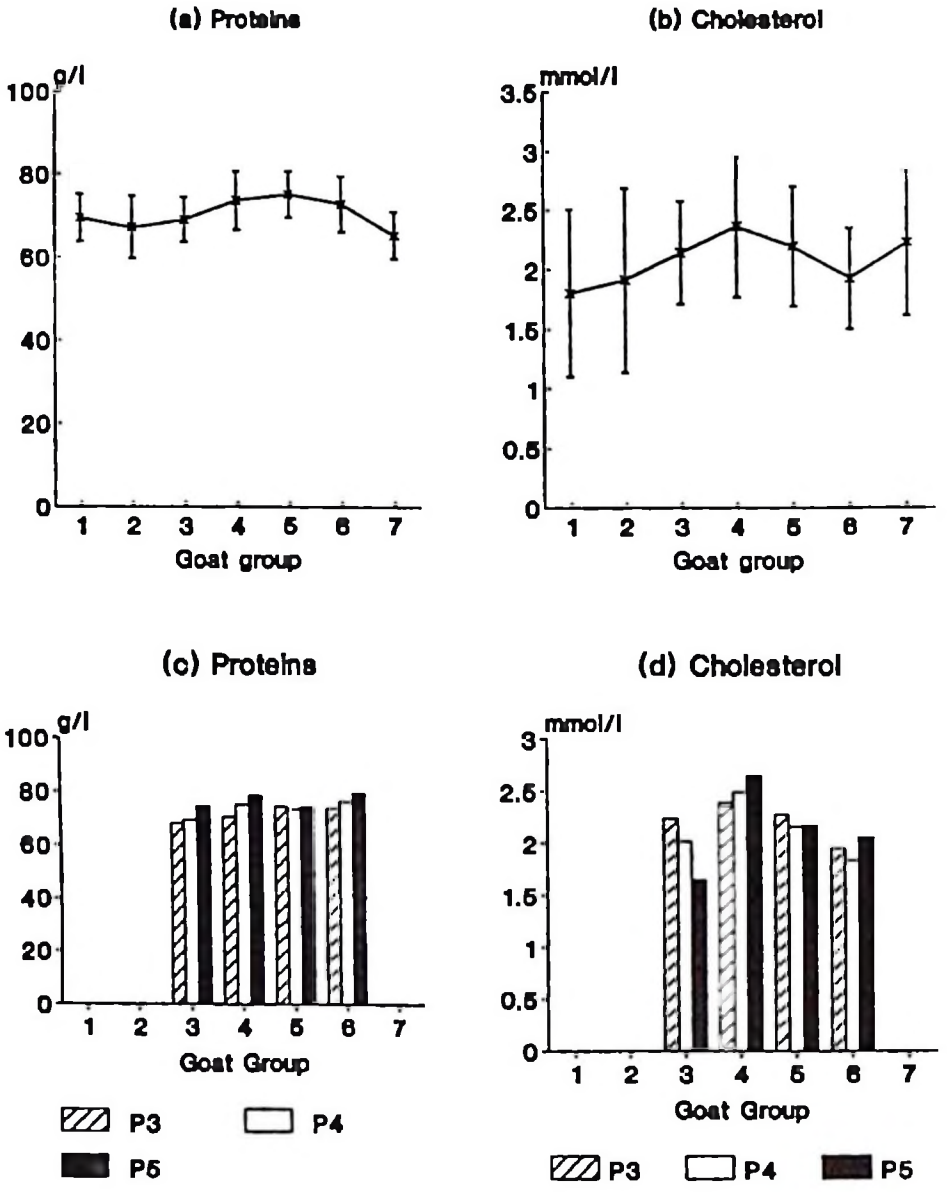

Figure 3a-b: The mean total serum proteins (a) and plasma cholesterol (b) levels with one standard deviation above and below (vertical lines with bars) in young non pregnant (group 1), young pregnant (group 2), advanced non lactating pregnant adult (group 3), early (group 4), mid (group 5) and late (group 6) nonpregnant lactating and non lactating nonpregnant adult (group 7) goats from all herds combined. **Figure 3c-d:** The mean total serum proteins (c) and plasma cholesterol (d) levels in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) and the same physiological state groups as in figure 3a and b.

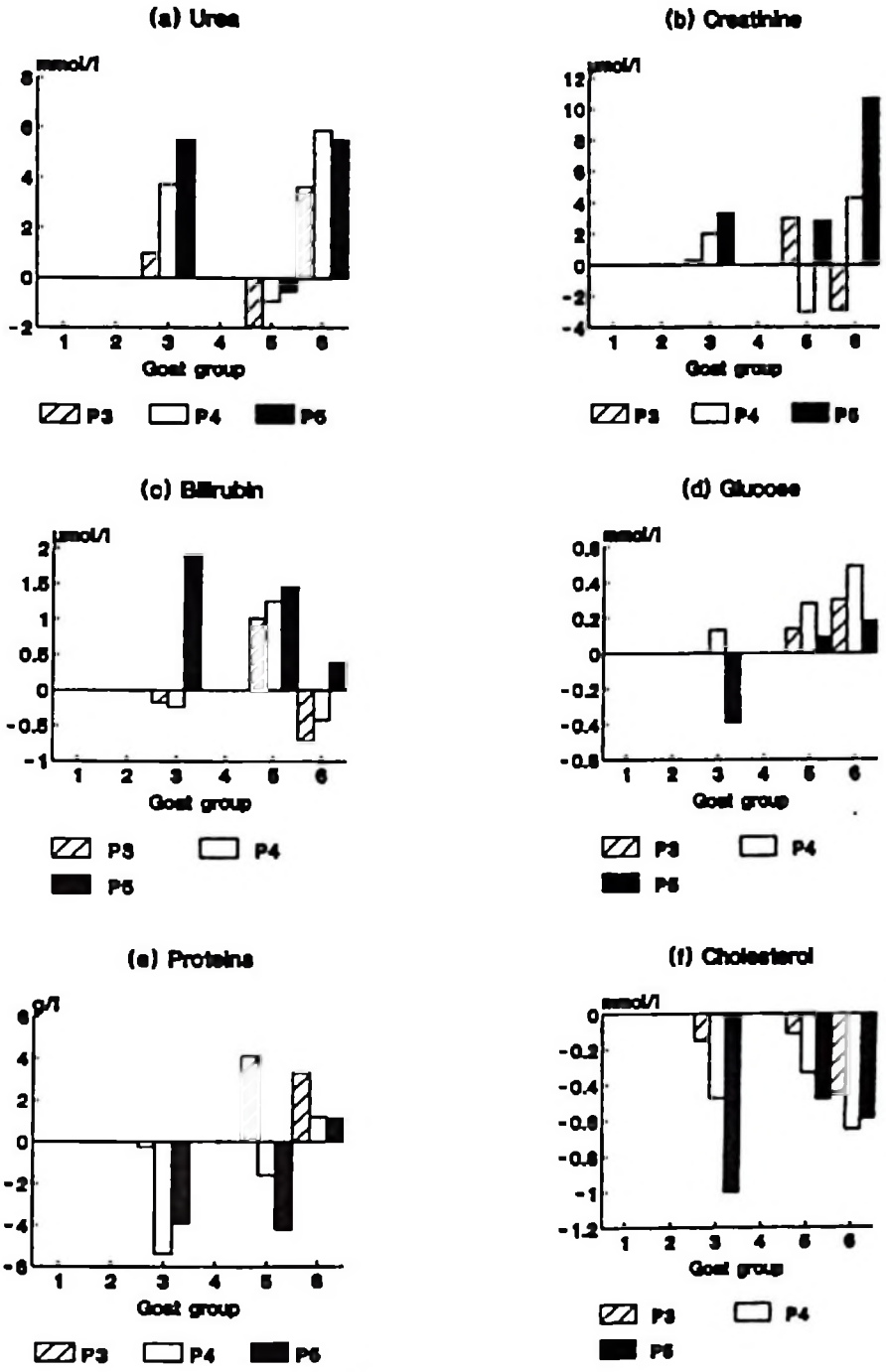

Figure 4: The differences ($\bar{x}_4$ - $\bar{x}_i$) and the direction of the changes in mean values between group 4 goats (reference group) close to parturition and those of other groups in herd A goats of parity 3 (P3), 4 (P4) and 5 and above (P5) for urea (a), creatinine (b), bilirubin (c) glucose (d) total serum proteins (e) and plasma cholesterol (f) levels. The physiological state groups are the same as for other figures.

Table 1: The median (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) values of biochemical parameters in pregnant, lactating and dry goats in all herds.

	Urea mmo/l		Creatinine µmol/l		Bilirubin µmol/l		Glucose mmol/l		Proteins g/l		Cholesterol mmol/l	
	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$
Group (n)												
1 (13)	8.9	8.5 ± 2.3	68.0	76.4 ± 22.4	4.0	3.9 ± 1.6	2.8	2.9 ± 0.3	68.0	69.4 ± 5.7	1.90	1.80 ± 0.70
2 (22)	7.6	7.2 ± 2.8^{bd}	64.0	63.5 ± 13.1^{a}	3.0	3.2 ± 1.0^{a}	3.0	2.9 ± 0.4	69.0	67.1 ± 7.5^{ac}	1.74	1.91 ± 0.77^{ade}
3 (76)	7.0	7.6 ± 3.0^{acde}	67.0	68.8 ± 13.1	3.1	3.2 ± 1.0^{a}	2.8	2.8 ± 0.4^{b}	69.0	69.0 ± 5.4^{b}	2.12	2.14 ± 0.43^{a}
4 (40)	5.8	6.2 ± 3.2^{ab}	65.0	67.8 ± 10.1	3.2	3.2 ± 1.1^{at}	2.8	2.8 ± 0.6^{a}	73.0	73.6 ± 7.1^{bcd}	2.31	2.36 ± 0.59^{abc}
5 (73)	4.2	4.6 ± 2.0^{abc}	68.0	67.8 ± 11.2	3.8	4.0 ± 1.4^{abcde}	3.0	2.9 ± 0.3	76.0	75.1 ± 5.5^{abcd}	2.16	2.19 ± 0.50^{bd}
6 (61)	9.7	9.2 ± 2.7^{abcde}	65.0	64.9 ± 13.6	2.7	2.7 ± 0.8^{acf}	3.1	3.1 ± 0.4^{ab}	73.0	72.8 ± 6.7^{acd}	1.86	1.92 ± 0.42^{bf}
7 (11)	6.2	5.9 ± 1.7^{d}	73.0	72.8 ± 11.7^{a}	2.6	3.2 ± 1.7^{b}	2.8	2.8 ± 0.4	65.0	65.3 ± 5.6^{d}	2.20	2.22 ± 0.61^{af}

n= number of goats. Similar superscripts= significantly different group means in columns ($p<0.05$). Groups; 1= nonpregnant 8 - 12 months old, 2= 1 - 2 years old, early pregnancy, 3= above 2 years of age, late pregnancy, 4= above 2 years of age nonpregnant, 14 - 20 days in lactation, 5= above 2 years of age nonpregnant, 60 days in lactation, 6= above 2 years of age, More than 90 days in lactation, 7= above 2 years of age, nonpregnant, nonlactating.

Table 2: The 5th (P_5) to 95th (P_{95}) percentile intervals of biochemical parameters in dry, pregnant and lactating goats from all farms combined.

Group	n	Urea mmol/l P_5 - P_{95}	Creatinine µmol/l P_5 - P_{95}	Bilirubin µmol/l P_5 - P_{95}	Glucose mmol/l P_5 - P_{95}	Proteins g/l P_5 - P_{95}	Cholesterol mmol/l P_5 - P_{95}
1	13	2.5 - 11.9	54.0-140.0^{W}	0.7 - 7.5	2.3 - 3.4	61.0-79.0	1.1 - 3.2
2	22	2.0 - 11.7	42.0-78.0	1.9 - 5.0	2.3 - 3.3^{W}	55.0-77.0	0.9 - 3.0
3	76	3.3 - 14.0^{W}	51.0-91.0^{W}	2.0 - 5.1^{W}	2.3 - 3.4^{W}	60.0-78.0	1.5 - 3.1
4	40	2.5 - 12.8	55.0-88.5	1.6 - 5.4	2.1 - 3.4^{W}	62.0-86.5	1.4 - 3.5
5	73	2.3 - 8.8^{W}	48.0-88.0	2.3 - 6.4^{W}	2.4 - 3.4	65.0-83.0	1.5 - 3.2
6	61	3.8 - 12.8^{W}	41.0-91.0	1.7 - 3.9	2.4 - 3.5	59.0-81.0	1.2 - 2.7
7	11	3.3 - 9.0	53.0-95.0	1.6 - 7.4	2.2 - 3.4	58.0-74.0	1.3 - 3.1

W= Non Gaussian distributions ($p<0.05$).

Table 3: Mean ± s of biochemical parameters in pregnant, lactating and dry goats of different parities in herd A.

Group	P[1] (n)	Urea mmol/l	Creatinine µmol/l	Bilirubin µmol/l	Glucose mmol/l	Proteins g/l	Cholesterol mmol/l
1	≤1 (0)	-	-	-	-	-	-
2	2 (0)	-	-	-	-	-	-
3	3 (38)	6.88±1.95[a]	65.8±8.37	3.18±0.82	2.83±0.34	68.0±4.43	2.24±0.44[ab]
	4 (25)	8.74±3.40[a]	66.9±10.0	3.00±0.80	2.72±0.27	69.5±4.46	2.02±0.36[a]
	>5 (3)	9.67±1.53	68.3±3.21	4.03±1.69	2.50±0.19	74.3±2.52	1.65±0.09[b]
4	3 (16)	5.91±1.99	65.5±7.91	3.35±1.21[a]	2.82±0.34	70.3±5.9[a]	2.39±0.65
	4 (8)	5.02±2.57	64.9±6.83	3.24±0.87	2.59±0.49	74.9±9.19	2.49±0.66
	>5 (5)	4.17±1.68	65.0±6.12	2.12±0.56[a]	2.89±0.33	78.2±6.46[a]	2.65±0.40
5	3 (34)	3.98±1.36	68.5±9.49[a]	4.36±1.53	2.96±0.28	74.4±5.41	2.28±0.54
	4 (14)	4.07±1.98	61.9±10.2[a]	4.49±1.29	2.87±0.33	73.3±6.17	2.16±0.47
	>5 (9)	3.62±1.27	67.8±13.3	3.58±0.49	2.98±0.24	74.0±4.64	2.17±0.47
6	3 (27)	9.55±1.39[a]	62.6±6.15[ab]	2.63±0.70	3.12±0.32	73.6±5.17	1.95±0.40
	4 (18)	10.9±2.13[a]	69.2±11.2[a]	2.83±0.64	3.08±0.42	76.1±4.32	1.84±0.35
	>5 (3)	9.67±1.95	75.7±8.74[b]	2.50±0.36	3.07±0.31	79.3±4.04	2.06±0.54
7	2 (0)	-	-	-	-	-	
	>5 (0)	-	-	-	-	-	-

[1]P = Parity and is proportional to age, (n) = number of goats. Same superscript = significantly different parity means within the groups ($p<0.05$).

Table 4: The mean ± standard deviation of biochemical parameters in pregnant, lactating and dry goats (group 1-7) from different herds (A-E).

Group	H (n)	Urea mmol/l	Creatinine µmol/l	Bilirubin µmol/l	Glucose mmol/l	Proteins g/l	Cholesterol mmol/l
1	D (3)	9.60±1.20	90.7±22.8	2.30±1.50	2.70±1.50	64.3±4.20	1.50±0.60
	E (10)	8.10±2.40	72.9±12.9	4.50±1.30	2.90±0.30	70.8±5.00	2.10±0.80
2	C (2)	8.70±0.23	64.5±2.12	2.85±1.34	3.29±0.01	69.0±11.3	2.78±0.35
	D (5)	6.50±1.93	71.2±11.1	2.76±1.00	2.89±0.30	64.4±3.78	1.69±0.50
	E (15)	7.31±3.24	60.9±13.9	3.44±0.96	2.89±0.45	67.8±8.23	1.87±0.83
3	A (66)	7.71±2.74^{b}	66.4±8.81^{a}	3.16±0.86^{a}	2.77±0.31^{a}	68.8±4.55^{a}	2.13±0.42
	C (5)	4.62±1.42^{ab}	74.6±25.1^{b}	3.68±0.88	3.68±0.58^{ab}	63.8±5.93^{ab}	2.16±0.28
	E (5)	9.05±5.73^{a}	95.6±17.2^{ab}	4.18±1.88^{a}	2.94±0.34^{b}	76.0±9.38^{ab}	2.22±0.66
4	A (30)	5.38±2.15^{bc}	65.2±7.14^{a}	3.11±1.11^{a}	2.77±0.38^{a}	72.8±7.41^{a}	2.46±0.61^{a}
	B (4)	3.88±1.49^{bd}	75.0±16.9	4.65±0.34^{ab}	3.44±1.50^{a}	74.5±0.58	1.73±0.44^{a}
	C (3)	11.4±2.54^{cd}	74.3±8.96	2.23±0.87^{b}	2.84±0.33	70.7±4.04^{b}	2.41±0.40
	E (3)	12.5±0.68^{ab}	78.0±18.2^{a}	3.40±0.75	2.99±0.03	83.0±4.58^{ab}	2.23±0.14
5	A (57)	3.94±1.50^{ab}	66.8±10.5^{a}	4.26±1.37^{a}	2.94±0.29^{ab}	74.1±5.42^{abc}	2.23±0.50
	C (5)	7.69±1.96^{cd}	75.8±9.44^{bd}	4.22±1.21^{bc}	3.05±0.32^{c}	76.2±3.27^{ad}	2.19±0.45
	D (3)	6.52±3.72^{bc}	57.7±9.07^{abc}	3.63±1.40^{ac}	3.14±0.13^{a}	76.3±3.46^{bc}	1.92±0.50
	E (8)	6.67±1.21^{ad}	74.0±14.0^{cd}	2.61±0.52^{b}	2.78±0.26^{bc}	81.1±3.56^{cd}	1.95±0.46
6	A (48)	10.0±1.81	65.9±9.31	2.69±0.66	3.10±0.35	74.9±5.00	1.91±0.38
	C (5)	4.84±1.62	70.8±13.5	3.64±1.11	3.16±0.17	70.4±3.97	2.02±0.53
	D (3)	10.3±1.12	87.7±12.0	2.40±1.13	2.66±0.53	66.0±2.64	2.16±0.79
	E (5)	5.01±3.18	36.4±5.46	2.48±0.72	2.65±0.03	58.6±2.61	1.75±0.54
7	C (5)	5.27±1.17	78.2±11.0	4.20±2.05^{a}	3.15±0.28	62.0±3.87^{b}	2.17±0.74
	D (3)	7.47±1.37	68.7±13.6	1.63±0.06^{a}	2.49±0.28^{a}	63.3±3.05^{ab}	2.51±0.32
	E (3)	5.59±2.40	68.0±11.1	3.30±0.70	2.69±0.42^{a}	72.7±1.53^{a}	2.02±0.71

n = number of goats. Similar superscripts = significantly different herd means in a column within groups ($P<0.05$).

herds for statistical tests because the samples in some were small and the same trend of metabolic profile was observed in all farms (table 1-2). Tests were also made for differences between herds for all parameters (table 4).

Urea levels increased in pregnancy, decreased in early and mid lactations and increased in late lactation (fig. 1a, 2a), a profile resembling that of cows (Rowlands *et al.*, 1975) and mares (Felbinger, 1987). Biagi *et al.* (1988a), however, observed only slight changes in urea concentration during pregnancy and lactation in Saanen goats. The differences may be because of the variable levels, proportional to dietary protein amount (Manston *et al.*, 1975; Blackwell and Libby, 1982; Pelletier *et al.*, 1985). Urea

levels are low on indoor winter rations but high under grazing conditions (Payne *et al* 1970), hence the differences among individuals and between herds.

Plasma creatinine concentration did not differ much between the pregnant and lactating landrace goats, it was only slightly higher in young and adult nonpregnant and those in late lactation (fig. 1b, 2b), in support of other observations (Cissik *et al.*, 1987). There were decreases during lactation in Saanen goats (Biagi *et al.*, 1988a). However, creatinine levels are constant in healthy goats, depending on the concentration of creatine (Finco, 1989), in turn depending on the diet, synthesis rate and on muscle mass.

Bilirubin was higher in young non pregnant than in adult nonpregnant dry goats indicating an age influence (fig. 1c, 2c) because the levels were identical in young and adult pregnant, and early lactating goats. There was a rise in bilirubin concentration during mid lactation, especially in 3 - 4 years old goats, a probable association with feeds (Bas *et al.*, 1980; Felbinger, 1987).

Plasma glucose was uniform for young and adult nonpregnant goats, indicating the little age influence in animals of above 8 months old (fig. 1d, 2d). Glucose levels decreased in the last stages of pregnancy, but increased following parturition and with advancing lactation. There were similar observations in Barbari (Vihan and Rai, 1987) and Baladi goats (Hassan *et al.*,1986) and mares (Felbinger, 1987). In cattle Payne et al. (1970) found that dry cows were hypoglycemic while glucose levels were high for low and low for high milk producers but Rowlands *et al.* (1975) observed lowest concentrations in the first month of lactation. Bostedt (1971) and Vihan and Rai (1987) found higher plasma glucose in pregnant than in lactating ewes and sows, but Bickhardt and Konig (1985) observed the opposite. In the present landrace goats there was clear fall of glucose level in advanced pregnancy and a rise during lactation. Since the thyroid hormone is elevated during lactation (Biagi *et al.*, 1988b), this might be an adjustment to mobilize glucose for lactogenesis. However, there are within and between days and weeks changes in glucose levels, low in the morning and high in the day (Bas *et al.*, 1980).

Total serum proteins were also not influenced by age in goats above 8 months old (fig. 3a). The concentrations increased in lactating landrace (fig. 3a, c) as found in Baladi (Hassan *et al.*, 1986), Barbari (Vihan and Rai, 1987) and Saanen goats (Biagi *et al.*, 1988a). Serum proteins were low in advanced pregnancy in the present goats, increased after parturition and with advancing lactation, consistent with findings in other goats (Vihan and Rai, 1987) and horses (Felbinger, 1987). The increase in total serum proteins is proportional to the lactation stage in Baladi goats (Hassan *et al.*, 1986) where albumin was lower for high than for low milk producers and vice versa for globulins. Serum protein levels in cows and goats are proportional to protein levels in the diet (Manston *et al.*, 1975; Blackwell and Libby, 1982) and hypoalbuminemia

occurs in low dietary intake (Payne *et al.*, 1970). Albumin decreases near calving to lowest levels in the first month of lactation while globulins are highest during that period (Rowlands *et al.*, 1975). Whether albumin or globulins dominate it appears that the increase in serum proteins during lactation results from increased synthesis and /or mobilization for secretion in the milk.

In landrace goats cholesterol levels were low in advanced pregnancy but increased after parturition to highest values in early lactation (fig. 3b, d), as found in sheep (Vihan and Rai, 1987) and mares (Felbinger, 1987). The increase in lactation is probably a demand for secretion in the milk. However, Rawal *et al.* (1987) and Biagi *et al.* (1988) observed increased cholesterol levels during pregnancy in sheep and Saanen goats respectively.

The differences in the level of biochemical parameters between the sources are enormous and result from nutrition, breed, environment, seasons, age, stage of pregnancy/lactation, milk yield differences in the goats, time of sampling and analytical methods (Bas *et al.*, 1980; Berglund and Oltner, 1983; Vrzgula *et al.*, 1985; Timet *et al.*, 1985; Hassan *et al.*, 1986; Mbassa and Poulsen, 1991). These, together with herd variations are the most important in cows (Payne *et al.* (1970), and appears so in goats too (table 4).

The influence of pregnancy and lactation on biochemical parameters was in all goats, but the magnitudes increased with increasing parity. Urea changes were inversely related to parity during pregnancy, but directly in lactation (fig. 2a). This may be due to a greater urea recycling in goats of higher parity than in young animals. The opposite was observed for glucose (fig. 2d) and cholesterol concentration (fig. 3d). Creatinine, glucose and serum protein concentration increased with respect to the number of lactations (fig. 2b, d, 3c-d). The positive direct relationship between total serum proteins and parity in the present lactating landrace goats supports similar observations in Saanen goats. Plasma glucose increases are also directly related to the number of lactation (Biagi *et al.*, 1988a; present studies).

The influence of environment on plasma and serum biochemical parameters was described by Payne *et al.* (1970), Hewett (1974) and Rowlands *et al.* (1975) in cattle and is a reflection of nutritional type and adequacy in different herds (Manston *et al.*, 1975). The present investigation have revealed them in Danish landrace dairy goats, therefore metabolic profile tests (Biagi *et al.*, 1988a) are specific to herds because nutrition varies between herds. The interactive influence of herd, age, parity, lactation and pregnancy increases the differences.

In conclusion, significant sustained alterations in plasma urea, creatinine, bilirubin, glucose, cholesterol and total serum protein levels occur in pregnancy and lactation, the extent of which depends on age and parity of the animal and on the environment.

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CHAPTER 13

CLINICAL REFERENCE RANGES FOR HEMATOLOGICAL VALUES

Summary

Hematological analysis was performed in Danish landrace goats of different ages from birth to over five years of age. The purpose was to determine the reference ranges for hematological parameters in this breed. Hemoglobin concentration, packed cell volume, number of erythrocytes and leukocytes were lower in neonates and juveniles than in goats of other ages. The levels of these parameters increased with age to maximum values at 8 - 12 months of age. The mean corpuscular volume and mean corpuscular hemoglobin were highest in neonatal kids and decreased with age concomitantly with increases in erythrocyte counts, hemoglobin concentration and hematocrit. The nonparametric reference range from 5th to 95th percentile intervals closely agreed and were within the parametric ranges of mean ± 2 standard deviation ($\bar{x}$ + 2s) in most parameters except for basophils, monocytes, eosinophils and band neutrophil counts. There were significant age differences in hematological parameters except for mean corpuscular hemoglobin concentration. Because of the strong age influence a distinction between kids (<4 months), juveniles (4-6 months) and adult goats is appropriate for correct interpretation of laboratory results. Sex differences were not significant in most parameters, being noted only for total leukocyte counts and mean corpuscular hemoglobin concentration at some ages only.

Introduction

There has been hematological studies in many breeds of goats (Masoni *et al.*, 1985; Wojcik *et al.*, 1986; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988). It is subsequently established that hematological values are influenced by breed (Nettleton and Beckett, 1976; Oduye, 1976), age (Facello *et al.*, 1983), season (Pospisil *et al.*, 1987), feeds, lactation and pregnancy (Mohy *et al.*, 1985; Hassan *et al.*, 1986; Oyewale and Olowookorun, 1986; Vihan and Rai, 1987; Biagi *et al.*, 1988; Fortagne and Schafer, 1989). Hematologic information for use in disease diagnosis therefore requires determination of age, and possibly breed specific reference values. Hematological reference ranges provide better comparisons of the clinical cases and facilitate easy interpretation of laboratory results. There is a paucity of information on the hematology of Danish landrace goats and the breed reference ranges are not readily

available, therefore it was found necessary to determine these values. Data for many blood parameters in healthy animals are frequently not normally distributed (Wu *et al.*, 1975). In data that are not normally distributed parametric statistics of mean ± 1 or 2 standard deviations do not properly describe the biological behavior of the system in question. In these cases the data must be converted to logarithmic scale to fit the Gaussian distribution or the nonparametric statistics (distribution free) have to be used (Solberg, 1983). The purpose of the present investigation was to establish the reference ranges for hematological values in Danish landrace goats by using both the nonparametric 5^{th} to 95^{th} interval and the parametric range of two standard deviations above and below the means which in a Gaussian distribution include about 95 % of all the observations.

Materials and Methods

The goats of this study were apparently clinically healthy Danish landrace breeds of goats of different ages; 27 (8 female, 19 male) 0 - 7 days, 28 (16 female, 12 male) 7 - 30 days, 25 (10 female, 15 male) 1 - 2 months, 74 (46 female, 28 male) 2 - 4 months, 63 (38 female, 25 male) 4 - 8 months, 22 (15 female 7 male) 8 - 12 months, 25 (22 female, 3 male) 1 - 2 years, 144 (138 female, 6 male) 2 - 3 years, 83 (74 female 9 male) 3 - 5 years, and 47 above 5 years (all females) and categorized in age groups 1 to 10 respectively referred to in the text. They were kept indoors throughout the year with only limited outdoor movements. In one herd only, goats were left outside for the entire summer and autumn. Nutrition was composed of green grass pellets and about 0.5 kg of barley or oat grains.

Blood samples were collected from the external jugular vein in vacuum tubes containing 0.12 ml, 0.34 M tripotassium ethylene diamine tetraacetate (K_3EDTA) (Becton-Dickinson vacutainers) between 8 and 10 am.

The numbers of red blood cells (RBC) were determined within 3 hours of blood sampling using model ZF Coulter counter with an aperture diameter of 100 μm and adjusted for compensation of coincident passages. Threshold setting was 6, attenuation 500 and the background counts kept at 200 maximum. Hemoglobin concentration (Hb) and total white blood cells (WBC) were determined in S560 Coulter counter with aperture diameter and length of 100 and 75 μm respectively. The packed cell volume (hematocrit, PCV) were determined in microhematocrit capillary tubes centrifuged at 12,000 G in a microhematocrit centrifuge for 5 minutes. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from standard formulae. Thin blood films stained with Leishman stain were used for differential leukocyte counts.

Parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles,

median) values were computed by using the univariate procedure of a statistical analysis system software (SAS, Cary, USA). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte in each age to the Gaussian distribution. The means of analytes were tested for statistically significant differences between ages with the general linear models procedure. Wilcoxon scores sums rank tests were performed for all parameters using average scores for ties. The Kruskal-Wallis test (Chi square approximation) and associated probability for testing differences were calculated. Tests between female and male goats indicated lack of consistent differences in mean values. The results for both sexes were therefore pooled and statistically analyzed together.

Results

The minimum to maximum values for RBC, PCV, Hb, MCV, MCH and MCHC are shown in table 1, while those for WBC, lymphocyte, segmented neutrophil, basophil, eosinophil and monocyte counts in table 2. The mean and median values of RBC, PCV, MCV, MCH, MCHC, Hb, WBC, lymphocyte and mature neutrophil counts in each age were close to each other (table 3, 4). The coefficient of skewness and the degree of kurtosis were small in most parameters and the Shapiro Wilk statistic (W) indicated that data for most parameters were distributed in a Gaussian manner except a few (W in tables 3, 4), and all data for basophil, monocyte, band neutrophil and eosinophil counts. The 5^{th} to 95^{th} percentile interval included all mean ($\bar{x}$) ± standard deviation (s), and in most parameters this was within the range of $\bar{x} \pm 2s$ in goats of all ages (table 5, 6).

RBC counts, Hb, PCV and WBC were lowest in kids, while the MCV and MCH were highest (fig. 1, 2). RBC counts, PCV and Hb values were at the highest peak in 8 - 12 months goats (fig. 1a, b, c), then decreased to adult levels. MCHC was not significantly different between goats of different ages (fig. 2b). The WBC counts were low in goats of less than 4 months of age (fig. 2c) and increased within 4 months of age to adult values, whereafter there appeared to be little fluctuations (fig. 2c). Most of the leukocytes in young goats up to 1 year of age were lymphocytes (table 4). After this age the number of lymphocytes and segmented neutrophils were about the same. There was no obvious trend in the number of basophils, eosinophils and monocyte, but band neutrophils tended to be numerous in young goats (table 2, 4, 7).

Both the parametric (t tests by general linear models procedure, table 8) and nonparametric (Wilcoxon scores sums rank tests shown in table 9) indicated that all the hematological parameters were strongly influenced by age. There were significant

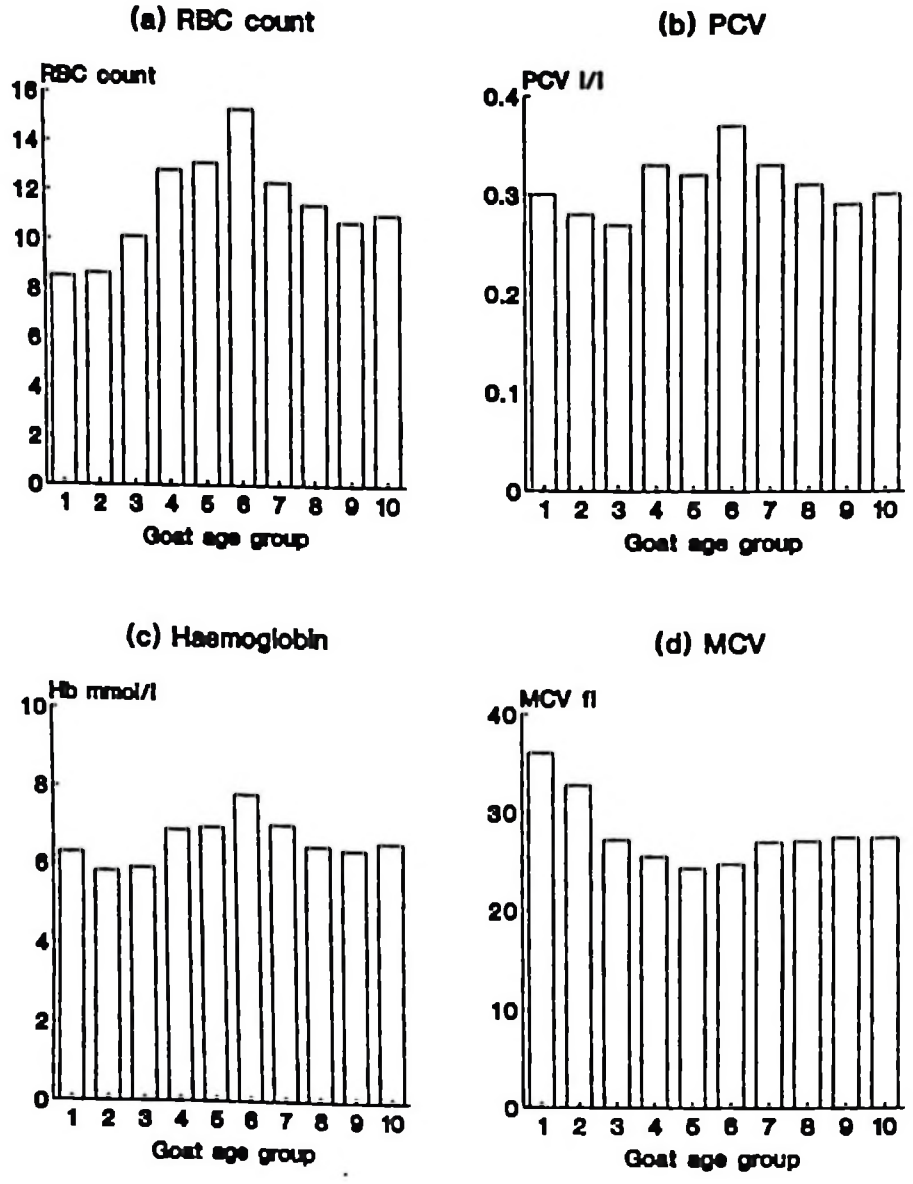

Figure 1: RBC counts ($x10^{12}/l$) (a), packed cell volume (b), hemoglobin concentration (c) and MCV (d) in Danish landrace goats of 0 - 7 days (1), 7 - 30 days (2), 1- 2 months (3), 3 - 4 months (4), 4 - 8 months (5), 8 - 12 months (6), 1 -2 years (7), 2 - 3 years (8), 3 - 5 years (9) and above 5 years of age (10).

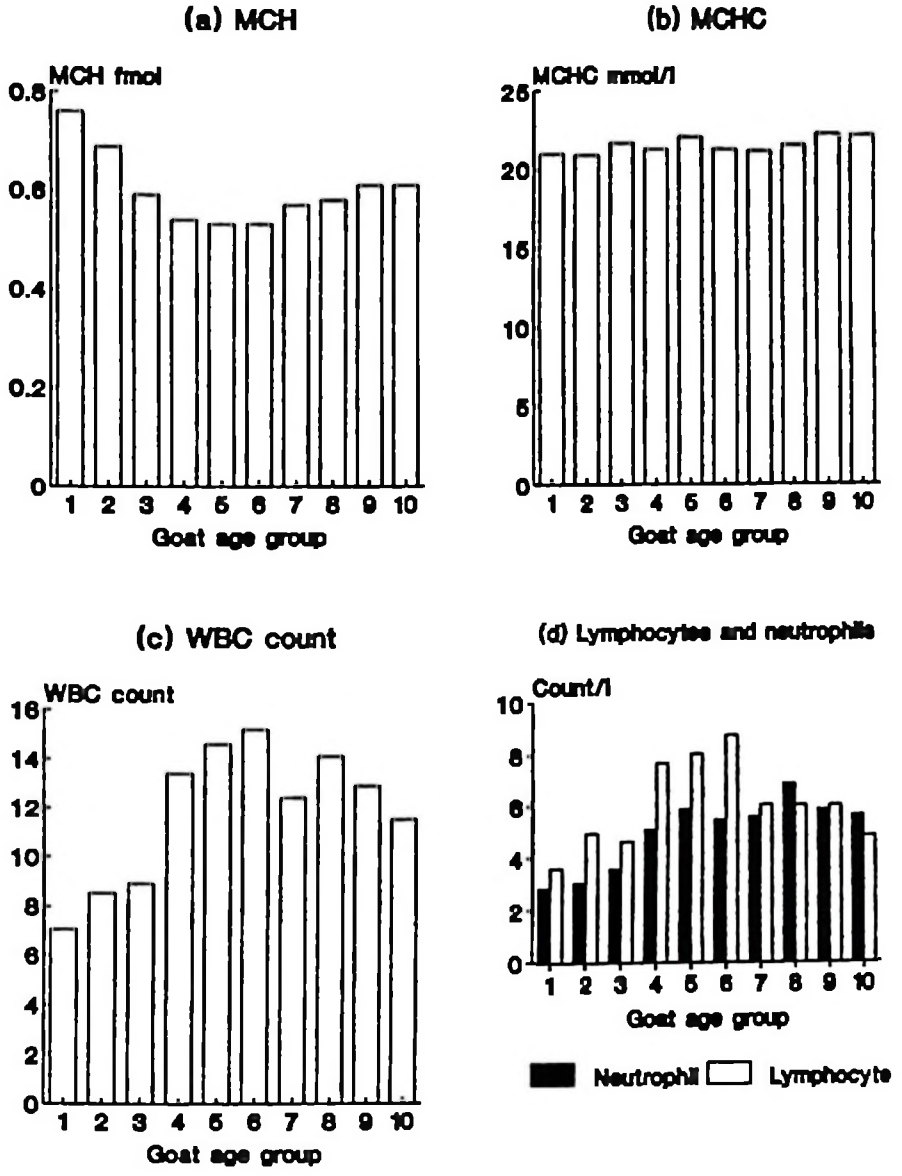

Figure 2: MCH (a), MCHC (b) and total WBC counts (x 10^9/l) (c) and lymphocyte and segmented neutrophil counts in Danish landrace goats of 0 - 7 days (1), 7 - 30 days (2), 1- 2 months (3), 3 - 4 months (4), 4 - 8 months (5), 8 - 12 months (6), 1 -2 years (7), 2 - 3 years (8), 3 - 5 years (9) and above 5 years of age (10).

Table 1: The minimum to maximum values (range) of hematological parameters in landrace goats of different ages.

Age	n	RBC $x10^{12}/l$	PCV l/l	Hb mmol/l	MCV fl	MCH fmol	MCHCmol/l
0-7d	27	5.56 - 11.6	0.20 - 0.39	4.30 - 8.00	25.1 - 47.8	0.50 - 1.00	17.3 - 22.6
7-30d	28	5.74 - 12.7	0.22 - 0.39	4.30 - 8.30	21.1 - 44.2	0.41 - 0.93	18.0 - 23.0
1-2m	25	8.38 - 12.1	0.18 - 0.33	4.70 - 6.80	19.6 - 33.8	0.46 - 0.70	20.0 - 33.3
2-4m	74	8.94 - 16.8	0.23 - 0.54	5.00 - 9.60	19.6 - 36.6	0.43 - 0.68	15.4 - 25.2
4-8m	63	9.74 - 17.4	0.20 - 0.49	5.10 - 9.80	15.7 - 34.9	0.41 - 0.65	13.4 - 38.2
8-12m	22	10.2 - 20.6	0.30 - 0.45	6.50 - 9.30	16.5 - 33.9	0.38 - 0.72	19.6 - 23.4
1-2y	25	8.89 - 16.5	0.20 - 0.42	4.50 - 8.30	21.6 - 31.7	0.45 - 0.68	18.2 - 23.7
2-3y	144	7.21 - 19.4	0.22 - 0.47	4.00 - 8.80	18.5 - 35.6	0.34 - 0.72	12.3 - 28.2
3-5y	83	5.59 - 19.0	0.20 - 0.39	4.90 - 8.20	16.9 - 44.7	0.37 - 1.00	14.9 - 30.0
>5y	47	8.56 - 20.0	0.22 - 0.40	5.00 - 8.40	17.0 - 39.1	0.36 - 0.80	15.7 - 28.6

n= number of goats, the same in all tables, d= days, m= months, y =years (the same for all tables).

Table 2: The minimum to maximum values (range) of total and differential leukocyte counts in landrace goats of different ages.

Age	Total WBC $x10^9/l$	Lympho cyte $x10^9/l$	S. neutro phil $x10^9/l$	Baso phil %	Eosino phil %	Mono cyte %	B. neut rophil %
0-7d	4.90 - 12.3	1.61 - 7.01	0.61 - 5.15	0 - 5	0 - 6	2 - 12	0 - 11
7-30d	4.60 - 16.9	0.78 - 13.2	1.10 - 11.5	0 - 7	0 - 7	1 - 12	0 - 3
1-2m	4.00 - 15.0	1.97 - 9.13	0.45 - 8.25	0 - 5	0 - 2	1 - 11	0 - 9
2-4m	4.00 - 20.7	3.13 - 14.6	0.20 - 13.3	0 - 3	0 - 6	0 - 11	0 - 3
4-8m	5.60 - 23.6	2.62 - 15.0	1.12 - 18.2	0 - 4	0 - 9	0 - 9	0 - 3
8-12m	8.30 - 21.4	2.33 - 14.1	0.86 - 12.2	0 - 4	0 - 6	1 - 11	0 - 3
1-2y	6.10 - 19.6	2.65 - 9.84	1.31 - 12.8	0 - 3	0 - 6	0 - 6	0 - 4
2-3y	5.70 - 23.6	2.17 - 14.6	1.03 - 13.5	0 - 4	0 - 26	0 - 14	0 - 7
3-5y	5.90 - 20.4	2.81 - 10.97	1.36 - 13.5	0 - 3	0 - 11	0 - 11	0 - 10
>5y	5.20 - 19.1	1.54 - 9.65	1.46 - 12.7	0 - 5	0 - 22	0 - 16	0 - 10

S.= segmented, B= band, d= days, m= months, y= years.

Table 3: The median (Q_2) and mean values ($\bar{x} \pm s$) of hematological reference values in landrace goats of different ages.

	RBC x10^{12}/l		PCV l/l		Hb mmol/l		MCV fl		MCH fmol		MCHC mmol/l	
Age	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q^2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$
0-7d	8.34	8.51±1.45	0.30	0.30±0.04	6.30	6.30±0.97	34.0	36.1±7.03	0.72	0.76±0.16	21.1	21.0±0.93
7-30d	8.51	8.64±1.61	0.28	0.28±0.04	5.65	5.83±1.00	31.8	32.8±4.76	0.68	0.69±0.11	21.0	20.9±1.10
1-2m	10.2	10.1±0.95	0.28	0.27±0.04	5.90	5.92±0.56	27.4	27.2±3.15	0.60	0.59±0.07	21.1	21.7±2.55
2-4m	12.9	12.8±1.48	0.33	0.33±0.06	6.70	6.90±1.03	25.0	25.5±3.60	0.54	0.54±0.06	21.6	21.3±1.60
4-8m	13.2	13.1±1.55	0.31	0.32±0.06	6.80	6.98±1.07	24.4	24.3±3.46	0.53	0.53±0.05	21.9	22.1±2.86^W
8-12m	15.4	15.3±2.61	0.36	0.37±0.04	7.80	7.83±0.68	24.5	24.7±4.59	0.51	0.53±0.10	21.1	21.3±0.97
1-2y	11.9	12.3±2.01	0.35	0.33±0.05	7.30	7.04±1.08	26.6	27.0±2.71	0.56	0.57±0.06	21.1	21.2±1.12
2-3y	11.3	11.4±1.62	0.31	0.31±0.04	6.60	6.50±0.88	27.0	27.1±2.99	0.59	0.58±0.07	21.9	21.6±2.19^W
3-5y	10.4	10.7±1.79	0.29	0.29±0.04	6.30	6.40±0.77	27.8	27.5±4.13	0.61	0.61±0.09	22.2	22.3±1.88^W
>5y	10.5	11.0±2.04	0.30	0.30±0.05	6.60	6.60±0.84	27.6	27.5±3.91	0.62	0.61±0.08	22.2	22.2±1.94

W= Non Gaussian distribution ($p<0.05$), d= days, m= months, y= years,

Table 4: The median (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) values of total leukocyte (WBC) and differential counts in landrace goats of different ages.

	WBC x10/l		LY x10^9/l		SN x10^9/l		Basop	Eosin	Mono	BN
Age	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	Q_2	$\bar{x} \pm s$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
0-7d	6.70	7.07±1.86^W	3.22	3.61±1.50	2.60	2.87±1.30	1.28	0.72	5.55	1.33
7-30d	7.95	8.51±3.18^W	4.36	4.94±2.44	2.70	3.03±1.99	1.15	1.70	4.75	0.90
1-2m	8.40	8.92±3.00	4.40	4.64±1.85	3.54	3.58±1.95	1.12	0.25	6.69	1.75
2-4m	13.4	13.4±3.34	7.33	7.68±2.80^W	4.94	5.10±2.46	0.59	0.64	3.30	0.81
4-8m	14.8	14.6±3.71	8.49	8.04±2.82^W	5.68	5.88±2.59^W	0.62	1.08	2.10	0.71
8-12m	15.2	15.2±4.11	9.46	8.75±3.05	5.44	5.46±2.88	0.60	1.43	3.70	1.13
1-2y	13.0	12.4±3.47	5.80	6.07±1.79	5.81	5.59±3.05	0.74	1.96	2.38	0.88
2-3y	13.5	14.1±3.44	5.67	6.02±2.00	6.56	6.89±2.63	0.93	3.44	3.16	1.15
3-5y	13.2	12.9±3.80^W	5.72	6.04±2.06^W	5.31	5.87±2.51	0.78	2.45	3.53	1.39
>5y	11.3	11.5±3.67	4.40	4.83±2.07^W	5.42	5.64±2.71^W	1.00	3.26	3.53	1.15

BN= band neutrophils %, SN= segmented neutrophils, LY= lymphocytes, baso= basophils %, eosin= eosinophils %, mono= monocytes %, d= days, m= months, y= years, W= non Gaussian distributions ($P<0.05$).

Table 5: Comparison between nonparametric ranges [percentile 5 (P_5) to 95 (P_{95} intervals] and parametric ranges ($\bar{x} \pm 2s$) for RBC counts, PCV and Hb in landrace goats of different ages.

	RBC x10^{12}/l				PCV l/l				Hb mmol/l			
Age	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s
0-7d	5.96	11.6	5.61	11.4	0.22	0.38	0.22	0.38	4.4	7.7	4.36	8.24
7-30d	6.11	12.1	5.42	11.9	0.22	0.35	0.21	0.36	4.5	7.5	3.83	7.83
1-2m	8.38	12.1	8.20	12.40	0.23	0.32	0.19	0.35	4.9	6.8	4.80	7.04
2-4m	10.6	15.0	9.84	15.8	0.24	0.43	0.21	0.45	5.4	8.5	4.84	8.96
4-8m	10.7	15.9	10.0	16.2	0.23	0.41	0.20	0.44	5.3	8.8	4.84	9.12
8-12m	11.2	19.1	10.1	20.5	0.32	0.45	0.29	0.45	7.0	9.1	6.47	9.19
1-2y	9.10	15.8	8.28	16.3	0.22	0.41	0.23	0.43	4.6	8.3	4.88	9.20
2-3y	9.41	14.0	8.16	14.6	0.24	0.37	0.23	0.39	4.9	8.0	4.74	8.26
3-5y	8.93	13.4	7.12	14.3	0.23	0.36	0.21	0.37	5.4	7.8	4.86	7.94
>5y	9.07	15.1	6.92	15.1	0.23	0.38	0.20	0.40	5.3	8.0	4.92	8.28

d= days, m= months, y=years.

Table 6: Comparison between nonparametric ranges [percentile 5 (P_5) to 95 (P_{95}) intervals] and parametric ranges ($\bar{x} \pm 2s$) for MCV, MCH and MCHC in landrace goats of different ages.

	MCV fl				MCH fmol				MCHC mmol/l			
Age	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s
0-7 days	25.2	47.0	22.0	50.2	0.54	1.00	0.44	1.08	19.7	22.4	19.1	22.9
7-30 days	25.5	40.1	23.3	42.3	0.46	0.92	0.47	0.91	19.3	22.3	18.7	23.1
1-2 months	23.1	31.8	20.9	33.5	0.48	0.67	0.47	0.71	20.4	22.8	16.6	26.8
2-4 months	20.6	30.4	18.3	32.7	0.46	0.65	0.42	0.66	17.6	23.3	18.1	24.5
4-8 months	20.2	29.4	17.4	31.2	0.44	0.62	0.43	0.63	20.0	24.6	16.4	27.8
8-12 months	17.8	30.4	15.5	33.9	0.38	0.71	0.33	0.73	20.2	23.4	19.4	23.2
1-2 years	22.9	31.1	21.6	32.4	0.49	0.66	0.45	0.69	19.8	22.7	17.6	24.8
2-3 years	22.1	31.9	21.1	33.1	0.42	0.69	0.44	0.72	18.6	24.4	17.2	26.0
3-4 years	20.3	32.3	19.2	35.8	0.49	0.69	0.43	0.79	20.0	24.8	18.5	26.1
>5 years	21.7	34.6	19.7	35.3	0.48	0.77	0.45	0.77	19.1	25.2	18.3	26.1

Table 7: Comparison between nonparametric ranges [percentile 5 (P_5) to 95 (P_{95}) intervals] and parametric ranges ($\bar{x} \pm 2s$) for total leukocytes and nonparametric ranges for lymphocyte and segmented neutrophil counts in landrace goats of different ages.

	Lymphocytes x10⁹/l		Segmented neutrophil x10⁹/l		Total Leukocytes x10⁹/l			
Age	P_5	P_{95}	P_5	P_{95}	P_5	P_{95}	$\bar{x}$-2s	$\bar{x}$+2s
0-7 days	2.08	7.01	0.61	4.92	5.20	11.7	3.35	11.8
7-30 days	1.80	11.4	1.12	4.52	4.70	15.7	2.15	14.9
1-2 months	1.97	9.13	0.45	4.28	5.30	14.7	2.92	14.9
2-4 months	4.09	13.7	1.65	9.21	8.70	19.1	6.72	20.1
4-8 months	4.01	12.6	2.57	8.43	9.40	20.4	7.18	22.0
8-12 months	2.33	14.1	0.86	12.2	9.60	21.4	6.98	23.4
1-2 years	3.21	9.58	1.39	9.89	6.70	17.1	5.46	19.3
2-3 years	3.39	9.90	3.06	11.7	9.30	20.4	7.22	21.0
3-5 years	3.12	9.45	2.54	10.6	6.60	18.7	5.30	20.5
>5 years	2.14	9.00	1.96	11.5	5.60	17.5	4.16	18.8

Table 8: Statistical tests for differences between means among the different age groups (* $p<0.001$ to $p<0.05$).

	Age group numbers where there were significantly differences						
Group	RBC	PCV	Hb	MCV	MCH	MCHC	WBC
1	3-10	4-7	4-10	3-10	3-10	5,9,10	4-10
2	4-10	4-7	4-10	3-10	3-10	5,9,10	4-10
3	4-8	4-8	4-10	1, 2, 5	1-2,4-5		4-10
4	1-3,6-10	1-3,6,8-10	1-3, 6-10	1-3,8-10	1-3,8-10	5, 10	10
5	6, 8-10	1-3,6,9	1-3, 6-10	1-3,7-10	1-3,8-10	1,2,4,7	1-4,7,9,10
6	1-5,8-10	1-5, 7-10	7-10		9, 10		10
7	1-6,8-10	1-6, 8-10	8, 9		9		
8	1-7,9	7, 9			10	9, 10	7, 9, 10
9							10
10							

Table 9: The rank sums scores (s) and mean scores (m) of Wilcoxon scores (Rank sums) for tests of differences between age groups

Age	Age group Scores																	
	RBC		PCV		Hb		MCV		MCH		MCHC		WBC		Lymphocytes		Neutrophils	
	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)
1	1194.5	66.4	3494.0	194.1	2802.0	155.7	6856.5	380.9	6189.0	343.8	2325.5	129.2	936.0	52.0	2035.5	113.1	1226.5	68.1
2	1627.0	81.3	3966.5	198.3	3383.5	169.2	7886.0	394.3	7622.5	381.1	2926.5	146.3	1845.5	92.3	3358.5	167.9	1323.0	66.1
3	2116.5	132.3	2358.0	147.4	2035.0	127.2	4128.0	258.0	4191.5	262.0	2838.0	177.4	1036.5	64.8	1870.0	116.9	647.5	40.5
4	22892.0	357.7	19125.5	298.8	18507.5	289.2	11726.5	183.2	9798.5	153.1	13309.0	207.9	17307.5	270.4	20675.0	323.0	8193.5	128.0
5	15916.0	346.0	11842.5	257.4	12796.0	278.2	6910.5	150.2	7054.5	153.3	12926.5	281.0	13539.0	294.3	15744.5	342.3	7554.0	164.2
6	5893.0	392.9	5961.5	397.4	6055.0	403.7	3348.5	223.2	2992.0	199.5	2642.5	176.2	3858.5	257.2	4694.5	313.0	3839.0	255.9
7	7437.5	297.5	7806.0	312.2	7544.0	301.8	6025.5	241.0	5313.0	212.5	4612.0	184.5	5726.5	229.1	6028.5	241.1	6029.5	241.2
8	33852.0	235.1	33951.5	235.8	33292.5	231.2	34433.0	239.1	34591.0	240.2	35618.0	247.3	40729.5	282.8	33365.5	231.7	43744.0	303.8
9	14638.0	176.4	15676.0	188.9	16976.0	204.5	21225.5	255.7	23472.5	282.8	23831.0	287.1	20349.0	245.2	19282.5	232.3	26690.5	321.6
10	8914.5	189.7	10299.5	219.1	11089.5	235.9	11941.0	254.1	13256.5	282.0	13452.0	286.2	9153.0	194.7	7426.5	158.0	15233.5	324.1

The Kruskal - Wallis tests (Chi square approximation) indicated significant differences between all age groups for all anlytes ($p<0.0001$).
If there is no significant difference between any two groups the average scores should be equal.

differences ($p < 0.05$) almost between each age group in all parameters (table 8). There were no significant differences between female and male goats except in the group of 8 - 12 months and 3 - 5 years old goats. In 8 - 12 months old goats, the total leukocyte count was higher ($p < 0.01$) in males (18.71 ± 2.11 $x10^9$/l) than in females (13.61 ± 3.82 $x10^9$/l). In goats between 3 and 5 years of age the total leukocyte count was higher ($p < 0.01$) in females (13.27 ± 3.86 $x10^9$/) than in males (10.42 ± 1.83 $x10^9$/l). In the same goats, the mean corpuscular hemoglobin concentration was higher in females (22.4 ± 1.93 mmol/l) than in males (21.2 ± 0.91 mmol/l).

Discussion

The results of the tests for Gaussian distribution in the present investigation indicate that data for in most parameters in each group followed a Gaussian distribution, therefore the models for parametric tests were satisfied. In some groups the data for some parameters did not follow a Gaussian distribution. However, all parameters in all age groups were tested by the nonparametric method of Wilcoxon scores sums rank procedure and the existence of any differences were confirmed.

Hemoglobin concentration, hematocrit and the number of erythrocytes in landrace goats of this investigation (tables 1, 3, 5) are comparable to the ranges reported in other goats breeds (Barakat and El-Guindi, 1967; Castro *et al.*, 1977; Bhargava, 1980; Domina *et al.*, 1982; Payne *et al.*, 1982; Facello *et al.*, 1983; Masoni *et al.*, 1985; Vrzgula *et al.*, 1985; Okorie and Anugwa, 1986; Pospisil *et al.*, 1987; Gray *et al.*, 1988). There were, however, differences in specific means. Erythrocyte counts as high as 32 $x10^{12}$/l were found in goats (Wesonga and Nandokha, 1989) compared to the present maximum of 20.6 $x10^{12}$/l.

The lower hemoglobin concentration, packed cell volume and number of erythrocytes in young goats than in adults observed in this study and the increase with age thereafter, supports earlier findings (Holman and Dew, 1964; Edjtehadi, 1978; Facello *et al.*, 1983; Löhle *et al.*, 1990). The differences found between the RBC counts, PCV and haemoglobin concentration between the landrace goats and those of other breeds are therefore attributed to variations in age, breed, season at the time of sampling, nutrition, lactation, pregnancy (Mazumder *et al.*, 1982: Unanian, 1986; Ginting, 1987; Löhle *et al.*, 1990) an probably analytical methods.

The ranges of MCV, MCH and MCHC in landrace goats (tables 1, 3, 8) derived from RBC, Hb and PCV values are also in agreement with those of other goats (Bhargava, 1980; Masoni *et al.*, 1985). In such cases the age trends are similar. The highest MCV values in new born kids (fig. 1) has been earlier reported (Holman and Dew, 1964; Edjetihadi, 1978; Facello *et al.*, 1983). Furthermore MCV is highly correlated with RBC diameters and the large cells are reported to contain foetal

hemoglobin type (Facello *et al.*, 1984). MCH values were also higher in young than in adult goats, showing a negative correlation with the number of erythrocytes (fig. 2a). The nearly constant MCHC levels for all ages (fig. 2b) indicates the independence of age for this parameter, as is known for in other goats between (Holman and Dew, 1964; Nangia *et al.*, 1968; Somvanshi *et al.*, 1987). The values for MCHC appear to fluctuate only little although they may be influenced by analytical methods because of hemoglobin and hematocrit values which are used for the calculations. The differences found in MCV and MCH between the present landrace goats and other breeds are also attributable to variations in age, breed and nutrition.

The ranges of total leukocyte counts in landrace goats (fig. 2, tables 2, 4, 7) are close to those of other breeds (Bhargava, 1980; Masoni *et al.*, 1985; Vrzgula *et al.*, 1985; Okorie and Anugwa, 1986; Pospisil *et al.*, 1987; Gray *et al.*, 1988). The lower total WBC counts in young goats than in adults and the increase with age is consistent with many other observations (Holman and Dew, 1964; Castro *et al.*, 1977; Kanemaki *et al.*, 1986; Somvanshi *et al.*, 1987). However, there are other studies that found the opposite trends (Nangia *et al.*, 1968; Nettleton and Beckett, 1976; Earl and Carranza, 1980; Upadhyay and Rao, 1985) and the reason for this opposite trend is obviously obscure. Earl and Carranza (1980) and Bialkowski *et al.* (1988) observed highest WBC counts in new born kids, and the values decreased with age. A study by Neto *et al.* (1986) could not reveal any differences between breeds and ages. The differences in these sources, particularly the number and age trends could be due to differences in techniques of sample collection, storage and laboratory analysis. The determination of reference values therefore may require standardization of the subjects for sampling as well as the sampling and analytical methods.

The majority of leukocytes in landrace goats are lymphocytes (table 7). Holman and Dew (1964) and Payne *et al.* (1982) found similar results. In some breeds of goats neutrophilic granulocytes predominate over other types of leukocytes (Nangia *et al.*, 1968; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988), a probable breed or the influence of prevailing environmental conditions influence. These leukocytic cells are produced independent of each other and according to body's demands, often triggered by internal or external factors.

The monocytes most numerous of the rare leukocytes in goats, followed by eosinophils, band neutrophils and basophils. Band neutrophils occur particularly in new born kids but become very rare within 1 - 2 weeks of age. Basophils are reported to be extremely rare in some tropical goat breeds (Wesonga and Nandokha, 1989). This is probably a result of specific physiological environmental conditions, which control their production.

There were some minor differences between female and male goats in RBC counts, PCV, Hb, MCV, MCH, MCHC, WBC, the number of lymphocytes and segmented

neutrophils as well as rare leukocytes which were not in particular age trend. Sex differences are reported in goats (Vaidya *et al.*, 1970), but the magnitudes are small (Jain, 1986).

In conclusion the reference ranges for hematological values in Danish landrace goats indicate similar wide intervals as for many other breeds, but the means and age trends are markedly different between the different investigations. All the parameters were strongly influenced by age and there is a large disparity of data between sources on the trends. Some investigators have observed increasing values others the opposite. Most differences might arise as a result of problems of aging of animals, variations in blood sampling, anticoagulants, storage techniques and laboratory analysis. The parametric values of two standard deviations below and above the means included the nonparametric interval from 5^{th} to 95^{th} percentile in most parameters. It is therefore appropriate to determine the reference ranges in different breeds specified to age.

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CHAPTER 14

REFERENCE RANGES FOR CLINICAL CHEMICAL VALUES

Summary

Plasma calcium, phosphorus, magnesium, potassium, sodium, alanine amino and aspartate amino transferases, creatine kinase, alkaline phosphatase, urea, bilirubin, creatinine, glucose, cholesterol and total serum proteins were determined in Danish landrace goats of different ages from the first day of life to over five years of age. The objective was to determine the reference ranges for these parameters in the breed. Parametric and nonparametric statistical methods were used for analysis and there were no apparent differences between them except for alkaline phosphatase. There was a very wide range of alkaline phosphatase activity. Except for this enzyme the intervals from the 5^{th} to 95^{th} percentiles were within two standard deviations of the mean in all parameters. Most clinical chemical parameters were strongly influenced by age. Calcium, inorganic phosphorus, alkaline phosphatase, urea, glucose and cholesterol were higher in young goats of less than 6 months of age than in adults. Sodium, magnesium, creatine kinase, bilirubin, creatinine, total serum proteins and alanine amino and aspartate amino transferases were moderately to significantly lower in juvenile than in old goats. Potassium was not significantly different between goats of different ages. As a result of the age differences a distinction between kids (< 4 months), juveniles (above 4 but less than 6 months of age) and adult goats is appropriate for correct interpretation of laboratory results. Sex differences were not significant in most parameters and were noted at some ages for urea, glucose, creatinine, bilirubin, cholesterol, aspartate aminotransferase and creatine kinase but not consistent for all ages.

Introduction

Clinical chemical analysis of blood in animals has become a useful tool in diagnosis and differential diagnosis of many diseases (Wilson *et al.*, 1986). Continuous assessment during the course of the syndrome is also possible. The determinations of concentrations of plasma or serum clinical chemical parameters reveals any existing pathophysiological states and leads to identification of the pathogenesis, and in turn their possible causes. They enable assessment of the body's ability to fight infections and predict the outcome of the disease (prognosis). Extension of this knowledge by determining the minimum and maximum levels in strictly healthy animals forms the

reference range. The laboratory analytical results of a clinically sick animal can then be compared to this range for better interpretation and subsequent diagnosis. Several expressions are used for reference ranges; the minimum to maximum observation in a set of data, the mean ±1 or 2 standard deviations and the nonparametric 2.5th to 97.5th or 5th to 95th percentile intervals (Solberg, 1983). The use of parametric statistics (mean ± standard deviation) has been widely criticized because the data of many blood parameters do not follow a Gaussian distribution (Wu *et al.*, 1975).

The reference ranges for plasma or serum biochemical values is reported in some breeds of goats (Sherman and Robinson, 1983). Many studies have indicated that clinical chemical parameters are affected by age (Bialkowski *et al.*,1988), breed (Chiofalo *et al.*, 1982; Pugliese *et al.*, 1982), nutrition and season (Bas *et al.*, 1980; Blackwell and Libby, 1982), sex (Chiericato *et al.*, 1986a), pregnancy and lactation (Garnier *et al.*, 1984; Cissik *et al.*, 1987; Biagi *et al.*, 1988). The use of generalized data for all goat categories without sorting them according to these factors could affect the diagnosis of clinical cases. Diagnostic information requires specification of breed and age. In addition to this, the type of nutrition and whether the animal is pregnant, lactating, intact male or castrated provides useful clues to laboratory results interpretation and therefore to the correct diagnosis of the disease. This is essential for the appropriate and prompt therapy. Information on the clinical chemistry of Danish landrace goats is lacking and literature studies could not reveal any established reference ranges for clinical chemical parameters. The present investigation was therefore conducted to establish these ranges in Landrace goats of different ages using both parametric and nonparametric values. Danish Landrace goats are large white, brown or black goats with a moderate to heavy under wool. They are the commonest milk goats in Denmark.

Materials and Methods

The goats of this study were apparently clinically healthy Danish landrace goats of different ages from five herds in Denmark consisting of 27 (8 female, 19 male) 0 - 7 days, 28 (16 female, 12 male) 7 - 30 days, 25 (10 female, 15 male) 1 - 2 months, 74 (46 female, 28 male) 2 - 4 months, 63 (38 female, 25 male) 4 - 8 months, 22 (15 female 7 male) 8 -12 months, 25 (22 female, 3 male) 1 - 2 years, 144 (138 female, 6 male) 2 - 3 years, 83 (74 female, 9 male) and 47 above 5 years (all females). The goats were categorized into 10 age groups. They were kept indoors throughout the year with only limited outdoor movements. In one herd only, goats were left in the field for the entire summer and autumn. Nutrition was composed of green grass pellet and about 0.5 kg of barley or oat cereals.

Blood samples were collected from the external jugular vein in vacuum tubes

containing sodium heparin (143 USP units) for most plasma analytes, sodium fluoride with sodium heparin (143 USP units) for plasma glucose and clot activator for total serum proteins (Becton-Dickinson vacutainers). The samples were collected between 8 and 10 am and the plasma or serum separated within 1 to 3 hours.

Plasma calcium and magnesium were determined by using model 5000 atomic absorption spectrophotometer (Perkin Elmer). Plasma sodium and potassium were determined by the electrolyte module of an autoanalyzer Cobas fara (Roche) using selective ion electrodes. Plasma inorganic phosphorus, urea, creatinine, bilirubin, cholesterol, glucose and total serum proteins were determined on the Cobas Fara analyzer. Plasma enzyme activities of alanine amino transferase (ALAT, EC. 2.6.1.2), aspartate amino transferase (ASAT, EC. 2.6.1.1), creatine kinase (CK, EC 2.7.3.2) were measured kinetically while alkaline phosphatase (ALP, EC. 3.1.3.1) by end point colorimetric method in the Cobas Fara according to the recommendations of the Scandinavian Committee on enzymes.

Parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles, median) values were determined by the univariate procedure of a statistical analysis system software (SAS, Cary, USA). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte in each group to the Gaussian distribution. The means of plasma and serum analytes were tested for statistically significant differences between ages by the general linear models procedure. Wilcoxon scores sums rank tests were performed for all parameters using average scores for ties, the Kruskal-Wallis test (Chi square approximation) and associate probabilities for testing differences were calculated.

Results

The minimum to maximum values of plasma calcium, magnesium, inorganic phosphorus, sodium and potassium concentrations are shown in table 1. The minimum to maximum values for plasma enzyme activities are shown in table 2, while those of other clinical chemical parameters in table 3.

The mean and median values for plasma electrolytes, ALAT, ASAT, glucose, cholesterol, urea, bilirubin and total serum proteins in each goat group were close to each other (table 4 - 6). Differences in these statistics were observed for ALP and at some ages for CK activities. The coefficient of skewness and the degree of kurtosis were small in most parameters and the Shapiro Wilk statistic (W) indicated, that data for most parameters were distributed in a Gaussian manner except a few (W in tables 1-3). The mean ± one standard deviation ($\bar{x} \pm s$) were within the 5^{th} to 95^{th} percentile intervals, which in turn were included in the $\bar{x} \pm 2s$ interval in all goat groups except

for ALP and at some ages for CK (table 7 - 11).

Plasma calcium and inorganic phosphorus were slightly higher in young goats of less than four months of age than in others. The concentrations were relatively even for the rest of the age groups (fig. 1 a). The calcium - Phosphorus ratios were 0.85, 0.87, 0.80, 0.99, 0.96, 1.07, 1.1, 1.18, 1.22 and 1.12 for age groups 1 - 10 respectively and tended to slightly increase with age. Plasma magnesium, was lower in kids less than 3 months of age (fig. 1b). Sodium and potassium concentrations were similar in the various age groups with only slight fluctuations (fig. 1b, c). ALAT and ASAT activities were lower in kids up to 3 months of age than in other goats (fig. 1d). ALP levels were very high in kids up to 4 months of age then decreased, while it was vice versa for CK (fig. 2a). Bilirubin was lower in juvenile goats (less than 6 months of age) than in adult goats. Plasma urea concentration was higher in juveniles than in adult goats (fig. 2c). Glucose and cholesterol levels were higher in very young kids than other goats (fig. 2d). The levels were very constant after one year of age.

Both parametric (t tests by general linear models procedure) and nonparametric (Wilcoxon sums rank scores) showed that all the clinical chemical parameters were strongly influenced by age. There were significant differences ($p<0.05$) almost between each age group (tables 12 -14). There were significant differences in means between female and male goats only in a few parameters and at some ages only, without a consistent sex trend, therefore results were pooled and statistically analyzed together. There were no differences in all goats younger than 4 months. In 2 - 4 months old goats plasma sodium concentration was higher ($p<0.001$) in females (152.7 ± 3.05 mmol/l) than in males (150.4 ± 2.58 mmol/l). In the same goats glucose levels were lower ($p<0.05$) in females (3.99 ± 0.76 mmol/l) than in males (4.36 ± 0.61 mmol/l), while urea was higher ($p<0.01$) in the former (5.29 ± 1.79 mmol/l) than in the latter sex (4.02 ± 1.68 mmol/l). In goats 4 - 8 months of age, calcium and urea concentrations were lower ($p<0.01$) in females than in males. The respective values for these were 2.37 ± 0.25 (2.52 ± 0.12) and 5.35 ± 1.75 (7.47 ± 2.30) mmol/l, the values in parentheses being for males. In the same goats plasma magnesium, sodium, creatinine and bilirubin concentrations and CK activity were higher in females than in males. The respective values for these were 1.04 ± 0.15 (0.93 ± 0.06), 150.8 ± 2.89 (146.9 ± 3.65) mmol/l, 64.2 ± 18.1 (74.4 ± 16.7) μmol/l, 5.07 ± 2.77 (3.24 ± 1.42) μmol/l and 4.59 ± 1.50 (3.21 ± 0.72) μkat/l, those in parentheses being for males.

In 8-12 months old goats, calcium, potassium, ASAT, creatinine and glucose were lower ($p<0.01$) in females than in males at respective values of 2.48 ± 0.15 (2.65 ± 0.05), 4.16±0.55 (4.93±0.38) mmol/l, 1.15±0.33 (1.49±0.13) μkat/l, 76.9±20.8 (102.9± 12.8) μmol/l and 2.93±0.35 (3.59±0.95) mmol/l, those for males in parentheses. Plasma urea levels were higher in females (8.16±2.39) than in males (6.14 ± 0.90) mmol/l.

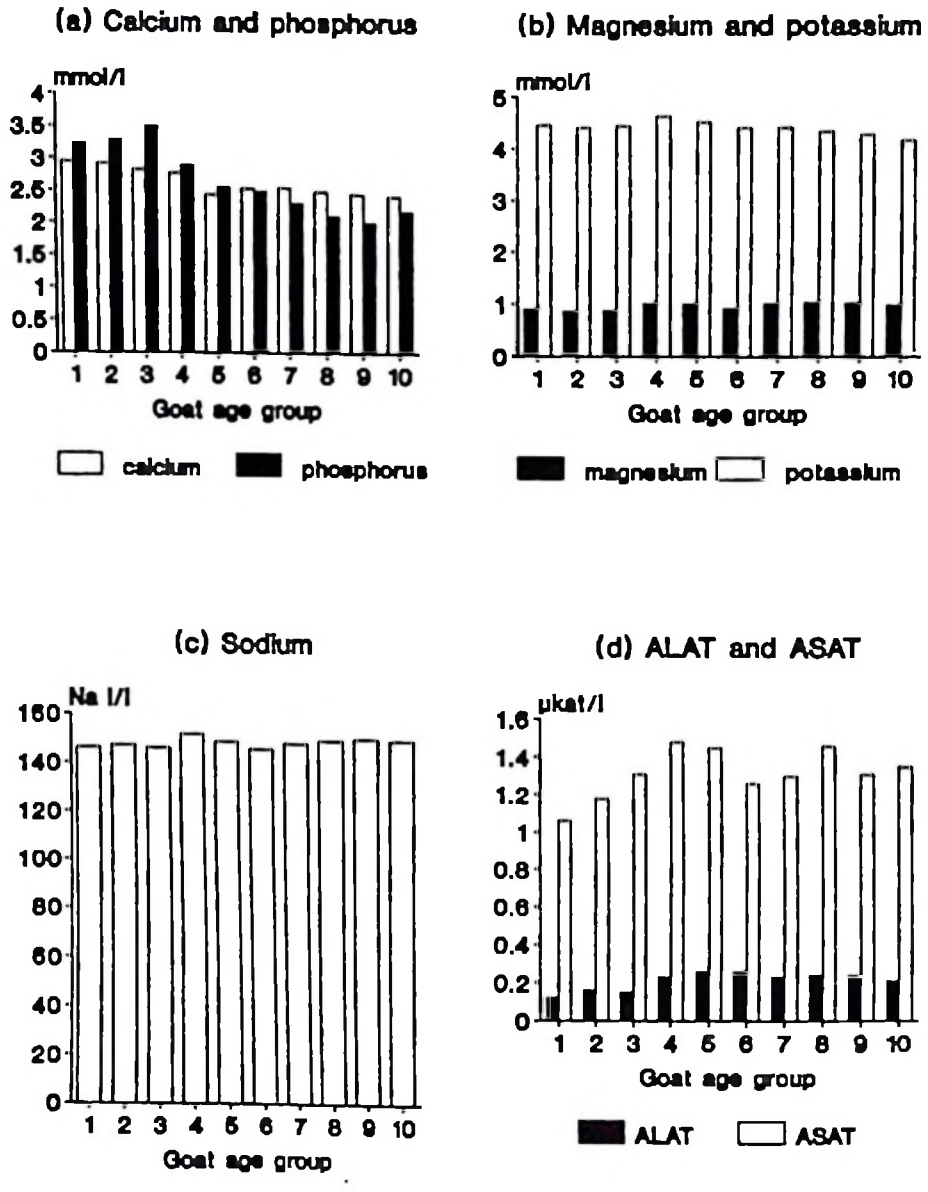

Figure 1: The plasma concentration of calcium and phosphorus (a), magnesium and potassium (b), sodium (c) and plasma ALAT and ASAT activities (d) in Danish landrace goats of 0 - 7 days (1), 7 - 30 days (2), 1- 2 months (3), 2 - 4 months (4), 4 - 8 months (5), 8 - 12 months (6), 1 -2 years (7), 2 - 3 years (8), 3 - 5 years (9) and above 5 years of age (10).

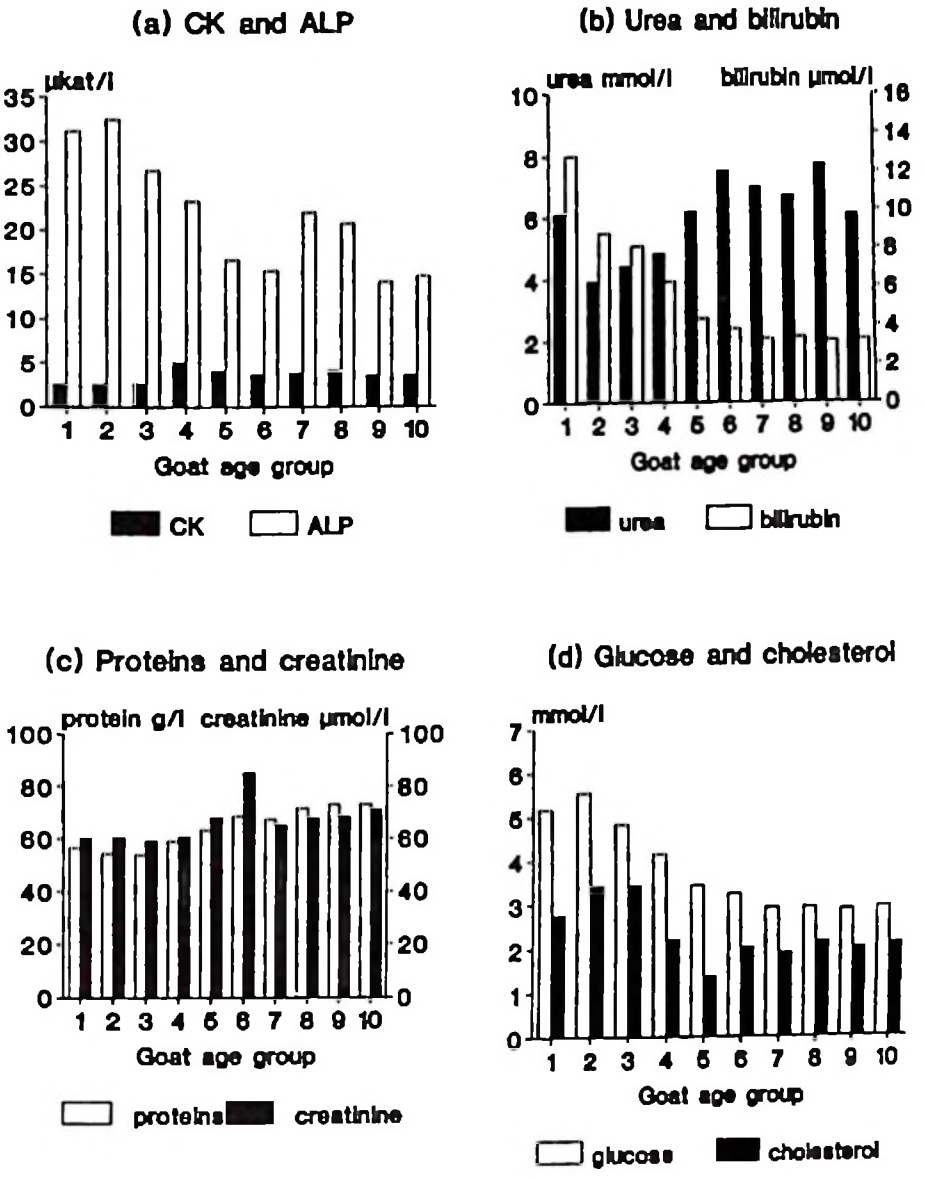

Fig. 2: The plasma activities of CK and ALP (a), plasma urea and bilirubin (b), total serum proteins and plasma creatinine (c) and plasma glucose and cholesterol (d) concentrations in Danish landrace goats of 0 - 7 days (1), 7 - 30 days (2), 1- 2 months (3), 3 - 4 months (4), 4 - 8 months (5), 8 - 12 months (6), 1 -2 years (7), 2 - 3 years (8), 3 - 5 years (9) and above 5 years of age (10).

Table 1: The minimum to maximum values of plasma electrolyte concentrations in landrace goats of different ages.

Age	n	Ca mmol/l	Mg mmol/l	P mmol/l	Na mmol/l	K mmol/l
0-7 days	27	2.55 - 3.15	0.71 - 1.10	2.07 - 3.95	142.6 - 150.2	4.05 - 5.63
7-30 days	28	2.41 - 3.61	0.68 - 1.13	2.65 - 3.99	144.8 - 149.5	3.53 - 5.25
1-2 months	25	2.52 - 3.08	0.61 - 1.03	2.46 - 3.97	144.2 - 152.6	3.90 - 5.04
2-4 months	74	2.19 - 4.35	0.76 - 1.67	0.60 - 4.67	145.8 - 162.7	3.31 - 5.57
4-8 months	63	1.50 - 2.77	0.76 - 1.44	1.32 - 3.57	141.9 - 158.8	3.49 - 5.53
8-12 months	22	2.15 - 2.73	0.81 - 1.16	0.61 - 3.85	137.6 - 154.5	2.80 - 5.67
1-2 years	25	2.13 - 3.20	0.68 - 1.38	0.94 - 3.78	137.5 - 152.7	3.78 - 5.60
2-3 years	144	1.92 - 3.36	0.65 - 1.40	0.82 - 3.99	139.3 - 164.8	3.33 - 5.63
3-4 years	83	2.06 - 3.05	0.78 - 1.33	0.49 - 3.63	142.7 - 159.0	3.37 - 5.19
> 5 years	47	1.74 - 3.36	0.77 - 1.32	0.46 - 4.62	137.4 - 156.2	3.19 - 5.60

n= number of goats, the same in all tables.

Table 2: The minimum to maximum values of plasma enzyme activity values in landrace goats of different ages.

Age	n	ALAT µkat/l	ASAT µkat/l	ALP µkat/l	CK µkat/l
0-7 days	27	0.02 - 0.27	0.56 - 1.59	3.22 - 86.5	1.11 - 6.77
7-30 days	28	0.09 - 0.23	0.82 - 1.66	2.44 - 64.8	0.55 - 11.5
1-2 months	25	0.04 - 0.37	0.80 - 2.25	10.2 - 66.7	1.17 - 4.97
2-4 months	74	0.10 - 0.58	0.85 - 2.92	2.12 - 74.7	1.23 - 14.3
4-8 months	63	0.11 - 0.48	0.89 - 3.48	0.92 - 81.8	1.84 - 8.76
8-12 months	22	0.13 - 0.46	0.73 - 2.01	1.05 - 42.6	1.17 - 6.07
1-2 years	25	0.12 - 0.37	0.60 - 2.43	1.18 - 79.0	1.26 - 13.7
2-3 years	144	0.05 - 0.39	0.82 - 3.25	1.40 - 110.2	1.17 - 10.5
3-5 years	83	0.10 - 0.47	0.79 - 1.94	0.64 - 66.9	1.16 - 5.95
> 5 years	47	0.10 - 0.42	0.82 - 2.72	0.75 - 54.2	1.51 - 11.6

n= number of goats.

Table 3: The minimum to maximum values of plasma clinical chemical parameters and total serum protein in landrace goats of different ages.

Age	Urea mmol/l	Creatinine µmol/l	Glucose mmol/l	Bilirubin µmol/l	Proteins g/l	Cholesterol mmol/l
0-7 days	3.19 - 14.7	39.0 - 140.0	2.80 - 9.67	4.50 - 29.7	40.0 - 71.0	0.95 - 4.34
7-30 days	1.58 - 5.90	39.0 - 89.0	4.40 - 7.88	4.70 - 17.1	43.0 - 70.0	1.16 - 6.99
1-2 months	2.59 - 7.98	40.0 - 88.0	3.38 - 6.67	4.50 - 14.8	47.0 - 78.0	1.58 - 8.04
2-4 months	1.14 - 9.63	37.0 - 100.0	2.39 - 6.01	1.20 - 16.6	45.0 - 85.0	0.58 - 7.05
4-8 months	1.35 - 12.5	34.0 - 123.0	2.29 - 5.37	1.50 - 10.8	44.0 - 75.0	0.46 - 2.37
8-12 months	2.53 - 11.9	54.0 - 140.0	2.28 - 5.00	0.70 - 7.50	60.0 - 79.0	0.87 - 3.60
1-2 years	1.84 - 12.0	40.0 - 97.0	1.60 - 3.49	1.80 - 5.90	48.0 - 78.0	0.93 - 4.07
2-3 years	2.02 - 14.0	30.0 - 118.0	2.06 - 4.04	1.50 - 9.50	56.0 - 88.0	0.85 - 3.60
3-5 years	0.29 - 16.0	30.0 - 120.0	1.70 - 4.56	1.40 - 7.50	57.0 - 94.0	0.81 - 3.70
> 5 years	1.58 - 15.9	31.0 - 120.0	2.28 - 5.68	1.50 - 7.40	57.0 - 90.0	1.21 - 3.13

Table 4: The median (Q_2) and $\bar{x}\pm s$ of plasma electrolyte concentrations in landrace goats of different ages.

Age	Ca mmol/l		Mg mmol/l		P mmol/l		Na mmol/l		K mmol/l	
	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q^2	$\bar{x}\pm s$
0-7d	2.99	2.94±0.16^W	0.90	0.89±0.11	3.42	3.23±0.54	146.3	146.5±1.6^W	4.36	4.46±0.36^W
7-30d	2.92	2.91±0.26	0.86	0.86±0.09	3.33	3.29±0.40	147.5	147.4±1.12	4.38	4.40±0.35
1-2m	2.80	2.82±0.16	0.90	0.87±0.13	3.54	3.50±0.37	146.9	146.4±1.86	4.35	4.44±0.33
2-4m	2.72	2.77±0.34	0.99	1.00±0.15^W	2.87	2.89±0.74	152.1	151.9±3.07	4.62	4.63±0.39
4-8m	2.46	2.43±0.22^W	0.96	1.00±0.13	2.53	2.55±0.56	149.1	149.2±3.73	4.61	4.52±0.42
8-12m	2.56	2.53±0.15	0.89	0.91±0.09^W	2.54	2.48±0.72	143.6	146.0±5.46	4.57	4.40±0.61
1-2y	2.52	2.54±0.27	0.99	1.00±0.13	2.34	2.30±0.59	149.5	148.3±5.72	4.28	4.41±0.49
2-3y	2.47	2.48±0.18	1.04	1.03±0.13	2.02	2.10±0.64	149.4	149.6±3.64	4.33	4.34±0.48
3-4y	2.44	2.44±0.18	1.01	1.01±0.10	1.89	2.00±0.66^W	150.2	150.3±2.81	4.34	4.28±0.42
>5y	2.41	2.42±0.29^W	0.98	0.98±0.11	2.15	2.18±0.60	150.2	149.7±4.3^W	4.14	4.17±0.52

W= non Gaussian distribution ($p<0.05$), d= days, m= months, y= years.

Table 5: The median (Q_2) and mean ± standard deviation ($\bar{x}\pm s$) of plasma enzyme activities, glucose and cholesterol in landrace goats.

Age	ALAT µkat/l		ASAT µkat/l		ALP µkat/l		CK µkat/l		Glucose mmol/l		Chol mmo/l	
	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm SD$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$
0-7d	0.11	0.12±0.05	1.08	1.06±0.24	24.9	31.2±22.1^W	1.84	2.56±1.55^W	5.41	5.17±1.26^W	2.91	2.76±0.86
7-30d	0.16	0.16±0.04	1.15	1.18±0.22	28.0	32.5±15.5	1.85	2.56±2.14^W	5.42	5.54±0.78	3.31	3.41±1.21^W
1-2m	0.13	0.15±0.09	1.21	1.31±0.35	21.3	26.8±15.7^W	2.37	2.59±0.83	4.63	4.81±0.80	3.14	3.42±1.51^W
2-4m	0.21	0.23±0.07	1.41	1.48±0.36	18.9	23.3±16.5^W	4.36	5.02±3.12^W	4.08	4.13±0.75	1.72	2.19±1.32^W
4-8m	0.25	0.26±0.07	1.38	1.45±0.35	11.0	16.6±16.4^W	3.58	4.05±1.42^W	3.36	3.42±0.57	1.39	1.36±0.38
8-12m	0.24	0.26±0.06	1.26	1.26±0.32	12.6	15.4±10.1^W	3.45	3.62±1.33	3.18	3.23±0.71^W	1.94	2.01±0.78
1-2y	0.21	0.23±0.07	1.25	1.30±0.38	17.7	22.0±16.7	3.00	3.80±2.60	2.99	2.90±0.40	1.84	1.89±0.75
2-3y	0.24	0.24±0.06	1.44	1.46±0.35^W	16.7	20.7±19.6^W	3.93	4.12±1.56^W	2.94	2.93±0.34	2.09	2.13±0.53
3-5y	0.21	0.24±0.08^W	1.29	1.31±0.26	9.29	14.1±15.0^W	3.56	3.53±1.10	2.87	2.88±0.42^W	2.04	2.01±0.55
>5y	0.21	0.21±0.07	1.24	1.35±0.35^W	13.0	14.8±13.4^W	3.32	3.56±1.55^W	2.84	2.94±0.50^W	1.82	2.09±0.56^W

W= non Gaussian distribution ($p<0.05$), Chol= cholesterol, d= days, m= months, y= years.

Table 6: The median (Q_2) and mean ± standard deviation ($\bar{x}\pm s$) of some plasma and serum biochemical parameters in landrace goats of different ages.

Age	Urea mmol/l		Creatinine µmol/l		Bilirubin µmol/l		Proteins g/l	
	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$	Q_2	$\bar{x}\pm s$
0-7 days	4.74	6.11±2.93^W	55.0	60.3±23.2^W	10.5	12.8±3.05	54.0	56.5±7.06
7-30 days	3.84	3.91±0.95	57.0	60.4±13.1	8.60	8.77±2.83	54.0	54.3±7.06
1-2 months	3.37	4.41±1.34	58.0	59.0±10.4	7.40	8.09±2.77	53.0	54.0±5.98
2-4 months	4.63	4.81±1.84	59.0	60.6±12.2	5.35	6.25±3.60	57.0	59.0±7.40^W
4-8 months	5.75	6.19±2.23	71.0	68.2±18.1	3.50	4.35±2.49^W	64.0	63.3±7.58
8-12 months	7.04	7.52±2.23	82.5	85.1±22.1	3.75	3.82±1.37	68.5	68.7±5.21
1-2 years	6.87	6.98±2.76	65.0	65.2±15.8	3.10	3.28±1.11	69.0	67.4±7.27
2-3 years	6.74	6.70±2.71^W	66.5	67.7±12.0	3.15	3.39±1.26^W	71.5	71.7±6.53
3-4 years	7.48	7.73±3.64	67.0	68.2±16.0^W	3.00	3.21±1.04^W	73.0	72.9±6.61
>5 years	5.38	6.09±3.23^W	71.0	71.0±15.6	3.10	3.26±1.33^W	74.0	73.0±7.22

Table 7: The 5th (P_5) to 95th (P_{95}) percentile and $\bar{x}$-2s to $\bar{x}$+2s intervals for plasma calcium, magnesium and phosphorus concentrations in landrace goats of different ages.

Age	Ca mmol/l P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	Mg mmol/l P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	P mmol/l P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s
0-7 days	2.65 - 3.12	2.26 - 3.26	0.71 - 1.05	0.67 - 1.11	2.07 - 3.95	2.15 - 4.31
7-30 days	2.41 - 3.20	2.39 - 3.43	0.74 - 1.00	0.68 - 1.04	2.67 - 3.82	2.49 - 4.09
1-2 months	2.57 - 3.08	2.50 - 3.14	0.65 - 1.03	0.61 - 1.13	2.91 - 3.93	2.76 - 4.24
2-4 months	2.35 - 3.21	2.09 - 3.45	0.80 - 1.33	0.70 - 1.30	1.77 - 3.97	1.40 - 4.37
4-8 months	1.96 - 2.66	1.99 - 2.87	0.83 - 1.21	0.74 - 1.26	1.63 - 3.54	1.43 - 3.67
8-12 months	2.27 - 2.71	2.23 - 2.83	0.81 - 1.06	0.73 - 1.09	1.66 - 3.84	1.04 - 3.92
1-2 years	2.15 - 3.15	2.00 - 3.08	0.87 - 1.27	0.74 - 1.26	1.63 - 3.05	1.12 - 3.48
2-3 years	2.21 - 2.80	2.12 - 2.84	0.84 - 1.23	0.77 - 1.29	1.16 - 3.30	0.82 - 3.38
3-5 years	2.16 - 2.72	2.08 - 2.80	0.85 - 1.16	0.81 - 1.21	1.01 - 3.37	0.68 - 3.32
>5 years	2.06 - 2.94	1.84 - 3.00	0.80 - 1.17	0.76 - 1.20	1.10 - 3.52	0.58 - 3.78

Table 8: The 5th (P_5) to 95th (P_{95}) percentile and $\bar{x}$-2s to $\bar{x}$+2s intervals for plasma sodium and potassium concentrations in landrace goats of different ages.

Age	Na mmol/l P_5-P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	K mmol/l P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s
0-7 days	143.9 - 149.0	143.3 - 149.7	4.09 - 5.21	3.74 - 5.90
7-30 days	145.8 - 149.4	145.2 - 149.6	4.03 - 5.00	3.70 - 5.10
1-2 months	144.8 - 150.8	142.7 - 150.1	3.98 - 5.04	3.78 - 5.10
2-4 months	146.8 - 156.1	145.8 - 158.0	4.02 - 5.24	3.85 - 5.41
4-8 months	143.1 - 154.5	141.7 - 156.7	3.69 - 5.15	3.68 - 5.36
8-12 months	137.9 - 152.2	135.1 - 156.9	3.45 - 5.09	3.18 - 5.62
1-2 years	138.6 - 154.8	136.9 - 159.7	3.82 - 5.23	3.43 - 5.39
2-3 years	144.3 - 154.9	142.3 - 156.9	3.51 - 5.24	3.38 - 5.30
3-5 years	145.6 - 154.8	144.7 - 155.9	3.60 - 4.90	3.44 - 5.12
Above 5 years	140.3 - 155.3	141.2 - 158.2	3.36 - 4.96	3.13 - 5.21

Table 9: The 5th (P_5) to 95th (P_{95}) percentile and $\bar{x}$-2s to $\bar{x}$+2s intervals for plasma ALAT, ASAT, ALP and CK activities in goats of different ages.

Age	ALAT µkat/l P_5 - P_{95}	$\bar{x}$-2s - $\bar{x}$+2s	ASAT µkat/l P_5 - P_{95}	$\bar{x}$-2s - $\bar{x}$+2s	ALP µkat/l P_5 - P_{95}	0 - $\bar{x}$+2s	CK µkat/l P_5 - P_{95}	$\bar{x}$-2s - $\bar{x}$+2s
0-7 days	0.02-0.20	0.02-0.22	0.56-1.57	0.63-1.59	8.96-77.4	75.4	1.17-6.23	0.00-5.66
7-30 days	0.09-0.23	0.08-0.24	0.84-1.54	0.70-1.62	11.4-61.7	63.5	1.22-6.84	0.00-6.84
1-2 months	0.08-0.24	0.01-0.29	0.85-1.81	0.61-2.01	14.0-64.2	58.2	1.53-3.62	2.42-2.76
2-4 months	0.13-0.39	0.05-0.41	1.04-2.25	0.76-2.22	4.78-62.5	56.3	1.98-9.21	0.00-11.3
4-8 months	0.15-0.39	0.12-0.40	1.04-1.83	0.75-2.15	1.37-52.9	49.4	2.15-6.44	1.21-6.89
8-12 months	0.15-0.39	0.10-0.42	0.79-1.63	0.62-1.90	5.98-34.5	35.6	1.99-5.89	0.96-6.28
1-2 years	0.12-0.37	0.09-0.37	0.75-1.93	0.54-2.06	1.83-45.7	55.4	1.59-7.19	0.00-6.40
2-3 years	0.13-0.34	0.12-0.36	0.99-1.98	0.76-2.16	1.92-56.2	59.9	2.11-6.62	1.00-7.24
3-5 years	0.11-0.38	0.08-0.40	0.94-1.73	0.79-1.83	1.52-46.0	44.1	1.17-5.45	1.33-5.73
Above 5 years	0.13-0.35	0.07-0.28	0.89-1.97	0.65-2.05	1.21-39.4	41.6	1.88-5.23	0.46-6.66

Table 10: The 5th (P_5) to 95th (P_{95}) percentile and $\bar{x}$-2s to $\bar{x}$+2s intervals for plasma ALAT, ASAT, ALP, CK, glucose and cholesterol levels in goats of different ages.

Age	Glucose mmol/l		Cholesterol mmol/l		Proteins g/l	
	P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s
0-7 days	3.03 - 5.84	2.65 - 7.69	1.20 - 4.04	1.04 - 4.48	48.0 - 69.0	42.4 - 70.6
7-30 days	4.57 - 6.83	3.98 - 7.10	1.58 - 5.91	2.00 - 5.09	45.0 - 63.0	40.2 - 68.4
1-2 months	3.83 - 6.46	3.21 - 6.41	1.58 - 3.93	0.40 - 5.72	48.0 - 59.0	42.0 - 66.0
2-4 months	3.02 - 5.35	2.63 - 5.63	0.88 - 4.54	0.00 - 4.83	52.0 - 77.0	44.2 - 73.8
4-8 months	2.50 - 4.41	2.28 - 4.56	0.80 - 1.88	0.60 - 2.12	47.0 - 73.0	48.1 - 78.5
8-12 months	2.28 - 4.47	1.81 - 4.65	0.87 - 3.60	0.45 - 3.57	61.0 - 76.0	58.3 - 79.1
1-2 years	2.35 - 3.30	2.10 - 3.70	0.95 - 3.03	0.33 - 3.45	55.0 - 77.0	52.9 - 81.9
2-3 years	2.37 - 3.44	2.25 - 3.61	1.36 - 3.06	1.07 - 3.19	60.0 - 82.0	58.6 - 84.8
3-5 years	2.37 - 3.44	2.04 - 3.72	0.98 - 2.92	0.91 - 3.11	62.0 - 82.0	59.7 - 86.1
> 5 years	2.45 - 3.41	1.94 - 4.94	1.44 - 3.07	0.99 - 3.19	60.0 - 85.0	58.7 - 87.4

Table 11: The 5th (P_5) to 95th (P_{95}) percentile and $\bar{x}$-2s to $\bar{x}$+2s intervals for some plasma biochemical parameters and total,serum proteins in goats of different ages.

Age	Urea mmol/l		Creatinine µmol/l		Bilirubin µmol/l	
	P_5 - P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	P_5-P_{95}	$\bar{x}$-2s to $\bar{x}$+2s	P_5-P_{95}	$\bar{x}$-2s to $\bar{x}$+2s
0-7 days	3.30 - 12.46	0.25 - 12.0	39.0 - 97.0	13.9 - 106.7	4.90 - 28.6	0.95 - 18.90
7-30 days	1.95 - 5.35	2.01 - 5.81	40.0 - 85.0	34.2 - 86.6	5.10 - 13.7	3.11 - 14.43
1-2 months	2.63 - 6.22	1.73 - 7.09	45.0 - 78.0	38.2 - 79.8	4.50 - 13.3	2.55 - 13.63
2-4 months	2.22 - 7.82	1.13 - 8.52	44.0 - 83.0	36.2 - 85.0	2.10 - 14.0	0.00 - 13.45
4-8 months	3.30 - 10.80	1.73 - 10.6	39.0 - 93.0	32.0 - 104.4	1.90 - 9.50	0.00 - 9.33
8-12 months	4.81 - 10.88	3.06 - 12.0	57.0 - 118.0	40.9 - 129.3	2.30 - 5.50	1.08 - 6.56
1-2 years	2.05 - 11.69	1.46 - 12.50	42.0 - 93.0	33.6 - 96.8	1.90 - 5.30	1.06 - 5.50
2-3 years	2.55 - 11.30	1.18 - 12.22	51.0 - 89.0	43.7 - 91.7	1.90 - 5.70	0.87 - 5.91
3-5 years	2.46 - 14.00	0.45 - 15.00	50.0 - 96.0	36.2 - 100.2	1.90 - 5.10	1.13 - 5.29
> 5 years	2.70 - 11.61	0.00 - 12.55	44.0 - 92.0	39.8 - 102.2	1.60 - 5.90	0.60 - 5.92

Table 12: Statistical tests for differences between means among the different age groups (* $p<0.001$ to $p<0.05$).

Group	Age group numbers with which there were significantly differences										
	Calcium	Magnesium	Phosphorus	Sodium	Potassium	ALAT	ASAT	ALP	CK	Urea	Creatinine
1	4-10	5, 7-10	4-10	4-5, 8-10	6, 10	4-10	4, 5, 7-10	4, 5-10	4-10	6-9	4-10
2	5-10	5-10	4-10	4-6, 8-10	6, 9-10	4-10	4, 5, 7-10	4-10	4-10	5-10	6-10
3	5-10	5-10	4-10	4-5, 8-10	6, 10	4-10	5-6, 8	5, 6, 8-10	4-5, 7-8	5-10	5-10
4	1-3, 5-10	5-10	5-10	1-3, 5-10	6-10	1-3,5,10	6, 9	1-2, 9, 10	1-3, 5-10	6-10	6, 8-10
5	7-8	1-4, 7-8	1-4, 9-10	1-4, 6-8	6, 8-10		6		1-4, 9-10	6, 7-9	1, 3, 6, 10
6				1-5, 7-10	1-5		10			8, 10	1-5, 7-9
7			9	9	10			9			
8					10		7, 9, 10	9, 10	9	9	
9										10	

Table 13: Statistical tests for differences between means among the different age groups (* $p<0.001$ to $p<0.05$).

Group	Age groups where there were significantly differences			
	Bilirubin	Glucose	Total proteins	Cholesterol
1	2 - 10	4 - 10	4 - 10	4 - 10
2	1, 3 - 10	3 - 10	4 - 10	3 - 10
3	1 - 2, 4 - 10	2, 6, 4-10	4 - 10	4 - 10
4	1 - 3, 5 - 10	1 - 3, 5-10	1 - 3, 6 - 10	1 - 3, 5
5	1 - 4, 7 - 10	6 - 10	1 - 3, 6 - 10	1 - 4, 6 - 10
6			9, 10	
7			8 - 10	

Table 14: The rank sums scores (s) and mean scores (m) of Wilcoxon scores (Rank sums) for tests of differences between age groups

Analyte	Age group Scores																			
	1		2		3		4		5		6		7		8		9		10	
	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)	(s)	(m)
Ca	7591	421.7	7838.5	391.9	6275.0	392.2	21477.0	335.6	7914.0	172.0	3334.0	222.3	5945.5	237.8	30779	213.7	15293	184.2	8034	170.9
Mg	2112	117.3	1910	95.5	1160	72.5	14624	228.5	11669	253.7	2610	174.0	6275	251.0	41320.5	286.9	22111.5	266.4	10688.5	227.4
P	7417	412.0	7908	395.4	6747.5	421.7	20067.5	313.5	12202	265.3	3578	238.5	5739	229.6	27199	188.9	14058	169.4	9565	203.5
Na	1681.5	93.4	2312.5	115.6	1363.5	85.2	22392	349.9	12706	276.2	2139.5	142.6	5526.5	221.1	33110.5	229.9	21413	258.0	11836	251.8
K	4531.5	251.8	5602.5	280.1	4519.5	282.5	20495.5	320.2	13301.5	289.2	2785.0	185.7	5855.5	234.2	31887.0	221.4	17264.0	208.0	8239.0	175.3
ALAT	1391.0	77.3	2089.5	104.5	1459.0	91.2	14642.5	228.8	13773.0	299.4	4156.0	277.1	6473.5	258.9	39383.0	273.5	21049.0	253.6	10064.5	214.1
ASAT	1866.5	103.7	2332.0	116.6	2278.5	142.4	17809.0	278.3	12107.0	278.3	2243.5	263.2	5383.5	149.6	40852.0	283.7	18820.0	226.7	10789.0	229.5
ALP	6261.5	347.8	7234.5	361.7	5308.5	331.8	17550.5	274.2	10499.0	228.2	3472.5	231.5	6470.5	258.8	33839.5	235.0	14866.5	179.1	8978	191.0
CK	1828.5	101.6	1439.5	72.0	1953.5	122.1	21073.0	329.3	13443.5	292.2	3540.5	236.0	5077.0	203.1	38044.0	264.2	18191.5	219.2	9890.0	210.4
Urea	3232.5	179.6	2301.0	115.0	1659.0	103.7	11785.0	184.1	9557.0	207.8	5115.0	341.0	7088.0	283.5	38440.0	266.9	24731.5	298.0	10572.0	224.9
Creat	1638.0	91.0	2912.0	145.6	1730.5	108.1	11698.5	182.8	10331.5	224.6	4899.0	224.6	6145.5	326.6	38601.5	245.8	22422.0	268.1	14102.0	300.0
Bilir	7714.0	428.6	8369.5	418.5	6574.0	410.9	19262.5	301.0	11235.5	244.2	3826.0	255.1	4738.5	189.5	28592.0	198.5	15485.0	186.6	8684.0	184.8
Gluc	7592.0	421.8	8975.5	448.8	6914.5	432.2	23560.0	368.1	13011.0	282.8	2731.5	182.1	4556.0	182.2	25875.0	179.7	13384.5	161.2	7881.0	167.7
Prot	1226.5	68.1	1323.0	66.1	647.5	40.5	8193.5	164.2	7554.0	164.2	3839.0	255.9	6029.5	241.2	43744.0	303.8	26690.0	321.6	15233.5	324.1
Chol	6915.0	384.2	7683.5	384.2	5438.0	339.9	11847.5	185.1	4350.5	94.6	3467.0	231.1	5036.0	201.4	38157.5	265.0	19858.5	239.2	11727.5	249.5

Creat = creatinine, Bilir = bilirubin, Gluc = glucose, Prot = proteins and Chol = cholesterol. The Kruskal - Wallis tests (Chi square approximation) indicated significant differences between all age groups for all anlytes ($p<0.0001$).

Plasma urea was higher ($p<0.01$) in female goats of above 1 but less than 2 years old (7.25±2.84) than in males (5.01±0.54) mmol/l. In 2-3 years old goats, magnesium, creatinine, were lower in females than in males, the respective values being 1.04±0.13 (0.86±0.07) mmol/l, 66.8±11.2 (87.3±13.8) μmol/l, in parentheses for males. Cholesterol and CK levels were higher in the female than in males ($p<0.001$), the values being 2.18 ± 0.32 (1.19 ± 0.32) mmol/l and 4.19±1.55 (2.50±0.78) μkat/l respectively, with those in parentheses being for males. In 3 - 5 years old goats calcium, phosphorus, bilirubin and cholesterol levels were higher in the former than in the latter sex with values of 2.46 ± 0.19 (2.31 ± 0.11), 2.05 ± 0.66 (1.57 ± 0.52) mmol/l, 3.32 ± 1.04 (2.34 ± 0.58) μmol/l and 2.12 ± 0.46 (1.10 ± 0.31) mmol/l, those in parentheses for males.

Discussion

The results of the tests for Gaussian distribution by the coefficient of skewness (g1), the degree of kurtosis (g2) and the Shapiro Wilk statistic (W) indicate, that values in most parameters in each group followed a Gaussian distribution, thus the models for parametric tests were satisfied. In some groups the data for some parameters did not follow a Gaussian distribution and were tested by the nonparametric method of Wilcoxon scores sums rank procedure (tables 12-14).

Plasma calcium, magnesium, inorganic phosphorus, sodium and potassium concentrations observed in this investigation (table 1, 4, 7) are comparable and in agreement with the ranges reported in other goats (Barakat and El-Guindi, 1967; Barlet *et al.*, 1971; Castro *et al.*, 1977a; Bogin *et al.*, 1981; Catarsini *et al.*, 1982; Payne *et al.*, 1982; Vrzgula *et al.*, 1985; Gray *et al.*, 1988). The conversion factors from conventional to SI units (Dybkær and Jørgensen, 1966) were used for comparisons between the different sources. The concentrations of calcium and sodium reported by Youssef (1985) were lower while magnesium levels much higher than in the present landrace and in most other goats, probably because of biological or analytical differences. Phosphorus concentration in landrace goats (fig. 1, table 1, 4, 7), however, appear to be slightly higher than those observed in other goats, 2.6 - 5.89 mg/dl or 0.84 - 1.90 mmol/l (Barakat and El-Guindi, 1967), 4.8 ± 0.9 mEq/l or 4.8 ± 0.9 mmol/l (Castro *et al.*, 1977a), 1.66 - 2.28 mmol/l (Vrzgula *et al.*, 1985) and 5.35 mg/dl or 1.73 mmol/l (Gray *et al.*, 1988). Plasma sodium concentrations in the present landrace goats are slightly higher than the values reported by Vrzgula *et al.* (1985) i. e. 118.0 - 146.8 mmol/l compared with 139.3 - 164.8, the widest range in this study (table 1). Plasma potassium concentrations of 2.43 mmol/l in Trinidad goats (Youssef, 1985) are lower than the range of values 3.19 - 5.60 mmol/l found in the landrace breed (table 1).

The factors that contribute the differences include age due to differences in basal metabolic rates (Bogin *et al.*, 1981), breed, season and nutrition, lactation, pregnancy or environmental influences. The higher calcium and phosphorus values in young goats observed in this study agree with earlier findings (Kumaresan and Ndzingu Awa, 1984). The lower magnesium levels observed in young than in adult goats are consistent with the results of investigations in other breeds of goats (Upadhyay and Rao, 1985).

Electrolyte levels are also subject to differences due to breeds and ambient temperature variations (Ridoux *et al.*, 1981). Magnesium and phosphorus concentrations are reported to decrease in cold conditions (Upadhyay and Rao, 1985). The effects of pregnancy and lactation shows that plasma calcium and magnesium concentrations are lower towards the end of pregnancy and in early lactation (Akinsoyinu, 1982). The lower values in goats above 2 years of age may therefore be due to pregnancy and lactation effect. Sex and age appear to have no profound effect on plasma potassium concentration in goats (Youssef, 1985).

ALAT and ASAT occur in very small quantities in cells of many tissues in ruminants, the latter about twice the amount of the former and in cytosol and mitochondrial isoforms (Kramer, 1989). They are both good indicators of soft tissue damage (Boyd, 1988). The ranges of plasma activity levels of these enzymes in landrace goats were similar to those in other goat breeds (Castro *et al.*, 1977b; Chiofalo *et al.*, 1982; Kramer and Carthew, 1985; Vrzgula *et al.*, 1985; Bialkowski *et al.*, 1988). ALAT and ASAT are subject to daily, weekly and seasonal variations (Bas *et al.*, 1980). They both increase slightly with age (Braun *et al.*, 1983; Raviart *et al.*, 1987, Bialkowski *et al.*, 1988) and are influenced by pregnancy and lactation (Garnier *et al.*, 1984).

The values for ALP varied greatly between individuals, there were too big ranges and coefficient of variations and in most age groups the data were not distributed in a Gaussian manner. The Wilcoxon rank test indicated major differences between ages ($p < 0.001$), associated with high ALP activity in young goats (fig. 2a) and confirms the negative correlation with age. Similar findings have been observed in other goat breeds (Bogin *et al.*, 1981; Kumaresan and Ndzingu Awa, 1984; Bhattacharyya and Daattagupta, 1987, Bialkowski *et al.*, 1988). The wide variations in ALP activity between individuals is reported in other goats (Ridoux *et al.*, 1981; Kramer and Carthew, 1985; Vrzgula *et al.*, 1985).

The data for CK activity at most ages were not distributed in a Gaussian manner (table 2), as a result of large differences between individual goats. This was also observed by Kramer and Carthew (1985). The values were in agreement with observations from other sources (Garnier *et al.*, 1984). CK activity is influenced by age, lactation and environment (Garnier *et al.*, 1984). CK activity levels are low in new

born kids and increase to almost constant values within four months of age (fig. 2a).

Plasma urea values in landrace goats (table 3, 6, 11) were comparable to those reported in other goat breeds; 21-35 mg/dl or 3.50 - 5.83 mmol/l (Barakat and El-Guindi, 1967), 22 - 24.4 mg/dl or 3.663 - 4.06 mmol/l (Castro *et al.*, 1977b) and 44.45 mg/dl or 7.40 mmol/l (Gray *et al.*, 1988). The values of 1.08 mg/dl or 0.18 mmol/l (Okorie Anugwa, 1986) are lower than in the present studies (table 3, 6, 11). The variations are based on breed, age, nutritional, location and analytical differences. Urea levels are higher in mature than in juvenile goats (fig. 2b), but the concentrations give good estimations of adequacy of dietary protein levels (Blackwell and Libby, 1982).

Creatinine concentrations in landrace goats ranged from 30.0 - 140.0 μmol/l, being the maximum range for all age groups. These are within similar limits as in previous studies where values were 0.87 - 1.38 mg /dl or 76.90 - 122.0 μmol/l (Barakat and El-Guindi, 1967) and means of 1.2 - 2.0 mg/dl or 80 - 106 μmol/l (Castro *et al.*, 1977b). Creatinine levels gradually increase with age to constant levels after six months from birth (fig. 2c; Finco, 1989). Castro *et al.* (1977b) had earlier observed decreases with age in pygmy goats. Creatinine concentrations are relatively stable, being dependent on total body content of creatine from where it is formed. Protein levels in the diet may influence the levels (Blackwell and Libby, 1982). Low energy intake results in decrease of serum levels.

Plasma bilirubin concentrations are higher in juvenile than in adult goats and increase with age to constant values after six months of birth (fig. 2b, table 3, 6, 11). The present values agree with the bilirubin concentration ranges of 2.05 - 11.79 μmol/l (Vrzgula *et al.*, 1985). The trends are, however, contrary to the observations in pygmy goats where bilirubin increase to maximum values in 1-2 years of age (Castro *et al.*, 1977b). In the present landrace goats there is a clear decrease from birth to 6 months of age (fig. 2b).

Plasma glucose concentrations in landrace goats are very close to those of other breeds; 40 -72 mg/dl or 2.22 - 4.00 mmol/l (Barakat El-Guindi, 1967; Castro *et al.*, 1977; Bogin *et al.*, 1981; Vihan and Rai, 1987). The low levels in young goats agree with the observed negative correlation with age (Upadhyay and Rao, 1985) and as in the present study (fig. 2d, table 3, 5, 10). Bas *et al.* (1980) noted within the day variations in glucose concentration, being low in the morning and increasing during the day. Results obtained later, however, showed high glucose levels in cooler months and days of the year (Upadhyay and Rao, 1985).

The ranges and trends of total serum proteins in landrace goat (table 3, 6, 10) are in agreement with those of other breeds of goats (Barakat and El-Guindi, 1967; Castro *et al.*, 1977b; Payne *et al.*, 1982; Vrzgula *et al.*, 1985; Vihan and Rai 1987; Gray *et al.*, 1988). They are lower in juvenile goats than in adults (fig. 2c), therefore significantly

correlated with age (Bhattacharyya and Dattagupta, 1987).

Plasma cholesterol levels in the present landrace goats were higher in young than in old goats (fig. 2d). The concentrations in adult goats were lower than those of 188-306 mg/dl or 4.86 - 7.91 mmol/l observed by Barakat and El-Guindi (1967).

There were no sex differences in ASAT, ALAT, ALP, CK, calcium, phosphorus, magnesium, potassium, glucose, cholesterol and urea. The results are consistent with findings in Boran goats (Chericato *et al.*, 1986b; Youssef, 1985). In some clinical chemical parameters differences due to sex are observed, but they follow no form of permanent trend. Female goats were noted to have lower creatinine, but higher total proteins and globulins (Chiericato *et al.*, 1986a). Bilirubin levels were higher in castrated males than in females (Castro *et al.*, 1977b) and lower in castrated than in intact males. Age is the most important component which affects the level of clinical chemical parameters.

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CHAPTER 15

COMPARISON OF HEMATOLOGICAL VALUES IN BLENDED AND EAST AFRICAN GOATS OF TANZANIA AND BREEDS REARED IN DENMARK

Summary

Erythrocyte counts, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte, lymphocyte, neutrophil, basophil, eosinophil and monocyte counts were determined in 202 blended, 14 indigenous East African 6 - 12 months old goats, 24, 15-45 days, 8 eight months and 27 above three years old pregnant Norwegian dairy goats reared in Tanzania. Comparisons were made with 24 dwarf and 57 Danish landrace goats at 6-12 months of age and 76 adult pregnant Danish landrace goats reared in Denmark. The purpose was to determine the reference ranges in Blended and indigenous East African breeds of goats and compare them with those of other breeds. The hemoglobin concentration, hematocrit, erythrocyte and white blood cells were lowest in Norwegian kids. Highest values were observed in 6-12 months old goats in all the breeds studied, whereafter they decreased to relatively low constant adult levels. The mean corpuscular volumes were highest in kids followed by adult pregnant Norwegian and Danish landrace goats, and lowest in 6-12 months old goats. East African and Blended goats had the smallest mean corpuscular volumes. The hemoglobin concentration, erythrocyte and leukocyte counts were highest in indigenous East African, followed by young Norwegian and Blended goats. The mean corpuscular hemoglobin concentration was highest in Blended while the mean corpuscular hemoglobin higher in pregnant than in other goats. The age and breed differences were statistically significant.

Introduction

Hematological and clinical chemical analysis of blood in animals is an indispensable tool in diagnosis and differential diagnosis of many diseases and knowledge of the reference level intervals of blood parameters in healthy animals is a primary requirement (Wilson *et al.*, 1986). Hematological data is available for many breeds of goats (Wojcik *et al.*, 1986; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988), but because of the influence of other factors apart from diseases, the data reveals

differences with respect to breeds, ages, environments seasons, nutrition, lactation and pregnancy. Interpretation of hematological information for diagnosis is consequently difficult. Accurate diagnostic information requires determination of breed and age specific reference values. Although some caprine hematological data is available in Southern (Nettleton and Beckett, 1976; Gray *et al.*, 1988), Central (Pospisil *et al.*, 1987), Western (Oduye, 1976; Okorie and Anugwa, 1986; Oyewale Olowookorun, 1986) and North Africa (Mohy *et al.*, 1985; Hassan *et al.*, 1986), only one study is known in East African breeds, the Galla goats (Wesonga and Nandokha, 1989). The present study was conducted to determine the hematological values in healthy Blended (a breed blend of Kamori, Boer, Galla and indigenous East African goats), indigenous East African (Sukuma/Masai/Gogo) and tropically adapted Norwegian dairy goats all kept in Tanzania, and compare these with the values of dwarf and Danish landrace goats reared in Denmark.

Materials and Methods

The goats of this study were apparently clinically healthy 202 (121 female, 81 male) 6-8 months old blended, 14 (8 female, 6 male) 8 months old indigenous East African, 24 (18 female, 6 male) 15 - 45 days old, 8 bucks eight months old and 27 above three years of age two months pregnant tropically adapted Norwegian dairy goats. These were located in Mwanza (Blended) and Morogoro (the rest), Tanzania. Goats were grazed outdoors throughout the year without any concentrate supplementation except the dairy Norwegian goats which in addition to pasture grazing, were provided with about 0.5 kg of maize bran and green fodder Guatemala grass in the stalls. Twenty four male West African dwarf 6 - 12 months old, 57 (51 female, 6 male) 6-12 months old and 76 above 3 years old pregnant Danish landrace goats located in Denmark all apparently clinically healthy were similarly examined. These were kept indoors throughout the year with slight degree of outdoor rearing.

Blood samples were collected from the external jugular vein in vacuum tubes containing 0.12 ml, 0.34 mol/l tripotassium ethylene diamine tetraacetate (K_3EDTA) (Becton-Dickinson vacutainers) between 8 and 10 am and analyzed with 4 hours. The number of erythrocytes (RBC) were determined in a hemocytometer after dilution with Hayem-Jørgensen solution (0.5 g mercuric chloride, 5 g sodium sulphate, 1 g sodium chloride in 200 ml distilled water) at a ratio of 1:500. The total leukocytes counts (white blood cells, WBC) were determined by hemocytometer after dilution with methyl violet acetic acid solution at 1:20 dilution. The number of lymphocytes, neutrophils, monocytes, eosinophils and basophils were determined microscopically from a count of 200 leukocytes on thin Leishman stained blood smears. Hematocrit (packed cell volume, PCV) was determined in microhematocrit capillary tubes in

model S 201 microhematocrit centrifuge (Sigma) at 12000 rpm for at least 10 minutes.

Hemoglobin concentrations (Hb) In East African and Norwegian goats were determined after conversion to cyanmethemoglobin with reaction solution (Hemoglobin Merckotest 3317) containing 1.00 mmol/l potassium cyanide, 0.60 mmol/l potassium hexacyanoferrate (III), 2.5 mmol/l phosphate buffer pH 7.2, 1.5 mmol/l sodium chloride and 0.05 % detergent. The dilution was 1:251 (20 μl blood to 5 ml diluent). The absorbance of the formed cyanmethemoglobin was measured in model 1211 Beckman spectrophotometer at λ = 544 nm. A standard cyanmethemoglobin (Hemoglobin merckotest 3298) and blank reaction solutions were run together and the necessary calculations made. This method was applied after correlation studies were performed in Denmark. The spectrophotometric hemoglobin measurements were compared with values of the same sample determined in model S560 Coulter counter (Coulter electronics England). The results were found to be highly correlated and there were no significant differences between the two techniques, therefore enabling performance of the biological comparisons. Hemoglobin concentrations in blended goats (Mwanza, Tanzania) were measured in a hemoglobinometer (Atago, Japan) by conversion to cyanmethemoglobin with the same reaction solution. The cyanmethemoglobin standard and the blank reaction solutions were run together for control of the results. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to standard formulae.

In West African dwarf and Danish landrace goats RBC counts were determined within 1-3 hours of blood sampling using model ZF Coulter counter with an aperture diameter of 100 μm, at threshold 6 and attenuation 500. The background counts were kept at 200 maximum and the instrument adjusted for compensation of coincident passages. Hemoglobin (Hb) concentration and total WBC were determined in S560 Coulter counter. PCV was determined in microhematocrit capillary tubes centrifuged at 12,000 rpm for 5 minutes in a microhematocrit centrifuge and MCV, MCH and MCHC calculated. Thin blood films stained with Leishman stain were used for differential leukocyte counts.

Preliminary studies were earlier conducted for comparisons between hemocytometric and electronic counters in blood cell counting, spectrophotometric and Coulter counter hemoglobin concentrations and PCV values after 5 and 10 minutes of centrifugation. The hemocytometric erythrocyte counts were 5.63 % higher while the leukocyte counts 2.79% lower than those of electronic counters and have been reported before. PCV values were determined after 5 and 10 minutes centrifugation in a microhematocrit centrifuge (Clay- Adams) at 12000 rpm in 152 goats. The differences between each pair were calculated and statistically tested for differences.

Parametric (means, standard deviations) and nonparametric (5^{th}, 95^{th} percentiles,

median) values of haemotogical parameters were determined by the univariate procedure of a statistical analysis system software (SAS, Cary, USA). The coefficient of skewness, degree of kurtosis and the Shapiro Wilk statistic (W) were calculated in order to determine the closeness of fit for the data of each analyte in each group to the Gaussian distribution. The means of hematological analytes were tested for statistically significant differences between sex (within same age and breed), age (in the Norwegian and Danish landrace breeds) and breeds by the general linear models procedure. Wilcoxon scores sums rank tests were performed for all parameters to eliminate the statistical influence on the results.

Results

Comparisons between PCV values after 5 and 10 minutes in the 152 goat blood samples indicated that the former were 12.31 % higher than the latter. The values being 0.3227 ± 0.06216 and 0.28296 ± 0.0422 for 5 and 10 minutes respectively with a mean difference of 0.03974 ± 0.03118.

The mean and median values of RBC, PCV, MCV, MCH, MCHC, Hb, WBC, lymphocyte and neutrophil counts in each goat group were close to each other (table 1-5). The coefficient of skewness and the degree of kurtosis were small and non significant in most parameters and the Shapiro Wilk statistic (W) indicated that data for most parameters were distributed in a Gaussian manner except a few (W in tables 1, 3), and all data for basophil, monocyte and eosinophil counts. The mean ± one standard deviation ($\bar{x} \pm s$) values in all parameters were within the interval from 5^{th} to 95^{th} percentile. The latter nonparametric ranges were within the parametric range $\bar{x} \pm 2s$ in all goat groups (table 1 - 5). In tables 1 and 2 it is shown that there are no sex differences for most parameters within the age of 6 - 12 months.

RBC counts, Hb, PCV and WBC were lowest while MCV highest in kids (fig. 1, table 3, 5), followed by adult pregnant Norwegian (Tanzania) and Danish landrace goats (Denmark). In pregnant goats the MCV and MCH were higher than in 6-12 months old goats (fig. 1, 2). RBC counts and hemoglobin concentrations were highest in 6-12 months old goats of all the breeds under study, even though there were differences between them (fig. 1). MCHC was highest in Blended goats while the MCH was highest in adult pregnant Norwegian goats. The total WBC counts were highest in the East African breed goats (fig. 2). There was a clear negative correlation between the number of erythrocytes with MCV and MCH (fig. 1a, d, 2a). MCV was higher in goats with very few erythrocytes (young Norwegian kids and adult pregnant Norwegian and landrace goats). MCV and MCH (thus the size of erythrocytes) were smallest in Blended, East African and Norwegian breeds of 6-12 months of age which apparently had the highest number of erythrocytes (fig. 1a, c, 2a). The same goats had

the highest concentration of hemoglobin per cell (MCHC) (fig. 2b).

The increasing trend of RBC count, hemoglobin levels, hematocrit, leukocyte counts, decreasing MCV and MCH and constant MCHC was observed in goats reared in Tanzania and Denmark. The highest values of parameters which increased with age were in 6-12 months old goats, those with reverse trends (MCV, MCH) were at lowest levels. After 12 months of age hematological values were relatively low. The MCV for 6-12 months old Norwegian goats were small compared to those of other ages in the breed (table 3). Most of the differences between the breeds were statistically significant (table 4, 5). Significant differences between female and male Norwegian kids and blended goats were not observed in all parameters (table 1 and 2). The number of lymphocytes was higher in female than in male East African breed and vice versa for neutrophils.

Discussion

The results of the tests for Gaussian distribution in the present investigation indicate that data of nearly all parameters for all groups followed a Gaussian distribution (table 1-2). Since the samples were taken randomly the assumptions for statistical models for the powerful parametric tests were fulfilled (Reed *et al.*, 1971; Wu *et al.*, 1975; Siegel and Castellan, 1988). The 5^{th} to 95^{th} percentile interval in most parameters were within the interval of $\bar{x} \pm 2s$, therefore parametric and nonparametric ranges did not appear to have significant differences ia all age groups.

The means of all parameters in the present investigation indicated some deviations from those of goats of other sources though the ranges were comparable. The differences may be attributed to variations in age (Edjtehadi, 1978), breed (Maru *et al.*, 1988), season of study (Domina *et al.*, 1982; Vrzgula *et al.*, 1985; Ginting, 1987), herds (Masoni *et al.*, 1985), nutritional quality and adequacy (Biswas *et al.*, 1986), lactation and pregnancy (Biagi *et al.*, 1988), ambient temperatures (Oyewale and Olowokoorun, 1986) and analytical method (Maru *et al.*, 1988). The influence of age on hematological values is very great and may account for a larger part of the reported variations. This is shown by the differences between values in Norwegian kids, those of 6-12 months old and adult goats as well as in Danish landrace goats of corresponding ages (fig. 1, 2, table 3, 5). Norwegian kids of the present investigation had the lowest values for RBC counts, PCV, hemoglobin concentration and WBC but the highest MCV and relatively higher MCH than in older goats. The highest values of RBC, PCV and hemoglobin concentration and lowest for MCV occurred at 6 - 12 months of age (table 5). This is a general trend in all breeds of goats (Edjtehadi, 1978; Facello *et al.*,1983) despite the reported reverse profiles in some breeds (Nangia *et al.*, 1968; Somvanshi *et al.*, 1987) Upadhyay and Rao, 1985; Bialkowski *et al.*, 1988).

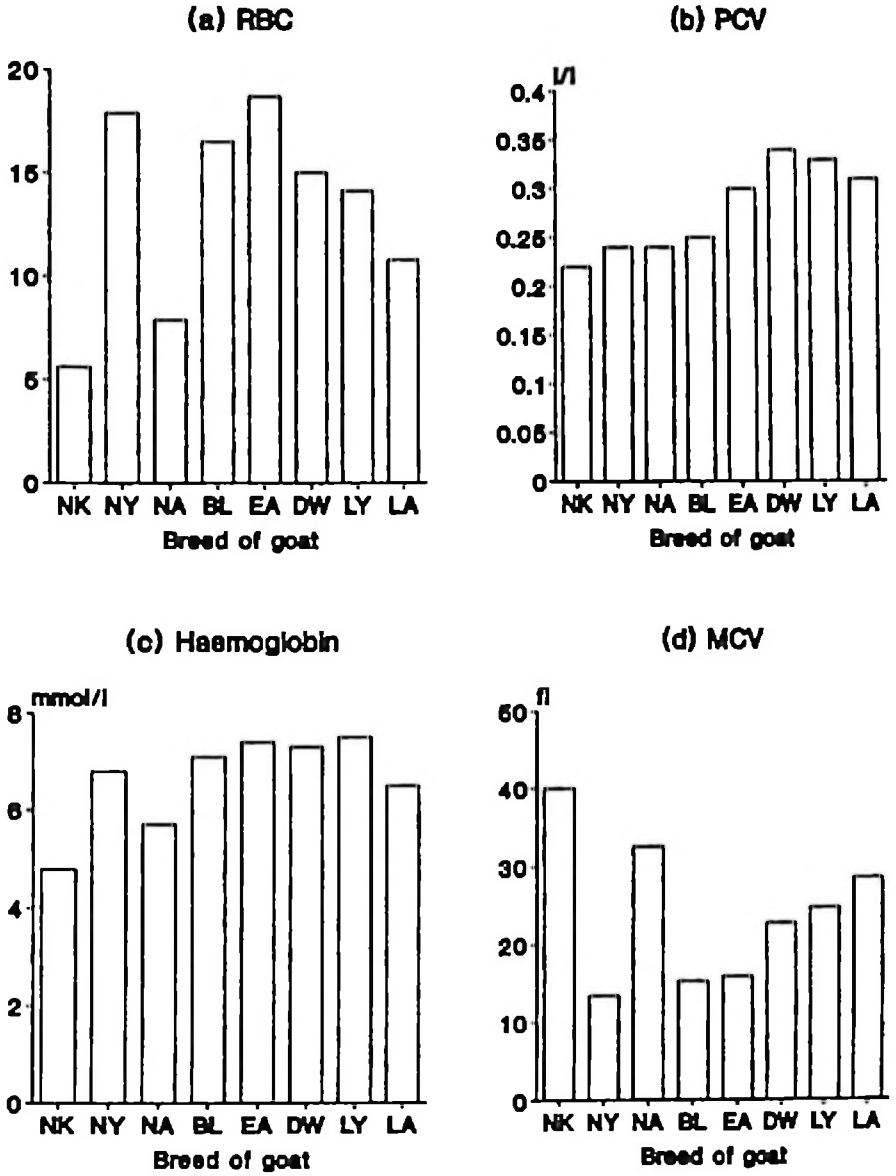

Figure 1: Hematological values in Tanzanian and Danish goats, RBC counts ($x10^{12}/l$) (a), packed cell volume (b), hemoglobin concentration (c) and MCV (d) in Norwegian kids (NK), Norwegian young 6-12 months bucks (NY), Norwegian adult pregnant (NA), 6-12 months old Blended (BL), 6-12 months old East African breed (EA), 6-12 months old Dwarf (DW), 6-12 months old young Danish landrace (LY) and pregnant adult Danish landrace (LA) goats.

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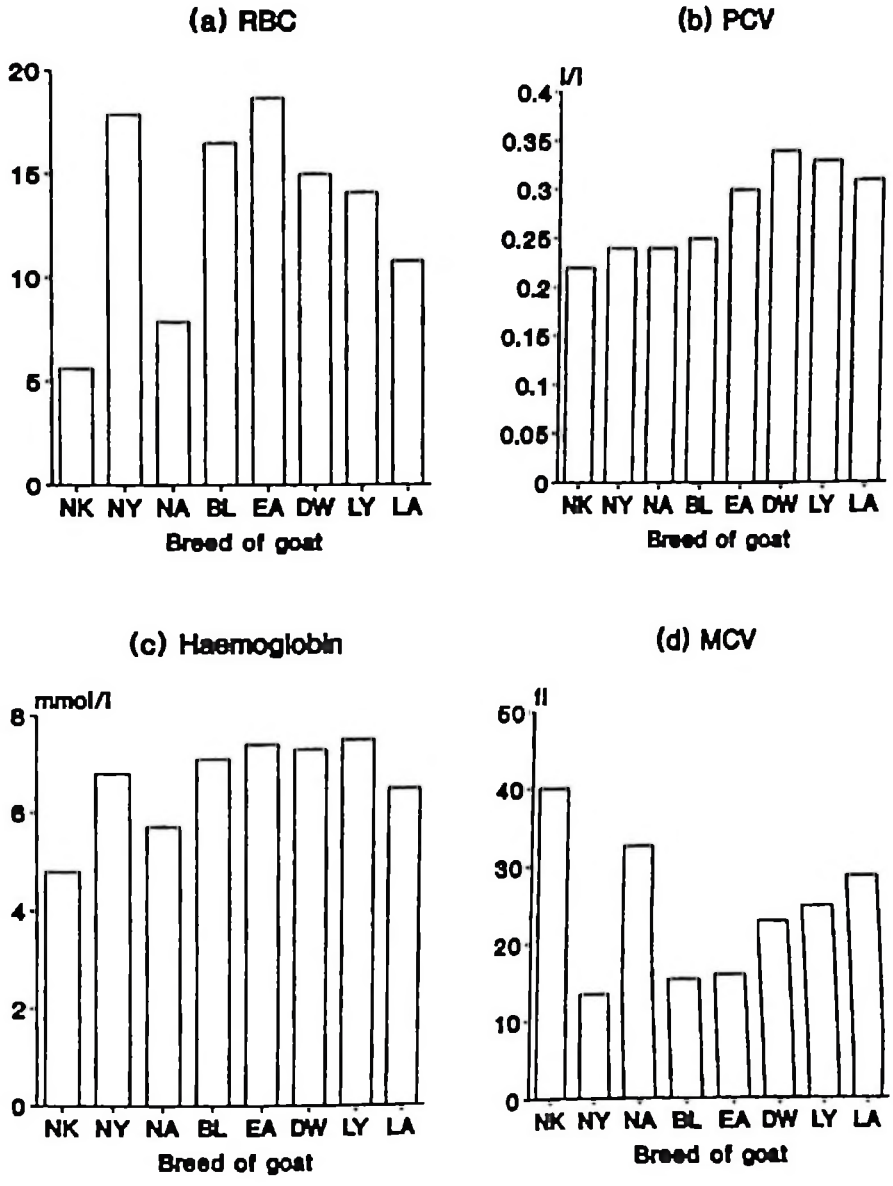

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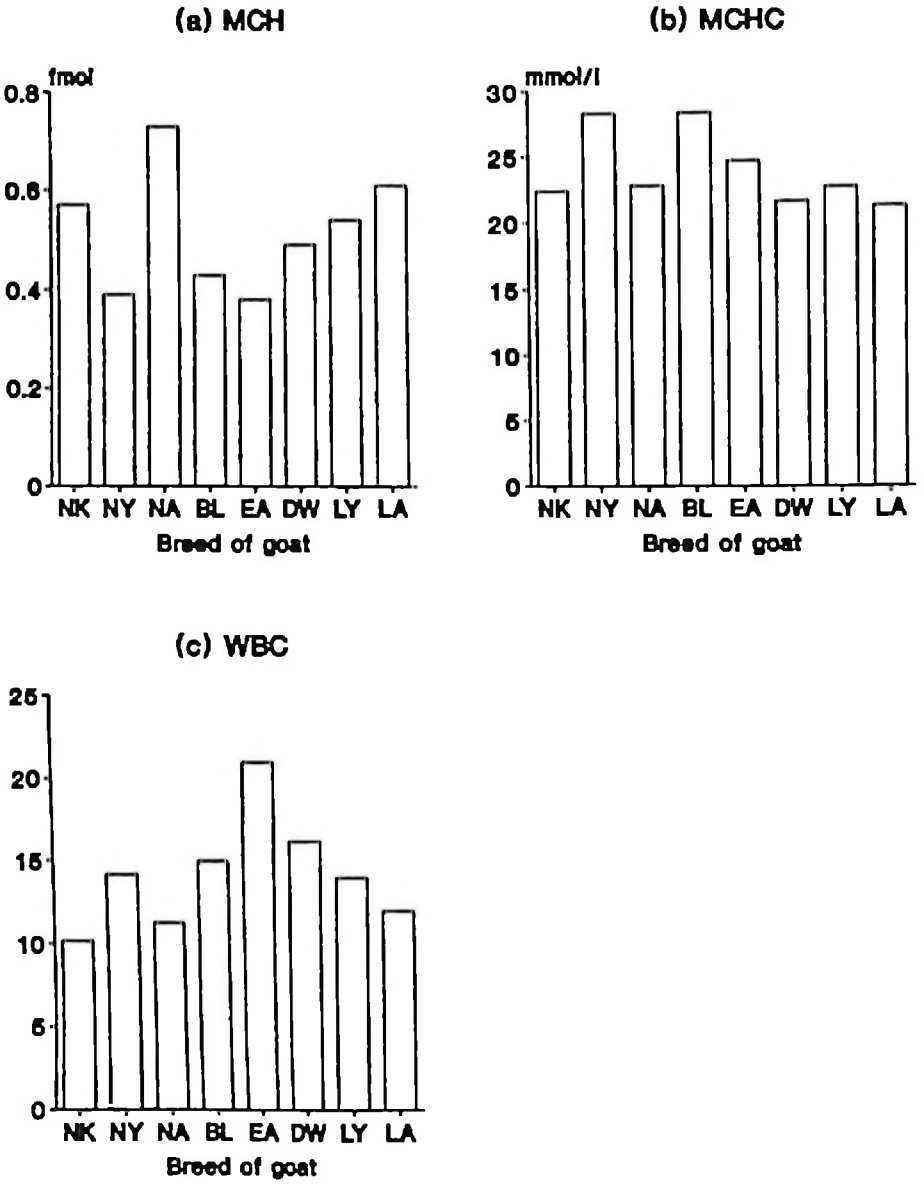

Figure 2: Hematological values in Tanzanian and Danish goats, MCH (a), MCHC (b) and total WBC counts (x 10^9/l) (c) in Norwegian kids (NK), Norwegian young 6-12 months bucks (NY), Norwegian adult pregnant (NA), 6-12 months old Blended (BL), 6-12 months old East African breed (EA), 6-12 months old Dwarf (DW), 6-12 months old young Danish landrace (LY) and pregnant adult Danish landrace (LA) goats.

Table 1: Comparisons between female and male goats in median (Q_2) and mean ± standard deviation values ($\bar{x}$ ± s) and the nonparametric interval from the 5th (P_5) to 95th (P_{95}) percentile of hematological parameters in 6 - 12 month old blended and East African goats.

	Female (n=121)			Male (n=81)			[1]Both (n=202)	
	P_5 - P_{95}	Q_2	$\bar{x}$±s	P_5 - P_{95}	Q_2	$\bar{x}$±s	P_5 - P_{95}	Q_2
Blended								
RBC x10^{12}/l	9.3-22.8	16.3	16.6±2.11	10.3-21.8	16.5	16.2±1.81	10.0-22.5	16.3
PCV l/l	0.21-0.29	0.26	0.25±0.03	0.20-0.29	0.24	0.24±0.03	0.20-0.29	0.25
Hb mmol/l	4.40-8.20	7.10	7.30±0.50	4.30-7.00	6.90	6.90±0.46	4.30-8.20	7.20
MCV fl	13.1-24.8	16.0	15.1±2.72[W]	14.7-24.9	14.5	14.8±2.10[W]	13.1-24.8	15.3[W]
MCH fmol	0.32-0.50	0.43	0.44±0.06[W]	0.31-0.50	0.42	0.43±0.04	0.32-0.49	0.44[W]
MCHC mmol/l	17.9-30.2	27.3	29.2±2.00	18.2-31.7	28.7	28.7±2.22[W]	17.9-35.4	28.8[W]
WBC x10^9/l	11.0-18.8	15.2	15.3±2.20	9.20-19.2	14.5	14.5±2.84	10.6-19.0	15.2
Lymphocyte %	39.0-61.0	47.0	47.8±5.96	42.0-58.0	48.5	49.2±5.00	39.0-61.0	48.0
Neutrophil %	35.5-59.0	50.0	49.2±6.71	36.0-57.0	46.5	46.9±6.24	35.5-58.0	49.0
Eosinophil %	0 - 15			0 - 4			0 - 15	
Basophil %	0 - 4			0 - 4			0 - 4	
Monocytes %	0 - 11			0 - 10			0 - 11	
East African	(n=8)			(n=6)			(n=14)	
RBC x10^{12}/l	11.6-28.3	17.9	18.1±5.47	11.0-25.8	22.4	19.5±6.11	11.0-28.3	19.7
PCV l/l	0.27-0.36	0.31	0.31±0.03	0.28-0.29	0.29	0.29±0.00	0.27-0.36	0.29
Hb mmol/l	6.8-7.90	7.70	7.50±0.43	5.60-7.70	7.40	7.20±0.77	5.60-7.90	7.40
MCV fl	10.2-25.9	17.1	17.8±5.23	11.2-25.5	13.0	16.2±6.06	10.2-25.9	25.9
MCH fmol	0.27-0.63	0.42	0.44±0.12	0.29-0.67	0.33	0.40±0.14	0.27-0.67	0.67
MCHC mmol/l	22.6-27.2	25.5	25.2±1.73	20.1-27.4	25.6	25.1±2.55[W]	20.1-27.4	25.5[W]
WBC x10^9/l	9.00-32.0	18.8	19.1±8.34	9.50-25.8	25.8	23.6±9.67	9.00-33.2	20.2
Lymphocyte %	47.5-72.0	61.0	60.4±8.57**	38.0-55.5	46.7	46.4±7.09**	38.0-72.0	53.2
Neutrophil %	25.0-47.5	37.8	38.1±8.12**	44.5-59.0	51.0	52.2±5.80**	25.0-59.0	46.2
Eosinophil %	0 - 3			0 - 1			0 - 3	
Basophil %	0 - 2			0 - 1.5			0 - 2	
Monocytes %	0 - 6			0 - 11			0 - 11	

Differences in means between sexes, *p<0.05, **p<0.01, ***p<0.001. Ranges given for rare leukocytes
W= Non Gaussian distribution (P<0.05). [1]See table 4 for $\bar{x}$ ± s.

Table 2: Comparisons between female and male 15 - 45 days old Norwegian kids in the median (Q_2) and mean ± standard deviation ($\bar{x} \pm s$) values and the interval from the 5th (P_5) to 95th (P_{95}) percentile of hematological values.

	Female (18)			Male (6)		
	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
RBC $x10^{12}/l$	3.40 - 7.50	5.70	5.48 ± 1.33	4.50 - 6.90	6.40	6.00 ± 1.01
PCV l/l	0.16 - 0.28	0.23	0.22 ± 0.03	0.17 - 0.24	0.20	0.20 ± 0.02
Hb mmol/l	3.80 - 6.10	4.80	4.87 ± 0.76	3.80 - 5.70	4.30	4.58 ± 0.72
MCV fl	32.0 - 59.6	40.0	42.1 ± 9.81	25.0 - 53.3	32.8	35.3 ± 1.07
MCH fmol	0.32 - 0.99	0.51	0.60 ± 0.28	0.25 - 0.99	0.33	0.45 ± 0.29
MCHC mmol/l	17.5 - 25.4	22.1	22.1 ± 2.37	19.1 - 24.7	23.1	22.6 ± 2.19
WBC $x10^9/l$	5.40 - 14.0	11.1	10.7 ± 2.81	5.20 - 12.9	8.30	8.63 ± 2.52
Lymphocyte %	42.0 - 73.5	58.2	56.6 ± 9.59	42.5 - 74.0	60.5	59.1 ± 15.2
Neutrophil %	23.5 - 57.0	40.0	41.5 ± 9.55	24.5 - 56.0	37.7	39.1 ± 15.1
Basophil %	0-1			0-2		
Eosinophil %	0-1.5			0-1.5		
Monocyte %	0-6			0-7		

Table 3: Comparisons between Norwegian goats of different ages reared in Tanzania for the interval from 5th (P_5) to 95th (P_{95}) percentile, median (Q_2) and $\bar{x} \pm s$ of hematological values.

	15 - 45 day old both sex (n=24)			6 - 12 moth old male (n=8)			2 months pregnant >3 years (n=27)		
	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$	P_5 - P_{95}	Q_2	$\bar{x} \pm s$
RBC $x10^{12}/l$	3.50-7.40	5.90	$5.61 \pm 1.26^{a***}$	15.8-19.5	18.0	$17.9 \pm 1.55^{ab***}$	5.80-12.8	7.00	$7.90 \pm 2.04^{b***}$
PCV l/l	0.16-0.26	0.22	$0.22 \pm 0.03^{ab*}$	0.19-0.28	0.24	$0.24 \pm 0.03^{a*}$	0.18-0.31	0.24	$0.24 \pm 0.03^{b*}$
Hb mmol/l	3.80-6.10	4.60	$4.80 \pm 0.75^{ab*}$	5.10-7.90	6.80	$6.80 \pm 0.55^{a*}$	4.80-7.20	5.70	$5.70 \pm 0.89^{b*}$
MCV fl	26.1-58.8	39.8	$40.0 \pm 10.2^{a**}$	11.2-15.6	13.4	$13.3 \pm 1.58^{ab***}$	20.3-44.5	32.6	$32.5 \pm 7.18^{b***}$
MCH fmol	0.26-0.99	0.43	0.57 ± 0.28^{W}	0.28-0.41	0.38	$0.39 \pm 0.03^{a**}$	0.40-0.99	0.73	$0.73 \pm 0.18^{a**}$
MCHC mmol/l	19.1-25.5	22.3	22.4 ± 2.29	17.6-28.2	28.2	28.3 ± 3.69	20.8-29.1	21.7	22.8 ± 2.68^{W}
WBC $x10^9/l$	5.40-14.0	9.70	$10.2 \pm 2.85^{a*}$	10.5-23.4	14.2	$14.2 \pm 2.10^{a*}$	6.60-22.0	10.5	$11.3 \pm 4.40^{b*W}$
Lymphocyte %	42.0-73.5	58.2	$57.2 \pm 10.9^{a*}$	43.0-59.5	50.7	$51.7 \pm 6.58^{a*}$	40.5-62.0	53.5	$52.6 \pm 7.17^{b*}$
Neutrophil %	24.5-56.0	40.0	40.9 ± 10.9	39.5-55.5	47.5	47.3 ± 6.39	35.0-59.0	45.0	45.0 ± 7.40
Eosinophil %	0 - 1			0 - 1			0 - 3		
Basophil %	0 - 1			0 - 3			0 - 3		
Monocytes %	0 - 3			0 - 5			0 - 7		

Similar superscripts indicate significant differences in means between ages (in rows), *$p<0.05$, **$p<0.01$, ***$p<0.001$. W = Non Gaussian distributions ($p<0.05$).

Table 4a: Comparisons of $\bar{x} \pm s$ of hematological parameters between goats of the five breeds at the same ages (6-12 months old).

Parameter	Blended (202)	E. African (14)	Norwegian (8)	[1]Dwarf (24)	[1]Landrace (57)
RBC $x10^{12}/l$	$16.50 \pm 2.00^{abc**}$	$18.7 \pm 5.56^{ad***}$	$17.9 \pm 1.55^{bd***}$	$15.0 \pm 1.12^{abd*}$	$14.1 \pm 1.35^{abd*}$
PCV l/l	$0.25 \pm 0.03^{a**}$	$0.30 \pm 0.02^{ab**}$	$0.24 \pm 0.03^{b**}$	0.34 ± 0.04	0.33 ± 0.04
Hb mmol/l	$7.10 \pm 0.49^{ab**}$	$7.40 \pm 0.60^{ac**}$	$6.80 \pm 0.55^{bcde*}$	7.30 ± 0.75^{ef}	7.50 ± 0.88^{ef}
MCV fl	$15.2 \pm 2.54^{cg*}$	$15.8 \pm 5.44^{bf*}$	$13.3 \pm 1.58^{ae*}$	22.7 ± 3.45^{abcdef}	$24.7 \pm 3.24^{abcfg*}$
MCH fmol	$0.43 \pm 0.05^{abcg*}$	0.38 ± 0.13^{ef}	$0.39 \pm 0.03^{ade*}$	0.49 ± 0.04^{abcef}	$0.54 \pm 0.10^{abfg*}$
MCHC mmol/l	$28.4 \pm 2.07^{ab**}$	$24.7 \pm 2.03^{a**}$	$28.3 \pm 3.69^{b**}$	$21.6 \pm 3.79^{a*}$	22.7 ± 2.71
WBC $x10^9/l$	$15.0 \pm 2.46^{a*}$	$21.0 \pm 8.90^{ab*}$	14.2 ± 2.10	$16.2 \pm 2.83^{b*}$	$14.0 \pm 3.20^{b*}$
Lymphocyte %	$48.3 \pm 5.65^{a*}$	54.4 ± 10.5	51.7 ± 6.58	54.9 ± 8.13	$59.6 \pm 5.75^{a*}$
Neutrophil %	$48.3 \pm 6.61^{a*}$	44.1 ± 10.0	47.3 ± 6.39	42.5 ± 6.80	$36.0 \pm 6.22^{a*}$

Similar superscripts indicate significant differences between breed means within rows, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, [1]Reared in Denmark.

Table 4b: Comparisons of Means of some hematological parameters between goats of five breeds at the same ages (6 - 12 months old) after correction for analytical methods (RBC[1], PCV[2] and WBC[3], with MCV, MCH, MCHC)

	Blended	E. African	Norwegian	Dwarf	Landrace	Remarks
RBC $x10^{12}/l$	15.57	17.65	16.89	15.00	14.1	Higher in tropical goats
PCV l/l	0.25	0.30	0.24	0.30	0.29	Lower in tropical goats
MCV fl	16.1	17.0	14.2	20.0	20.6	Lower in tropical goats
MCH fmol	0.46	0.42	0.40	0.49	0.54	Lower in tropical goats
MCHC mmol/l	28.4	24.7	28.3	24.3	25.9	Higher in tropical goats
WBC $x10^9/l$	15.0	21.0	14.2	15.8	13.6	Higher in tropical goats

[1]Hemocytometric count for Blended, E. African and Norwegian (thus -5.63%)
[2]PCV for Dwarf and Landrace goats is based on 5 minute centrifugation (thus -12.31%)
[3]WBC for Dwarf and Landrace goats based on Coulter counter results (thus - 2.79%)

Table 5: Comparisons between hematological values between 6-12 months old Dwarf and landrace, and adult pregnant Landrace goats examined in Denmark, the interval from the 5th (P_5) to 95th (P_{95}) percentile, median (Q_2) and means.

	Dwarf (24)			Landrace (57)			Pregnant adult Landrace (76)		
	P_5 - P_{95}	Q_2	$\bar{x}$	P_5 - P_{95}	Q_2	$\bar{x}$	P_5 - P_{95}	Q_2	$\bar{x}\pm s$
RBC x 10^{12}/l	10.9-18.0	14.9	15.0^{a*}	10.2-20.6	14.0	14.1^{b*}	8.98-13.1	10.6	10.8±1.35^{ab*}
PCV l/l	0.22-0.40	0.35	0.34	0.23-0.45	0.33	0.33	0.25-0.37	0.30	0.31±0.04
Hb mmol/l	5.10-8.40	7.20	7.30	5.30-9.30	7.30	7.50^{a*}	4.80-8.20	6.50	6.49±0.88^{a*}
MCV fl	15.7-34.9	22.7	22.7^{b*}	20.1-33.9	25.3	24.7^{a*}	24.9-35.3	27.9	28.5±3.24^{ab*}
MCH fmol	0.41-0.56	0.49	0.48^{a*}	0.47-0.72	0.55	0.54	0.40-0.72	0.61	0.61±0.10^{a*}
MCHC mmol/l	13.4-38.2	21.0	21.6	19.6-24.6	21.6	21.7	13.9-23.8	22.0	21.3±2.71
WBC x10^9/l	7.70-21.9	16.1	16.2^{a*}	9.40-21.4	13.5	14.0^{b*}	6.00-17.2	12.2	12.0±3.20^{ab*}
Lymphocyte %	53.2-75.5	54.0	54.9	44.4-65.4	62.0	59.6	48.7-53.1	45.7	48.4±5.75
Neutrophil %	30.8-55.0	41.4	42.5^{a*}	27.7-57.0	35.6	36.0^{ab*}	36.3-51.0	41.1	42.9±6.22^{b*}

Similar superscripts indicate significant differences within rows, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

The values then decline to relatively low stable adult levels after 12 months of age.

The observations that WBC counts are higher in young than in old goats (Nettleton and Beckett, 1976; Earl and Carranza, 1980; Bialkowski *et al.*, 1988) is due to grouping of goats less than 12 months old as young and those above as mature. Caprine neonatal hematological studies have revealed minimum WBC counts at birth (Kanemaki *et al.*, 1986; Somvanshi *et al.*, 1987). These findings appears to be unequivocal. WBC counts then increase with age to maximum values at 6-12 months of age then decrease to adult levels. They were highest in 6 - 12 months old Blended and East African goats, probably as a result of exposure to various degrees of parasitic infestations that occur in the tropics.

RBC values were found to be lower in blended than in indigenous East African and Norwegian goats of the same ages (table 4), probably because of breed influence. Breed variations in hematologic values are observed in goats (Unanian, 1986). These are probably modified by environmental conditions such as temperatures, humidity, rainfall, nutrition and air conditions of the place where goats are kept. Blended goats of this study had the highest MCHC, and it was observed that the higher the RBC counts the smaller the MCV and the higher the MCHC. In this study East African and Blended goats had the smallest MCV (fig. 1). However, different breeds are observed to have similar levels of blood parameters if reared under the same environment and fed on identical nutrition (Neto *et al.*, 1986). It may thus be deduced from these

acquired similarities that the breed influence is not as large as that of age. Though it contributes to the differences in various data sources, it is rather subject to environmental modifications.

There appears to be no consistent differences in all parameters between male and female goats in all the breeds studied indicating that sex has little influence in the level of blood parameter (Oduye, 1976; Somvanshi *et al.*, 1987). In adult female goats, however, the levels of parameters are altered during pregnancy and lactation (Mohy *et al.*, 1985; Biagi *et al.*, 1988) and might be reflected as sex differences when compared with male goats. This is also the case for castrated male goats (Castro *et al.*, 1977). There are a few reports of sex differences in RBC counts, PCV, Hb and MCV in some goat breeds (Jain, 1986).

The method of analysis is another factor that may have contributed to the differences. Hemocytometric RBC counts are higher while WBC lower than those of electronic counters not specifically adapted to small MCV because of the pipette and chamber errors in the former and failure of compensation for coincident particle passages in the latter method. This is important in goats because of the large number of erythrocytes that are small in size. The higher the number, and also the smaller the size of cells the greater the coincident passages because this follows a Poisson law so that electronic counters are accurate for solutions with low number of cells and vice versa. RBC and WBC counts are therefore subject to inter laboratory variations and this was observed to be the case by Lorenz *et al.* (1978). Furthermore the small size of goat erythrocytes reduces the sedimentation rate, thus the determination of hematocrit requires centrifugation time longer than 5 minutes (Maru *et al.*, 1988; table 4b). Jain (1986) recommends a minimum time of 10 minutes for obtaining a constant PCV value in goats. Most of the reported PCV values are based on 5 minute centrifugation (Wesonga and Nandokha, 1989). Correction of analytical methods brings the values somehow close together indicating their large influence in reported levels (table 4b). This factor causes large differences in PCV, MCV and MCHC.

The PCV values in dwarf and Danish landrace goats appear to be larger than in other breeds despite having relatively low RBC counts (fig. 1, table 4a). This is because they were determined after 5 minutes centrifugation and the difference was close to being eliminated after analytical corrections (table 4b). The RBC and WBC counts and MCHC were confirmed to be higher while the MCV lower in tropical (Tanzanian) than in Temperate (Danish) goats. The observed differences in hematological values between the present goats and other sources are therefore due to the above factors.

In some breeds of goats there appears to be more neutrophils than lymphocytes at birth, but the reverse is true in mature goats. In the latter 50 - 70 % of leukocytes are lymphocytes and 30 - 50 % are neutrophils (Payne *et al.* 1982). Other sources,

however, indicate reversed profiles (Nangia *et al.*, 1968; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988). The present results in indigenous East African, Blended, Dwarf, Landrace and Norwegian goats show that most leukocytes are lymphocytes at most ages. Band neutrophils, eosinophil, basophil and monocyte counts are very low in all ages.

Hematological values in blended, indigenous East African and Norwegian goats were found to be within the wide range intervals reported in many breeds. The specific means were different between the breeds (in similar age goats) and ages (within the breeds). Reference hematological and clinical chemical values must be carefully determined for different breeds specified to age, and for specific metabolic studies to herds.

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CHAPTER 16

GENERAL DISCUSSION AND CONCLUSIONS

Introduction

This chapter discusses the factors which affect the levels of hematological and clinical chemical values in goats. The factors are also relevant to other species. The results of this investigation which are presented in chapters 3-15 are discussed in comparison to the reported literature values. The chapters are serially referred to as I to XIII. The influences of age, breed, sex, herd, pregnancy, lactation and parity were evaluated. In addition to these, the effects of nutrition, season, ambient temperatures and drugs are discussed. The conclusions are based on the current studies.

The influence of Age

Supravital staining of blood of newborn Dwarf and Landrace goats with new methylene blue have revealed large basophilic polychromatophilic erythrocytes and punctate or aggregated reticulocytes (macrocytes) in addition to mature erythrocytes of normal sizes (II[1]). The numbers of the former two erythrocytic cells are highest during the first week of life and diminish fast from the circulation. On the basis of osmotic resistance Perk *et al.* (1964) and Facello *et al.* (1985) demonstrated decreasing numbers of erythrocytes of high resistance and an increasing fragile erythrocyte population. Deductions can therefore be made with high confidence that the two erythrocyte populations consist of the supravitally stainable polychromatophilic erythrocytes and reticulocytes on the one hand, and mature erythrocytes on the other. The Romanowsky stained blood smears showed polychromasia and anisocytosis. The differences in sizes and staining intensities are due to the presence of immature cells at different stages of erythropoiesis. Since immature hematopoietic cells are larger than those at more advanced hematopoietic stages, these morphological findings support the observations of large erythrocyte diameters and mean corpuscular volumes at birth and their decrease with age (II, III; Facello *et al.*, 1983; Löhle *et al.*, 1990).

It is well established that the number of erythrocytes is inversely proportional to the volume, and mammals with large numbers of erythrocytes (sheep and goats) show the smallest cellular sizes. This negative correlation between the number of erythrocytes, PCV and hemoglobin concentration on one side, and the MCV and

[1]Chapters 3 to 15 of this thesis are referred to in this discussion as I-XIII.

MCH on the other is confirmed by the finding that RBC counts, Hb and PCV were shown to be lowest in kids, while the MCV and MCH highest (III). The MCV and MCH were also higher in adult pregnant than in 6 - 12 months old goats. The MCV and MCH were smallest in Dwarf, Landrace, Blended, East African and Norwegian goat breeds of 6 - 12 months of age which had the largest number of erythrocytes with the highest concentration of hemoglobin in each (MCHC). The increase in RBC counts with age has been observed in other breeds of goats (Holman and Dew 1964; DeShaw *et al.*, 1969; Ejtehadi, 1978; Löhle *et al.*, 1990). Highest RBC counts, thus lowest MCV and RBC diameters occur in the period from 6-12 months of age. In goats above one year old, erythrocyte counts decrease (Homan and Dew, 1963). The RBC count, PCV and Hb were found to be higher in young adult goats 8 - 12 months and 1 - 2 years old compared to those above this age. The MCV, MCH and MCHC are lower in the young (1 - 2 years old) than adult goats of above 2 years of age (VIII). The results agree with those reported for other goats (Holman and Dew, 1965a; Nangia *et al.*, 1968; Edjetihadi, 1978; Somvanshi *et al.*, 1987). It is frequently reported that erythrocyte counts are highest at birth and decrease with age (Nangia *et al.*, 1968; Oduye, 1976; Upadhyay and Rao, 1985; Somvanshi *et al.*, 1987; Bialkowski *et al.*, 1988). These findings are not, however, supported by physiological or morphological observations. The decrease in number would imply that the cells become larger in size with increasing age, but morphological studies have unequivocally shown the opposite (II, III; Facello *et al.*, 1983). A large proportion of hemoglobin in neonatal goats is of the fetal type which occur in large erythrocytes (Facello *et al.*, 1984), which are presumably immature macrocytes. This provides further evidence for low rather than high erythrocyte counts at birth. There is therefore enough evidence in support of the conclusion that the erythrocyte counts in kids are very low at birth and increase with age, particularly in the first 6 months, with gradual changes in subsequent growth periods.

The MCV, MCH and erythrocyte diameters are highest in neonates of all the goat breeds of the present study, being inversely proportional to RBC counts, therefore following the opposite trend to that of RBC counts (III, XIII). They are found to decrease with age to lowest levels at 6 - 12 months of age also in other goats (Holman and Dew, 1964; Löhle *et al.*, 1990).

Hematocrit and hemoglobin concentration values are low at birth in dwarf and landrace kids, decreasing further in the first two weeks of life before increasing with age to maximum values at 6 - 12 months of age in both breeds (III) and in others (Holman and Dew, 1964; 1966a; Edjetihadi, 1978). The changes in MCHC are little and non significant in Dwarf and Landrace goats, supporting results obtained earlier (Holman and Dew, 1964). The influence of age was not observed in Pashmina goats (Mazumder *et al.*, 1982), but this was probably due to the wide age group intervals (in

years).

There are great variations among sources on the leukocyte profile from birth to maturity. The total leukocyte counts are low at birth in Dwarf and Landrace goats and increase with age to maximum at 6-12 months of age, whereafter they decreased (IV). The total leukocytes and total lymphocyte counts are, however, still higher in young adult goats of 8-12 months and 1-2 years of age compared with those above 2, but it was vice versa for neutrophil counts (VIII). The majority of leukocytes are lymphocytes and neutrophils. The number of band neutrophils is high in newborn goats, whereas monocyte, basophil and eosinophil counts fluctuate little with age. In many goat breeds there are minimum counts of total leukocyte counts at birth, as in the present Dwarf and Landrace goats (IV), increasing with age to maximum values at 6-12 months (Holman and Dew, 1965b; DeShaw *et al.*, 1969; Castro *et al.*, 1977a; Upadhyay and Rao, 1985; Kanemaki *et al.*, 1986; Somvanshi *et al.*, 1987). In others, WBC counts are higher in young than in old goats (Wilkins and Hodges, 1962; Nangia *et al.*, 1968; Vaidya *et al.*, 1970; Nettleton and Beckett, 1976, Earl and Carranza, 1980; Mazumder *et al.*, 1982; Bialkowski *et al.*, 1988). Other studies revealed no particular age trends (Neto *et al.*, 1986). These disagreements are probably due to differences in breeds and specific environmental or prenatal conditions. The number of leukocytes in pregnant goats increase near parturition (Wojcik *et al.*, 1986; Fortagne and Schafer, 1989), the factors triggering this increase may probably affect WBC counts in the kid due to the nature of maternal-fetal circulation relationship. The high number of leukocytes in the neonate may also be due to the sequence of parturition events and the duration in individual goats. The present Dwarf, Landrace and Norwegian goats, however, showed minimum counts at birth and increasing profile with age for leukocytes of all types.

Plasma electrolyte levels are reported to be higher in young than in adult goats due to high metabolic rates, milk source and fast osteogenesis, particularly for calcium and phosphorus (Castro *et al.*, 1977c; Bogin *et al.*, 1981; Kumaresan and Ndzingu Awa, 1984; Upadhyay and Rao, 1985; Vrzgula *et al.*, 1985; Bhattacharyya and Duttagupta, 1987; Gray *et al.*, 1988). The results in Dwarf and Landrace goats are in agreement with the trend of high birth values for calcium, magnesium and inorganic phosphorus concentrations and their decrease with age (V). Magnesium concentration in Dwarf and Landrace kids briefly increase before decreasing with age. Calcium, magnesium and phosphorus levels have been observed to increase after colostral intake (Braun *et al.*, 1983), but thereafter they decrease with age. Sodium concentration follows a similar pattern but the changes are slight, whereas potassium increases slightly with age (V).

Plasma alkaline phosphatase activity is reported to be higher in young than in adult goats because of bone formation (Bogin *et al.*, 1981; Bhattacharyya and Duttagupta,

1987; Bialkowski *et al.*, 1988; Kramer, 1989) and in pregnant goats due to fetal demands (Kumaresan and Ndzingu Awa, 1984). In support of these findings higher ALP activity was observed at birth than at other ages in Dwarf and Landrace goats and the values decreased during growth (VI). Plasma ALAT, ASAT and CK activities are low at birth, then increase with age probably as a result of increasing body mass, metabolic and muscle activities (Kramer, 1989). Earlier studies had observed no difference in plasma ALAT activity between young and old goats (Bogin *et al.*, 1981) but slight increases within the first week of birth were noted (Braun *et al.*, 1983). ASAT activity shows an increasing profile from birth to constant values after two months of age (VI; Bialkowski *et al.* (1988). CK activity was observed to increase gradually from the low neonatal values to constant levels within 8 - 12 months of age (VI). There are high individual goat variations in CK activity which are probably due to its dependence on the muscle mass (Ridoux *et al.*, 1981; Kramer and Carthew, 1985).

Urea concentration in Dwarf, Landrace and other breeds of goats decrease in the second week but gradually increase with age afterwards (VII; Braun *et al.*, 1983). The concentrations of creatinine are minimum at birth and increase with age (VII). Creatinine values are constant in healthy adult goats because they depend on total body content of its sole precursor, creatine. The amount of the latter in turn depends on the diet, rate of synthesis from renal, intestinal and pancreatic arginine and glycine and on muscle mass (Finco, 1989). Bilirubin values are higher in neonatal Dwarf and Landrace goats than at other ages (VII), then follows a decreasing profile with age to become constant within 12 months. Transient decreases are reported to occur in neonates (Braun *et al.*, 1983) but were not observed in the present study.

Glucose concentrations are higher in neonatal goats than in other age groups, showing decreasing trend with age to constant levels within 6 months of age (VII). A further increase occurs immediately after colostral intake (Raviart *et al.*, 1987). Similar age profiles have been reported in other goat breed (Bogin *et al.*, 1981; Upadhyay and Rao, 1985; Pereka and Riis, 1987; Bialkowski *et al.*, 1988).

Total serum protein concentrations are low at birth in Dwarf and Landrace goats, and increasing with age (VII). An immediate increase occurs following colostral ingestion (Braun *et al.*, 1983; Kanemaki *et al.*, 1986; Raviart *et al.*, 1987). A slight decrease in total serum proteins in the first to 6th week of life was observed in Dwarf, Landrace (VII) and Polish (Bialkowski *et al.*, 1988) goats. This is probably due to disappearance of colostral proteins (Braun *et al.*, 1983). Serum proteins show an increasing profile with age in Dwarf and Landrace (VII) and most goat breeds (Bogin *et al.*, 1981; Upadhyay and Rao, 1985; Bhattacharyya and Duttagupta, 1987). This is likely to be because of build up of immunoglobulins.

Cholesterol levels are low in dwarf and landrace kids in the first week of life,

increasing dramatically within 6 weeks, whereafter they decreased (VI). The initial increase is probably due to colostral intake (Raviart *et al.*, 1987). Cholesterol levels in adult goats are highest in the first week following parturition (X), it is likely that goat colostrum has high content of cholesterol. Cholesterol levels in the present Dwarf and Landrace breeds were higher in young goats (above two weeks of age) than in goats of 6 months and above. The values decrease with age to adult levels (X) as found in other breeds (Castro *et al.*, 1975; Bialkowski *et al.*, 1988).

The influence of sex

There are no statistically significant differences in most hematological and clinical chemical values between female and male goats (II - VII). In parameters where differences are observed, there are no consistent trends as to a value being higher or lower in one sex over the other for all ages (I-VI; Holman and Dew, 1966b; Oduye, 1976, Unanian, 1986). The number of reticulocytes were observed to be higher in female than in male two weeks old Dwarf and Landrace goats but not at later ages (II). The values for PCV, Hemoglobin concentration, MCV, MCH, MCHC, RBC, lymphocyte, neutrophil, eosinophil, basophil and monocyte counts were not significantly different in nearly all age groups of goats between female and male goats of Dwarf, Landrace, Blended, East African and Norwegian goats (III, IV, XI, XII, XIII) and also other breeds (Wilkins and Hodges, 1962; Oduye, 1976; Somvanshi *et al.*, 1987). Although significant sex differences in RBC and total leukocyte counts, PCV, MCV and MCHC at some ages (III, IV; Vaidya *et al.*, 1970; Pospisil *et al.*, 1987), they do not follow any particular trend.

The concentration of plasma calcium, inorganic phosphorus, magnesium, sodium and potassium, ALP and ALAT were not significantly different between female and male Dwarf and Landrace goats at some ages (V, VI), Trinidad (Youssef, 1985) and Boran goats (Chiericato *et al.*, 1986a). At some ages, however, urea, glucose, creatinine, bilirubin, cholesterol, ASAT and CK values were observed to differ significantly between female and males (XI), concurring with results of other investigations (Castro *et al.*, 1977b; Chiericato *et al.*, 1986b). Plasma sodium, urea, bilirubin, proteins and cholesterol tended to be higher, while creatinine, ASAT and CK lower in female than in male goats (VII, VIII). Earlier, reports indicated cholesterol levels to be higher in male than female goats (Castro *et al.*, 1975). The patterns appear to be non predictable. Creatinine, total serum proteins and globulins were also earlier reported to be higher in female than in male Boran goats (Chiericato *et al.*, 1986b). Bilirubin levels are higher in castrated males than in female and non castrated male pygmy goats (Castro *et al.*, 1977d).

In adult female goats, specific metabolic activities related to pregnancy and

lactation might further have influences on blood parameters and reflected as sex differences in comparisons with male goats. Despite the observation of some sex differences in some clinical chemical and hematological parameters at some ages, it can generally be stated that there appears to be no permanent trend of differences between female and male young goats, adults may be affected by the confounding influence of pregnancy and lactation.

Influence of the breed

A genotypic influence on clinical chemical parameters is manifested by differences between breeds. Significant differences are observed in RBC, reticulocyte and WBC counts PCV, hemoglobin concentration, MCV, MCH, MCHC, urea, glucose, bilirubin, creatinine and serum proteins, plasma enzymes and electrolytes between Dwarf and Landrace goats at some ages (II - VII). The differences are not uniformly consistent for all ages, that a specific trend of values in one breed being higher or lower than those of the other. In Saanen and Alpine goats breed differences are observed for total proteins, urea, creatinine, bilirubin, calcium, inorganic phosphorus, ALAT, ASAT and ALP (Ridoux *et al.*, 1981). In Tibetan dwarf and Tibetan - Maltese cross breeds, significant differences are observed for proteins, albumin, lipids, glucose, cholesterol and urea, although they were also under influenced by season (Pugliese *et al.*, 1982). In many goat breeds differ significantly in various parameters (Davies and Sims, 1983). Differences between breeds occur in many parameters, for example erythrocyte fragility (Mahanta *et al.*, 1983; Failey *et al.*, 1988). Erythrocytes in pygmy goats are more susceptible to hemolysis than those of Toggenburg goats (Failey *et al.*, 1988), the differences being attributed to RBC membrane composition differences. However, pygmy goats under comparison were 7-13 months old whereas Toggenburg goats were aged 1-5 years, therefore age might account for differences as well.

Although breed variations are reported in goats, there also investigations where breed differences were not significantly different. The concentrations for calcium, phosphorus, potassium, magnesium and sodium are not significantly different in Tibetan dwarf and Tibetan - Maltese crosses (Catarsini *et al.*, 1982). Most hematological values in Anglonubian, Bhuj, Caninde, Moxoto and Sem Raca definida breeds of goats do not appear to differ significantly (Unanian, 1986), which were in agreement with results obtained in another study in Toggenburg, Faun, Saanen and Camoun goat breeds (Neto *et al.*, 1986).

There are also great individual goat variations in many plasma enzyme activities in may breeds (VII, Bas *et al.*, 1980; Chiofalo *et al.*, 1982) masking the differences between them. Higher values for ALP are observed in Landrace than in Dwarf goats at most ages (VII). Urea, creatinine and bilirubin concentrations varied more in

Dwarf than in Landrace goats during growth and were significantly higher in the former than in the latter at most ages of the developmental period (VII).

Glucose and total serum proteins levels were noted to be significantly higher in Landrace than in Dwarf kids at some ages. The lack of a specific pattern for the herd differences throughout all ages suggest that the influences of the breed on blood values is weak and is subject to environmental moderation. Breed differences in hematological and clinical chemical values ought to exist because of genetic control of breed traits.

The Influence of herd

There were differences in hemoglobin concentration, PCV, MCV, MCH, MCHC and RBC total leukocyte, lymphocytes and neutrophils counts (III, IV, VIII), plasma electrolytes (V, IX), enzyme activities (V, IX) and other clinical chemical parameters (VII, X) in Landrace goats of similar ages from different herds at some ages. The influence of the herd on hematological and clinical chemical parameters appears to be of higher magnitude than those of sex and breed. This factor together with age and analytical methods (I) account for the major part of the observed differences from various sources (Davies and Sims, 1983; Masoni *et al.*, 1985). All parameters were significantly different in goats of the same age and breed (Landrace) from different herds (III - X, XIII). The differences between places are enormous and most probably result from specific environmental meteorological effects in addition to differences in nutrition (Bas *et al.*, 1980; Vrzgula *et al.*, 1985; Hassan *et al.*, 1986). Herd and age variations are the most important sources of differences in goats (III - IX; Masoni *et al.*, 1985; Biagi *et al.*, 1988a).

The influence of Lactation, Pregnancy and Parity

Pregnancy and lactation are increasingly being identified as factors having major influence on the hematological and clinical chemical reference values. During pregnancy the RBC and WBC counts, PCV, hemoglobin concentration decrease, while MCV, MCH and MCHC increase in Landrace (VIII, IX), other goats (Holman and Dew, 166b), French (Masoni *et al.*, 1985), Baladi (Mohy *et al.*, 1985), Saanen (Biagi *et al.*, 1988a) and Dwarf breeds (Löhle *et al.*, 1990) and revert to original non pregnant values during lactation. The extent of the effects in individual goats differ according to the age and number of pregnancies (parity). Values for RBC counts, PCV and hemoglobin concentration are much lower while MCV, MCH and MCHC much higher in pregnant goats of higher than of lower parity. MCH and MCHC are maximum around birth. The WBC, lymphocyte and neutrophil counts are low during pregnancy,

increase near parturition period, then decrease during lactation in Landrace goats, the effects being directly proportional to parity (IX). Leukocyte counts are maximum within the first to second week of parturition. The low number of leukocytes during pregnancy and the increase at parturition and early lactation is probably a response to parturition trauma, infections, puerperal conditions the subsequent and uterine involution. This is observed in many breeds of goats (Holman and Dew, 1966b) including Baladi, Saanen and Dwarf goats (Mohy *et al.*, 1985; Biagi *et al.*, 1988; Fortagne and Schafer, 1989).

In some goat breeds hematological trends opposite to the above have been observed i.e. increases in RBC and WBC counts, MCV during pregnancy (Perreira *et al.*, 1987), with higher MCV and MCH, but lower RBC and WBC counts in older than in younger animals. In others there are no statistically significant difference between pregnant and nonpregnant goats (Pospisil *et al.*, 1987). These are, however, exceptions because decreases of hemoglobin concentration, PCV and RBC and WBC counts, increases in cell size and haemoglobin contents are frequently reported in pregnant goats and cows, with leukocytes increasing at parturition and early lactation (VIII; Payne *et al.*, 1975). The differences between investigations are probably due to nutrition as Hb and PCV are correlated with dietary crude protein intake, stage of lactation (Pelletier *et al.*, 1985) and the levels of nutrition (Biswas *et al.*, 1986). The RBC, hemoglobin concentration and hematocrit decreases are proportional to milk yield and parity (Payne *et al.*, 1975; Hassan *et al.*, 1986; IX).

The increase in WBC, lymphocyte and neutrophil counts following parturition was distinct and in support of results of Vihan and Rai (1987). The decline in leukocytes in pregnancy and lactation is more pronounced in old adult than in young goats of low parity. The latter, however, have higher initial WBC count values than the former in adult animals (Nangia *et al.*, 1968). The further decrease in leukocyte counts in goats of high parity might be an additional effect of the age.

Plasma calcium and inorganic phosphorus concentrations decrease during advanced pregnancy through parturition to mid lactation directly proportional to the number of lactations (IX). There is an initial low level of calcium, magnesium and phosphorus at the onset of lactation, declining trends of which are already apparent at the end of pregnancy. The concentrations increase up to the end of lactation (IX; Akinsoyinu, 1982). These increases of calcium, magnesium and potassium in lactating goats are only slight in Saanen goats (Biagi *et al.*, 1988a). Magnesium, sodium and potassium tend to increase only slightly with increasing parity in both pregnancy and lactation. High phosphorus levels at parturition and postparturient periods were observed in Barbari goats (Vihan and Rai, 1987) but contrary to the findings in Landrace goats (IX) there are no differences in the levels of calcium and magnesium between pregnant and non pregnant animals. The results between sources vary because of

nutritional, breed, herds, seasonal, age and parity differences of the goats (Bas *et al.*, 1980; Ridoux *et al.*, 1981; Vrzgula *et al.*, 1985), and in analytical methods (I).

The influence of pregnancy and lactation on electrolytes becomes higher with increasing parity. During pregnancy much lower calcium, magnesium and potassium but higher phosphorus were observed in goats of high than of low parity. This trend resembles that seen in cows (McAdam and O'Dell, 1982) where cows with many lactations have much higher risks of post parturient hypocalcemia than young ones.

ALAT and ASAT decrease in pregnancy but increase close to parturition and during lactation, while CK activity is low in lactating Landrace goats (IX). ASAT levels are highest in early lactation. ALAT, ASAT and CK activities are highest at around parturition in young goats with low parity. The decrease of ALAT and ASAT activities in the last stages of pregnancy and the increase following parturition occurs also in Barbari goats (Vihan and Rai, 1987). During pregnancy and early lactation ALAT, ASAT, ALP and CK are lower in goats of higher than those of lower parity (IX). Plasma CK activity slightly decreases at the onset of lactation but increases thereafter with lactation (IX; Garnier *et al.*, 1984).

ALP activity levels in goats are unpredictable and vary so greatly that the conclusions on the metabolic profile of this enzyme are equivocal (IX, Kramer and Carthew, 1985). It is, however, unequivocally agreed that ALP activity is higher in young than in old animals (Kumaresan and Ndzingu Awa, 1984; Kramer, 1989) and this is confirmed in growing Dwarf and Landrace kids (VII). There is also evidence that ALP activity is higher in pregnant than in non pregnant goats (Kumaresan and Ndzingu Awa, 1984; IX). A slight ALP rise during lactation is observed in Saanen goats (Biagi *et al.*, 1988a).

Plasma urea, creatinine and bilirubin concentrations are observed to be higher in young and adult nonpregnant goats (X). The changes in these biochemical metabolites during pregnancy are more pronounced in goats with higher number of lactations or pregnancies and they are probably due to fetal excretory processes. Urea increases during pregnancy but decreases in early lactation. The levels increase again in late lactation. In Saanen goats there are only slight changes in urea concentration during pregnancy and lactation (Biagi *et al.*, 1988a). The variable dietary urea and proteins in the rations obviously introduce differences among individual goats and between herds, in addition to the pregnancy and lactation influence (Blackwell and Libby, 1982; Pelletier *et al.*, 1985). Pastures are higher in urea content than indoor rations (Payne *et al.*, 1970; Vrzgula *et al.*, 1985).

Bilirubin concentration increases during pregnancy and decreases after parturition. The levels increase in mid lactation, more in goats of low parity but decrease again in late lactation stages. Creatinine concentration in goats increases in late lactation. The levels are only slightly higher in young and adult nonpregnant and those at late

stage lactation (X; Cissik *et al.*, 1987). Decreased values are reported during lactation in Saanen goats (Biagi *et al.*, 1988a).

Glucose levels decrease in the last stages of pregnancy, but increase following parturition and with advancing lactation, especially more in goats of higher than of low parity. There were similar observations in Barbari (Vihan and Rai, 1987) and Baladi goats (Hassan *et al.*,1986). Since the active thyroid hormone forms T_3 and T_4 are also elevated during lactation (Biagi *et al.*, 1988b), this might be an adjustment to mobilize glucose for lactogenesis.

Total serum protein concentrations are low in advanced pregnancy, increase after parturition and during lactation in Landrace (X), Baladi (Hassan *et al.*, 1986), Barbari (Vihan and Rai, 1987) and Saanen goats (Biagi *et al.*, 1988a). The increase during lactation is more in goats of high than of low parity and proportional to the stage of lactation (Hassan *et al.*, 1986; Biagi *et al.*, 1986b). The increase is probably due to increased synthesis for secretion in the milk. Serum protein levels in goats are also proportional to protein levels in the diet (Blackwell and Libby, 1982).

Cholesterol levels decrease in pregnant goats, but increase to highest levels during early lactation, changes that are directly proportional to parity (X). These fidings are similar to those observed in cattle (Prakash and Tandon, 1979) and sheep (Vihan and Rai, 1987). The increase of plasma cholesterol levels during lactation probably results from synthesis, the balance of which is coupled with thyroid hormone, estrogens, oxytocin and adrenocorticotrophic and thyrotrophic hormone levels, which play roles in pregnancy and lactation. Increased thyroid and estrogen hormones lower plasma cholesterol levels (Prakash and Tandon, 1979). Both hormones increase towards parturition and fall thereafter. The observed slight increase in cholesterol levels during pregnancy in sheep (Rawal *et al.*, 1987) and Saanen goats (Biagi *et al.*, 1988a), contrary to the finding in Landrace goats (X) is likely to be because of nutritional interactions in the herds. It can therefore be concluded that sustained alterations in the levels of hematological and clinical chemical values occur during pregnancy and lactation.

The influence of nutrition

Nutritional type, quality and quantity greatly influence blood plasma and serum values of many parameters. As it is impossible to state the effect of each type and level of nutrition, a few examples are mentioned to highlight the diverse and large influence of nutrition. The RBC and WBC counts, hemoglobin concentration, hematocrit, MCH and MCHC are higher in well nourished than in poorly fed goats (Biswas *et al.*, 1986). Ali *et al.* (1984) observed decreases in hemoglobin concentration, packed cell volumes, erythrocyte, eosinophil and lymphocyte counts, total serum proteins and calcium concentrations after food restriction in goats for 168 hours.

During the same period, there were elevations in ASAT activity, lactate, nonesterified fatty acids and bilirubin but there were no effects on plasma glucose, sodium and potassium levels. This is in agreement with results of earlier investigations (Chatterjee *et al.*, 1979).

The total serum proteins, creatinine, bilirubin, ALP, CK, ALAT, calcium, potassium, hemoglobin concentration, hematocrit, MCV, MCH, MCHC and WBC are significantly dependent on the dietary protein levels (Blackwell and Libby, 1982). It has been shown that lower protein rations result in elevation of hematocrit, Hb and erythrocyte counts in dry and lactating cows (Esievo and Moore, 1979). In poor nutritional balance under poor husbandry practices the concentrations of total serum proteins in goats are higher, while cholesterol levels lower than those under better management systems (Fiocre *et al.*, 1986), mainly due to hyperglobulinemia. The albumin concentration is low. Similar biochemical conditions are reported in goats with low grade chronic gastrointestinal and hepatic parasitic infections (Saad *et al.*, 1984). Studies on leukocyte counts did not reveal any differences between goats under confinement and those under semi confinement management systems (Marques Junior, 1983).

Tirkey *et al.* (1987) reported decreased enzyme activities, particularly acetylcholinesterase in goats fed on a widely distributed tropical plant *Ipomoea carnea*. Although most hematological and clinical chemical parameters were less affected, this finding emphasizes the nutritional influence on blood values and the importance in clinical diagnosis and comparative studies. These examples allow the conclusion that the type, level and quantity of nutrition have a profound influence on blood parameters.

The influence of season

Extensive changes in hematological and clinical chemical values due to seasonal changes are reported in goats (Holman and Dew, 1966b; Mazumder *et al.*, 1982; Vrzgula *et al.*, 1985; Ginting, 1987). RBC and WBC counts, hemoglobin concentration, hematocrit are higher in warm than in cold months (Holman and Dew, 1966b). Calcium, magnesium, phosphorus, sodium, potassium, chlorine decrease in winter while hemoglobin concentration, hematocrit, bilirubin and vitamin E decrease at the end of grazing season (Vrzgula *et al.*, 1985). Seasonal variations are also observed in RBC, WBC and lymphocyte counts, hematocrit, hemoglobin concentration in Indian (Vaidya *et al.*, 1970), Tibetan dwarf and Tibetan - Maltese cross breeds (Domina *et al.*, 1982) and Cameroon goats (Pospisil *et al.*, 1987). There are also daily, weekly and monthly variations in lipids, ASAT, urea and glucose concentration (Bas *et al.*, 1980). The type of changes in blood values are actually a reflection of the type and quality

of nutrition fed to animals in the different seasons and meteorological effects. In the tropics the seasonal dispositions of the prevalence of internal and external parasites play roles. It can be concluded that large seasonal influences on hematological and clinical chemical values are expected in goats. The determination of the exact changes requires multiseasonal investigations.

The influence of ambient temperatures

The influence of ambient temperatures can be viewed as short term effect of season on blood values. It is a component among others such as rainfall and humidity. Variations have been observed in RBC, and WBC counts, hemoglobin concentration and hematocrit during the day and presumed to be due to changing ambient temperatures (Oyewale and Olowookorun, 1986). RBC count, hemoglobin concentration, hematocrit, total leukocyte count, serum proteins, inorganic phosphorus and magnesium decrease, whereas glucose and calcium increase with increasing ambient temperatures (Upadhyay and Rao, 1985), the RBC counts being positively correlated also to relative humidity. Glucose concentration was also noted to steadily increase during the day but decreasing in the evening (Bas *et al.*, 1980). Controlled experiments are obviously required for full determination of the influence of temperatures on blood values. The available data show that the temperature under which the animal is subject to before the blood sample is taken has influence on the laboratory results.

The influence of drugs

The influence of drugs on hematological and clinical chemical values constitute an important aspect on clinical diagnosis. Blood samples are frequently submitted for laboratory analysis when the animal has been under drug therapy for a variable period of time (sometimes repeated or long exposed). Only a few studies have been performed on the effects of drugs in domestic animals, but there are a number on man especially recently due to sports problems of doping. This is obviously a relevant factor in horses. Cationic phenothiazines acting through the RBC membranes change erythrocyte shapes from discoids (discocytes) to stomatocytes and spherostomatocytes (tomato shaped) while anionic ones produce echinocytes and speroechinocytes (crenated spheres) (Smith *et al.*, 1982). Drugs also change the osmotic fragility of erythrocytes. Chlorpromazine and lysolecithin are proved to be stomatocytic and echninocytic agents (Jain and Kono, 1989). The erythrocytes of goats are reported to occur in numerous shapes including biconcave, flat discs, poikilocytes of triangular, pear shaped, fusiform, sickles and spindle forms (Jain and Kono, 1977). The various

shapes offer some resistance to some of the drug induced changes especially in goats, camels and llamas. For this reason it is important that alteration of shapes due to drugs are identified for appropriate interpretation of erythrocyte morphology in healthy and diseased goats because the morphology of cells are important guides to classification of anemia in domestic animals. Stomatocytes and spherostomatocytes appear in hemolytic anemia while schistocytes form salient features of disseminated intervascular coagulation, microangiopathic anemia and severe sepsis.

Xylazine and ketamine which are commonly used anesthetics cause hyperglycemia in goats lasting for several hours after the operation (Mgasa *et al.*, 1987). Exogenous testosterone and estrogens in male and female goats respectively produces hyperglycemia, especially in starved animals but reduces aldolase (Chatterjee *et al.*, 1979). Furthermore dexamethasone which is used as an antinflammatory drug causes hypocalcemia, hypokalemia, hypophosphatemia, hyperglycemia, hyponatremia and hypochloremia in goats if it is administered 2 to 3 days before samples are taken (Maddux *et al.*, 1988). It is hence wealthy noting these and other drugs effects on hematological and clinical chemical values to avoid misinterpretation of laboratory results brought about by drug therapy.

Conclusions

(1) The reference ranges and mean values of some hematological and clinical chemical parameters for Danish landrace goats have been adequately elaborated in this investigation. It is unequivocally concluded that the main factors affecting the values are age and herds (environment) followed by pregnancy and lactation. The influence of breed and sex are of small magnitudes. The influence of herd may be due to nutritional differences between farms.

(2) Alterations in plasma electrolytes levels (calcium, phosphorus, magnesium, sodium and potassium) and enzyme activities (ALAT, ASAT, CK and ALP) occur due to pregnancy and lactation, the degree of which depends on age and parity, influenced also by environment.

(3) Sustained changes in hematological values (RBC, total and differential WBC counts, hemoglobin concentration, hematocrit, MCV, MCH, MCHC) and clinical chemical values (urea, creatinine, bilirubin, glucose, total serum proteins and cholesterol) occur during pregnancy and lactation in goats, the magnitude of changes depends on age and parity, and vary between herds.

(4) Because of the strong age influence a distinction between kids (<4 months), juveniles (4-6 months) and adult goats is appropriate and essential for correct interpretation of laboratory results.

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