THE IMPACT OF HELMINTH INFECTIONS IN FREE-RANGE CHICKENS WITH SPECIAL FOCUS ON THE PATHOGENICITY OF *TETRAMERES AMERICANA*

BY

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ABSTRACT

The distribution of helminth infections in free-range chickens is known to be wide spread and some of the species occur with high prevalence and intensities, but the impact of helminth infections has not been adequately quantified. Two on-farm studies were conducted: the first was to determine the effect of natural helminth infections and the second was to evaluate different treatment regimens in free-range chickens. In addition two on-station experimental infections of *Tetrameres americana* were conducted to determine the pathogenic effect of this parasite. Twelve farms were involved in the on-farm studies where anthelmintics were applied strategically. In the first experiment a single dose of mebendazole was applied fortnightly and the chickens were followed for six months. In the second experiment the chickens were followed for 12 months where single, seasonal, and monthly treatments were tested. Mortality, weight, and egg production were recorded every fortnight for the entire experimental period. For the on-station studies the pathogenic effect of different infective doses of *T. americana* were assessed for 12 weeks. The parameters measured were; mortality, weight gain, and the effect on haematological profiles. The results of the on-farm studies showed that natural helminth infections in free-range chickens reduced growth rates by 22% and delayed sexual maturity by eight weeks. The following helminth species were noted to cause mortality: *Ascaridia galli* (one death) and *Syngamus trachea* (15 deaths) and *Heterakis gallinarum* (17 deaths) through transmission of histomonosis. Monthly treatment was a better regimen but seasonal treatment was very promising. In the experimental infection *T. americana* produced transient anaemia; lymphocytopenia,

heterophilia, eosinophilia, and elevated blood pepsinogen levels. The key findings from the present studies are that natural mixed helminth infections cause a subclinical disease resulting in slow growth rate and delayed sexual maturity in free-range chicken. For the first time *T. americana* has been observed to elevate the blood pepsinogen level as well as causing low weight gains and that a mean of 8.7 female *T. americana* intermittently caused anaemia.

DECLARATION

I, HENRY BUDODI MAGWISHA, do hereby declare to the Senate of the Sokoine University of Agriculture that this thesis is my own original work and that it has not been submitted for a degree award in any other University.

Signature .....

Date 04 - 8 - 2003

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ABBREVIATIONS

CP	Crude protein
DLC	Differential leucocyte counts
dpi	Days post infection
G	Gauge
GIT	Gastrointestinal tract
Hb	Haemoglobin concentration
HP	High protein diet
I/H	Intermediate host
L_3	Third stage larvae
L_4	Fourth stage larvae
LP	Low protein diet
PCV	Packed cell volume
pH	Hydrogen ion concentration
RCBD	Randomised complete block design
rpm	Revolutions per minute
RVAU	Royal Veterinary and Agricultural University
SD	Standard deviation
SEM	Standard error of the mean
SFRB	Scavenging feed resource base
sp.	Species (singular)
spp.	Species (plural)

SUA	Sokoine University of Agriculture
VEO	Village Executive Officer
VEW	Village Extension Worker
wpi	Weeks post infection
wt	Weight

CHAPTER ONE

1.0 INTRODUCTION

The contribution of free-range chickens to the economy and human nutrition in Tanzania is immense as the industry constitutes 98.3% of the estimated 26,065,000 chickens population (MoA, 1995; FAO, 2002). The industry provides petty cash to the resource poor farmers and almost all of the eggs and chicken meat requirements in the rural areas and 13-20% of the urban requirements (Kabatange and Katule, 1990).

However, the mortality rate in free-range chickens is known to be very high especially at the early age and during outbreaks of viral or bacterial diseases such as Newcastle disease (Minga *et al.*, 1989) and fowl typhoid (Sa'idu *et al.*, 1994). Despite the high mortality at early age, thirty percent of the hatched chicks are known to grow to adult stage (Minga *et al.*, 1989). Kuit *et al.* (1986) and Bell *et al.* (1990) noted that majority of the free-range chickens die before they are three months old. Predators such as mongoose, hawks and eagles (Negesse, 1993) and diseases such as viral, bacterial, fungal and parasitic (endo and ecto-parasites) are among the causes of mortality (Sa'idu *et al.*, 1994).

Furthermore, the productivity of free-range chickens is said to be low owing to slow growth rate and production of few eggs per year. The reasons being genetic constitution (Kabatange and Katule,1990), poor nutrition (Gunaratne *et al.*, 1993; Tadelle, 1996), diseases (Minga *et al.*, 1989; Sa'idu *et al.*, 1994), management (Negesse, 1993) and helminth parasites (Yadav and Tandon, 1991; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001). Postmortem examinations have shown that free-range

chickens with high worm numbers tend to have poor body conditions (He *et al.*, 1990; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001). There is paucity of information on the effect of helminth parasitism in free-range chicken: most reports are on the helminth type, prevalence, intensity, and the pathology they cause in relation to sex, age, breed, season and/or climate but very little information is available on the economic impact of these parasites.

Helminths exert their effects on the host by different ways such as blood sucking, tissue destruction during larval migration, feeding, mechanical or chemical irritation of contact surfaces, liberation of toxic metabolites and obstruction of excretory ducts, air passages or blood vessels (Nielsen, 1976; Kassai, 1999). They therefore have variable pathogenic effects depending not only on the worm type and burdens but also on the environmental and management factors e.g. nutrition, climate, and management system (Permin *et al.*, 1997a, 1997b). Management system plays an important role in the prevalence and intensity of helminths in poultry. For example, chickens reared in battery cage are less parasitised compared to those reared in deep litter system which in turn are better off if compared to free-range chickens (Hussain, 1967; Humphrey, 1979; Hemalatha *et al.*, 1987; Oyeka, 1989; Abebe *et al.*, 1997; Permin *et al.*, 1999).

In most cases chickens under free-range management system are found infected with multiple species of helminth parasites (Banage, 1968; Fatihu *et al.*, 1991; Mpoame and Agbede, 1995; Abebe *et al.*, 1997; Permin *et al.*, 1997b; Poulsen *et al.*, 2000; Mukaratirwa *et al.*, 2001; Magwisha *et al.*, 2002). The mixed infections have been observed to have ≥ 10 species (Humphrey, 1979; Vattanodorn *et al.*, 1984; Permin *et al.*,

1997b; Poulsen *et al.*, 2000; Magwisha *et al.*, 2002). However, the infected organs vary considerably in their ability to compensate for the loss of functional tissue cells, for example, the gastrointestinal tract (GIT) can tolerate a substantial number of helminth parasites than does the trachea (Soulsby, 1976). However, heavy worm counts have been implicated to cause poor body conditions in free-range chickens (He *et al.*, 1990; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001) and in severe conditions they are reported to cause deaths in poultry (Flatt and Nelson, 1969; De Rosa and Shivaprasad, 1999). There is a need, therefore, to determine the impact of natural helminth infections on the survival rate and productivity of free-range chickens.

Of the proventricular worms *Tetrameres americana is* very prevalent in free-range chickens (Msanga and Tungaraza, 1985; Otaru and Nsengwa, 1985; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001), yet its importance on the health and productivity of the infected chickens has not been given due attention. Studies have shown that massive *T. americana* infections were associated with emaciation in pigeons in which the parasite was implicated to interfere with the digestive functions of the host (Flatt and Nelson, 1969). The pathogenic effect of helminths is aggravated in case of nutritional deficiency. Earlier on, Cram (1931) had observed that *T. americana* infection was severe in vitamin A deficient chickens. So far most of the natural helminth infections in free-range chickens are subclinical (Banage, 1968; Fabiyi, 1972; Ssenyonga, 1982a; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001). The effect of subclinical helminth infections in free-range chicken is however not well known. Albeit, there is regrettably very little information on the level of infections that could cause significant losses in free-range

chickens. The effect of insidious chronic helminth infections in free-range chickens need to be assessed in order to provide basic information in planning for control programmes. The low productivity of free-range chickens could partly be directly or indirectly influenced by helminth infections (Sa'idu *et al.*, 1994), inadequate nutrition (Gunaratne *et al.*, 1993) and/or genetic potential (Msoffe *et al.*, 2001). It is conceivable that control of helminth parasites in free-range chickens might improve the survival rate of young chicks, improve the health and productivity of free-range chickens that in turn would improve the health and economic status of the resource poor farmers.

The overall objective of this study was therefore to determine the impact of natural helminth infections in free-range chickens and the pathogenic effect of *T. americana* in chickens.

The specific objectives were:

1. To determine the effect of helminth parasites on the productivity of free-range chickens
2. To assess the effectiveness of different deworming strategies in free-range chickens
3. To determine the pathogenic effect of *T. americana* in chickens
4. To determine the effect of *T. americana* in chickens fed high and low protein diets

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry production systems

In the developing world most chickens are raised under free-range system where the chickens are left to fend for themselves (Hussain, 1967; Humphrey, 1979; Hemalatha *et al.*, 1987; Oyeka, 1989). The free-range chicken flock sizes are usually small (10-20 chickens) and rarely supplemented with food and depend on field gleanings, kitchen refuse, and insects as a sole source of nutrition (Cumming, 1992; Abebe *et al.*, 1997; Mwalusanya *et al.*, 2001). Another small scale management system is the backyard system where chickens are provided with shelter, water and feed full time (Hedge *et al.*, 1973; Abebe *et al.*, 1997). In the developed world, where chickens are raised commercially, a large number of chickens are housed at one time and raised under clean conditions (all in all out management system) whereby chickens rarely come in contact with disease causing agents (Permin *et al.*, 1999).

However, for the past two decades in Europe a free-range/organic farming system has become popular where chickens are raised on the range around a restricted area and are not routinely treated against endoparasites to avoid drug residues in eggs and meat (Thamsborg *et al.*, 1999). In this case chickens under organic farming have been observed to have higher prevalence and intensity of helminth parasites compared to those under intensive management system (Permin *et al.*, 1999).

2.1.1 Nutrition of free-range chicken and helminth infections

Insects is one of the most important source of protein for free-range chickens (Mwalusanya, 1998). Proximate analysis of insects show that they contain high crude protein level (63-75%) compared to plants e.g. soya beans (40-45%) (Scott *et al.*, 1969). However, recent studies have shown that the scavenging feed resource base (SFRB) is inadequate for optimal production (Roberts, 1992; Gunaratne *et al.*, 1993; Tadelle, 1996). The limiting factor for growth in free-range poultry is protein level (Scott *et al.*,1969; McDonald *et al.*, 1992; Magwisha, 1997). Williamson and Payne (1987) recommended that growing free-range chickens should consume 12-15% crude protein (CP) as compared to the commercial chicken that need 18-23 % CP. Analyses of crop contents have consistently revealed about 10 % CP in various countries. In Sri Lanka Gunaratne *et al.* (1993) recorded the CP of crop contents to be 9.4 %. Another study in Ethiopia, Tadelle (1996) reported 8.7 % CP. In Tanzania Mwalusanya (1998) recorded 10.8% CP.

Nutritional deficiencies have marked effects on the physiology of the host and hence indirectly influence the establishment and burdens of helminth parasites in livestock (Van Houtert *et al.*, 1995). In their studies Kambara *et al.* (1993) and Van Houtert *et al.* (1995) noted that protein supplementation reduced the adverse effects of helminth parasites when compared to un-supplemented controls. In addition, high protein level is known to improve the tolerance of the host to helminth parasites (Ackert and Beach, 1933; Ackert, 1947). However, most insect families such as ants, grasshoppers, cockroaches, and beetles; and other invertebrates such as earthworms and

snails have high level of protein content but are also suitable intermediate hosts of some poultry helminths (Ackert, 1917; Ackert, 1919; Cram, 1931; Jones and Horsfall, 1935; Sugimoto and Nishiyama, 1937; Horsfall, 1938; Avancini and Ueta, 1990). Thus, chickens enjoy the nutritive value of these invertebrates but in turn acquire helminth infections by ingesting infective forms of the parasites (Nadakal *et al.*, 1973; Lim, 1975; Ramaswamy and Sundaram, 1979).

2.2 Helminth infections in chickens

The epidemiology of helminths in chickens is influenced by many factors such as the life cycle of the helminth species, climatic and ecological condition of the area, and management system of the chickens (Birova *et al.*, 1990; Terregino *et al.*, 1999). Climate plays a significant role on the prevalence and intensities of different helminth species (Permin *et al.*, 1997b). Temperature and humidity being the most important factors. Most parasites are abundant in the humid and warmer areas (Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001).

The eggs deposited by adult female worm in an infected host are passed out in the faeces to the environment where they may directly develop to infective stage (Ruff and Norton, 1997) or they maybe ingested by a suitable intermediate host and develop to infective larvae (Reid and McDougald, 1997). The duration required for the eggs to reach infective stage vary with species and environmental factors. Eggs of *Ascaridia galli,* for example, require a minimum of 5 days at 32° to 34 °C to become infective (Reid, 1960). In case of lower ambient temperature below 32°C development becomes

slower and takes 19 days at 16°C. It was reported that below this temperature the development is arrested. On the other hand, eggs are destroyed at temperature above 35°C (Reid, 1960). Once chickens are infected they may start passing *A. galli* eggs in the faeces 30 days post infection (dpi) (Kerr, 1955).

Ackert (1917) demonstrated that the fowl nematode, *Heterakis papillosa*, synonymously known as *Heterakis gallinarum* can either have a direct life cycle or sometimes utilises a transport host, 'the dung earthworm'. The development of *H. gallinarum* egg to infective stage has been reported to be as short as 2 weeks (Ruff and Norton, 1997). And for those nematodes with direct life cycle the final host becomes infected by ingesting embryonated eggs containing a second stage or free second stage larvae (Ruff and Norton, 1997). On the other hand, for those species with an indirect life cycle the final host is infected after ingesting an infected arthropod (Reid and McDougald, 1997). When the intermediate host is itself eaten, the infective larvae are liberated and enter the gastrointestinal tract (GIT) and migrate to their predilection sites within the definitive host (Ramaswamy and Sundaram, 1981a). It is known that about half of the poultry nematode species have direct life cycle (Ruff and Norton, 1997).

Tetrameres fissispina is a common fowl parasite (Poulsen *et al.*, 2000) but it is also known to parasitise mallards and domesticated ducks (Birova *et al.*, 1990; Fedynich and Pence, 1994). Recently, a chicken crop nematode, *Capillaria contorta*, has been reported to parasitise and kill a vulture guinea fowls (De Rosa and Shivaprasad, 1999). Therefore, the importance of wild birds in the epidemiology of helminth infections in free-range chickens cannot be overemphasized.

All cestodes and trematodes have indirect life cycles utilising insects, crustaceans, earthworms, slugs, snails or leeches as intermediate hosts depending upon the species of the worm (Ackert, 1919; Jones and Horsfall, 1935; Horsfall, 1938). Most of the cestode larvae need at least 2 weeks to become infective and the prepatent period of 14 days is common in the vast majority of cestodes (Reid and McDougald, 1997). Most cestodes are host specific (Matthews, 1998) and in this regard, mixing of unrelated birds have been reported to reduce the environmental contamination (Nonaka *et al.*, 1991). In most instances, trematodes are known to be less specific and have more complicated life cycles whereby they often need two intermediate hosts; and completion of a two-intermediate-host life cycle depends upon an unique set of ecological conditions (Reid and McDougald, 1997).

2.2.1 Prevalence of helminth infections in free-range chickens

The distribution, prevalence, and intensity of helminth parasites in free-range chickens have been reported in many tropical countries. The infected chickens are usually observed to carry multiple helminth species as reported by Kaushik and Deorani (1968) in India, El Khawad (1977) in the Sudan, Ssenyonga (1982a, b) in Uganda, Otaru and Nsengwa (1985) in Tanzania, Shamsul-Islam (1985) in Zambia, Fakae *et al.* (1991) in Nigeria, Mpoame and Agbede (1995) in Cameroon, Abebe *et al.* (1997 in Ethiopia, Permin *et al.* (1997b) in Tanzania , Poulsen *et al.*(2000) in Ghana and Mukaratirwa *et al.*(2001) in Zimbabwe. The geographical distribution of nematodes and cestodes in free-range chicken is wide (Edgar, 1953; Round, 1962; Banage, 1968; Mpoame and

Agbede, 1995; Mukaratirwa *et al.*, 2001; Magwisha *et al.*, 2002). The prevalence and intensity of trematodes (*Prosthogonimus ovatus*, *P. pellucidus*, *P. macrorchis*, *Echinostoma revolutum*, *Postharmostomum gallinae*) and the eye nematode, *Oxyspirura mansoni*, are high in the Asian continent (Matta and Ahluwalia, 1981; Padhi *et al.*, 1987; Khan and Chishti, 1989; Mir, 1992; Luong-Van-Huan, 1997). Additionally, the prevalence and intensity of trematodes are exceptionally high in ducks in Bangladesh (Islam *et al.*, 1988).

Previous reports by Msanga and Tungaraza (1985), Otaru and Nsengwa (1985), Yongolo (1996), Permin *et al.* (1997b) and Magwisha *et al.* (2002) show that the prevalence of helminth parasites in free-range chicken in Tanzania range from 74 to 100%. From these reports it has been noted that nematodes are more prevalent followed by cestodes whereas trematodes are rare. Most of the helminth species were noted to have a high prevalence but with moderate worm numbers (Msanga and Tungaraza, 1985; Permin *et al.*, 1997b). Similar conditions have been observed in Ethiopia (Negesse, 1993), Cameroon (Mpoame and Agbede, 1995) and Nigeria (Fabiyi, 1972; Fatihu *et al.*, 1991). In controlled studies moderate worm burdens have been observed to cause reduced weight gain in poorly fed chickens (Ikeme, 1971c; Permin, 1997a).

The prevalence of individual helminth parasites in free-range chickens vary appreciably from country to country or area to area within the same country. Among the most widely distributed and highly prevalent chicken nematodes, studies have shown that *T. americana* is among the nematodes that occurs with high prevalence (Fatihu *et al*, 1991; Permin *et al.*, 1997b; Poulsen *et al.*, 2000; Mukaratirwa *et al.*, 2001; Magwisha

et al., 2002), followed by other species like *H. gallinarum*, *A. galli*, and *Capillaria* spp in that order. The available literature show that nematodes and cestodes have a wide distribution across the world especially in the tropical areas like Africa. On the other hand, the prevalence of trematodes seem to be rare in the African continent when compared to Asia (Table 1).

Table 1. Prevalence (%) of selected helminth species in free-range chickens

Reference	Country	*Ascaridia galli*	*Capillaria* spp.	*Cheilospirura hamulosa*	*Gongylonema ingluvicola*	*Heterakis gallinarum*	*Tetrameres* spp.	*Davainea proglottina*	*Raillietina echinobothrida*	*Raillietina tetragona*	Trematodes
Fabiyi (1972)	Nigeria	50	-	16.7	47.7	-	43.3	-	60.0	53.3	-
Humphrey (1979)	Papua New Guinea	17.2	21.1	10.2	53.9	7.8	32.8	25.0	51.6	39.8	1.6
Ssenyonga (1982a)	Uganda	32.7	10.9	-	3.6	83.6	-	-	-	-	3.6
Vattanodorn *et al.* (1984)	Malaysia	10	76.7	3.3	20.0	43.3	30.0	43.3	76.7	43.3	10
Padhi *et al.* (1987)	India	47.2	8.3	19.4	-	63.0	53.7	0.9	29.6	57.4	36
Fatihu *et al.* (1991)	Nigeria	-	0.5	5.2	13.3	6.2	66.7	-	18.1	38.6	-
Permin *et al.*(1997b)	Tanzania	32.3	25.0	19.3	17.7	78.7	60.3	5.7	46.3	25.3	-
Abebe *et al.* (1997)	Ethiopia	71.6	-	-	-	21.1	-	-	29.5	26.3	-
Poulsen *et al.* (2000)	Ghana	24.0	60.0	25.0	62.0	31.0	58.0	-	81.0	59.0	1.0
Mukaratirwa *et al.* (2001)	Zimbabwe	32.9	3.0	4.4	60.1	15.2	64.1	-	-	-	-
Magwisha *et al.* (2002)	Tanzania	29.0	68.0	26.0	39.0	84.0	82	2.0	62.0	21.0	-

2.2.2 *Tetrameres* spp.

2.2.2.1 Background

The family Tetrameridae comprises of nematodes that are parasites of the proventriculus of birds. There are two closely related genera that are commonly found in birds namely; *Tetrameres* and *Microtetrameres*. *Microtetrameres* is sometimes regarded as a subgenus of *Tetrameres* but differences in larval and adult morphology justify its recognition as a distinct genus (Anderson, 1992). *Tetrameres* spp differ from *Microtetrameres* spp in that the adult female *Tetrameres* is spindle to globular in shape and has four longitudinal furrows that divide the body into four equal (tetra) sectors (meres) hence the name *tetrameres*. On the other hand, adult female *Microtetrameres* has no longitudinal furrows instead has 2-3 spirals along the body axis. The furrows in female *Tetrameres* spp correspond to the median and lateral lines of spines in males.

The genus *Tetrameres* was first coined by Creplin in 1846 and is synonymous to *Tropisurus* Diesing, 1835; *Tropidurus* Wiegmann, 1935; *Tropidocerca* Diesing, 1851; *Astomum* Schlotthamber, 1859; *Acanthophorus* Linstow, 1876 (Cram, 1927).

Tetrameres spp occur in the glandular stomach (proventriculus) of many birds such as chicken, turkey, pigeon, duck, quail, grouse, and many wild birds (Cram, 1927). The female is brilliant red in colour with two short pointed projections, the tail in one end and the head region on the other side (Cram, 1927; Yamaguti, 1958). The female is found embedded in the proventricular glands of birds whereas the male, filiform in shape, is found free in the lumen of the proventriculus, but it may follow the stationary female into the gland temporarily for copulation (Cram, 1927).

The species that occur in chicken include *Tetrameres fissispina* Diesing, 1861, *T. americana* Cram, 1927, *T. confusa* Travassos, 1917 and *T. mohtedai* Bhale Rao and Rao, 1944 (Soulsby, 1982; Anderson, 1992). These *Tetrameres* species lay embryonated small, oval, with thin , smooth shells and operculated eggs. The transmission occurs after the eggs are ingested by suitable intermediate hosts and hatch to first stage larvae, then moult to second stage larvae that feed and grow before moulting to the infective third stage larvae. The time required for the larvae to reach the infective stage differs from one species to another. It is 41 days or probably shorter for *T. americana* (Cram, 1929), 8 - 18 days for *T. fissispina*, 9 - 10 days for *T. confusa* (Anderson, 1992) and 16 - 21 days for *T. mohtedai* (Sundaram *et al.*, 1963).

Transmission of species of *Tetrameres* takes place either in water e.g *T. fissispina* or on land e.g. *T. americana*, *T. confusa* and *T. mohtedai*. In the former, intermediate hosts are aquatic crustaceans and in the latter , mainly terrestrial insects and isopods. The various species do not seem highly specific in their use of intermediate hosts (Anderson, 1992). *T. americana* has been reported from the American, Asian and African continents, *T. fissispina* from the European and African continents, whereas *T. confusa* has been reported in the Southern American and *T. mohtedai* from the Asian continent and India in particular. According to Anderson (1992) *T. americana* and *T. mohtedai* may be synonyms of *T. confusa*. The observable morphologic characteristics of each species are shown in Table 2.

Table 2. Morphological characteristics of *Tetrameres* species described in chicken

	Details	*T. americana*	*T. confusa*	*T. fissispina*	*T. mohtedai*
Male	Length x width	5 - 5.5 mm x 116 - 133 µm	4.5 mm	3-6 mm x 140-150 µm	4.27 - 5.8 mm
	Buccal capsule	27 µm x 4.5 µm	24 - 80 µm	8 µm x 3 µm	8 µm x 23 µm
	Oesophagus	1361 µm x 1725 µm	990 - 1240 µm	780 µm	965 - 1780 µm
	Cervical papillae	183 - 199 µm		150 µm	268 - 290 µm
	Long spicule	290 - 312 µm	291 µm	280-490 µm	397 - 430 µm
	Short spicule	100 µm	69 µm	82-150 µm	142 - 160 µm
	Cloaca	232 - 290 µm	70 µm	130-250 µm	290 - 344 µm
Female	Length x width	3.5 - 4.5 mm x 3.3 mm	3 - 5 mm	1.6 x 6.0 mm	3.2 x 5.6 mm
	Buccal capsule	35 - 38 µm x 10 µm	20 x 14 µm	21 x 10 µm	17 - 18 x 14 µm
	Oesophagus	1700 - 2715 µm x 50-125 µm	2400 - 2800 µm	1460 µm	1417 - 1450 µm
	Anterior projection	903 µm long	-	-	-
	Posterior projection	860 µm long	-	70 µm	-
	Anus	332 µm from tail tip	250 µm	71 µm	180 - 335 µm
	Vulva	631-664 µm from tail tip	near anus	130 µm	320 - 600 µm

2.2.2.1 *Tetrameres americana* Cram, 1927

i. Morphology

The male *T. americana* is white and threadlike (filiform) and is armed with double row of posteriorly pointed spines lying sub-medially and in lateral lines extending the entire length of the parasite from the head to the tail (Yamaguti, 1958). The cervical papillae are known to be located at different levels, one is slightly higher than the other at 183 μm whereas the second one is at 199 μm from the anterior end. The nerve ring is just posterior to the caudal papilla (Cram, 1927). Spicules are unequal as shown in Table 2. Also they are not heavily chitinised and the tail is long and slender with a cloacal aperture located 232 to 290 μm from the posterior end (Cram, 1927).

The female, on the other hand, is globular or fusiform blood-red in colour (when alive) with anterior and posterior appendages, buccal cavity, oesophagus, anus, and vulva located as shown in Table 2. The nerve ring is at 183 μm from the cephalic extremity. The intestines are saccular filled with black detritus. The uteri and ovaries are very long with numerous coils filling the whole body cavity containing thin-shelled embryonated eggs at different stage of development (Cram, 1927; Stoddard, 1946; Yamaguti, 1958).

ii. Distribution

Occurrence of *T. americana* has been reported in the USA by Cram (1927) and Edgar (1953), in Hawaii and Mexico by Alicata (1938). Whereas in the Asian continent, the worm has been reported from southern Thailand by Ayudhya *et al.* (1997).

While *T. americana* seems to be rare in the Asian continent it is widely

distributed in free-range chickens across the African continent. The worm has been observed to have high prevalence in Zimbabwe(Mukaratirwa *et al.*, 2001). Tanzania (Msanga and Tungaraza, 1985; Otaru and Nsengwa, 1985; Permin *et al.*, 1997b; Magwisha *et al.*, 2002), Nigeria (Fakae *et al.*, 1991; Fatihu *et al.*, 1991), Cameroon (Mpoame and Agbede,1995), Sudan (El Khawad *et al.*, 1977), and in Zambia (Shamsul-Islam, 1985).

T. americana has been reported to parasitise different species of poultry (Cram, 1931) chickens being the first natural host to be reported (Cram, 1927; Otaru and Nsengwa, 1985; Fatihu *et al.*, 1991; Mpoame and Agbede, 1995; Permin *et al.*, 1997b; Mukaratirwa *et al.*, 2001). Other natural hosts include the bobwhite quail (Cram, 1931; Beaudette *et al.*, 1933; Stoddard, 1946), pigeons (Mathey *et al.*, 1956; Raggi and Baker, 1957; Flatt and Nelson, 1969; Young, 1981; Toro *et al.*, 1999), doves (Panigraphy *et al.*, 1982) and turkeys (Beaudette *et al.*, 1933; Shamsul and Shamsul-Islam, 1985). In addition, the parasite infects zoo and wildlife birds like flamingoes (Aguirre *et al.*, 1991) and brown-headed cowbird (Cooper *et al.*, 1973). Other poultry that were experimentally infected by *T. americana* were ducks and ruffed grouse (Cram, 1931; Cram *et al.*, 1931).

iii. Life cycle

Five members of orthopterans, four species of grasshoppers; *Melanoplus differentialis, Melanoplus femurrubrum, Scyllina cyanipes* (Cram, 1929, 1931) and *Conocephalus saltator* (Alicata, 1938) and one species of cockroach *Blatella germanica* (Cram, 1929) have been reported to transmit *T. americana* in poultry. Stoddard (1946) reproduced the

life cycle of *T. americana* using the grasshopper *M. differentialis*. However, the American cockroach, *Periplaneta americana* and the oriental cockroach, *Periplaneta orientalis* were refractory to infections (Soulsby, 1982). Other experimental intermediate hosts of *T. americana* include beetles; *Dendrophilus* spp, *Dermetes vulpinus*, *Epitragus diremptus*, and *Gonocephaus saltator*; Earwig, *Euborellia annulipes*, and the sandhopper, *Orchestia platensis* (Cram, 1937; Alicata, 1938). Furthermore, Raggi and Baker (1957) isolated *T. americana* from pigeons that were previously exposed to pillbugs (*Armadillidium* spp.) and sowbugs (*Porcellio* spp.). Twenty four years later, Young (1981) successfully infected racing pigeons with *T. americana* after forcefully feeding them with naturally infected pillbugs. *Cylisticus convexus*.

Studies conducted by Cram (1929) showed that grasshoppers are more suitable intermediate hosts (I/H) than cockroaches. *T. americana* eggs are known to contain first stage larvae (L_1) (Cram, 1929). When ingested by a suitable intermediate host the eggs of *T. americana* hatch to release the second stage larvae (L_2) that develop in the body cavities of the I/H to an infective third stage larvae (L_3) 42 days post-infection (Cram, 1929). The L_3 are known to migrate into various parts of the body of the I/H and loosely encyst in thin-walled vesicles. The common sites for encystment being the thorax, abdomen, head and muscles of the legs (Cram, 1931; Olsen, 1962). The infected I/H usually becomes weak, droopy, inactive making it easy prey for scavenging fowls and many of the I/H do not survive long (Cram, 1931).

The final host is infected through ingestion of the I/H harbouring the L_3 (Cram, 1931). In the final host the L_3 are freed and penetrate the mucosa of the proventriculus

and spend at least 11 days in the gastric mucosa before moulting to the fourth stage larvae (L_4) (Cram, 1929). Early mature males and females migrate into the gastric glands between 16 - 19 days post infection where they copulate (Cram, 1931). After copulation, the males leave the glands and return to the lumen of the proventriculus (Cram, 1931). The prepatent period of *T. americana* is 45 days and a female reaches its full size when it is three months old (Cram, 1931). The female *T. americana* is found in the gastric (proventricular) glands of the chicken and lay thin-shelled embryonated eggs which pass out of the vulva and with the glandular secretions are carried into the lumen of the stomach (Cram, 1927; Wehr, 1934; Yamaguti, 1958). The eggs are passed out in faeces and hatch after they have been ingested by a suitable orthopteran intermediate host (Fig. 1).

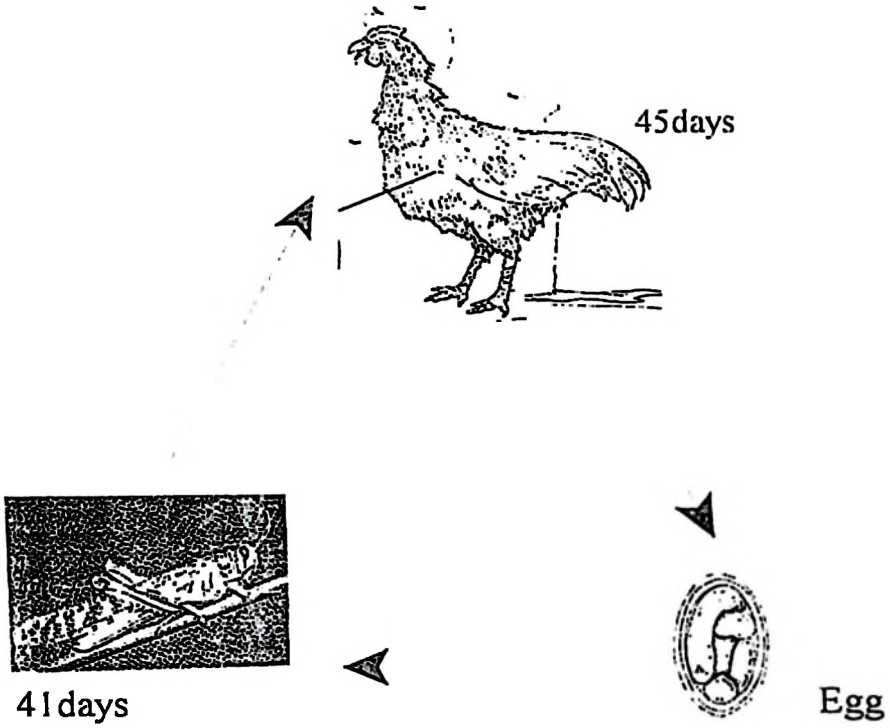

Figure 1. Life cycle of *Tetrameres americana*

2.3 General helminth infections

2.3.1 Pathogenicity

Helminth infections cause a variety of physiological disturbances in chickens depending on their location, mode of attachment and size (Boomaiah *et al.*, 1984). For example, infection in the mouth, oesophagus, and the crop may affect the functional ability of mucous and enzyme secretions (Hodges, 1974; Yasugi and Mizuno, 1981; Shibata *et al.*, 1991; Denbow, 2000). The proventriculus is made up of many compound tubular glands lined by oxynticopeptic cells that produce both the pepsinogen and hydrochloric acid (Imai *et al.*, 1991). The basal proventricular secretion is 15.4 ml/hour and contains 93 mEq/litre of acid and 247 Pu/ml of pepsin with a pH of 2.6 (Crevieu-Gabriel, 1999). The secretions from the oxynticopeptic cells make the pH of the gland acidic between 2.0 to 3.5, that is optimal for pepsin function in chickens (Keilova *et al.*, 1977; Crevieu-Gabriel *et al.*, 1999; Denbow, 2000). Higher pH is detrimental to the enzymatic activity of the proventriculus. The pH is, however, normally above 2.6 due to presence of ingesta (Coles, 1986; Denbow, 2000). The chicken proventriculus is equivalent to the abomasum in ruminants and is the place where chemical digestion of protein begins (Denbow, 2000).

Tetrameres spp (proventricular worm), *Gongylonema ingluvicola* (crop worm), and *Cheilospirura hamulosa* (gizzard worm) are always found embedded in tissues of their predilection sites (Soulsby, 1982; Ruff and Norton, 1997). A female *Tetrameres* spp. is found embedded in the gastric glands of the host (Flatt and Nelson, 1969). The head is embedded deep in the mucosa with its tail projecting into the canal of the gland

(Stoddard, 1946). The parasite is blood-red in colour because of its haematophagous nature (Cram, 1931; Ramaswamy and Sundaram, 1981a; Appleton, 1983). The engorged/gravid female cause distention of the glandular lumina culminating into compression atrophy on the glandular secretory epithelium (Flatt and Nelson, 1969; Appleton, 1983; Fatihu *et al.*, 1992a). Although the occurrence of adult female worms in the proventricular glands is so striking, the greatest damage is known to occur when the immature *T. americana* are migrating into the wall of the proventriculus causing marked irritation, inflammation and leaving migratory tracks (Cram, 1927).

The extent of damage caused by the adult female will depend on the intensity and the duration of infection, age, health, and nutritional status of the bird (Nielsen, 1976). In heavy infections the secretory epithelium of the gastric glands are extensively damaged by the worm and even the drainage of the secretion to the lumen is sometimes obstructed by compressed ducts (Fatihu *et al.*, 1992a). Massive *Tetrameres* infections in pigeons (Flatt and Nelson, 1969), guineafowls and chickens (Fatunmbi and Adene, 1979) have been reported to cause diarrhoea, emaciation, anorexia, and finally death. However, male *T. americana* are primarily found superficially on the mucosa of the proventriculus and sometimes they may be found together with the females deep in the glands causing little effect compared to females (Flatt and Nelson, 1969).

Different helminth species vary considerably in their pathogenicity; *Dispharynx nasuta* infection for example, results in thickening of the proventricular walls with ulceration of the mucosa, causing pinpoint haemorrhages, emaciation and jaundice (Goble and Kutz, 1945a; Mbise *et al.*, 1983; Otaru and Nsengwa, 1985; Vassilev and

Jooste, 1991). *Cheilospirura hamulosa* when found embedded in the pliable muscles of the gizzard is known to cause yellowish tumour like swelling that tend to weaken the mechanical digestion of the gland (Fatihu *et al.*, 1992a).

Massive infections in the intestines with some of the larger nematodes such as *A. galli*, have been reported to block the intestinal lumen of the infected bird. Furthermore, additional damage is caused during migration of immature stages (Ramadan and Abou Znada, 1991). For instance, though the habitat of *A. galli* is the small intestine, especially the duodenum and jejunum, in rare cases larvae have been found in the liver, lungs and trachea (Ackert, 1923).

It is further not uncommon to find adult *A. galli* in the crop, proventriculus, gizzard (Fabiyi, 1972), or in the cloaca, oviduct and even incorporated in the chicken egg (Schell, 1970; Pesti, 1982). Tissue stage of *A. galli* cause extensive petechial to ecchymotic haemorrhages in the intestinal mucosa as well as enteritis (Fatihu *et al.*, 1992b). The worm has been reported to reduce weight gain by 8-20% (Ackert and Herrick, 1928; Hurwitz *et al.*, 1972).

Capillaria spp. are found in the oesophagus, crop, intestines and the caeca and when found in high numbers they have been observed to cause diarrhoea, vomiting, anorexia that culminate into death of the bird (De Rosa and Shivaprasad, 1999).

Of the pathogenic cestodes, *Davainea proglottina* is found embedded deep in the mucosa of the small intestines causing extensive damage of the intestinal epithelium hence resulting to impaired absorptive capacity of intestine and hence reduced weight gain (Levine, 1938; Owen, 1951), *Raillietina echinobothrida* causes granuloma in the

lamina muscularis mucosa resulting in impaired function of the intestines (Nadakal *et al.*, 1973; Samad *et al.*, 1986). The structural alterations caused by the parasites will result in impaired organ function. Though *Raillietina cesticillus* is considered to be of mild pathogenicity, experimental infection resulted in reduced weight gain by 2.3 - 32.8% (Harwood and Luttermoser, 1938). On the other hand, *Hymenolepis carioca* did not appear to affect growth rate of the fowl at the level of infection used by Luttermoser (1940). Extensive studies by Crompton (1984) reported that the host food intake is reduced depending on either the infective dose given to the host or the number of established parasites. It was further noted that the species of parasite involved and the development stage may determine the amount of food to be consumed.

In case of infection with *Heterakis isolonche* the caecal walls have been observed to show nodular inflamation (Phyllis and Clapham, 1937). *Heterakis gallinarum* is considered to be nonpathogenic in chickens (Soulsby, 1982). However, heavy infections have been observed to cause haemorrhage and granulomas in the liver and caeca in chickens (Riddell and Gajadhar, 1988) and in guinea fowls (Khan *et al.*, 1994). Apart from causing liver and caecal granulomas, *H. gallinarum* is also associated with transmission of histomonosis an economically important protozoan disease of turkeys and sometimes chickens (Ruff *et al.*, 1970). Although *H. gallinarum* is not a blood sucker, heavily infected poultry have been observed to be clinically anaemic with pale comb and legs (Hubbard and Kelm, 1984) and at postmortem examinations, the infections show remarkable thickening of the caecal walls (Kaushik and Deorani, 1969).

Syngamus trachea causes irritation in the trachea that result into massive

secretions which usually culminate into difficult breathing, gasping, and sometimes death (Goble and Kutz, 1945b).

2.3.2 Effect of parasitism and clinical signs

The impact of helminth infections on the health of free-range chickens is exacerbated by the chronic nature of infections (Permin *et al.*, 1997b). The chronic multiparasitism usually occurs concurrently with malnutrition (Roberts, 1992; Tadelle, 1996; Mwalusanya, 1998). The nutritional deficiency in infected chicken can directly be due to inadequate feed supply, reduced feed intake and/or poor feed utilisation (Ackert and Herrick, 1928, Tornøe, 1985). Conversely, light infections with *A. galli* have been observed to enhance appetite (Ikeme, 1971c; Mir, 1992). The voracious appetite in moderately infected chickens have a compensatory effect to the harmful effect of helminth parasites (Ikeme, 1971b; Nickim, 1983).

Helminths with mild pathogenicity such as *Raillietina cesticillus* have been observed to show transient reduced appetite (Tornøe, 1985). The consequences of heavy infections with pathogenic species such as *A. galli* and *Capillaria* spp. in poultry is almost always associated with reduced feed intake (Ackert and Herrick, 1928) that at times results to death (De Rosa and Shivaprasad, 1999). The voluntary feed intake in other animal species such as sheep when infected with helminths is significantly reduced (Parkins *et al.*, 1973; Kyriazakis *et al.*, 1994). However, the digestibility of ingested feed is not altered by infections (Parkins *et al.*, 1973) and it has been reported that animals fed low protein diet tend to succumb to moderate helminth infections (Abbott *et al.*,

1986; McDonald *et al.*, 1992).

Massive helminth infections in poultry are characterised by anaemia, reduced weight gain and sometimes weight loss (Ackert and Herrick, 1928). Acute infections, for example, with *A. galli* (Phyllis and Clapham,1937; Ikeme, 1971a; Fatihu *et al.*, 1992b; Permin *et al.*, 1997a) or *T. americana* (Hubbard and Kelm, 1984) usually result into wasting, loss of appetite, weakness (lethargic), constipation, diarrhoea and finally death. In some cases infected chickens may show transient clinical signs of reduced feed intake and drowsiness and later recover completely (Ackert and Herrick, 1928; Mir, 1992); or may show ruffled feathers, droopy wings, and increased mortality (Ackert and Herrick, 1928). These clinical signs are more pronounced in young chickens (Mönnig, 1941). A morbidity of 50% in a flock with mixed infections of *Capillaria obsignata*, *H. gallinarum* and *A. galli* have been reported to cause anaemia, listlessness, watery diarrhoea and low grade daily mortality (Eleazer, 1969). However, some species such as *R. cesticillus* and *Hymenolepis* spp have been observed to be less pathogenic even if found in numbers above 100 (Botero and Reid, 1969). In some cases of heavy infections hosts pass the worms in the faeces (Kassai, 1999).

2.3.3 Immunity against helminth infections

Parasites differ enormously in their ability to stimulate host immune defences (Tizard, 1996). Chandra (1984) noted that malnutrition impairs immunity. For example, Mansour *et al.* (1991; 1992) observed that calves fed low protein diet and infected with *Ostertagia ostertagi* had higher antibody titers that was not protective when compared

with those fed high protein diet. A similar observation was made by Michael and Bundy (1992) after infecting mice with *Trichuris muris*.

Effects of helminth parasites on weakening the immune protection against other diseases was speculated by Fatunmbi and Adene (1979) and Vikraman and Paily (1986) after observing high mortality rates in chickens with concurrent infections of helminth parasites and Newcastle disease. However, most notably, cell-mediated immunity (CMI) is the most important factor in the survival of the host (Chandra, 1984). Immunity against helminth infections have been observed to differ with breeds and strains of chickens (Ackert, 1935; Permin and Ranvig, 2001; Gauly *et al.*, 2002). Both the major histocompatibility complex (MHC) and non-MHC genes have been associated with disease susceptibility and disease resistance in chickens (Lillehoj, 1991). During the early patent helminth infections, the CMI plays a major role towards elimination of the parasites (Malviya *et al.*, 1988; Lillehoj, 1991).

In chickens, IgA and IgM are the most predominant immunoglobulins in the external intestinal secretions (Lillehoj, 1991). During heavy infections the infected hosts are capable of expelling (self-cure) some of the parasites load (Permin and Ranvig, 2001). However, the expulsion of helminth parasites is not related simply to humoral responses (Michael and Bundy, 1992). Instead, the phagocytic cells such as heterophils and monocytes play the first line of defence towards elimination of invasive parasites and their effects are pronounced in case of presence of endotoxins (Andreasen *et al.*, 1991). Eosinophils are known to kill the antibody coated parasites and the mast cells are responsible for hypersensitivity reaction that triggers self-cure (Tizard, 1996). Self cure

has been proven to occur in chickens infected with *A. galli* (Ackert and Herrick, 1928; Permin and Ranvig, 2001). However, it has been observed that immunocompromised hosts are not capable of expelling the parasites (Johnson *et al.*, 1974). The parasite infected hosts were observed to have reduced T- lymphocytes that occurred concomitantly with alteration of the T-cells subsets (Chandra, 1984). It was further observed that mucosal antibody response is blunted, complement activity decreased, and the microbiocidal capacity of phagocytes is reduced (Chandra, 1984). Such changes in the host resistance are important determinants of the final outcome of the host-parasite interactions.

For example, Cannon (1966) superimposed worms with coccidia parasites and observed significantly higher nematode establishment in chickens with superimposed infections than in the negative controls. Again, Ackert *et al.* (1935) in a series of experiments managed to demonstrate that age of the host and the infective dose of the parasite play a significant role on the resistance to the helminth parasites. Older chickens were observed to be more resistant to infections when infected with high doses of *A. galli* than younger chickens on the same dose, the difference was more pronounced when older chickens were compared to young chickens infected with low dose.

Furthermore, host species, age, and sex have been noted to influence the prevalence and intensity of certain poultry helminths. For instance, Ransom (1921) noted a species difference towards susceptibility to *S. trachea* infection whereby chickens were observed to be more resistant to viability and growth of the parasite than were turkeys. Another specie difference was reported by Ackert and Eisenbrandt (1935) who noted

that turkeys were more resistant to *Ascaridia lineata* (synonymous to *A. galli*) infection than were chickens.

Chickens older than three month of age have been observed to be resistant to *A. galli* infections (Ackert *et al.*, 1935).

Whitlock (1937) observed high prevalence of *Syngamus trachea* in female Hungarian partridges that were maintained in breeding pairs. In another study Todd and Hollingsworth (1951) found high mean worm counts in males than in female chicks older than nine weeks of age. Ackert and Dewhirst (1950) demonstrated that the female hormone diethyl-stilboestrol tends to increase the resistance against *A. galli* infection.

In general, birds infected with helminth parasites are known to mount both humoral and cellular responses (Malviya *et al.*, 1988; Lillehoj, 1991). Tsvetaeva (1960) reported massive cellular infiltration in ducks infected with *T. fissispina*. Whereas a study conducted by Ramaswamy and Sundaram (1981a) working with *T. mohtedai* reported a transient cellular inflammatory reaction which subsided as the female worms matured in the proventricular glands. The mechanisms by which helminths evade the immune system and survive in their natural host have been explained by Cohen (1976).

2.4 Diagnosis of gastrointestinal helminth infections

For diagnosis of helminth infections it is indispensable to isolate and quantify the number of parasites. Moderate helminth burdens in infected birds is characterised by subtle clinical signs and difficult to be diagnosed (Flatt and Nelson, 1969). In case of massive infections the clinical signs are overt and cannot be missed because they may

be associated with expulsion of parasites in the faeces (Permin and Ranvig, 2001). The possible diagnostic techniques available include; post mortem worm counts, detection of parasite ova in the faeces by either direct examination of faecal smear, floatation, or sedimentation technique (Permin and Hansen, 1998). Serology and other immunological techniques for helminth diagnosis in poultry are currently not available (Permin and Hansen, 1998).

2.4.1 Faecal examinations

Faecal examination for diagnosis of poultry helminth parasitism is an essential diagnostic tool though it has some limitations (Henriksen and Aagaard, 1976). Oyeka (1989) managed to establish the types and intensity of helminth parasites in Nigeria by faecal examination. The quick method for screening a flock is by qualitative faecal examination to determine the presence of parasite eggs or alternatively quantification of the egg output is done by conducting egg counts per gram of faeces (EPG) (Permin and Hansen, 1998). However, detection of helminth eggs from chicken faeces has limitations. For example, consistence of the faeces, low fecundity, immature stage of worms, high male to female sex ratio have been reported to negatively affect the EPG (Henriksen and Aagaard, 1976). The appropriate way to determine the intensity of infections is to quantify the number of eggs per gram of faeces (EPG) using a modified McMaster technique (Henriksen and Aagaard, 1976).

However, the sensitivity of the techniques vary. For example, during diagnosis of poultry helminths by faecal examinations, Hemalatha *et al.* (1987) observed that

sedimentation was better than floatation technique. Furthermore, the resemblance of some eggs such as that of *A. galli* with that of *Heterakis gallinarum* and those of *T. americana* when compared to those of *G. ingluvicola, Dispharynx nasuta, Cheilospirura hamulosa* and *Strongyloides avium*, which are not only similar but also difficult to find, make the technique deficient (Soulsby, 1982; Ruff and Norton, 1997). For example, Hubbard and Kelm (1984) could not find the parasite eggs in faeces from pigeons infected with *T. americana*. The presence of helminth eggs in the faeces would lead to tentative diagnosis that might require confirmation and quantification at necropsy of a few sacrificed cases.

2.4.2 Haematological picture

In ruminants the use of haematological pictures such as PCV, Hb concentration and blood protein levels in assessing the pathogenicity of helminth infections have been shown to be of great value (Ross and Armour, 1960). In calves PCV levels have been observed to be negatively correlated with worm burdens (Van Aken *et al.* 1997). Clinical and subclinical infections in ruminants with stomach worms i.e. *Ostertagia ostertagi, Haemonchus contortus* or *Trichostrongylus axei* is associated with elevated blood pepsinogen levels (Satrija *et al.*, 1996; Claerebout *et al.*, 1997). Plasma pepsinogen concentration of ≥3 International Units (IU) of tyrosine have been reported to be diagnostic of ostertagiosis in cattle (Selman *et al.*, 1977;Claerebout *et al.*, 1997). Shaw *et al.* (1997) noted that levels over 5.5 IU of tyrosine represent clinical gastritis due to nematode infection. Very little is known about pepsinogen levels in free-range chickens

infected with *T. americana* that is known to cause compression atrophy in the proventricular glands and is at the same time haematophagous (Cram, 1927).

In chickens, normal levels of serum/plasma total protein range from 3-6 g/dl, levels below 2.5 g/dl are known to be detrimental to the health and survival rate of the affected bird (Coles, 1986). Low PCV and low serum protein may suggest possible blood loss anaemia (Campbell, 1995) whereas low PCV and normal serum protein indicate depression anaemia or a haemolytic anaemia. Elevated PCV and serum protein have been observed to be associated with haemoconcentration (Campbell, 1995). Reddy and Ratnam (1985) observed a low albumin/globulin ratio in chickens experimentally infected with *A. galli*.

2.4.3 Postmortem differential worm counts

Postmortem differential worm count is used to establish the type and intensity of worms present in a flock and can also assist on recording the pathological lesions inflicted by the helminths. Sometimes few pathogenic worms such as *S. trachea* could lead to significant deleterious effect on the health and productivity of poultry (Jordan and Pattison, 1996). During post mortem identification and enumeration of helminth parasites it makes easy to estimate the influence of the burden to the pathology caused by the parasites found (Dhar and Raina, 1987). High counts of nonpathogenic species could nevertheless have the same adverse effect as the presence of moderate number of pathogenic species (Nielsen, 1976).

2.5 Control of helminth infections

Multiple parasitism in free-range chickens is a common phenomenon (Humphrey, 1979; Ssenyonga, 1982a, 1982b; Shamsul-Islam, 1985; Tager-Kagan *et al.*, 1992; Abebe *et al.*, 1997; Permin *et al.*, 1997b; Terregino *et al.*, 1999; Poulsen *et al.*, 2000; Mukaratirwa *et al.* 2001; Magwisha *et al.*, 2002). Furthermore, the infection pattern of most species does not show any seasonal variation (Virk *et al.*, 1987; Yadav and Tandon, 1991). Helminths with indirect life cycle may be controlled by manipulating the ecology or controlling the intermediate host (Raggi and Baker, 1957). For nematodes with a direct life cycle they may be controlled by improving sanitary condition in and around the chicken house. The change of management from free-range system could be an alternative control measure (Udo *et al.*, 2001).

2.5.1 Chemotherapy

Free-range chickens are constantly exposed to an environment contaminated with helminth parasites. Anthelmintics have been advocated to remain as the cornerstone of worm control in livestock (Van Wyk, 2001). For instance, application of coumaphos cleared the infection, stopped the mortality, and improved the general conditions of the flock previously infected by *Capillaria obsignata*, *Heterakis gallinarum* (Eleazer, 1969), and similar result was observed by Sharma *et al.* (1990) when they applied ivermectin in chickens experimentally infected with *A. galli*. Furthermore, coumaphos was reported to be effective against *A. galli* when given in feed for 10 days (Eleazer, 1969). Broad spectrum anthelmintic with long acting activity would appreciably minimize the

frequency of treatment and reduce the environmental contamination (Rickard *et al.*, 1991).

Most anthelmintics have limited activity against helminth species. Praziquantel for example, has been observed to be efficacious against poultry cestodes (Rajendran and Nadakal, 1988; Nurelhuda *et al.*, 1989). Flubendazole is effective against *Raillietina cesticillus* (Tornøe, 1985), *A. galli*, *H. gallinarum* and *S. trachea* when applied at a dose of 20mg/kg body weight (Ssenyonga, 1982b); Mebendazole has been reported to control *T. americana* in pigeons (Young, 1981: Hubbard and Kelm, 1984). Hygromycin B was observed to be efficacious when applied at a high dose for a prolonged period of time (time-dose dependent) in killing both *A. galli* and *H. gallinarum* in chickens (Shumard *et al.*, 1958).

Similarly, piperazine salts doses below 200 mg/kg body weight required longer treatment periods whereas higher doses were noted to be effective against both adult and immature *A. galli* after single treatment (Horton-Smith and Long, 1956; Nilsson and Alderin, 1988). Similar study by Verma *et al.* (1991) demonstrated the inability of the drug to control the immature *A. galli*.

Of the imidathiazole group, levamisole and tetramisole are effective against *A. galli* (Pavlicek and Dykova, 1976; Verma *et al.*, 1991). Whereas the efficacy of pyrantel tartrate depended on the stage of the worm and dose level of the anthelmintic, immature worms were appreciably reduced but not completely eliminated (Okon, 1975). Therefore, the use of anthelmintic that is capable of reducing the parasite burden to a level that is not harmful to the host might be of paramount importance.

Williamson and Payne (1987) recommended that anthelmintics be applied to chickens only once at the sixth week of age, arguing that at this age the immune system of a chicken is developed enough to evoke a strong and solid immune response. This argument could be based on the idea that an adaptive response usually confers lifelong protective immunity to reinfection with the same pathogen (Janeway *et al.*, 2001).

Most poultry helminth parasites have been reported to develop in the environment and reach infective stage within two weeks (Mönnig, 1941) whereas *A. galli* under favourable conditions can take 5 -10 days only (Ackert 1931; Roberts, 1937; Reid, 1960). Frequent deworming practice have produced controversial findings towards anthelmintic resistance. Recently, Van Wyk (2001) has proposed that the parasite in refugia (the free living parasites that are not exposed to anthelmintics) may possibly be important for selection for anthelmintic resistance than the frequency of treatment or underdosing. In poultry, some strains of *A. galli* have been reported to be resistant against piperazine (Pavlicek and Dykova, 1976).

2.5.2 Management systems

Management systems have been observed to be one of the risk factors for the prevalence and intensity of helminth parasites in chickens (Hussain, 1967). For example, chickens reared in battery cage are less parasitised compared to those reared in deep litter system which in turn are better off if compared to those on the range (Hussain, 1967; Humphrey, 1979; Hemalatha *et al.*, 1987; Oyeka, 1989; Abebe *et al.*, 1997; Permin *et al.*, 1999). However, worms with direct life cycle such as *A. galli*, *H. gallinarum* and

some *Capillaria* spp have been observed to have high prevalences in deep litter system especially if the litter is not changed frequently (Hedge *et al.*, 1973).

Helminth species with indirect life cycle are rarely found in intensively (deep litter and battery cages) reared poultry because birds are usually precluded from contact with the intermediate hosts (Reid and McDougald, 1997). However, it is not uncommon to find intensively kept poultry being parasitised by cestodes that have indirect life cycle if sanitation is poor (Tornoe, 1985; Wilson *et al.*, 1994; Abebe *et al.*, 1997). Hemalatha *et al.* (1987) in their study isolated *Raillietina echinobothrida* and *Raillietina tetragona* whereas Abebe *et al.* (1997) recorded *Raillietina cesticillus* and *Choanotaenia infundibulum* in domestic fowls reared on deep litter and battery cage systems.

During the past two decades in Europe, a free-range/organic farming system has become popular where chickens are allowed to free-range around restricted areas and are not routinely treated against endoparasites in order to avoid drug residues in eggs and meat (Thamsborg *et al.*, 1999). In this case, chickens under organic farming have been observed to have higher prevalence and intensity of helminth parasites than those in the intensive management system (Permin *et al.*, 1999).

Another alternative control measure was noted by Nonaka *et al.* (1991) that when mixed species of poultry are raised in one pen the contamination level is lowered than in a pen with a single species. This is because some of the helminth species will be ingested by the dead host and stop further development.

On the other hand nutrition has been observed to mask or reduce the effect of helminth parasites (Reddy and Ratnam, 1985). Of late, chickens on high protein diet

experimentally infected with *A. galli* were observed to perform better though they carried significantly higher mean worm burdens at the 10% significance level than their counterparts on low protein diet (Permin *et al.*, 1998). In another study chickens fed high protein diet were observed to tolerate a substantial number of worms (Ikeme, 1971c). However, some scientists argue that the prodigal over-formulation of diets with high protein contents will result in high cost of the feed and at the same time excretion of the excess amino acids will ultimately serve as a source of pollution (McNab, 1994). The advantage of high protein in feed remain an alternative control method as the effect of parasitism will be covered up.

2.5.3 Breeding for resistance

Breeding for genetic resistance to diseases has long been recognised as a valid strategy for disease prevention. It avoids the use of drugs and vaccines and, once achieved, may provide long-term protection. This alternative control measure against helminth parasites is being sought owing to current anthelmintic resistance of some nematodes and the increasing awareness on the drug residues in the treated animals' meat, milk and eggs (Thamsborg *et al.*, 1999). Studies on breeding for resistance to diseases is on the increase in all animal species probably due to improved technique to detect/study innate genetic resistance (Lillehoj, 1991). In cattle, for example, the N'Dama breed in West Africa is genetically tolerant against trypanosomosis (Roelants *et al.*, 1983; Taiwo and Ogunsanmi, 2000).

The centre for all genes coding for resistance, susceptibility, and production

traits is known to be the major histocompatibility complex, MHC, (Lillehoj, 1991; Tizard, 1996). Recent findings support the occurrence of genetic resistance against poultry helminths where Lohman Brown has been observed to be resistant against *A. galli* primary infection when compared to Danish Landrace (Permin and Ranvig, 2001). However, the Danish Landrace harboured fewer worms after a challenge infection. It is also possible that some breeds are resistant to more parasites than is actually known. More studies are required to determine the chicken breeds that are genetically resistant to gastrointestinal nematodes. However, the selection at DNA level is known to be labour intensive. It is also not known as to whether the expression of the quantitative trait is caused by a single gene or by combined effect of several genes (Lillehoj, 1991).

2.5.4 Vaccination

Following drug resistance to helminth and protozoal diseases in poultry, alternative control methods have been sought and to date coccidiosis can be controlled by vaccination (Chapman, 1984). Vaccines against avian coccidiosis are being used in parent stock chickens in Europe and the United States of America (Dalton and Mulcahy, 2001). Although Reddy and Rao (1984) successfully used irradiated larval vaccine (ILV) to vaccinate chickens against *T. mohtedai*, the vaccine has never been commercially produced. Therefore, control of helminth parasites in chickens using vaccines is yet to be practised. Few irradiated larvae vaccines (ILV) have been successfully used against some ruminant nematodes such as *Haemonchus contortus* (Smith and Christie, 1979) and Dictol® for *Dictyocaulus viviparus* (Dalton and Mulcahy, 2001).

Massive production of protective and cost-effective helminth vaccine is a difficult task (Kassai, 1999). The main obstacle to the development and industrial production of effective helminth vaccines is the identification and isolation of the protective antigens (Kassai, 1999). Hopefully practical problems concerning parasite vaccines will be solved ultimately and this alternative control method may be available for use in the near future (Van Wyk, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

In this chapter, the four studies that were conducted are described. The first two studies were conducted on-farm in one village where 12 randomly selected farmers were involved in each study. The last two studies were conducted on-station where confined chickens were experimentally infected with a single dose of *Tetrameres americana* and followed-up for 12 weeks post infection.

3.1 Study I. Determination of the effect of helminth infections on the productivity of free-range chickens

A study was conducted in free-range chickens for six months to determine the effect of helminth infections under free-range condition. Two groups, the treated and untreated controls, of both sexes were used. The following production parameters were recorded every fortnight for six months i.e. mortality, weight gain, and egg production.

3.1.1 Study area

The study was conducted in Kibwaya Village, Mkuyuni Division, Morogoro Region of Tanzania which lies between longitudes 37^0 45'- 38^0 00' E, latitudes 6^0 45'- 7^0 00' S and at an elevation of 1200 m above sea level. The area receives an average annual rainfall of 1850 mm with bimodal pattern, the first rains (vuli) start in October and end in late December or early January whereas the second rainy season (masika) starts in late

February or early March with a peak in April and ends in late May. The ambient temperature is usually between 18 to 28 °C around the year. The village is spread into three topographical zones, lowland, the hillside and hill top with a total of 500 households and about 2000 inhabitants. Four hundred of these households kept chiekens whereas only 80 households kept goats and there were neither cattle nor pigs kept in the village at the time of this study. This was a follow-up study following a previous study on prevalence and intensity of helminth infections by Magwisha *et al.* (2002) who observed moderate helminth infections with a fair mixture of nematodes and cestodes.

3.1.2. Determination of the number of clusters/farms to be sampled

A two stage cluster sampling method was used as described by Thrusfield (1995). In this case therefore, the study intended to take samples from a few randomly selected households and within each household to sub-sample the chickens.

The number of clusters (farms) to be sampled were calculated basing on a fixed total sample size (Ts) formula. The total sample size was fixed at 120 chickens. Again another common phenomenon was considered that in nature the parasite burdens in a population follows negative binomial distribution whereby many hosts tend to have few parasites. In this regard it is considered that only a small proportion of poultry is heavily infected and carries a disproportionate parasite burden that is most likely to cause overt disease to the infected host (Matthews, 1998).

Therefore, the number of clusters (g) was calculated as follows:

$$g = \frac{Z^2 \times Ts \times Vc}{d^2 \times Ts - Z^2 \times P_{exp}(1 - P_{exp})}$$

Where: g = Number of clusters/farms

Z = Value for two tailed 95% confidence interval (1.96)

Ts = Total number of animals to be sampled (120)

Vc = Variance between clusters (0.0225)

d = Desired absolute precision (10%)

P_{exp} = Prevalence of chickens expected to have high worm burdens (10%)

A 95% confidence interval (CI) was applied thus the $Z = 1.96^2$, the total number of chickens to be sampled (Ts) was set at 120 chickens, the variance between clusters(Vc) was derived from the assumption that standard deviation between clusters was 15% and then the variance was obtained by squaring the standard deviation (0.15), hence Vc was 0.0225, absolute precision (d) set at 10% and expected prevalence (P_{exp}) for chickens to have high worm burdens that may cause concern was set at 10% with reference to previous works by Banage (1968), Permin *et al.* (1997b) and Poulsen *et al.* (2000). Therefore, the number of clusters or farms required to be sampled in the study was calculated to be 12 farms and each farmer was supposed to be given 10 chicks to look after, five being in the treated group and the other five as untreated controls.

3.1.3 Selection of farmers

The process of selecting farmers involved the village administration where the Village Extension Worker (VEW) was asked to arrange a meeting with a group of farmers from the village. The VEW contacted the village authority and the Village Executive Officer (VEO) produced a list of families (sampling frame) in the village. Four farmers from each of the three topographic zones were called for the meeting. The farmers were informed about the objective of conducting a study in their village. Farmers were supplied with chicks and their task was to look after the chickens and to record daily mortality and egg production. In addition, farmers were paid a monthly wage of equivalent to US$ 7 for looking after the chickens.

Hatches in any of the flocks was going to be appropriated by the farmers. Each farmer had a chicken housing separate from the family house so as to facilitate catching the birds during the sampling days (the selected farmers had their own chickens between 0 to 14). Again the farmers were requested to be ready to keep up with the timetable that was preset at that meeting. Farmers who failed to abide with the above conditions were dropped.

3.1.4 Study design

3.1.4.1. Experimental chickens and their general management

A flock of 56 Morogoro ecotype parent stock chickens was reared and fed breeders mash at the Sokoine University of Agriculture (SUA) in order to provide chicks of similar genetic background and avoid confounding with genetic variability. The parent stock

comprised of 50 hens and six roosters, the eggs from hens were collected and incubated in an electric incubator (Funki®, Denmark). After hatching, the chicks were raised in an electric brooder at (SUA) farm until they were six-week-old. During this period the chicks were fed chicken mash and given water *ad libitum*. Before taking them to the village, the chicks were weight matched using an electronic scale (Tefal®, Jelly) with a precision of 1 gram and were identified using leg rings and metallic wing tags.

The experimental chickens were randomly distributed to 12 farmers and were allotted to two groups of equal size, treated and untreated controls. All farmers were given ≥ 18 chickens although the required number was 10 chicks per farmer. The high number of chicks given to farmers considered the high mortality that is known in free-range chickens (Kuit *et al.*, 1986; Minga *et al.*, 1989; Bell *et al.*, 1990; Gunaratne *et al.*, 1993).

A total of 318 six-week-old chicks were sent to the village in the rainy season from March to May 2000. In case of high losses chicks were replaced just to make sure that at the end of the experiment there were enough chickens for statistical analysis. The chicks were allowed to stay there for two weeks to acclimatise before the start of the experiment at the age of eight weeks. In addition, all the experimental chickens were vaccinated against Newcastle disease using (LaSota®, Sanofi) at the age of 3, 8, and 32 weeks as recommended by Jordan and Pattison (1996). The vaccination included also farmer's own chickens.

Chickens were released in the morning together with farmer's own chickens to fend for themselves and were housed during the night. In general, there was no feed

supplementation in most of the flocks.

Treatment with mebendazole (Kukuzole®, Interchem Pharma). at 50 mg/kg live weight as recommended by Brander *et al.* (1991) was done fortnightly on individual basis. The outcome of anthelmintic application was followed for six months. All chickens were autopsied seven days after the last treatment and the differential total worm burden determined.

Table 3. Study design to determine the effect of natural helminth infections on chicken production

Number of flocks	Group	Number of chickens	Mebendazole (50 mg/kg)	Parameters assessed
12	Control	60	NO	Mortality, weight gain, egg production, worm counts.
	Treated	60	YES	Mortality, weight gain, egg production, worm counts.

3.1.4.2 Clinical assessment

Farmers were visited every fortnight in order to record mortality, individual body weight, age at the point of lay, and weight of eggs. The weighing of individual chickens and eggs was done using an electronic scale (Tefal®, Jelly). This was conducted in the morning before 0900 h when the chickens had not eaten anything after which they were released to start free-ranging. In case of an outbreak of any disease the entire flock was

treated against the disease in question. For example, during the course of the experiment there was outbreak of fowl pox and coccidiosis is four farms.

The chickens were routinely treated against ectoparasites using carbaryl (Sevin®, Rhone Poulenc). Dead chickens were stored in a kerosene operated Electrolux® refrigerator/freezer that was placed in the village and were collected during the sampling days or other visits for postmortem examinations.

3.1.4.3 Weight gains

Individual weights were recorded at the beginning of the experiment and at 14 days interval until the end of the study. The cumulative weight gain for each animal was calculated by subtracting the initial weight from the weight on the day of sampling.

Cumulative weight gain = Weight at day n – weight at day 0

Where:

n = Day of sampling

0 = Day of starting the experiment

3.1.4.4 Parasitological examination of the autopsied chickens

Seven days after the last treatment all chickens were autopsied and total worm counts were determined. The slaughtered chickens were eviscerated and the entire GIT was put in a tray and divided into four portions; the oesophagus to proventriculus, the gizzard, small and large intestines, and the caeca. Embedded worms from the crop (*G.*

ingluvicola), proventriculus (*T. americana*), and gizzard (*C. hamulosa*) were isolated after teasing the tissue by scalpel blade. Isolation of gizzard worm was preceded by removing the keratin layer.

For the worms that are found in the lumen of the GIT, each portion was washed separately under running tap water. The contents were washed through two test sieves of 500 µm and 100 µm. The deposits were transferred to a petri dish for examination. All worms were picked, recorded and stored in 70% ethyl alcohol until identification.

Identification of helminth species followed a standard technique described by MAFF (1986)

The prevalence of helminth species identified was calculated using a formula as described by Thrusfield (1995);

$$\text{Prevalence} = \frac{\text{Number of chickens found with the parasite}}{\text{Total number of chickens examined}} \text{x}100$$

The efficacy of the anthelmintic used was calculated as previously described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) in the guidelines for testing against anthelminthic efficacy (Duncan *et al.*, 2002).

$$\text{Eff.} = \frac{\text{Mean number of worms in control group} - \text{mean number of worms in treated group}}{\text{Mean number of worms in control group}} \times 100$$

3.2 Study II. Assessment of the effectiveness of different deworming strategies in free-range chickens

In this study, three different treatment regimens were tested. The treatments were compared against each other and the untreated controls. This study was conducted in order to establish the most suitable treatment regimen in free-range chickens.

3.2.1 Study area

The study was conducted in Kibwaya Village in the Morogoro Region of Tanzania as described previously (3.1.1). The experiment was carried out from the middle of the rainy season in early April 2000 to September 2001.

3.2.2 Cluster determination and selection of farmers

In this study the number of farms was determined using a two stage cluster sampling formula as described in section 3.1.2. Five farmers from the first experiment continued with the second experiment and seven new ones who were randomly selected were added on. However, two new farmers were dropped during the course of the experiment because of unacceptably high mortality rate of chicks in their flocks due to predation and repeated outbreak of coccidiosis. The dropped farmers were replaced by other two farmers.

3.2.3 Study design

3.2.3.1 Experimental chickens

Chicks were hatched at the SUA farm and raised in an electric brooder up to four-weeks of age. Chicks were identified using metallic wing tags and plastic leg bands of different colour codes corresponding to treatments before being distributed to farmers. In addition, chicks were weight matched and sent to the village at four weeks of age and left to acclimatize for two weeks. All chickens were vaccinated against Newcastle disease as described in the first experiment using (LaSota® Sanofi) strain. In case of disease outbreaks the entire flocks were treated against the disease.

Each farmer was given ≥ 20 chicks. The chickens were divided into four equal groups, namely; monthly treatment, seasonal treatment (chickens treated at the beginning of the dry and at the beginning of the rainy seasons), single treatment (at the sixth-week of age only), and the untreated controls.

Treatment of respective chickens was conducted on individual basis using mebendazole (Kukuzole®, Interchem Pharma) at 50 mg/kg live body weight. Unfortunately, mebendazole was withdrawn from the market therefore it was replaced with a single dose of a combination of oxyclosanide and levamisole (Fasiola®, Coopers). The Fasiola®, Coopers was used in the last four months. The dose of 25 mg/kg body weight was calculated with reference to levamisole concentration of the drug (Brander *et al.*, 1991).

Table 4. Study design for the deworming strategies in free-range chickens

Group (n=30)	Treatment	Parameters assessed
1	Monthly	Mortality, wt, egg production, worm count
2	Seasonal	Mortality, wt, egg production, worm count
3	Single	Mortality, wt, egg production, worm count
4	Control	Mortality, wt, egg production, worm count

3.2.3.2 Clinical and parasitological assessment

The effect of treatment was assessed every fortnight by recording mortality , live weight gain, age at the point of lay, number of eggs laid and their weights. The weighing was conducted as described in section 3.1.4.3. Seven days after the last treatment, all chickens were autopsied and the PM total worm burden determined in the same way as described in section 3.1.4.4.

3.3 Study III. Determination of the pathogenic effect of *T. americana* in chickens

The pathogenic effects of *T. americana* was determined in chickens experimentally infected with different dose levels by recording both the health and production parameters of the chickens.

3.3.1 Study period and area

The experiment was run for a period of 12 weeks at the Royal Veterinary and Agricultural University (RVAU), Copenhagen, Denmark.

3.3.2 Sample size

The sample size was determined by fitting values of the mean weight gain of untreated control, mean weight gain of treated chickens and the overall standard deviation (S) obtained from the first experiment that was done on-farm. The first experiment had proven that helminth infections had negative effects on the growth performance in free-range chickens. The sample size was determined on the basis of one parameter available, weight gain, and thereafter trusting that this sample size would be sufficient for the other parameters. Therefore, the required small sample size was calculated according to Martin *et al.* (1987) as follows:

$$n = 2 \times \left[\frac{(Z\alpha - Z\beta)S}{\bar{X}_e - \bar{X}_c} \right]^2$$

Where; n= Sample size

Z_α = Type I error at 95% Confidence Interval (1.96)

Z_β = Type II error at 20% (-0.84)

S = Common standard deviation (200)

$\bar{x}e$ = Mean weight gain in exposed group (931 g)

$\bar{x}c$ = Mean weight gain in unexposed group (1136 g)

Therefore, the calculated group sample size was 14.92444973 that is approx. 15 birds.

3.3.3 Study design

Sixty, day-old-female Lohman Brown chicks were housed in two pens of 4 x 2 m size. At the age of two weeks all chickens were wing tagged and identified using plastic leg rings of different colour codes. The chickens were weight matched into 4 groups of 15 each and randomly distributed into two pens where feed and water were provided *ad libitum*.

3.3.3.1 Source of the third stage larvae

The life cycle of *T. americana* was adapted and maintained at the National Pest Laboratory in Lyngby, Denmark. The gravid female *T. americana* were sent from Morogoro, Tanzania, and kept in physiological saline under refrigeration temperature

(+4 °C) until the day of infection. A group of 100 adult migratory locusts, *Locusta migratoria*, and 50 cockroaches, *Blatella germanica*, were reared at 30 °C for propagation of *T. americana* life cycle. Fifty migratory locusts and 25 cockroaches were kept as uninfected controls. Each locust was housed in a plastic container (20 cm wide by 20 cm long by 25 cm high) with holes at the bottom and top sides for ventilation.

The locusts were daily fed on grass. Cockroaches were laboratory reared in smaller plastic containers and were infected by plastering the crushed *T. americana* with peanut butter. The locusts were fasted for one day before infection. The infection was conducted by putting a female *T. americana* on a grass and fed to a locust and this procedure was closely watched to make sure that the locusts ate the worms. Thereafter, more grass was provided to the experimentally infected locusts. The third stage larvae of *T. americana* were isolated from *L. migratoria* and *B. germanica* 41 days post infection (dpi).

At 41 dpi *L. migratoria* and *B. germanica* were dissected in a petri dish filled with physiological saline. Isolation of the third stage larvae (L_3) was conducted under a dissecting microscope. During dissection larvae escaped from the locusts and were seen freely swimming in the physiological saline. The larvae were stored overnight at +4 ^{0}C in three different doses; 25, 100, and 400 L_3.

3.3.3.2 Experimental infection

The dose-response trial involved three dose levels, the first three groups were experimentally infected with 25, 100, and 400 L_3 whereas the fourth group was left as

uninfected control. The experimental infection of the respective groups was conducted using plastic Pasteur pipette. Each pipette was used to drench one chick and the pipettes were repeatedly rinsed with physiological saline to make sure that all of the larvae had been given to the chickens.

Table 5. Dose response trial to determine the pathogenicity of *Tetrameres americana*

Group (n=15)	Dose of *T. americana*	Measurements monitored
1	25	Weight, pH, digestibility of crude protein (CP), pepsinogen & differential leucocyte counts (DLC), worm counts
2	100	as above
3	400	as above
4	Control	as above

3.3.3.3 Clinical assessment

Chickens were observed three times a day (in the morning at 0800 h, afternoon at 1400 h, and evening at 1700 h) to study any abnormal signs after infection such as reduced activity, reduced feed intake, consistence of the faeces. The outcome of the experimental infections was assessed every two weeks for 12 weeks by measuring the live weights using a portable electronic scale with a precision of 0.5 gram (Tefal®, Jelly).

3.3.4 Haematological study and examination

3.3.4.1 Collection of blood samples

The chickens were restrained manually for venipuncture. Two to five millilitres of blood were collected from the wing vein (*Vein ulnaris*) using 22 gauge (22G. 1½ inch) needle into vacutainer tubes containing vitamin K3E as an anticoagulant (BD Vacutainer, UK). A drop of blood was taken for making blood smear for differential leucocyte counts (DLC). The remaining blood was centrifuged as described by Campbell (1995) and plasma was collected and stored at -20 °C until analysed for pepsinogen level.

3.3.4.2 Staining for differential leucocyte counts

The staining of blood slides was conducted using Wright's stain as described by Campbell (1995). Counting of white blood cells included heterophils, eosinophils, basophils, lymphocytes and monocytes which were counted at the monolayer part of the stained microscope slide. A multiple counter was used to count the first 200 white blood cells on the examined field and recording the proportion of each cell type.

3.3.4.3 Determination of blood pepsinogen levels

It has been proven by Berghern *et al.* (1987) that the level of pepsinogen in plasma and serum stays constant during storage at -20 °C, and that the duration of storage does not alter the concentration of pepsinogen level of the sample. The plasma samples were sent to Sveriges Lantbruks Universitet (SLU), Uppsala, Sweden for analysis. The pepsinogen level was determined by standard method described by Berghen *et al.* (1987). The

technique involves incubation of the plasma or serum using albumin substrate to release tyrosine. The amount of tyrosine liberated was measured spectrometrically at 650 nm. The pepsinogen concentrations were measured in International Units of tyrosine, i.e. micromoles of tyrosine per litre of plasma per minute, (Berghen *et al.*, 1987).

3.3.5 Collection of faecal samples and determination of digestibility of CP

Faecal samples were collected fortnightly. Chickens were separated by treatments one day before collecting the faecal sample and they spent a night in the boxes. The faecal samples were colleted the following morning between 0700 - 0730 h. The pooled fresh droppings were then stored at -20 °C until the day of analysis. The minimum quantity collected was five grams per group.

The apparent digestibility of the crude protein (CP) was determined by Kjeldahl method and the digestibility coefficient computed using the formula by McDonald *et al.* (1992). This formula is neglecting the amount of nitrogen in the faeces that is believed to be 2% and thus referring to crude protein in the droppings (McNab, 1994).

$$\text{Apparent digestibility of CP} = \frac{(\text{Amount of CP in feed} - \text{Amount of CP in droppings})}{\text{Amount of CP in feed}} \times 100$$

3.3.6 Gastric pH

The gastric pH was determined at necropsy of the slaughtered chickens. The electrode of an electronic pH meter (pH-Meter CG840®, Schott) was inserted into the proventriculus five minutes after slaughter and the reading on the display window was recorded after the machine had stabilised.

3.3.7 Post mortem worm counts

At the end of the study all chickens were autopsied and the total worm counts determined. Since female *T. americana* are implicated to be the most pathogenic compared to the males, the numbers reported in the results exclude the males. All worms were collected, recorded and stored in 70% ethyl alcohol

3.4 Study IV. Determination of the effect of *T. americana* on chickens fed different levels of protein diets

The effect of parasitism on chickens fed different levels of protein diet was studied. Two levels of protein were involved and in each of the protein level studied. half of the chickens were infected with a single dose of 100 L_3 of *T. americana*. Haematological and production parameters were measured to assess the impact of infection

3.4.1 Study chickens, period and area

The experiment to determine the pathogenic effect of *T. americana* in growing chickens was conducted using female Lohman Brown (LB) for 12 weeks at the Royal Veterinary and Agricultural University (RVAU), Copenhagen. Denmark.

3.4.2 Sample size

The sample size used in this experiment was the same as described in section 3.3.2. Therefore, 15 birds were used in each group.

3.4.3 Study design

3.4.3.1 Experimental chickens

Sixty, day-old Lohman Brown female chicks were put into two groups of 30 chicks each receiving either high protein (HP) or low protein (LP) diets. The HP diet contained 17.8% crude protein (CP) whereas the LP diet contained 13.3% CP. Chickens in the

same level of nutrition were further subdivided into infected (+) and uninfected (-) subgroups of 15 chickens each.

The compositions of the 100 kilogram bag of chicken feed of the high (HP) and low (LP) protein diets were as shown in Table 6.

Table 6. Composition of the experimental diets

Ingredient	High Protein (HP) diet	Low Protein (LP) diet
Protein	17.8%	13.3%
Energy	1091 MJ	1104 MJ
Fat	4.8%	4.6%
Calcium	1.68%	2.25%
Phosphorus	0.96%	0.72%
Ash	9.10%	9.60%
Cellulose	5.70%	5.00%

3.4.3.2 Grouping of chickens

At the age of one week all chickens were identified using metallic wing tags and plastic leg rings. The experimental infection was carried out on individual basis at the age of two weeks by giving 100 L_3 of *T. americana*. The dose of 100 L_3 was chosen so that in the end the results could be extrapolated to low and high dose levels. The infected and uninfected chickens under the same protein level were housed together. All chickens were provided with feed and water *ad libitum* for a period of 12 weeks by which time females of *T. americana* are known to reach their full size (Cram, 1929, 1931). This

study also intended to determine the effect of female *T. americana* when they have reached their full sizes. The study set up is shown in Table 7.

Table 7. Study design of *Tetrameres americana* infected chickens fed different levels of protein diets

Group (n=15)	Level of CP	Dose of *T. americana*	Assessed Parameters
HP+	17.8%	100	Weight gain, PCV, Hb concentration, Blood pepsinogen, and worm counts
HP-	17.8%	0	as above
LP+	13.3%	100	as above
LP-	13.3%	0	as above

3.4.3.3 Clinical assessment

Response of chickens to the treatments were assessed every 14 days. Chickens were clinically observed three times a day and the weight was measured using a portable electronic scale as described in section 3.3.3.3.

3.4.4 Assessment of haematological parameters

Blood was collected from *Vein ulnaris* as described in section 3.3.4.1 Determination of pepsinogen levels was done as described in section 3.3.4.3 whereas determination of PCV and Hb are described hereunder.

3.4.4.1 Determination of packed cell volume

PCV level was determined by drawing the blood into haematocrit capillary (Wintrobe®) tubes after which one end of the tube was sealed with Crista sealant. The Wintrobe tubes were then centrifuged at 12,000 g for 5 minutes in a Hawskley micro-haematocrit high speed centrifuge (Campbell, 1995; Cooper *et al.*, 1996). The PCV was read using a Hawskley Micro-Haematocrit Reader (Hawskley and Sons Ltd, Sussex, UK) and the values were expressed in (SI) units, that is, litres per litre (Blood *et al.*, 1985).

3.4.4.2 Determination of haemoglobin concentration

Haemoglobin concentration was determined photometrically by directly analysing the whole blood using a modified azidemethemoglobin reaction. This involves the disintegration of the red blood cells (RBC) by sodium deoxycholate and releasing the haemoglobin. Sodium nitrite converts the haemoglobin iron from ferrous to ferric state to form methemoglobin which then combines with azide to form azidemethemoglobin.

In this photometer carboxyhemoglobin originating from ruptured leucocytes, leucocytosis, and turbidity do not interfere with the HemoCue haemoglobin measurement (HemoCue®, AB®, Angelholm, Sweden). The Hb concentration of the sample is directly read from the machine.

3.4.5 Parasitological examination of slaughtered chickens

At the end of this experiment all birds were autopsied for worm counts. The slaughtered chickens were eviscerated. Embedded female *T. americana* were isolated and stored after teasing the tissue using a scalpel blade as described in section 3.3.7

3.5 Statistical analyses

Data were analysed using a computer package SAS System for Windows Version 8, (SAS, 1999). A Kaplan-Meier test was run to compare the survival rates between groups.

A normality test was run before analysis was done in order to choose the right test for comparison of means. Data on weight gains, PCV, Hb, leucocyte counts were found to be normally distributed and therefore were not transformed and thus analysed straight away. Whereas data on the worm burdens were skewed with many birds having light infections and few having heavy infections and could not be transformed thus a non-parametric, Kruskal-Wallis, test was used to measure the difference between group mean ranks. Analysis was mainly done as factorial designs under completely randomised block design (CRBD).

A mixed model procedure was applied to analyse the effect of treatments and their interactions on how much they influenced the production parameters. The tests were conducted at 95% confidence interval and whenever difference was detected a least significant difference (LSD) comparison of means was used to identify the different groups.

Data were analysed basing on the following general statistical model:

$$\sum X_{ijk} = \mu + Ti + Bj + (TB)ij + Eijk$$

Where:

$\sum X_{ijk}$= Sum of parameter X for i^{th} treatment, j^{th} farmer/pen and k^{th} chicken

μ= Overall mean

T *i* = Treatment effect

B *j* = Farmer/Pen (block) effect

(TB) *ij* = Interaction of treatment and farmer/pen effect

E *ijk* = Random error

CHAPTER FOUR

4.0 RESULTS

4.1 The effect of helminth parasites on the productivity of free-range chickens

4.1.1 Mean worm burdens of treated versus control groups

A total of 19 helminth species were isolated in this study, 12 being nematodes and 7 cestodes. Treatment using mebendazole showed broad spectrum of activity by controlling all of mature GIT nematodes including the tracheal worm, *Syngamus trachea*. In this study mebendazole was highly effective with efficacy > 98% towards control of *Ascaridia galli*, *Capillaria bursata*, *Cheilospirura hamulosa*, *Tetrameres americana*, *Hymenolepis cantaniana* and *Raillietina cesticillus*.

Mebendazole was also effective with efficacy between 80-97% against *Capillaria anatis*, *Capillaria contorta*, *Capillaria obsignata*, *Gongylonema ingluvicola*, *Heterakis gallinarum*, *Strongyloides avium*, *S. trachea*, *Amoebotaenia cuneata*, *Hymenolepis carioca*, and *Raillietina echinobothrida*.

However, the drug was ineffective against *Choanotaenia infundibulum*, *Raillietina tetragona*, and immature *H. gallinarum* that were predominant among the immature nematodes that were isolated. The mean worm burdens were relatively moderate with few exceptions. Furthermore, the prevalence of most helminth species was lower in treated group when compared to untreated controls (Table 8).

Table 8. Prevalence (prev.) and mean worm burdens in mebendazole treated and control free-range chickens

Helminth species	Treated (n=51)		Control (n=51)		Efficacy (%)
Nematodes	Prev.	Mean	Prev.	Mean	
Ascaridia galli	0.0	0.00	15.7	0.5	100
Capillaria anatis	13.7	0.30	51.0	7.4[a]	95.9
Capillaria contorta	3.9	0.04	13.7	0.5	92
Capillaria bursata	2.0	0.02	21.6	1.8	98.8
Capillaria obsignata	11.8	0.30	58.8	8.1[a]	96.3
Cheilospirura hamulosa	0.0	0.00	13.7	1.2	100
Dispharynx nasuta	2.0	0.02	0	0.0	0
Gongylonema ingluvicola	13.7	0.30	52.9	1.9[a]	84.2
Heterakis gallinarum	45.1	2.30	76.5	29.5[a]	92.2
Strongyloides avium	3.9	0.10	11.8	4.3	97.7
Syngamus trachea	2.0	0.04	3.9	0.2	80
Tetrameres americana	3.9	0.04	62.7	3.8[a]	98.9
Cestodes					
Amoebotaenia cuneata	15.7	1.2	49	15.1[a]	92.3
Choanotaenia infundibulum	5.9	0.2	9.8	0.7	71.4
Hymenolepis cantaniana	0.0	0.0	13.7	13.7	100
Hymenolepis carioca	5.9	0.1	17.6	2.2	91.2
Raillietina echinobothrida	9.8	0.3	39.2	3.4[a]	91.2
Raillietina tetragona	25.5	1.7	29.4	2.8	39.3
Raillietina cesticillus	0.0	0.0	2	0.1	100

[a] Mean worm burden significantly higher than in the treated group ($p<0.05$).

4.1.2 Clinical observations

During this study few chickens were observed to show clinical helminth infections such as gasping within the first month of exposure. Fortunately most of the chickens were those receiving treatment and hence they recovered after treatment. One grower from control group died of *S. trachea* infection. In the second month post exposure one chicken became lethargic, anaemic, droopy, losing weight and started passing soft faeces and ultimately died; postmortem (PM) examination revealed presence of 98 adult *A. galli* worms that were blocking the intestinal lumen and the carcass was emaciated.

4.1.3 Mortality among experimental chickens

A total of 318 chicks were sent to the village but during the acclimatisation period 70 chicks died before the beginning of the study due to mismanagement such as lack of health care and inclement weather. This number was equal to 32.4% of the total loss. In addition, during the experimental study 146 chickens were lost making a total loss of 216 chickens and only 102 (32.1%) chickens survived to the end of the experiment.

The flock sizes that survived to the end of the experiment ranged from 5 to 12 chickens. The major causes of mortality during this study were observed to be predators. mismanagement/inclement weather, diseases and other causes in that order. Predation was very common during the day and rarely occurred at night. Various diseases contributed to 16.2% of total losses. Other causes such as chickens being run over by a car or a bicycle, theft and just disappearance contributed to 18%. The total mortality was observed to be 67.9%. The mortality was higher at the beginning when chicks were

young and decreased as they grew older. The loss due to various causes are shown in Table 9. The trend of decreasing number of chickens is shown in Fig.2.

i. Predators

Predation was noted to contribute 33.4% of total mortality rates. Birds and beast of prey were the common causes of deaths. The birds of prey such as eagles, crows, hawks, and buzzards were responsible for losses mostly in chicks and mainly during the daytime whereas beasts of prey such as mongoose, stray dogs, cats and badgers were reported to cause mortality especially in growers and adults. Badgers were reported to break into chicken houses at night and kill the victims. Two cases were reported to be caused by snake bites one inside the chicken house at night and the second case during the daytime while chickens were scavenging.

ii. Diseases

Diseases contributed 16.2% of total mortality. During the course of this study there were outbreaks of fowl pox in two farms. The outbreaks killed 2 chickens, one from each farm. In addition, diseases such as coccidiosis killed 11 chickens, pneumonia (6), histomonosis (3). helminthosis (2) one chicken died of syngamosis and another one of ascaridiosis, and tumours of unknown origin and inconclusive diagnosis were responsible for death of 11 chickens. Diagnosis of mortality due to bacterial infections was not looked into. Three farmers had a habit of enclosing the experimental chickens and supplementing them with maize bran, that in turn resulted in outbreaks of caecal

coccidiosis due to accumulation of *Eimeria tenella* oocysts. Histomonosis which occurred simultaneously with liver and caecal lesions was confirmed by histopathological examinations (Fig. 3).

iii. Other causes

Other causes contributed to 18% of total mortality. Theft and accidents were also responsible for losses. Before the start of the experiment one chick was ran over by a car and two chicks were ran over by bicycles. In addition, during the experimental period four chickens died from accidents. Furthermore, 35 chickens just disappeared without explanations whereas theft was reported on two farms just at the time of concluding the experiment where one rooster was lost from each of the two flocks.

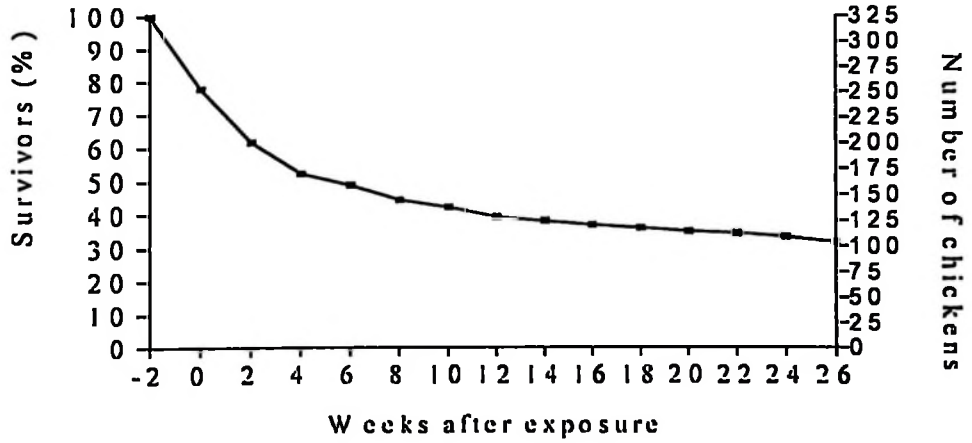

Figure 2. Survival rate of free-range chickens over time.

Table 9. Contribution of various causes of mortality in free-range chickens

	Causes of mortality				
Group	Mismanagement	Predators	Diseases	Other causes	Total
Control	38 [a]	39 [a]	17 [a]	16 [a]	110 [a]
	(17.6%)	(18.1%)	(7.9%)	(7.4%)	(51%)
Treated	32 [a]	33 [a]	18 [a]	23 [a]	106 [a]
	(14.8%)	(15.3%)	(8.3%)	(10.6%)	(49%)
Total	70	72	35	39	216
	(32.4%)	(33.4%)	(16.2%)	(18%)	(100%)

[a]Values in a column with a common superscript are not significantly different ($p>0.05$)

4.1.4 Management practice of experimental chickens

During the course of the study some farmers attempted to confine the experimental chickens up to 1100 h to avoid predation. Others confined the experimental chickens and fed them with maize bran. In those farms where chickens were released late the chickens grew slowly and they could not reach sexual maturity even at the conclusion of the experiment when the chickens were 34 weeks old. While on those farms where experimental chickens were confined and fed maize bran there were several outbreaks of coccidiosis. These farmers were asked to follow the normal way of managing the free-range chickens as their own chickens but were very slow in response. In addition, there was an outbreak of Newcastle disease in the village whereby the experimental and farmer's own chickens survived. However, two of the experimental chickens died of Newcastle disease.

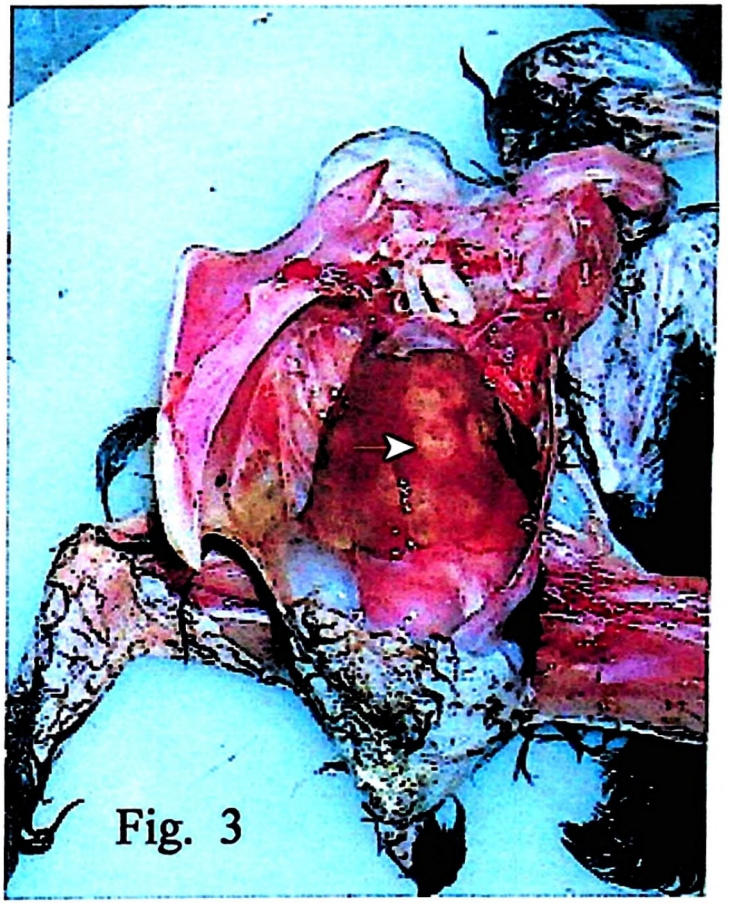

Figure 3. Circular necrotic lesions (arrow) with central depression on the liver of a chicken that had died of histomonosis.

4.1.5 Weight gains

4.1.5.1 Mean weight gains among farms

It was noted that some farmers were located in areas that were rich of insects, plant shoots, flowers and seeds; whereas others were staying at the hill top where chickens had to walk quite a distance to find consumable materials. Those flocks that were located at the hill top were growing relatively slower compared to the flocks at the foot of the hills.

Considerable weight gain was observed in treated chickens when compared to untreated controls. This difference was consistent in 11 out of the 12 farms. However, one flock showed discrepance where the control group had higher weight gain than treated chickens. The weight gains of treated versus controls in different farms/flocks are shown in Fig. 4.

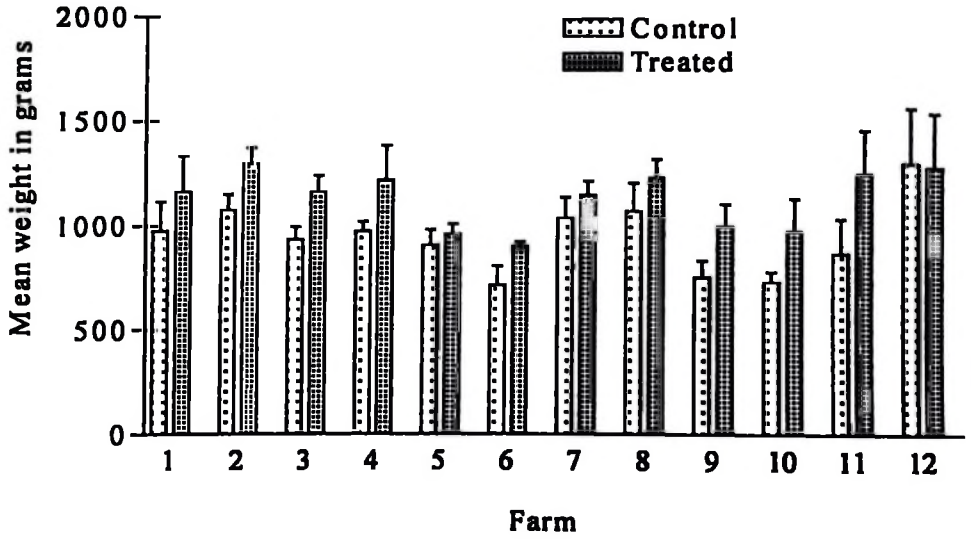

Figure 4. Mean weight gain (Error bars = SEM) in treated chickens compared to untreated controls in 12 experimental farms.

4.1.5.2 Mean total weight gains of treated and control groups

At the end of the experiment the accumulated mean total weight gain of treated chickens was observed to be significantly higher ($p<0.05$) than that of the untreated controls (Fig.5). The treated group gained a total of 1136 g compared to 931 g of untreated controls. The treated chickens therefore had 205 grams more than the untreated controls which is equivalent to 22% higher weight gain. The difference was statistically significant ($p<0.05$).

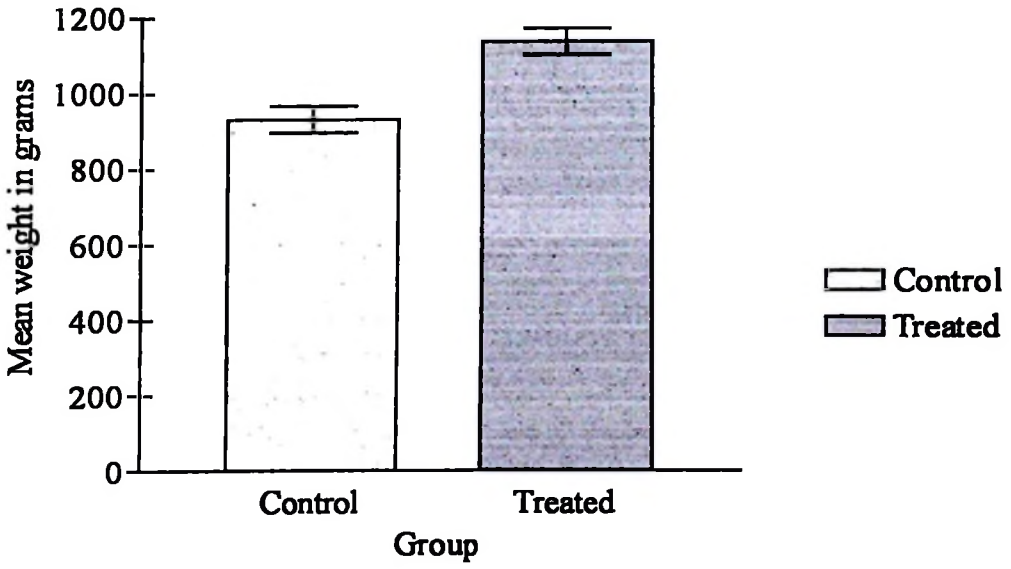

Figure 5. Mean total weight gain (±SEM) in treated chickens compared to untreated controls.

4.1.5.3 Mean weight gain with regard to sex of the chicken

The effect of treatment was significant when treated males and females were compared to untreated counterparts ($p<0.05$) see Fig. 6. Analysis for interaction between sex and treatment was carried out and could not show any added advantage of treating either a hen or a rooster ($p>0.05$). There was therefore no interaction effect.

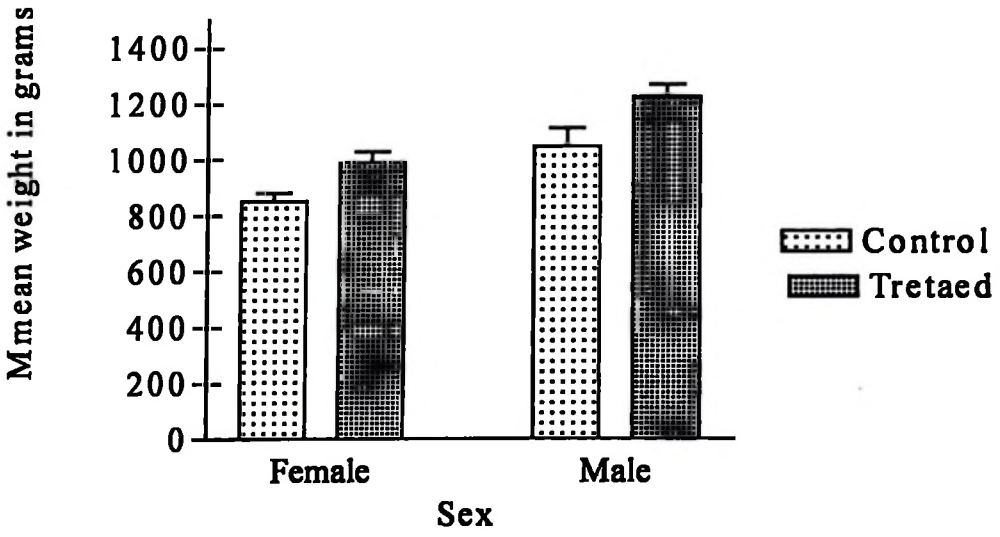

Figure 6. Mean weight gain (±SEM) in treated and untreated controls in both sexes.

4.1.5.4 Cumulative weight gain

At the early stage of exposure, from day 0 to 14, all chickens showed similar weight gains. From week four to six post exposure there was a slight difference between treated and untreated groups. Thereafter, the difference was noticeable and became significant from 12 weeks post exposure. The treated chickens started gaining more weight than the

controls from the sixth week post exposure. The weight gain was significantly different when the treated group was compared to untreated controls from 12 weeks post exposure ($p<0.05$). The performance of treated and control groups over time are shown in Fig. 7.

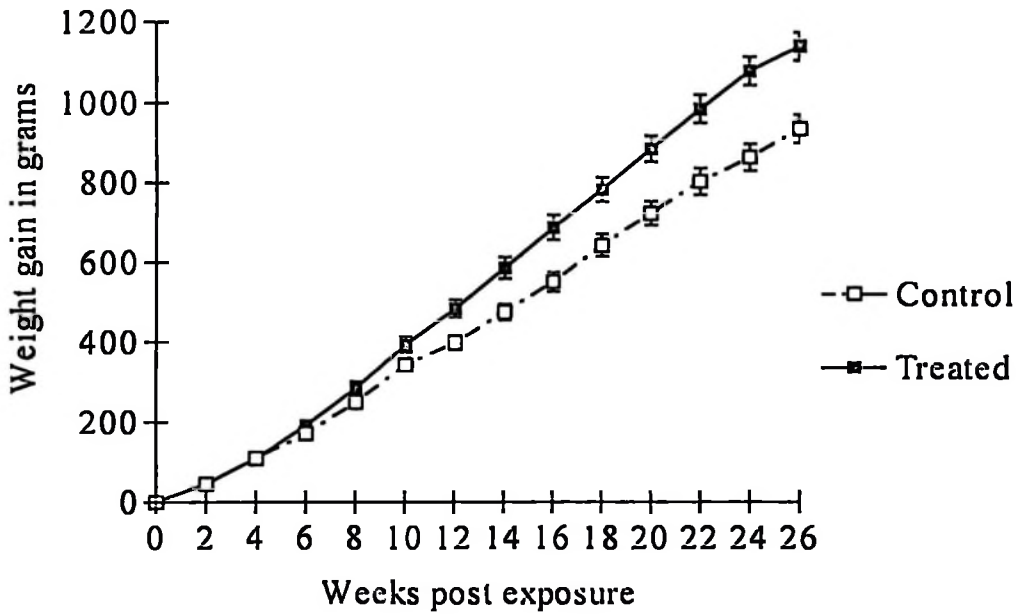

Figure 7. Mean cumulative weight gains (±SEM) of treated compared to untreated controls.

4.1.6 Hen's mean age at the first egg

The mean age at the point of lay of treated hens was 31.5 weeks (range 29 - 34) whereas that of controls was 32.8 weeks (range 30 - 34) and was not statistically different ($p>0.05$).

Twelve treated hens were able to start laying at this age and layed an average of 7.75 eggs (range 2 - 15) whereas only six control hens started laying eggs and produced an average of 5.2 eggs (range 2 - 13). However, at the point of lay hens were observed to have similar mean body weight in the two groups. There was no significant difference on mean egg weights between treated 40.6 grams, range (36-47) grams and controls mean 41.7 grams, range (38-46) grams (Table 10) ($p>0.05$).

Table 10. Mean age and body weight at the first egg and mean egg weight

Group	Age at first lay (weeks ± SD)	Body weight (grams ± SD)	Egg weight (grams ± SD)
Control (n=6)	32.83 [a] ± 1.8	1380.7 [a] ± 113.2	41.7 [a] ± 3.6
Treated (n=12)	31.50 [a] ± 1.8	1406.3 [a] ± 194.3	40.6 [a] ± 2.7

[a] Means with the same superscript within a column are not significantly different ($p>0.05$).

4.2 Effectiveness of different deworming strategies in free-range chickens

4.2.1 Postmortem worm burdens in different deworming strategies

A total of 15 species were isolated, 11 being nematodes and 4 cestodes. The PM total worm counts revealed substantial higher worm counts in untreated controls and in the group that received single treatment. These worms were: *Heterakis gallinarum*, *Amoebotaenia cuneata* and *Hymenolepis cantaniana*. Moderate worm burdens were observed in case of *Capillaria anatis* and *Capillaria obsignata* (Table 9). The overall counts were relatively lower in terms of number of species and the individual parasite counts when compared to previous prevalence studies conducted in the same area a few years ago.

The results showed substantial higher counts of *Capillaria* spp in untreated control and in the single treatment groups. In addition, *C. anatis, C. obsignata, and H. gallinarum* showed significantly higher counts ($p<0.05$) in controls and sixth-week-treated groups when compared to seasonal and monthly treatment groups. The mean worm burdens of *G. ingluvicola* and *Heterakis brevispiculum* were significantly higher ($p<0.05$) in control and groups treated at sixth week of age when compared to seasonal and monthly treatments, respectively. The seasonal treatment however showed satisfactory effect against majority of poultry helminths. The mean worm burden of species isolated are shown in Table 11.

Proventriculus infected with *T. americana* showed dark spots on the serosa surface (Fig. 8a) and on teasing the tissue, female worms were recovered that were spindle to globular shape and blood-red in colour (Fig. 8b). On the other hand, the male

T. americana were isolated from the lumen of the proventriculus and were filiform in shape (Fig. 8c).

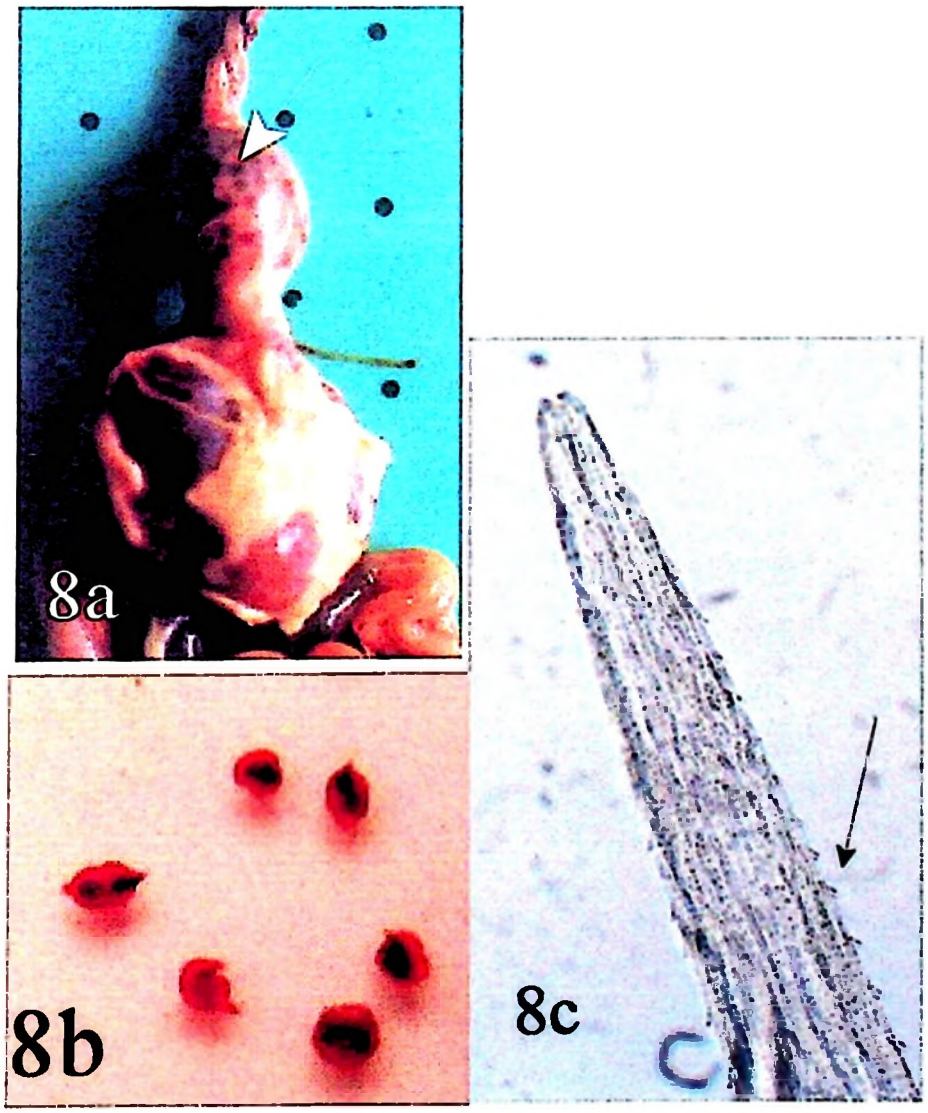

Figure 8a. *Tetrameres americana* are observed as slightly raised dark spots (2-3 mm) on the surface of the proventriculus (white arrow). 8b. Spindle to globular shaped blood-red female *T. americana*. 8c. Anterior end of a male *T. americana*, arrow showing spines.

Table 11. Mean worm burdens in chickens treated with a mixture of oxyclosanide and levamisole (25 mg/kg) at different intervals

Helminth species	Control (n=21)	Sixth week (n=23)	Seasonal (n=23)	Monthly (n=19)
Nematodes				
Ascaridia galli	1.55[a]	2.19[a]	0.04[a]	0.00[a]
Allodapa suctoria	0.00[a]	0.52[a]	0.00[a]	0.00[a]
Capillaria anatis	5.65[a]	7.07[a]	0.26[a]	0.22[a]
Capillaria bursata	0.05[a]	0.71[a]	0.00[a]	0.00[a]
Capillaria obsignata	12.6[a]	9.91[a]	0.39[b]	0.56[b]
Cheilospirura hamulosa	0.70[a]	0.33[a]	0.00[a]	0.00[a]
Gongylonema ingluvicola	5.40[a]	2.29[ab]	0.13 [b]	0.55 [b]
Heterakis brevispiculum	4.00[ab]	13.91[a]	6.83[ab]	0.33[b]
Heterakis gallinarum	81.3[ab]	83.52[a]	21.22[b]	16.50[b]
Syngamus trachea	0.00[a]	0.19[a]	0.09[a]	0.28[a]
Tetrameres americana	4.65[a]	2.05[a]	1.70[a]	2.67[a]
Cestodes				
Amoebotaenia cuneata	8.40[a]	0.24[a]	0.65[a]	0.22[a]
Hymenolepis cantaniana	0.00[a]	0.00[a]	0.30[a]	0.00[a]
Raillietina echinobothrida	0.45[a]	1.33[a]	1.30[a]	0.67[a]
Raillietina tetragona	2.40[a]	1.81[a]	0.65[a]	1.94[a]

[a-c] Means in a row not sharing the same superscript are significantly different from one another by Kruskal-Wallis One-Way Analysis of Variance and comparison of mean ranks at ($p<0.05$).

4.2.2 Mortality of chickens under different deworming strategies

A total of 411 chicks were sent to the village at the age of four weeks and 146 (35.5%) died within the first two weeks before commencement of the experiment. During the experiment an additional 179 chickens died from similar causes as described in the first study. At the time of concluding this experiment (one year later) only 86 (20.9%) chickens had survived. However, mortality showed no significant difference ($p>0.05$) among the groups as shown by a Kaplan-Meier survival chart (Fig. 9).

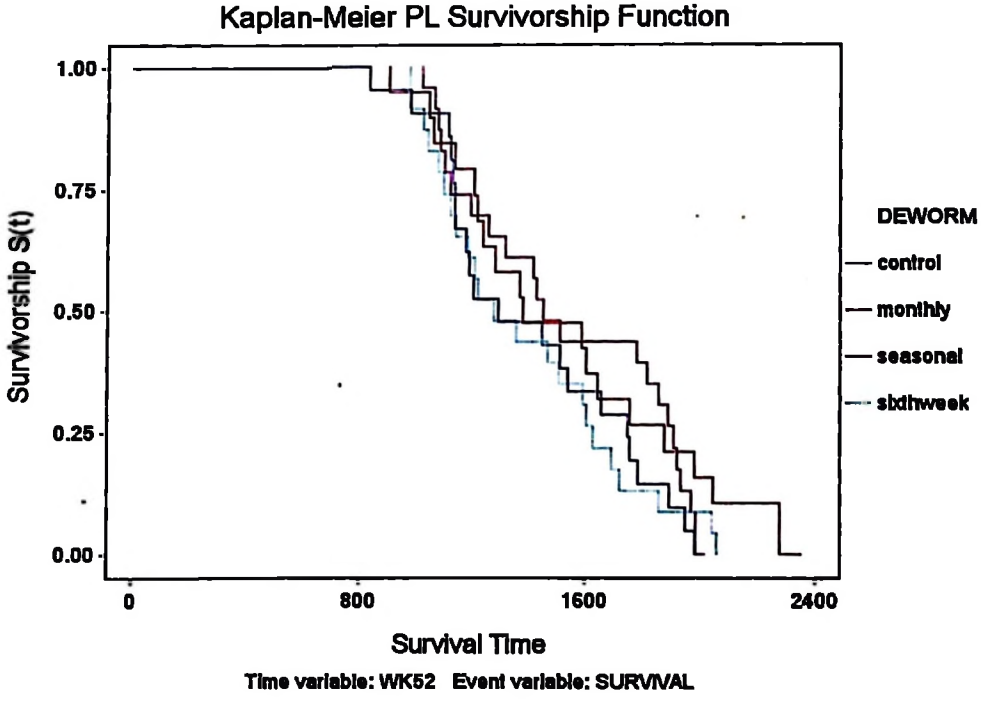

Figure 9. Survival rate of chickens from four different treatment groups.

Necropsy could only be carried out on some birds due to problem with storage facilities. The few birds autopsied showed that 14 chicks died of *Syngamus trachea* before they were 12 weeks of age and had abundant mucous secretions in their trachea while fourteen died of histomonosis. The general trend of survival/mortality rate is shown in Fig. 10 whereas *A. galli* and *S. trachea* that were observed to cause mortality are shown in Figure 11.

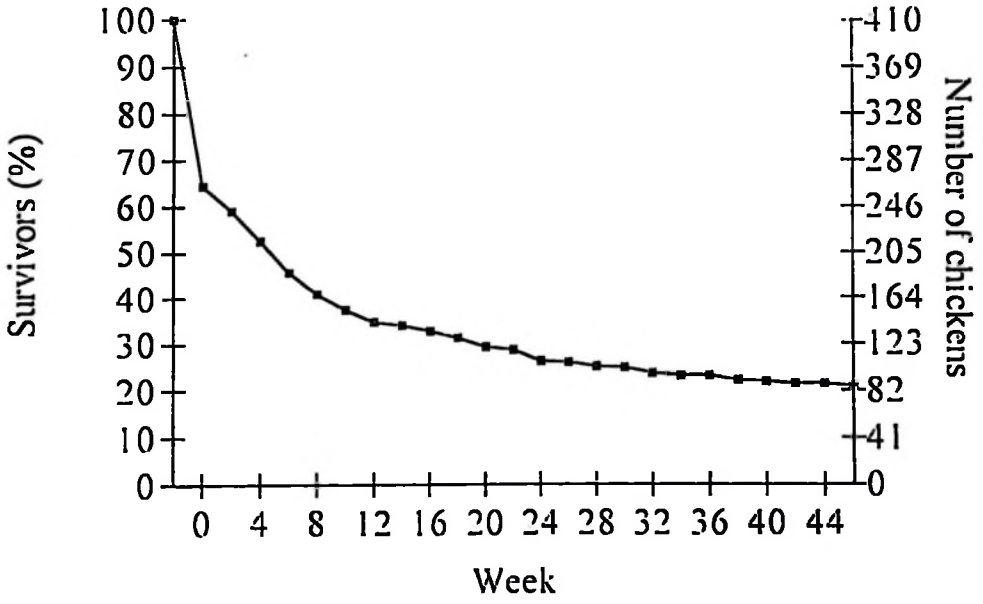

Figure 10. Survival rate of free-range chickens over one year.

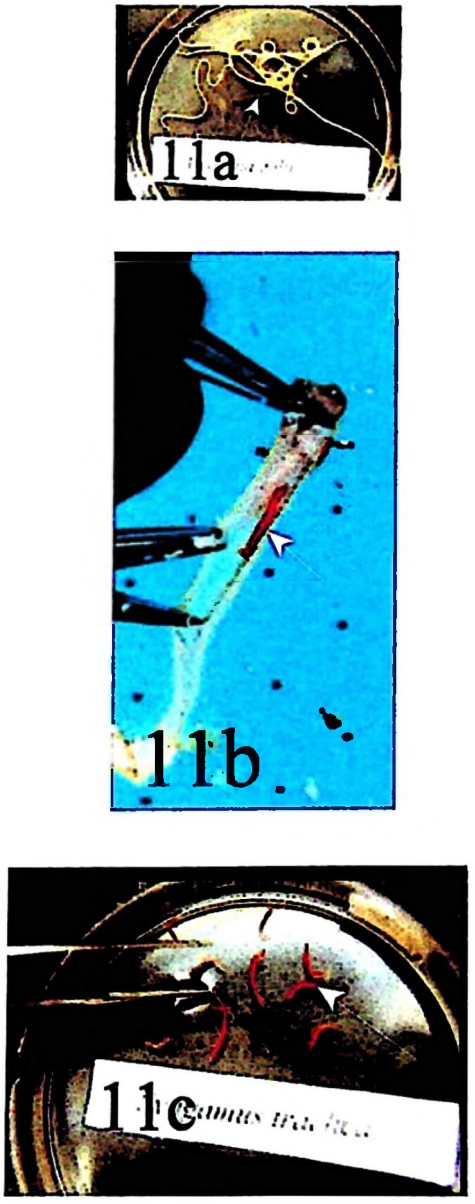

Figure 11. Helminth species that caused mortality in the free-range chickens; 11a is *Ascaridia galli*, 11b is *Syngamus trachea* in situ and 11c is *Syngamus trachea* in a petri dish (arrow).

4.2.3 Cumulative mean weight gains of different treatment regimens

The effect of treatment on weight gain was significantly higher ($p<0.05$) in chickens treated monthly when compared to untreated controls and those chickens which were treated only once at the age of six weeks. The mean total weight gain of seasonally treated chickens was not significantly different ($p>0.05$) when compared to all other treatments (Fig. 12).

During the period between week 35-37 and week 40-43, the monthly treated group showed reduced weight gains. Similar observation was made between week 42-44 in the group treated only once. On the other hand, the control group showed low weight gains throughout the experiment. The mean weight gain of chickens that were given single treatment increased between week 44-48 and decreased towards the end of the experiment (Fig. 13).

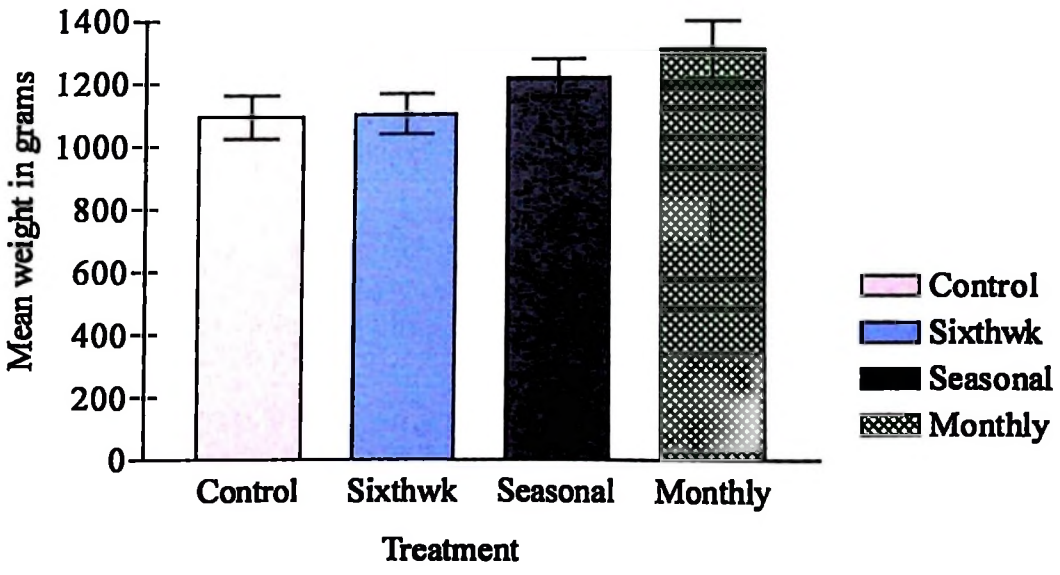

Figure 12. Mean total weight gain in different treatment groups

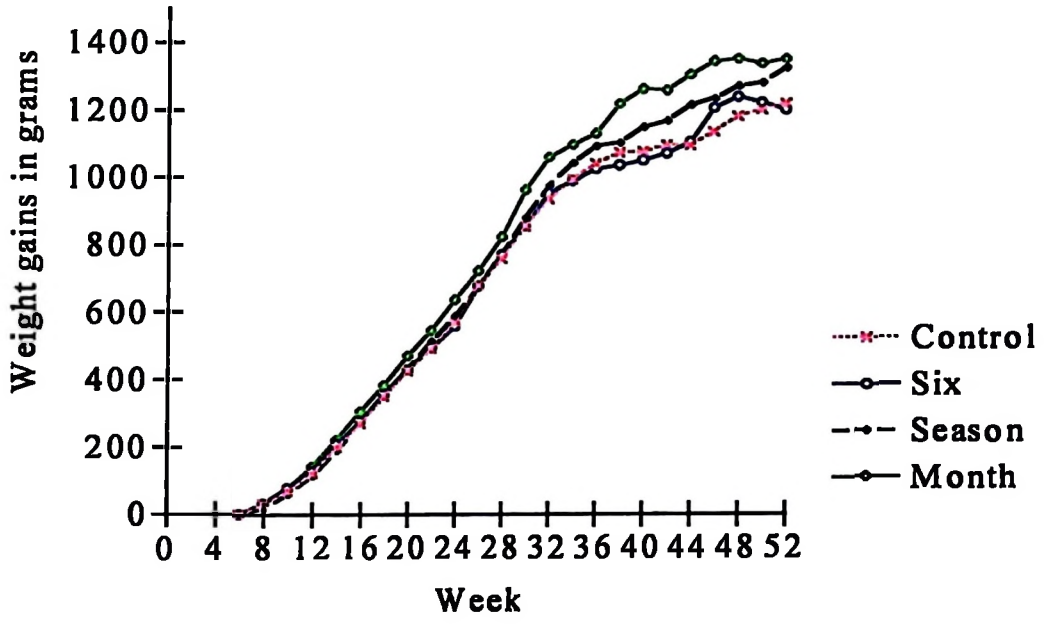

Figure 13. Mean cumulative weight gain of chickens in the different treatment groups

4.2.4 Mean age and body weight of hens at sexual maturity

The number of treatments had effect on the age at which hens reached sexual maturity and started laying. Monthly treatment significantly hastened hens to reach sexual maturity age ($p<0.05$) when compared to controls and those hens that were treated only once at sixth week of age. Though monthly treated hens had their first egg earlier than all other groups the delay was not significantly different when were compared to those treated seasonally ($p>0.05$).

The mean ages at which hens started laying their first egg were; 33.8 weeks for the monthly treated group, 38.7 for the seasonal treated group, 40.1 for those treated at the sixth-week of age only, and 42.2 weeks for the control group. At their point of lay

hens had attained the following body weights: monthly treated hens had mean live body and mean egg weight of 1205 g and 37.1, respectively, for seasonally treated hens (1326 g and 38.5 g), hens that were treated only at sixth-week of age (1233 g, 39.1 g) and the controls (1199 g and 40.1 g). The hens that were monthly treated started laying their eggs of the second batch from week 48 up to the end of this study whereas non of the other groups had their second batch eggs laid.

The hatchability of eggs was markedly different from flock to flock and from hen to hen within the same flock and treatment regimen. Hatchability ranged from 21.4 to 100% with a mean of 65.8% but did not differ significantly among treatments ($p>0.05$). Mean number of eggs and hatchability per treatment regimen are shown in Table 12. However, the study seemed to be too short to have enough number of eggs produced.

Table 12. Mean age and weight at sexual maturity of hens and the number of eggs laid and their hatchability

Treatment	Age in weeks	Maturity weight in grams	Egg weight in grams	Number of eggs per clutch	Hatchability
Control	42.2 [a]	1199 [a]	40.1 [a]	4 [a]	58.1% [a]
Sixth week	40.1 [a]	1233 [a]	39.1 [a]	7 [a]	60.8% [a]
Seasonal	38.7 [a]	1326 [a]	38.5 [a]	7 [a]	66.2% [a]
Monthly	33.8 [b]	1205 [a]	37.1 [a]	5 [a]	73.6% [a]

[a-b] Means in a column not sharing the same superscript are significantly different from one another ($p<0.05$).

4.3 The pathogenic effect of *Tetrameres americana* in chickens

4.3.1 Establishment rates of *T. americana* in experimentally infected chickens

All infected grasshoppers and cockroaches were found with L_3 whereas the uninfected controls were negative of infection. Grasshoppers had an average of 700 infective larvae though some had ≥ 1000 L_3 whereas infected cockroaches had an average of 200 L_3 with up to 500 L_3. Uninfected controls did not have any helminth larvae. Infected grasshoppers looked inactive and there was high mortality of ≥ 80% in both infected and uninfected controls for both grasshoppers and cockroaches.

All control chickens were negative of *T. americana* infection and the infected groups had no parasites other than *T. americana*. Chickens infected with low, 25 L_3, and moderate, 100 L_3, doses of *T. americana* larvae had significantly higher establishment rates compared to the group infected with 400 L_3 (Table 13). All birds that were infected with 400 L_3 got infected whereas two of the 100 L_3 and one of the 25 L_3 no worms were found.

The mean worm counts of the groups infected with 100 L_3 and 400 L_3 doses were not significantly different ($p>0.05$) but were both significantly different ($p<0.05$) from the group infected with 25 L_3 and uninfected controls. High dose had higher variability on worm counts (Fig. 14) as indicated by high standard deviation value (Table 13).

Table 13. Establishment of *T. americana* in chickens infected with different dose levels

Parameter	Infection dose			
	400 L_3	100 L_3	25 L_3	0 L_3
Number of chickens	15	15	15	14
Chickens with worms	15	13	14	0
Mean worm burden (± SD)	15.4 ±11.08[b]	11.2 ±7.02[b]	2.93 ±2.05[a]	0[a]
Establishment rate	3.9%	11.2%	11.7%	0
Range	2-33	0-20	0-6	0
Median	16	11	3	0

[a-b] Means in a row not sharing the same superscript are significantly different ($p<0.05$).

The establishment rate of 400 L_3 was relatively low and showed greater variability as shown by large standard deviation (SD) when compared to other doses (Table 13 above). The intensity of infection recovered from the experimental chickens are shown in Fig. 14.

About one third of the chickens infected with 400 L_3 had worm counts less than 10, the second third had between 14 -20 and the last third had 24-33. On the other hand, those chickens infected with 100 L_3 had normally distributed counts ranging from 0-20 and those infected with 25 L_3 had a clumped distribution between 0-6 (Fig. 14).

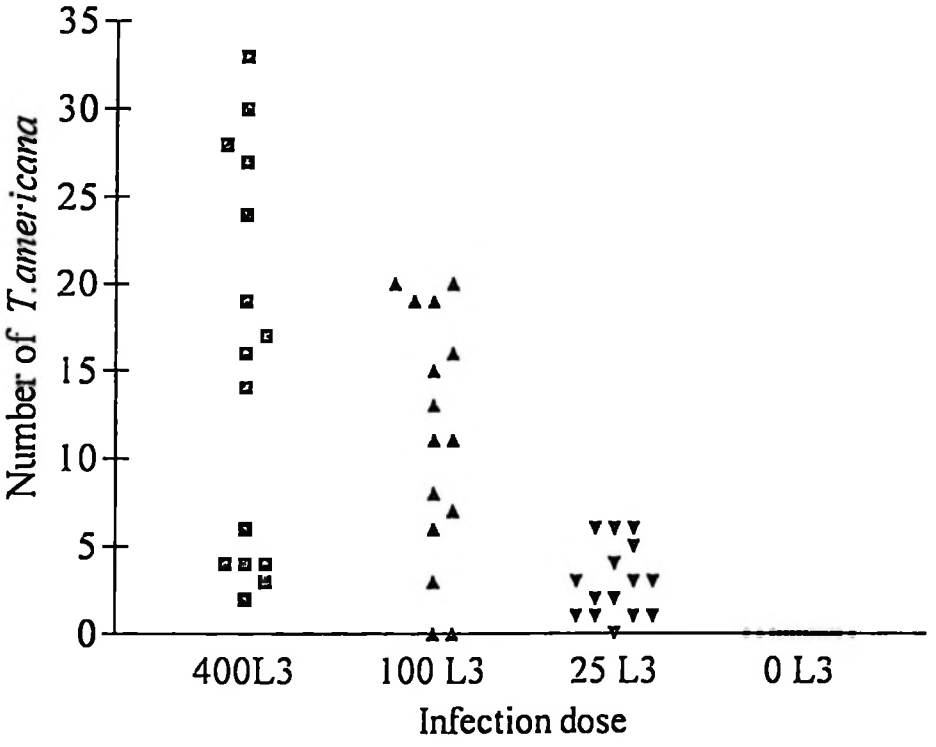

Figure 14. Distribution of *Tetrameres americana* in chickens experimentally infected with different doses

4.3.2 Clinical appearance

During the course of the experiment all chickens were apparently healthy, alert, active, eating and drinking normally. No mortality was recorded owing to *T. americana* infection. However, one chicken from the control group died of accidental suffocation. Furthermore, chickens that were infected with 400 *T. americana* L_3 passed soft faeces (diarrhoea) and at the same time showed the habit of excitement by trampling on other chickens when given food and they were eating relatively faster than others.

4.3.3 Differential leucocyte counts

During this study it was noted that all chickens (infected and uninfected controls) had predominantly higher counts of lymphocytes (37.5-90%) followed by heterophils (6-45%), monocytes (0-18%), basophils (0-15%) and eosinophils (0-10%). At two weeks post infections (wpi) it was clear that chickens infected with 400 L_3 had significantly higher number of heterophils and eosinophils ($p<0.05$) when compared to chickens infected with 25 L_3 and the uninfected controls.

Again at two weeks post infection the proportion of lymphocyte counts in the group infected with 400L_3 was significantly lower ($p<0.05$) when compared to all other groups. Furthermore, the group infected with 100 L_3 had significantly higher numbers of heterophils and basophils ($p<0.05$) when compared to 25 L_3 and uninfected controls.

Thereafter, there was no significant difference in the number of any cell type among the groups until 10 wpi when a significant low lymphocyte counts and higher heterophils counts were observed in the chickens infected with 400 L_3 The difference was significant ($p<0.05$) when compared to uninfected controls. The detailed chronological cellular responses is shown in Table 14.

Table 14. Cellular responses in chickens following infection with *T. americana*

Cell type	Weeks post infection (wpi.)	Dose $400L_3$	Dose $100L_3$	Dose $25L_3$	Control
Monocytes	2	9.4^{a}	5.3^{a}	5.2^{a}	5.5^{a}
Monocytes	4	4.4^{a}	5.4^{a}	3.9^{a}	4.4^{a}
Monocytes	6	6.0^{a}	6.7^{a}	5.7^{a}	5.2^{a}
Monocytes	10	5.0^{a}	4.0^{a}	4.1^{a}	4.1^{a}
Monocytes	12	6.1^{a}	4.9^{a}	5.3^{a}	7.5^{a}
Lymphocytes	2	49^{a}	57.1^{b}	59.1^{b}	58.1^{b}
Lymphocytes	4	60.5^{a}	61.8^{a}	64.8^{a}	64.5^{a}
Lymphocytes	6	64.4^{a}	59.0^{a}	57.4^{a}	62.2^{a}
Lymphocytes	10	67.7^{a}	71.6^{ab}	71.3^{ab}	74.8^{b}
Lymphocytes	12	64.4^{a}	71.5^{a}	71.5^{a}	65.1^{a}
Heterophils	2	33.4^{a}	29^{a}	27.8^{b}	26.5^{b}
Heterophils	4	26.5^{a}	23.9^{a}	24.3^{a}	23.9^{a}
Heterophils	6	21.3^{b}	24.9^{ab}	28.4^{a}	23.6^{ab}
Heterophils	10	21.1^{a}	19.2^{ab}	19.2^{ab}	16.1^{b}
Heterophils	12	23.0^{a}	17.7^{a}	18.6^{a}	22.3^{a}
Eosinophils	2	5.7^{a}	4.1^{ab}	3.0^{b}	2.5^{b}
Eosinophils	4	2.1^{a}	2.4^{a}	1.6^{a}	2.5^{a}
Eosinophils	6	2.4^{a}	3.0^{a}	2.6^{a}	3.3^{a}
Eosinophils	10	1.9^{a}	2.0^{a}	2.0^{a}	1.4^{a}
Eosinophils	12	2.3^{a}	2.5^{a}	1.13^{a}	1.3^{a}
Basophils	2	6.0^{ab}	6.9^{a}	4.8^{b}	4.7^{b}
Basophils	4	6.5^{a}	6.5^{a}	5.3^{a}	5.0^{a}
Basophils	6	6.0^{a}	6.3^{a}	8.5^{a}	5.7^{a}
Basophils	10	4.3^{a}	3.2^{a}	3.5^{a}	3.6^{a}
Basophils	12	4.2^{a}	3.4^{a}	3.8^{a}	4.0^{a}

$^{a-b}$ Means in the same row not sharing the same superscript are significantly different from one another by one way Analysis of Variance (ANOVA) and comparison of means by the least significant difference (LSD) ($p<0.05$).

4.3.4 Pepsinogen levels in experimental chickens

The pepsinogen levels were observed to be higher at early age in all groups which tended to decrease as the chickens grew up. However, the levels were significantly higher ($p<0.05$) in chickens infected with 400 L_3 at 4 and 6 weeks post infection when compared to uninfected controls. At 8 weeks post infection (wpi) all infected groups had significantly higher ($p<0.05$) plasma pepsinogen levels when compared to uninfected controls.

Furthermore, chickens infected with 100 L_3 and 400 L_3 continued to show significantly higher($p<0.05$) pepsinogen levels when compared to controls at 10 wpi. However, at the end of the experiment, i.e. 12 wpi, the plasma pepsinogen levels were not significantly different ($p>0.05$) among groups (Fig. 15). From these results the level of pepsinogen was between three to nine Units of tyrosine. Statistical differences were observed at weeks 4 - 10 ($p<0.05$).

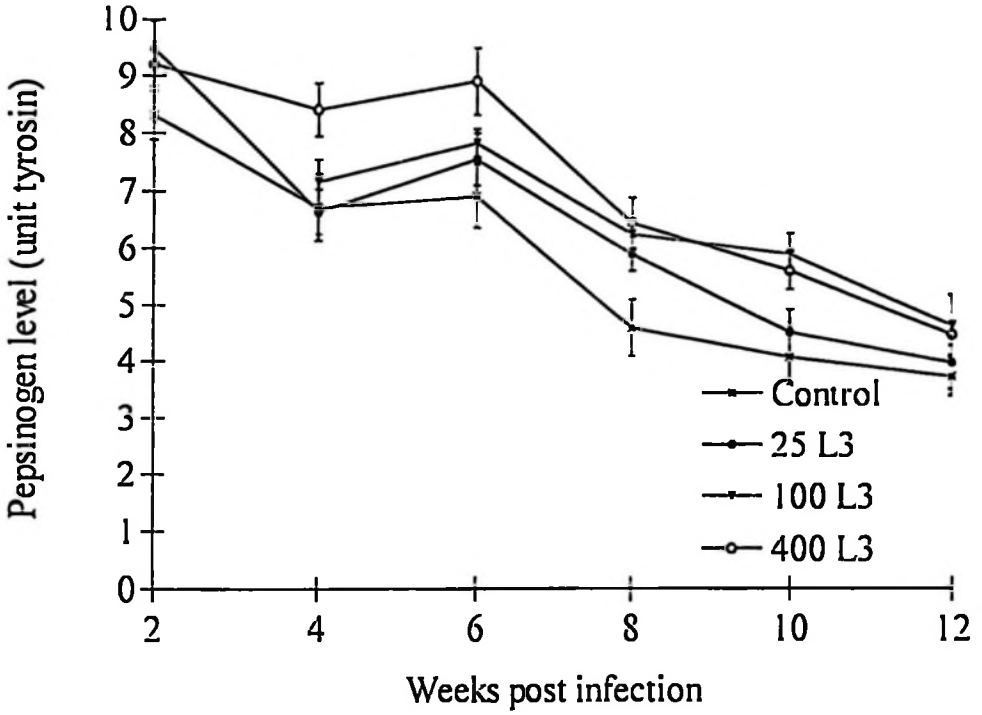

Figure 15. Mean (Error bars = SEM) plasma pepsinogen levels in chickens infected with different doses of *T. americana* compared to uninfected controls.

4.3.5 Mean pH of the proventriculus and digestibility of CP

At slaughter the mean proventricular pH levels were within the normal range (2.5 - 3.5) in all groups. However, the pH of chickens infected with 400 L_3 of *T. americana* was significantly higher than the other groups ($p<0.05$). In this study the digestibility of CP was not affected by the observed pH and the infection levels therefore there was no significant difference ($p>0.05$) among groups (Table 15).

Table 15. Digestibility of CP following *T. americana* infections

Weeks post infection (wpi)	Parameter	400 L_3	100 L_3	25 L_3	0 L_3
4	Digestibility of CP	78.0	-	77.2	78.6
6	Digestibility of CP	75.5	80.4	78.5	77.2
8	Digestibility of CP	80.8	81.4	81.4	83.0
10	Digestibility of CP	80.2	81.4	83.0	79.9
12	Digestibility of CP	76.6	79.4	76.7	77.8
12	Number of worms	15.4^{b}	11.2^{b}	2.9^{a}	0^{a}
12	pH	3.23^{b}	2.79^{a}	2.64^{a}	2.72^{a}
12 from ileum	Digestibility of CP	81.4^{a}	85.4^{a}	84.5^{a}	82.3^{a}

$^{a-b}$ Means in a row not sharing the same superscript are significantly different ($p<0.05$)

4.3.6 Weight gains of chickens infected with different doses of *T. americana*

The mean cumulative weight gains of chickens experimentally infected with different doses of *T. americana* were as follows; controls gained a mean total weight of (mean±SD) 1244.9 ± 95.2 grams, 25 L_3 group gained 1270.2 ± 106.4 g, 100 L_3 group gained 1256.5 ± 105.5 g and 400 L_3 group gained 1265.7 ± 118.7 g.

The mean weight gains were not significantly different ($p>0.05$) from one another (Fig.16). The variation (SD) in weight gains was lowest in controls and highest in the 400 L_3 group.

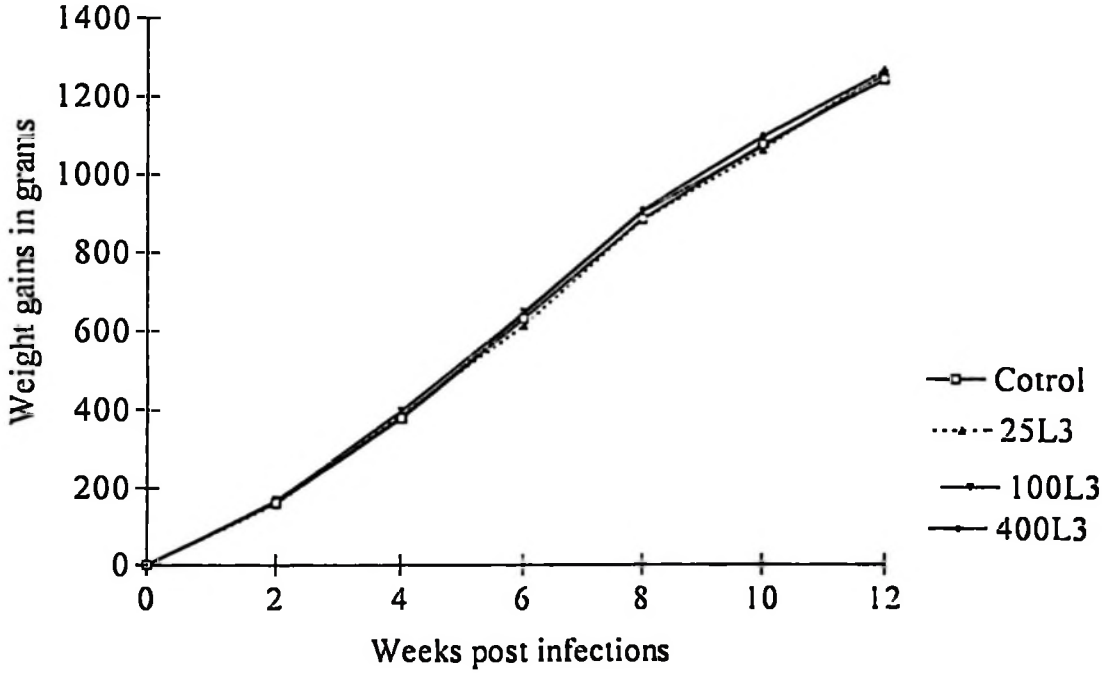

Figure 16. Mean cumulative weight gains in chickens infected with different doses of *T. americana*.

4.4 The effect of *Tetrameres americana* in chickens fed low and high protein diets

4.4.1 Effects of protein diet on worm establishment

The artificially infected chickens had no parasites other than *T. americana* whereas all of the control chickens were completely free from worm infection. The establishment rate of *T. americana* was observed to be higher, though not statistically significant ($p>0.05$), in chickens fed high protein diet compared to those fed low protein diet. Furthermore, the variability was twice as high in chickens fed high protein diet as shown by the values of standard deviation (Table 16).

Table 16. Establishment rates, mean, and median of *T. americana* in chickens fed different levels of protein diets

Parameter	LP-	LP+ 100 L_3	HP-	HP+ 100 L_3
Number of birds	15	15	14	15
Number infected	0	15	0	13
Establishment	0	8.7%	0	11.2%
Range	0	1 - 14	0	0 - 20
Mean (±SD)	0	8.7 [a]±3.43	0	11.2 [a]±7.02
Median	0	9	0	11

[a] Mean (±SD) worm counts in chickens experimentally infected with *T. americana* and fed different protein levels were not significantly different ($p>0.05$). The counts were significantly higher when compared to uninfected controls ($p<0.05$). LP- is uninfected group fed low protein, LP+ is infected group fed low protein, HP- is uninfected group fed high protein and HP+ is infected group fed high protein diet.

4.4.2 Effects of protein levels and infections on blood parameters

Analysis of the effect of protein level and or parasite infection and their interactions on the packed cell volume (PCV) and haemoglobin (Hb) concentrations showed inconsistent results. There were noticeable interaction effect of protein diet and infections at two weeks post infection on PCV level. *T. americana* infection showed reduced PCV during week six and reduced Hb concentrations on the second and sixth weeks post infection. The difference was statistically significant ($p<0.05$).

The effect was more pronounced in infected chickens fed low protein diet. Protein level influenced the PCV values which were significantly higher ($p<0.05$) in chickens fed high protein diet at week four and 10 post infection. Hb concentration was significantly higher ($p<0.05$) at week 10 (Table 17).

Table 17. Effect of protein and infection on the haematological picture

Week	Parameter	Effect	Parameter	Effect
2	PCV	Interaction effect	Hb	Ctrl* > Infected
4	PCV	HP* > LP	Hb	n.s.d.
6	PCV	Ctrl* > Infected	Hb	Ctrl** > Infected
8	PCV	n.s.d.	Hb	n.s.d.
10	PCV	HP* > LP	Hb	HP* > LP
12	PCV	n.s.d.	Hb	n.s.d.

Key: * = significant at ($p<0.05$), ** = significant at ($p<0.01$), and > = higher than, n.s.d. = no significant difference, LP = low protein diet, HP = high protein diet.

4.4.3 Packed cell volume and haemoglobin concentration

All chickens showed low PCV and Hb concentrations at young age and the values increased with age. The PCV levels in infected chickens fed low protein diet (LP+) were significantly lower ($p<0.05$) during the first 10 wpi when compared to either LP- group (uninfected chickens fed low protein diet) or HP- group (uninfected chickens fed high protein diet). On the other hand, infected chickens fed high protein diet (HP+) showed significantly lower PCV at the sixth, eighth, and 10 weeks post infection ($p<0.05$) when compared to uninfected controls of corresponding protein levels. However, there was no significant difference at week 12 post infections among the groups ($p>0.05$).

Chickens experimentally infected with *T americana* and fed low protein diet (LP+) showed significantly lower ($p<0.05$) haemoglobin (Hb) concentrations at week two and six when compared to LP- and HP-, respectively.

At the second and sixth week post infection the uninfected chickens fed low protein diet (LP-) had a significantly higher Hb concentration when compared to the infected counterparts the LP+ ($p<0.05$). Similarly the unifected chickens fed high protein diet (HP-) had significantly higher Hb concentration ($p<0.05$) when was compared to infected chickens on low and high protein diets (LP+) and (HP+) as shown in Table 18.

Table 18. Haematological profile of chickens experimentally infected with *Tetrameres americana* and fed different levels of protein diets (n=15).

Week	Parameter	LP-	LP+	HP-	HP+
2	PCV (L/L)	0.273^{a}	0.249^{b}	0.259^{ab}	0.264^{ab}
	Hb (mmol/L)	6.13^{a}	5.71^{b}	5.83^{ab}	5.7^{ab}
4	PCV (L/L)	0.274^{ab}	0.265^{b}	0.283^{a}	0.279^{ab}
	Hb (mmol/L)	5.69^{a}	5.53^{a}	5.76^{a}	5.75^{a}
6	PCV (L/L)	0.275^{a}	0.266^{b}	0.281^{a}	0.265^{b}
	Hb (mmol/L)	5.77^{ab}	5.44^{c}	5.9^{a}	5.56^{bc}
8	PCV (L/L)	0.312^{a}	0.292^{b}	0.298^{a}	0.293^{b}
	Hb (mmol/L)	6.01^{a}	5.82^{a}	5.93^{a}	6.02^{a}
10	PCV (L/L)	0.331^{a}	0.317^{b}	0.323^{a}	0.311^{b}
	Hb (mmol/L)	6.21^{a}	6.01^{a}	6.29^{a}	6.03^{a}
12	PCV (L/L)	0.305^{a}	0.301^{a}	0.305^{a}	0.300^{a}
	Hb (mmol/L)	6.13^{a}	6.06^{a}	6.25^{a}	6.18^{a}

$^{a-c}$ Means in the same row not sharing the same superscript are significantly different from one another by one way Analysis of Variance (ANOVA) and comparison of means by the least significant difference (LSD) ($p<0.05$). LP- is an uninfected group fed low protein diet, LP+ is an infected group fed low protein diet, HP-is uninfected group fed high protein diet, and HP+ is an infected group fed high protein diet.

4.4.4 Pepsinogen levels in chickens fed different protein levels

In this study, infection seemed to influence the blood pepsinogen level. The pepsinogen levels of infected chickens was significantly higher ($p<0.05$) in chickens fed low protein diet from week 4 post infection throughout the experiment when compared to uninfected counterparts.

Furthermore, the level of protein in the diet tended to influence the blood pepsinogen levels. Chicken fed high protein diet had lower pepsinogen levels when compared to those fed low protein diet. The effect was pronounced when infected chickens fed low protein diet were compared to uninfected control that were fed high protein diet (Fig. 17).

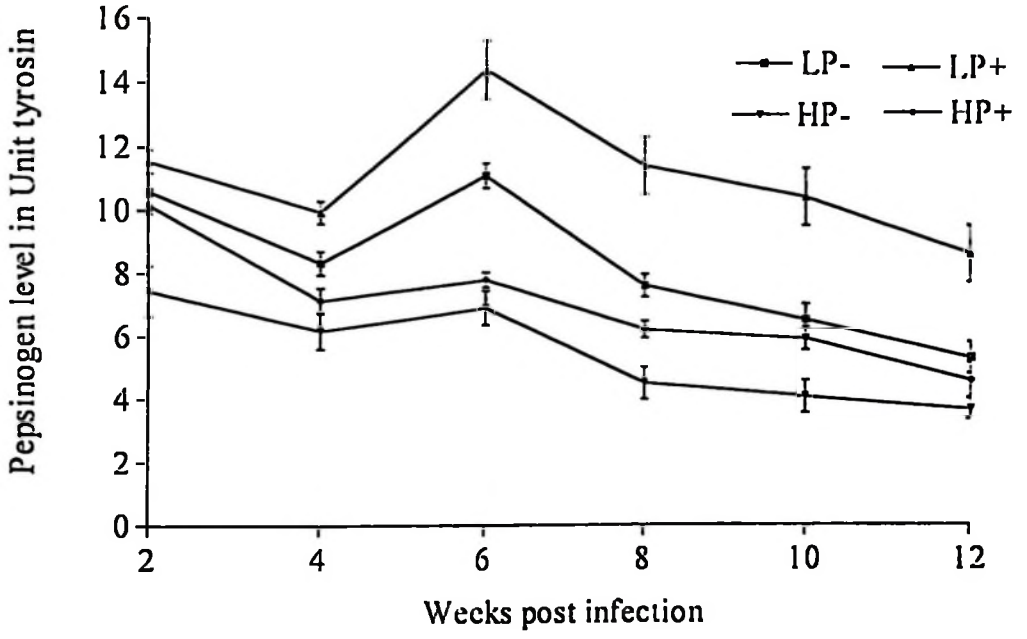

Figure 17. Mean pepsinogen levels (Error bars = SEM). Effect of nutrition and infection with *T. americana* on pepsinogen levels in chickens. LP- is uninfected group fed low protein diet, LP+ is an infected group fed low protein diet, HP- is uninfected group fed high protein diet, and HP+ is an infected group fed high protein diet.

4.4.5 Weight gains of *T. americana* infected chickens fed different protein levels

Chickens on high protein diet (HP) showed significant higher ($p<0.05$) weight gain when compared to those on low protein diet (LP). The infections did not show any significant effect ($p>0.05$) on weight gain in either of the protein levels. In addition, there was no interaction effect of infection and nutrition on weight gain ($p>0.05$).

However, uninfected chickens that were fed low protein diet (LP-) started gaining more weight compared to their counterparts from week six post infection and the growth was parallel from week eight post infection (Fig. 18).

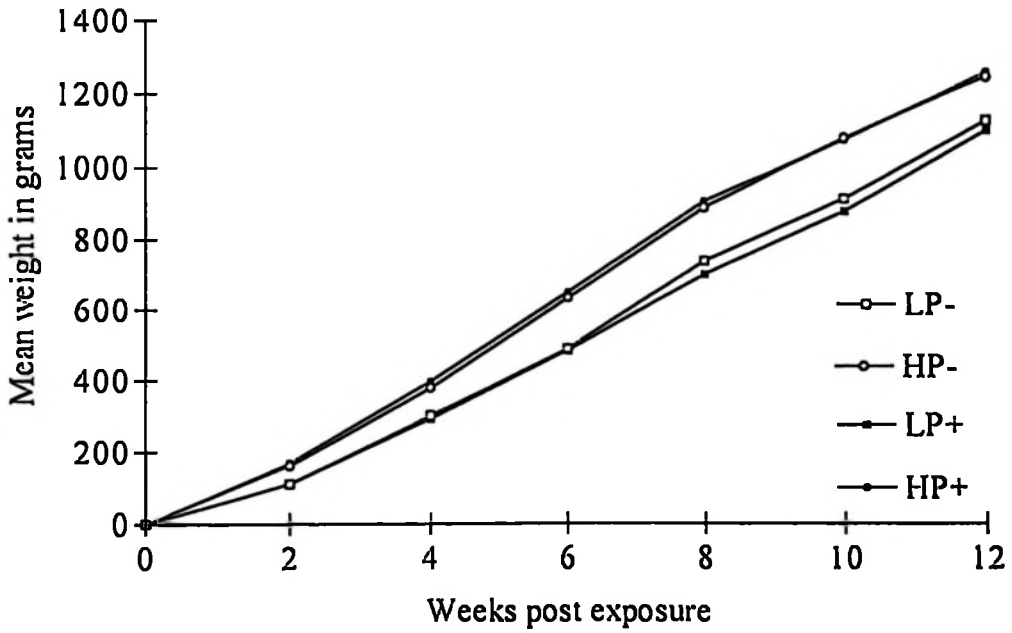

Figure 18. Mean cumulative weight gains of *T. americana* infected and uninfected chickens fed different levels of protein diets. HP+ is a infected group fed high protein diet, HP- is uninfected group fed high protein diet, LP- is uninfected group fed low protein diet, and LP+ is an infected group fed low protein diet.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The effect of helminth parasites on the productivity of free-range chickens

5.1.1 Efficacy of mebendazole and mean worm burdens

The marked efficacy of mebendazole against most of the poultry helminths observed in this study resulted to significantly lower mean worm counts in treated group. This finding demonstrates that mebendazole is a potential anthelmintic of choice against chicken helminths. Similar recommendation for using mebendazole against poultry GIT nematodes, cestodes and *Syngamus trachea* was given previously by Permin and Hansen (1998). The remarkable efficacy extend the guidelines outlined by the World Association for the Advancement of Veterinary Parasitology (WAAVP) that an anthelmintic with an efficacy of 80-100% could be used in livestock (Van Wyk, 2001). The presence of immature *H. gallinarum* and cestodes in treated chickens could be due to the fact that mebendazole may have had limited efficacy when given as a single dose than when given over several days (Brander *et al.*, 1991). It may also have been due to reinfection of the treated chickens seven days after the last treatment.

In the present study, a single dose of mebendazole was used because of the free-range management system where chickens fend for themselves and the method of administration required catching every chicken. Therefore, it was found necessary to avoid catching the birds for three consecutive days as that would have caused more stress in the treated group. Despite the fact that mebendazole was effective against most of the helminth species seen in this study, it was however less effective against

Choanotaenia infundibulum and *Raillietina tetragona*. The low efficacy of mebendazole against *C. infundibulum* and *R. tetragona* observed in this study demonstrated the need to use higher doses when dealing with such parasites, or that the dose must be spread over several days. Otherwise the anticestodal drugs such as praziquantel should be used (Rajendran and Nadakal, 1988; Nurelhuda *et al.*, 1989).

It is generally advocated that in order to control the immature nematodes and cestodes, the application of mebendazole need to be given at a lower dose but repeated over several days (Brander *et al.*, 1991). Furthermore, the absence of interspecies competition between helminths for the same habitats and nutrients in the GIT could have determined the substantial elevated number of *C. infundibulum* and *R. tetragona* population in the treated chickens.

Although the female *T. americana* is found embedded in the host tissue the efficacy of mebendazole against this parasite was remarkably high. Similar effect against this parasite has been reported in pigeons by Young (1981). It may well be that the high efficacy was influenced by the blood sucking behaviour of *T. americana* such that the parasite is directly affected by the concentration of the drug in the blood.

Syngamus trachea is also a blood sucker but the reduction was fairly low compared to *T. americana*. The worm has been observed to be killed by higher doses of benzimidazole derivatives (Ssenyonga, 1982b).

It is therefore recommended that mebendazole should be used to control *T. americana* and where *S. trachea* is also prevalent higher doses may be used to eliminate this gapeworm.

5.1.2 Mortality

In the present study it was observed that the prevailing poor management, in particular the lack of proper health care, inadequate nutrition and housing, pneumonia, coccidiosis, predators, accidents, and unknown causes led to a an overall mortality of 67.9%. Although these chicks were not hatched in the village and instead were taken to the village after being reared on-station up to the age of six weeks, the mortality rate observed in this study is in agreement with findings reported by Wilson (1979) in the Sudan, Kuit *et al.* (1986), Wilson *et al.* (1987) in Mali, Minga *et al.* (1989) in Tanzania, Bell *et al.*(1990) in Morocco, and Gunaratne *et al.* (1993) in Sri Lanka.

The cause of such high mortality in this study could be attributed to the new environment and change of management system from deep litter to free-range management where chicks had to fend for themselves. In addition, the chicks did not have a brooding hen to take care of them as they normally become independent after 12 weeks of age (Mwalusanya *et al.*, 2001). Furthermore, during the rainy days especially immediately after arrival, on the farms, some chicks were soaked to an extent of becoming pneumonic and died few days later, the rainy season therefore may have contributed much more on the mortality because of the chilly weather that ended up causing fatal pneumonia.

Thereafter, predators were observed to have highest contribution on the mortality rate of free-range chickens whereas diseases had the lowest contribution on the mortality rate of the population at risk. Most of the chicks fell easy prey before they were four months old. Similar findings have been reported in Ethiopia by Negesse (1993). This

could be due to the fact that chicks fall victim to both bird and beast of prey whereas adults succumb mainly to beast of prey and rarely to bird of prey.

It is apparent, therefore, that if predation could be controlled, the productivity of free-range chickens could be raised. In the present study Newcastle disease was controlled by vaccination but still other diseases were noted to be the cause of mortality of about 16%. The contribution of bacterial diseases was not looked into. Similar levels of mortality have been reported by Bell *et al.* (1990) in Morocco. Helminth infections featured as the cause of death in a few heavily parasitized chicks by either *S. trachea* or *A. galli*. However, a few of the lightly *S. trachea* infected chickens died due to the sequella of mucous secretions and not due to the worm burdens *per se*.

Histomonosis, a protozoan disease that is known to be transmitted by a fully embryonated *Heterakis gallinarum* ova (Ruff *et al.*, 1970) or intact male worms (Springer *et al.*, 1969) caused three deaths in this study. From these results it can be concluded that subclinical helminth infections have little effect on mortality in free-range chicken. However, failure of some farmers to follow the preset instructions resulted to incredibly high mortality rate such that it is advised that the on-farm studies need to be followed up closely.

5.1.3 Mismanagement of experimental chickens

In this study inadequate nutrition, poor housing and lack of proper health care were commonly encountered in some farms. Farmers who were confining the experimental chickens up to 1100 h and who fed them maize bran for the fear of predators, such

practices resulted into slow growth rate due to inadequate nutrition. The practice resulted to frequent outbreaks of coccidiosis that might have confounded the ultimate performance of the chickens in this study. It is therefore assumed that, had it been all chickens were reared under the ordinary free-range system the results of this study could have been markedly improved.

5.1.4 Effects of worms on weight gain and egg productions

Anthelmintic treatment improved the growth rate of free-range chickens by 22% when compared to infected controls. The 22 % weight loss in the infected controls was in agreement with the previously estimated meat loss in native chickens with mixed infections in Indonesia by He *et al.* (1990). In the present study, the sub-clinically infected chickens were able to grow normally up to 10 weeks post exposure indicating that a build up of worm burden is essential in order to cause noticeable harmful effects. The results further demonstrated that helminth infections were responsible for retarding the growth rate in young growing free-range chickens.

In this study the infections were moderate but still caused noticeable reduction in growth rate. Hence, this finding complements the observation made by Reid and Carmon (1958) and Brewer and Edgar (1971) who noted significant decrease in weight gain as the number of worms increased. In their controlled study Reid and Carmon (1958) went further by calculating the impact of every recovered worm as being able to cause a mean weight loss of 1.39 grams. In this study, it was observed that all chickens had mixed infections, therefore, it was difficult to estimate the loss caused by every

single parasite found due to differences in the parasites' pathogenicity.

The 22% weight loss in infected controls for the six months can be extrapolated, in Tanzania which has 26 million free-range chickens with a ratio of chick: grower: adult of 10:5:6 (Minga *et al.*, 2000) could be an economically important disease as will be responsible for a loss of 1.36 million kg of growers per year. Given that one kilogram of grower is sold at Tanzania shillings 1200 (equivalent to US$ 1.3), the annual loss is therefore equivalent to US$ 1.768 million. This estimate implies that the widespread subclinical helminth infections in free-range chickens has considerable economic losses.

The hens started laying their first egg between 29 and 34 weeks of age. This observation is in accordance with findings by Wilson (1979) and Minga *et al.* (1989). Although treated hens started laying their eggs two weeks earlier when compared to untreated counterparts, the egg number and sizes did not differ significantly from the infected group. It was further noted that eggs from the infected groups were slightly heavier, though not statistically significant, than those from treated hens. This result fails to indicate that egg production was reduced by the parasitism, neither in numbers nor weight of the eggs. Ackert and Herrick (1928) observed that parasitized hens laid more eggs than the treated hens by 22.3% in a period of 10 weeks. Similarly, Szelagiewicz and Sokol (1991) did not observe any adverse effect on weight gain and egg production in chickens with mixed infection of *A. galli* and *Capillaria* spp.

In this study the important finding was the enhanced time to reach the point of lay for the treated hens but once the infected hens started laying, the egg weight and numbers were not affected. It is therefore thought that mature laying hens are able to

overcome the side effects of subclinical helminth infections possibly due to hormonal influence as reported by Ackert and Dewhirst (1950).

5.2 Effectiveness of different deworming strategies in free-range chickens

5.2.1 Worm burdens

The mean worm burden observed in this study was relatively low when compared to the previous study possibly owing to reduction of environmental contamination due to the prolonged use of anthelmintics in the study area. The use of a mixture of oxyclosanide and levamisole in this study was an unplanned alternative after using all the stock of mebendazole in the first experiment and that the mebendazole formulation for poultry was no longer on the market.

The high efficacy of treatment against most poultry helminths that was observed in this study when a mixture of oxyclosanide and levamisole was used showed that these drugs could be used in chickens with mixed infections.

The presence of immature *H. brevispiculum* and *H. gallinarum*, and adult female *T. americana* in treated chickens indicated that the efficacy of the mixture of levamisole and oxyclosanide was limited to mature nematodes and cestodes than to immature helminths or female *T. americana* that are found embedded in the host tissue. Under the conditions of this study, helminth burden was reduced even in the controls, an observation also made by Rickard *et al.* (1991) who noted appreciable (67-98%) reduction of environmental larval nematode contamination in the pasture grazed by the treated cattle. It is possible, therefore, that the intensive use of anthelmintics in the study

area could have reduced the environmental contamination of free living larval nematode and the infected intermediate hosts.

5.2.2 Mortality

The high mortality rate observed in this study is in accordance with the report made by Wilson (1979) in the Sudan. The observed high mortality rate at the beginning of the study was possibly due to the age of the chicks. Therefore, it is recommended that while conducting an on-farm experiment like the present study, four-week-old chicks should not be sent to the village due to the low survival rate. Other risk factors include confining and supplementing the chicks with maize bran in wet environment, the rain, diseases and predators accounted for additional losses.

Confining of chicks and supplementing them with maize bran could have resulted into accumulation of coccidia oocysts in the environment. In this case the observed survival rate of 20% is a challenge to researchers to find solutions for improving the survival rate of free-range chickens.

It is therefore advocated that farmers keep their chickens in shelters during harsh weather such as rains then the survival rate may be improved a great deal. As far as mortality rate is concerned all groups were equally susceptible to predation and mismanagement.

Helminth infection, *S. trachea* in particular, caused mortality in 14 chicks by blocking the trachea when ≥ 20 pairs of *S. trachea* were found. In cases of heavy burdens the deaths of birds were caused by the worm burden *per se* but in light infection

death was due to massive mucous secretions in the trachea. In addition, histomonosis was noted to be an important killer disease in free-range chickens.

From these results it is evident that helminth parasites are able to directly or indirectly cause mortality in free-range chickens through massive infections or the consequences of helminth related disease such as histomonosis. In this study it was difficult to control the disease because of the necessity of having the untreated controls staying together with the treated group at all times. Treating the control group to eliminate the disease could have defeated the purpose of this study though it would have reduced the mortality rate.

It is apparent, therefore, that syngamosis and ascaridiosis are important helminth parasites in young free-range chickens. Similar findings have been reported by Devada and Sathianesan (1989) who noted high *S. trachea* infection rates in chicks of one to two months old. On the other hand, heterakiosis was a threat in all age categories. From these results, it can be concluded that helminth parasites have their direct and indirect contribution to mortality of free-range chickens.

5.2.3 Weight gains and egg productions

The monthly treated chickens showed significantly higher weight gain when compared to untreated controls and those that were treated only once. In addition, monthly treatment hastened the sexual maturity. Similar results have been reported by Ramaswamy and Sundaram (1981b) after infecting chickens with *T. mohtedai* and or *Acuaria spiralis* where the infected chickens took 33 to 51 days more to attain sexual

maturity. Similar observation was made by Ackert and Wisseman (1944).

The higher number of eggs laid by the seasonally and singly treated chickens observed in this study is in agreement with the results made by Ackert and Herrick (1928). From these findings it is apparent, therefore, that moderate worm burdens may be tolerated and would not affect the production of the infected hosts. It may well be that lower worm numbers are required to cause reduced weight gain than for reduced egg production. Another possible explanation could be associated with the low nutrient requirement by laying hen with exception of energy feed, calcium, vitamin A and D when compared to growing hens, pullets (Scott *et al.*, 1969).

In addition, the failure to see a clear difference between different treatment regimens could be attributed to the low number of chickens used for analysis as the majority of them died during the course of the experiment. The high mortality might have negatively affected the power of the statistical analysis. It is therefore postulated that some of the findings in this study may have been statistically significant had more chickens survived to the end of the experiment. Monthly treatment was the only treatment regimen that demonstrated significant effect on both weight gain and hastening sexual maturity followed by seasonal treatment. However, treating the chickens twelve times a year had no statistical advantage in terms of weight gain and egg production over seasonal deworming that seems to be a less expensive control method. With this type of results it is recommended that anthelmintics should be applied to all subclinically infected chickens.

Chickens from different farms performed differently, for example, in farms

where chickens were let out late in the morning, they could not reach sexual maturity at the time of terminating the experiment, when the chickens were 12 months old. On the other hand, the confinement of the chickens and feeding them with maize bran resulted into high losses compared to those supplemented but not confined flocks. Confining the chickens and feeding them with maize bran were however not common practices in the village but just evolved during the study. It could have evolved in order to impress the researcher. It may, however, be noted that confined flocks required more treatments against coccidiosis because of contaminated environment that resulted in a build up of the coccidia oocysts. It could therefore be argued that the on-farm studies are good for screening interventions within the context of the management systems but it was too complicated, costly and sometimes frustrating owing to high mortality partly due to failure of farmers to follow the instructions.

5.3 The pathogenic effect of *T. americana* in chickens

5.3.1 Establishment rate and worm burdens

In this study the absence of infection in the control chickens suggests that *T. americana* does not have a direct life cycle (Cram, 1929). The parasitological results showed that the high dose had the lowest establishment rate whereas the low and medium doses had comparable establishment rates. The present finding could be due to nutrient competition between parasites and the host. The establishment rate observed in this study differ from those reported by Ramaswamy and Sundaram (1983) who observed fairly similar establishment rates when they infected White Leghorn chickens with 200,

400, and 600 L_3 of *T. mohtedai* and the establishment rates were 34, 28.5, and 34%, respectively. In this study the highest dose produced substantial higher mean worm counts but comparable to medium dose (Table 13).

The present findings may be attributed to several factors such as chicken breed difference, helminth species difference, and/or the infection method. Ramaswamy and Sundaram (1983) infected White Leghorn chickens within two hours after isolation of the infective larvae. In contrast, in the present study female Lohman Brown chickens were used and the doses of 25, 100, and 400 L_3 of *T. americana* were given to chickens after 15 hours of storage in a refrigerator. The low establishment rate observed in this study could be attributed to the breed of the chickens, dose rate and storage of the L_3 that could have reduced the infectivity of the third stage larvae.

5.3.2 Differential leucocyte counts in *T. americana* infected chickens

The heterophilia and eosinophilia in chickens infected with high dose of *T. americana* when compared to controls 2 weeks post infection; and the absence of difference on all cell types between infected and uninfected controls during the period of 4-6 weeks post infection is in agreement with observations by Ramaswamy and Sundaram (1981a). A similar finding was reported by Dahl *et al.* (2002) in chickens superimposed with *Pasteurella multocida* and *A. galli*. Similarly when *A. galli* was given alone it was reported to produce the same results (Kaushik and Sen, 1978) and chickens with eosinophilia tended to have lower worm numbers.

This observation differs from the one made by Singh *et al.* (1988) who observed

lymphocytophilia, eosinophilia, and heteropenia in chickens harbouring 10 to 18 *T. mohtedai*. In the present study, it was observed that from 6 weeks post infection there was no cellular response. This observation is in agreement with the one made by Appleton (1983) who noted the absence of cellular response in chronic case of tetrameriosis.

This study demonstrated that it was the early stage of infections that produced the most acute cellular responses and by the time the worm had matured the reaction becomes localised and became more chronic probably indicating the development of host-parasite equilibrium. Heterophilia was also observed in this study as was in bacterial infections by Andreasen *et al.* (1991). This suggests that heterophils may also be responsible for elimination of parasitic infections. The lymphocytopenia observed during the second and 10 weeks post infection is in agreement with the observation made by Ramaswamy and Sundaram (1983), Dahl *et al.* (2002) and that by Chandra (1984) but the latter further noted alterations of the T-cell subsets.

Lymphocytopenia at a later stage of infection suggests that *T. americana* at this stage might be secreting /excreting toxic substances that are suppressive to lymphocytes multiplication. The decreased number of lymphocytes and increased number of heterophils observed in study have also been reported by Gross and Siegel (1983) to be associated with increased response to stressor factors such as corticosteroids. This is further supported by the fact that minimum tissue reaction apart from compression atrophy is observed in case of chronic tetrameriosis (Appleton, 1983) indicating that absence of cellular response during chronic phase of infection could be linked to

immuno-suppressive effect of *T. americana* infection.

The early heterophilia and eosinophilia could explain the response of the host immune system towards eliminating the early patent parasitic infections. It is possible that the cellular responses studied in this experiment, which is a result from a single infection, could also occur in trickle infections which more closely mimic the natural infections in free-range chickens. The basophilia observed in moderately infected chickens during early infections is difficult to explain. It may well be that when stimulation is strong enough then eosinophil will dominate over the basophil counts. However, more work is required to determine the importance of basophils in the immune system of chicken against parasitic infections.

5.3.3 Pepsinogen level in *Tetrameres* infected chickens

The high pepsinogen levels observed in experimentally infected chickens in this study may be a proof of damaged glandular tissue during infection. The pathogenesis of increased blood pepsinogen in cattle infected with ostertagiosis is associated with destruction of parietal cells that produce hydrochloric acid (HCl) that would convert pepsinogen that is produced by chief cells to pepsin (Satrija *et al*., 1996; Kassai, 1999). The death of parietal cells results to elevated abomasal pH to 6-7 that ceases protein digestion. Deficiency of HCl and pepsin leads to accumulation of pepsinogen and some leaks back to the blood vessels and plasma pepsinogen rises (Kassai, 1999).

In chicken both HCl and pepsinogen are produced by the oxynticopeptic cells, in that regard the mechanism of elevated blood pepsinogen is different from that

observed in ruminants.

In the present study, the elevated blood pepsinogen level could be the result of leakage of the accumulated pepsinogen in the obstructed excretory ducts due to compression exerted by infected nearby lobules or it may well be that pepsinogen was absorbed through the damaged tissue. However, the mechanism by which blood pepsinogen is elevated is not known with certainty.

In this study chickens infected with high level of parasites had correspondingly higher blood pepsinogen levels at the early stage of infection substantiating the assumption that the tracks/damages caused by migrating larvae were the focal points for the pepsinogen to leak back into the blood.

The emphasis on worm burden in this context is necessary since the pepsinogen level seemed to correlate with the infective dose. However, the effect seemed to be transient as the level of pepsinogen in infected chickens dropped to the levels of uninfected chickens at 12 weeks post infection. The transitory effect of elevated blood pepsinogen levels corresponded with the time when the immature worms are known to be migrating to the predilection site. This suggests that the migratory tracks form the focal point for pepsinogen to leak back into the blood circulation.

From the current findings there is a need, therefore, to establish the normal range of blood pepsinogen level in healthy free-range chicken before the diagnostic level can be established. Under natural infections, the concurrent occurrence of *Acuaria spiralis* (synonym *Dispharynx nasuta*) and *T. americana* in the proventriculus is likely to complicate the situation. From the present results it is therefore inescapable to say that

T. americana infection in this study caused elevation of blood pepsinogen in chicken.

Again it is currently not known as to whether the pepsinogen level in poultry vary with age, sex, season, or nutritional status of the bird, therefore, further work is required to elucidate the difference among individuals.

5.3.4 Proventricular pH and the digestibility of crude protein (CP)

The pH of the infected and control chickens were within the normal range of 2.5 to 3.5 (Denbow, 2000). The elevated pH in chickens infected with 400 L_3 of *T. americana* was towards the higher level within the normal range of gastric pH.

The proventriculus is known to be made of multiple compound glands, the present result could be attributed to the low number of infected glands. It is therefore argued that except in cases of high worm numbers the moderate infections should not greatly affect the secretory ability of the proventriculus. It was unlikely, therefore, to affect the digestibility of protein diet.

It may well be that the pH was not affected owing to the fact that oxynticopeptic cells produce both pepsinogen and hydrochloric acid. It is assumed that in case of massive infection such as in trickle infections and if the period of the experiment was extended hopefully it could be possible to observe the effect of *T. americana* infections on the pH.

In the present study the digestibility of CP in infected chickens did not differ from that of the uninfected controls. The overall percentage of protein digestion tended to be lower in chickens infected with high dose of *T. americana*. In a similar study,

Hurwitz *et al.* (1972) noted that protein digestion was compensated for in the duodenum in case of GIT parasitic infections. The absence of difference on protein digestion in the present study could possibly be explained by the fact that worm burdens were not high enough to cause harmful effects under the condition of this experiment. It may well be that *T. americana* might have caused dysfunction of the proventricular glands but the effect could have been compensated for in the intestinal protein digestion. Under the condition of this study *T. americana* infection did not support the hypothesis that infected chickens would show suppressed CP digestion owing to infection of the proventriculus.

5.3.5 Weight gains

As far as weight gain is concerned, infected chickens were not affected by the number of *T. americana* recorded. The mean worm count of 15 *T. americana* was not harmful to the chickens fed 17.8% CP diet *ad libitum*. So far no weight loss was observed in infected chickens despite the fact that the number of adult worms in the glands ranged from 0 to 33. Such counts have been reported to cause mortality in bobwhite quail (Stoddard, 1946). However, the present results differ from the observation made by Ramaswamy and Sundaram (1983) who recorded higher parasite counts that led to adverse effect on the growth performance of infected chickens.

In the present study the absence of difference in weight gain could be due to the fact that the pathogenicity of *T. americana* was masked by the low worm numbers and possibly the nutrition could have been optimal. It is also clearly known that chickens

with moderate number of helminth parasites eat more (Ntekim, 1983; Fatihu *et al*., 1992b). This voracious appetite was first reported by Ackert and Herrick(1928) where the lightly infected chickens ate more and with time gained weight more rapidly than did the controls.

In this study the failure to note a significant detrimental effect of parasitism on weight gain is not an indication that such detriment did not occur but rather that most of the factors which affect both parasite level and weight gain could not be quantified. Such factors include the amount of food eaten by each chicken was not measured, dietary quality could have been good enough to mask the effect of helminth infection, the infection method did not produce the expected establishment rate, the dose and frequency of infection could have been low, the genetic potential of the birds against *T. americana* infection is not known and the experiment was conducted in female birds only whereas the pathogenic effect in males remains unclear. Controlled experimental infection studies using *T. mohtedai* (Ramaswamy and Sundaram, 1981a, b) have documented adverse effect on weight gain but the parasite levels were different from those seen in this study.

Furthermore, *T. mohtedai* is a different species from *T. americana* and may thus exhibit different pathogenicity. It is also still not clear as to what would have happened if the chickens were infected repeatedly with the same dose for a longer period of time. It may well be that the effect of repeated infections might have similar effect as of a single infection but it is expected that in case of repeated infection the migrating immature parasites are likely to cause marked growth depression due to repeated

inflammatory reaction to the host caused by the immature parasites (Ramaswamy and Sundaram, 1981a). However, this hypothesis needs further study.

5.4 The effect of *T. americana* in chickens fed low and high protein diets

5.4.1 Establishment rate and worm burdens

The absence of infection in the control chickens that were housed together with infected contemporaries is in agreement with the observation made by Cram (1929) and this suggests that *T. americana* does not have a direct life cycle. The observation made in this study demonstrates that chickens become infected with *T. americana* after ingesting insects presumably cockroaches, grasshoppers, and/or sowbug which contain the infective larvae.

The question remains of how do the grasshoppers and cockroaches become infected with the larval stage of *T. americana* as Fink (2002) was not able to demonstrate the infective larvae in grasshoppers, cockroaches nor sowbugs in an area where tetrameriosis had a prevalence of >80%. It is possible that the method used and the number of insects examined could be the reason for failure to recover the infective larvae from those insects. Unfortunately the habits of the grasshoppers and cockroaches are not well known and there is no evidence in the literature that these insects eat chicken faeces. Therefore, it does not eliminate the possibility that these insects are seasonally infected by eating contaminated materials within their vicinity.

The establishment rates were 8.7% and 11.2% for low protein diet and high protein diet, respectively. This finding differs from the observation made by

Ramaswamy and Sundaram (1983) who observed 28 - 34% establishment rate. In this study the infective larvae were stored in a refrigerator over night before infection, the low establishment rate therefore could be attributed to storage that might have reduced the infectivity of the larvae.

Furthermore, in this study the establishment rate of *T. americana* was not influenced by the protein level in the diet though chickens on high protein diet had substantially higher mean counts compared to those chickens fed low protein diet. The low worm numbers observed in chickens fed low protein diet when compared to high protein diet suggests that *T. americana* develop better in chickens fed high protein diet. It may well be that in case of optimum nutrition the parasites would use the extra nutrients for establishment. In this regard there is a need to examine the dietary requirements of *T. americana*.

5.4 2 Packed cell volume and haemoglobin concentration

In the present study the experimental chickens were fed *ad libitum*, the inconsistent effect on PCV and Hb. was not an unexpected finding. Soulsby (1976) described the effect of infection with haematophagous worms that there was a tendency for the host to have increased absorption of iron and incorporate it immediately into the blood cells. Similar findings were reported by Abbott *et al.* (1986) in sheep parasitised by *Haemonchus contortus*, a blood sucking worm, whereby there was increased red blood cell production and absorption of dietary iron in lambs on high protein diet than those on low protein diet.

The results in this study differ from observations made by Ramaswamy and Sundaram (1983) who noted consistently low PCV and Hb concentrations in chickens infected by *T. mohtedai*. The present findings indicate that the intermittent low level of packed cell volume and haemoglobin concentrations in chickens fed low protein diet could be due to compensatory mechanism against parasitism owing to increased erythropoiesis and absorption of dietary iron (Abbott *et al.*, 1986).

The mean worm numbers recorded by Ramaswamy and Sundaram (1983) of 68 *T. mohtedai* could have caused the permanent anaemia. The mean worm counts recorded in this study differ from many reports made on *T. americana* in which the mean worm count range from 10 to 22 (Msanga and Tungaraza, 1985; Mpoame and Agbede, 1995; Magwisha, 1997; Permin *et al.*, 1997b; Magwisha *et al.*, 2002). In that regard the absence of consistent effect could be attributable to the species of the *Tetrameres* or the low mean worm counts recorded in this study.

From the present findings it may, however, be noted that *T. americana* infection even if occurring in low numbers (8-11) can result in considerable blood loss in the infected host and the effect is pronounced in chickens on low protein diet. In case of free-range chickens where protein level has been observed to be a limiting factor *T. americana* infection is likely to aggravate the situation and cause chronic anaemia.

5.4.3 Pepsinogen level in chickens fed different levels of protein

Generally, low protein diet was associated with higher blood pepsinogen level when compared to high protein diet. The pepsinogen levels in infected chickens were

consistently higher compared to their controls. The levels were highest in chickens that were fed low protein diet when compared to chickens fed high protein diet and the difference was marked when infected chickens fed low protein diet were compared to uninfected chickens fed high level of protein diet.

The high pepsinogen levels observed in chickens fed low protein diet in this study could be due to a loss of integrity of the glandular epithelium hence become permeable to pepsinogen. However, the exact mechanism of elevation of blood pepsinogen level is not known to the best of my knowledge thus needs further investigation. The effect of parasitism in chickens fed low protein diet persisted up to 12 weeks post infection when the experiment was terminated. The elevated blood pepsinogen level in infected chickens could be due to obstruction of the excretory ducts that culminated into leaking back of pepsinogen into the blood circulation.

From the present findings it is evident that low protein caused elevated blood pepsinogen. It may well be that under free-range chickens where the crude protein is known to be sub-optimal (Gunaratne *et al.*, 1993; Tadelle, 1996; Mwalusanya, 1998) the effect of *T. americana* would probably exacerbate the condition. In this study it was demonstrated that chickens with high blood pepsinogen levels were growing slowly. It can therefore be concluded that efficient production will not be attained while there is any factor such as infection or low protein diet that would lead to elevated blood pepsinogen

5.4.4 Weight gain

Weight gain in this trial was influenced by protein level in the diet. chickens fed high protein diet had remarkable growth rate compared to those on low protein diet. The results therefore demonstrated that infected chickens fed low protein diet suffered substantially more compared with uninfected counterparts. However. chickens on high protein diet performed comparably the same regardless of infections.

Infected chickens fed low protein diet had substantially lower weight gain though not statistically different from their uninfected counterparts. Similar results have been reported by Ackert and Wisseman (1944), Ikeme (1971b), and Permin *et al.* (1998) when they infected chickens with *Ascaridia galli* and fed different levels of protein diets.

The observations made in this study suggest that chickens fed low protein diet were less able to withstand the harmful effects of infection with 100 L_3 of *T. americana* than chickens fed high protein diet. despite the substantial lower worm burdens. It is worth mentioning that the protein levels in this experiment were not optimal. However, protein levels were within the husbandry conditions of practical farming in Denmark

Comparable growth rates of infected chickens on high protein when compared to their contemporaries and the slight difference on weight gain of infected chickens fed low protein diet when compared to uninfected ones suggests that the level of infection used in this study did not have marked effects on growth rate. Lack of conclusive evidence on the effect of *T. americana* on weight gain could be attributed to the low worm numbers and possibly also the nutrition provided to the experimental chickens was sufficient on both protein levels.

The observation in this study indicates that if all ingredients required by growing chickens are supplied, the young fowls can tolerate considerable numbers of *T. americana* without serious effects. Under natural conditions free-range chickens tend to get ≤ 10% CP (Gunaratne *et al.*, 1993; Tadelle, 1996; Mwalusanya, 1998) and the mean worm number in growers is about 20 *T. americana*, it is probable that the effect of *T. americana* on weight gain could be observed.

In this study the protein level of the low protein diet (13.3%) was unlikely to have been sufficiently low to have had such an effect *per se*. Under natural conditions it would be expected that the most significant effects in infected chickens would be most pronounced owing to the fact that free range chicken usually get sub-optimal level of protein (≤ 10% CP). Furthermore, under natural condition there is trickle infections throughout the year. The combination of low plane of protein and the constant exposure of free-range chickens to helminth infection is likely to result into overt effect. Hence it is expected that marked effect on growth rate could be observed in free-range chickens getting sub-optimal plane of protein.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

1. From the present findings it is apparent that a 22% decrease in weight gain was due to helminth infections.

2. Helminth parasites had adverse effect on the growth performance of free-range chickens as shown by the consistent higher weight gains in the treated chickens when compared to controls in 11 out of 12 farms.

3. Monthly treatment against helminth infections in free-range chickens was superior but seasonal treatment was comparable and would be less expensive with regard to cost-benefit implications and might also delay anthelmintic resistance due to less frequent exposure of the worms to anthelmintics.

4. The study demonstrated that the establishment rate of *T. americana* was lowest when chickens were infected with 400 L_3 compared to 100 and 25 L_3 doses; and that low protein diet and infection resulted to elevated pepsinogen levels.

5. *Tetrameres americana* is capable of causing transient anaemia in *ad libitum* fed chickens which was compensated for by probably sufficient plane of nutrition.

6.2 RECOMMENDATIONS

1. The presence of one clinically infected chicken suggests the presence of other developing clinical cases in the flock. Treatment measures therefore must be directed to the entire flock in order to reduce the environmental contamination of the parasites to a minimum. It is generally known that low worm burdens have no detrimental effect to the productivity of the host.

2. The use of isotopically labelled reagents such as Chromium (Cr) and Iron (Fe) may be required to study the red blood cell removal and production and the iron kinetics in order to understand the blood loss per parasite and the pathogenesis of anaemia caused by *Tetrameres americana* in the host, or for that matter, alterations caused by the host to its parasites.

3. Further work is required to study the consequences of *Tetrameres americana* infection in chickens of different sex, age, and at varying nutritional, physiological, and immune status and the effect of damage after the chickens are repeatedly infected with different dose levels.

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