LABORATORY EVALUATION OF EFFECTS OF QUINESTROL AND LEVONORGESTREL ON FERTILITY AND REPRODUCTIVE PERFORMANCE OF MULTIMAMMATE RATS *MASTOMYS NATALENSIS*

GINETHON GABRIEL MHAMPHI

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ABSTRACT

This study was carried out to evaluate the effects of fertility control agents (quinestrol and levonorgestrel) on reproduction performance of multimammate rats (*Mastomysnatalensis*). A total of 316 individual *Mastomysnatalensis*were used to evaluate consumption rate of bait and their effects on reproductive performance under laboratory conditions. Animals were fed with contraceptive baits for seven days, paired and sacrificed for histological observations. Results indicated that acceptance of the control bait was not statistically different ($p< 0.001$) from that of levonorgestrel at 10 ppm. Acceptance of bait treated with quinestrol at three concentrations (10 ppm, 50 ppm and 100 ppm) was significantly lower ($p< 0.001$) than plain bait and that of levonorgestrel treatments. Significant interaction effects of treatment and sex were observed ($F_{(16,392)} =10.007$, $p<0.001$), with higher acceptance of treated bait for both levonorgestrel and quinestrol in female rats than male rats. Quinestrol, levonorgestrel and quinestrol/levonorgestrel combination significantly reduced testes, epididymis and seminal vesicles weight. Sperm concentration and motility were significantly decreased with increased abnormal sperms morphology. In female animals there were significant differences ($F_{(2, 86)} =3.28$, $p = 0.0423$) in pregnancy rate compared to the control group. There were no observed differences in uteri and ovaries weights. All treatment concentrations of quinestrol, levonorgestrel and quinestrol/levonorgestrel combinations had significant effects on pregnancy compared to the control ($p< 0.05$). The most effective compound was quinestrol which reduced spermatogenesis at all concentrations. Levonorgestrel alone was less effective on male and female reproduction status. These results indicate that quinestrol and/or quinestrol/levonorgestrel combination have anti-fertility effects on *M. natalensis*.

DECLARATION

I, Ginethon Gabriel Mhamphi, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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Ginethon Gabriel Mhamphi Date

(MSc. Candidate)

The above declaration is confirmed by

....

Prof. Makundi, R.H. Date

(Supervisor)

....

Prof. Massawe, A. W. Date

(Supervisor)

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DEDICATION

To my brother and sister Jonathan and Loy Gabriel Mhamphi who laid the foundation of my education, and to my parents '*Mzee'* Gabriel Seng'hondoMhamphi and his wife the late Hagar MeshackMbeswa for their moral support. I also dedicate this work to my beloved wife Regina and my children Gabriel, Grace and Godwin for their prayers, patience and encouragement.

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LIST OF ABBREVIATIONS AND SYMBOLS

$	dollar
%	percentage
<	less than
×	times
≤	less than or equal
µ	micro
^{0}C	degree celcius
Fig	figure
gm	gram
LNG	levonorgestrel
mg/kg BW	mill gram per kilogram body weight
ml	mill litre
ppm	parts per million
QE	quinestrol
Se	standard error
SPMC	SUA Pest Management Centre
spp	species
SUA	Sokoine University of Agriculture

CHAPTER ONE

1.0INTRODUCTION

1.1 Background Information

Rodents are mammals characterized by the presence of diastema, continuous gnawing and constant growth of their incisor teeth. They constitute the largest and the most diverse group of mammals with great variations in size from 10 g to more than 66 kg (Feldhamer*et al*., 2007; Wolf and Sherman, 2007). Rodents are among the most important vertebrate pests in agriculture. They are responsible for significant damage of crops worldwide. In addition they consume and contaminate stored grain and also transmit diseases mostly in rural communities (Fiedler, 1988; RATZOOMAN, 2006). In eastern and southern Africa, rodents are the leading pests of cereal crops (Makundi and Massawe, 2011; Mulungu*et al*., 2012). Losses caused by rodents are enormous, and it is estimated that more than one billion people are suffering from chronic hunger caused by rodents (Singleton *et al*., 2003). In Tanzania more than two million people are affected by rodents due to loss of 412.5 tonnes of cereals per year (Makundi *et al.,* 1999) and loss of 15% of maize output has been reported (Leirs, 2003).

In Tanzania, the roof rat, *Rattusrattus*, is the most abundant and widespread commensal rodent species, while *Mastomysnatalensis, Musmusculus, Cricetomysgambianus*and *Arvicanthisniloticus*are some of the field pest species in the country (Kilonzo, 1976). *Mastomysnatalensis*and *A. niloticus*are peri-domestic species found in fallow and cultivated lands, up to 2000 m above sea level (Makundi*et al*., 1999). Other species including *Lemniscomysgriselda*, *Acomysspinosissimus, Otomys*spp., *Grammomysdolichurus*and *Rhabdomyspumilio*are also common but less abundant in many parts of Tanzania (Makundi*et al*., 1991).

The Multimammate rats of the genus *Mastomys* are indigenous and are the most abundant pests of pre-harvested crops in eastern and southern Africa (Fiedler, 1988; Massawe*et al*., 2011; Mulungu*et al*., 2013). In Tanzania, regular outbreaks of *M. natalensis* have reported (Mwanjabe*et al*.,2002) and are considered a threat to cereal crops at early stages of growth and are also a public health concern (Mulungu*et al*., 2012; Katakweba*et al*., 2012; Makundi*et al*., 2005, Singleton *et al.*,2010). Rapid reproduction influenced by abiotic and biotic factors are associated with outbreaks (Leirs, 1992). The population of *M. natalensis* is higher in cultivated than uncultivated land (Kasso, 2013). The feeding behaviour of *M. natalensis* and their ecological adaptation to savannah grasslands enable them to predominate in agricultural landscapes where cereals are grown (Leirs*et al*., 1989; Worknehe*t al.,* 2004; Mulungu *et al*., 2013).

Rodent management strategies in agricultural systems vary from one country to another depending on which species exist as the main pests, type of crops, method of cropping, availability and affordability of the methods to be used to control rodents (Singleton and Petch, 1994).

In Tanzania, rodent control methods rely mostly on the use of rodenticides (Makundi*et al.*, 1999; Ngowo*et al.*, 2005). Acute and chronic rodenticides have been used extensively during rodent outbreaks (Ngowo*et al*., 2005).Traditional methods such as killing of rodents by trapping, hunting, flooding and gassing have been applied in many places but rarely had significant reduction effects on the rodent populations (Smith, 1994; Thankur*et al*., 2013). Rodents are able to multiply fast and re-colonise the farms after rodent control operations (Leirs*et al.,* 1997). Moreover, outbreaks are unpredictable and poisons have short-run impacts due to the rapid immigration of rodents from nearby natural areas; furthermore, poisons are often too expensive to be affordable by poor farmers and can

also affect non-target species (Stensethet *al.*, 2003; Davis *et al.,* 2004; Makundiet *al*., 2005; Skonhoftet *al.,* 2006; Kilonzo, 2006; Mulungu*et al.,*2014). In addition, bait shyness and palatability cause rodents to shift to other food sources instead of feeding on the treated bait (Myllymäki, 1987; Cowan and Townsend, 1994). For these reasons, the use of fertility control chemicals is proposed as a potential biological control method. This method of controlling rodents has been studied and practiced on several species of rodents in China (Liu *et al*., 2012; Liu *et al*., 2013). There have been no studies on the effects of fertility control agents on African rodent pest species**.** Therefore, this study aim at evaluating the effects of fertility control compounds in order to develop and adopt this technology to control the African multimammate rats, *Mastomysnatalensis,* in Tanzania.

1.2 Objectives

1.2.1 Main Objective

To assess the effectiveness of fertility control agents (quinestrol (QE)/levonorgestrel (LNG) on the reproduction of *M. natalensis.*

1.2.2 Specific objectives

i. To evaluate bait acceptability of QE, LNG and their combination by *M. natalensis.*

ii. To evaluate the effects of QE, LNG and their combination on body weights of *M. natalensis.*

iii. To evaluate the effects ofQE, LNG and their combination on the reproductive performance of *M. natalensis.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Distribution of Multimammate Rats

The multimammate mouse,*M. natalensis,* is characterized by possessing two rows of nipples each with 8-12 nipples (Happold, 2013). It is a widespread African rodent, which belongs to the family *Muridae* and it is the most common rodent in sub- Saharan Africa. It is the most abundant and most widely spread field rodent in Tanzania (Massawe *et al*., 2003; Kilonzo, 2006). In Tanzania, *M. natalensis* was indigenous house rat before the introduction of the larger and more aggressive *Rattusrattus* which drove the former out and forced it to adopt semi- domestic behaviour (Kilonzo, 2006).

2.2 Ecology and Biology of Multimammate Rats

Rodents are the most successful and abundant mammals on earth. They are able to live in diverse climatic and geographic environments including savannahs, woodland, peri-domestics, fallow and cultivated land where they thrive primarily on wild plants and crops (Fiedler and Fall, 1994; Kingdon, 1997; Stenseth*et al*., 2003).

The multimammate rat is characterised by its high reproduction rate and dispersal that makes it to be aserious pest. The litter size of *M. natalensis* can be up to 27 young (Duplantier*et al.,* 1996) with fecundity rates of up to 68 young per adult female. Its gestation period does not exceed 25 days (Leirs*et al*., 1994; Lund, 1994). Under favourable conditions, the litter size of *M. natalensis* can reach an average of 11 young compared to the mean of 4-6 in other small rodents (Leirs, 1995).

A matured male rodent is able to produce a large number of young by inseminating numerous females at short intervals and female reproductive performance is limited by the

length of the gestation period (Leirs*et al*., 1994).The young become reproductively active after abundant rainfall and vegetation growth and when such conditions prevail they may start reproducing at the age of three months (Happold, 2013).

The breeding season and growth of *M. natalensis* in Tanzania are much influenced by rainfall patterns, which in turn influence food availability in maize dominated cropping and farm-fallow mosaic landscape (Leirs*et al.,* 1990). However, in irrigated rice cropping system, breeding of *M. natalensis* occurs throughout the year due partly to the availability of food and water (Mulungu*et al.*, 2013).

2.3 Outbreaks ofRodents

Rodent outbreaks have been reported worldwide, where cultivation of agricultural crops is practiced (Singleton and Redhead, 1999;Stenseth*et al*., 2003; Singleton *et al*., 2010). Abundance of rodent pests has been influenced by rainfall, environment features and agricultural practices or anthropogenic factors (Leirs, 1995; Leirs *et al*., 1997; Singleton, *et al.,* 2010; Thakur *et al*., 2013; Mulungu *et al.,* 2014).Rodent species responsible for outbreaks vary according to the predominant species of a specified region. For instance, in Australia, *Musdomesticus* is the main pest species responsible for outbreaks and about 800 mice per hectare have been reported. In sub-Saharan African countries, *Mastomys*spp. are the major outbreak species (Leirs*et al*., 2010). According to Leirs*et al*. (2010) outbreaks of *M. natalensis* can exceed 1000 animals per hectare, although damage and economic losses are significant even in years with low population densities. Tanzania has experienced several irregular outbreaks of *M. natalensis* in maize and rice fields in many regions including Lindi, Morogoro, Dodoma, Singida and Tanga (Mwanjabe*et al*., 2002; Mulungu*et al*., 2012). In 1989, a widespread outbreak of the multimammate rats was reported in Lindi region where the densities were estimated to reach up to 1,400 rats per hectare, causing a yield loss of 48% in maize fields. Due to this outbreak, the government of Tanzanian had to supply food to residents threatened by famine (Mwanjabe*et al*., 2002).

In 2004, there was an outbreak of *M. natalensis* in irrigated low land rice fields in Mvomero District, Morogoro region (Mulungu*et al.,* 2012).

2.4 Rodent Behaviour

Understanding rodent behaviour is one of the most important aspects in rodent management operations. Behaviour affects population density, dispersal and sexual maturation. Social behaviour such as reproduction and movement influences birth, death and dispersal rates and hence changes in population density (Krebs *et al*., 2007).

The most important aspects of rodent behaviour in relation to management operations are movement, feeding and socialization. These behavioural characters ensure the survival of both individuals and populations and have to be fully exploited to make rodent control successful (Sridhara and Babu, 2006). Rodents usually explore the area in their habitat in order to be familiar with any new objects in their surroundings. They learn the details of their pathways, obstacles, hiding places, location of foods and water. These processes significantly aid survival of individuals in their living environments (Kilonzo, 2006, Sridhara and Babu, 2006). Movement pathways would quickly lead rodents into traps, or result in their eating poisoned bait. Accordingly, rodents primarily tend to avoid any strange object that is encountered in familiar surroundings. This behaviour is called "*neophobia"* or *"New object reaction"* (Kilonzo, 2006; Sridhara and Babu, 2006).

2.5 Economic Importance of Rodents

2.5.1 Agricultural importance

Agriculture is the sector most affected by rodents worldwide, in which an average damage of $30 billion worth of cash crops and cereal grains each year has been reported (Feldhamer*et al.,* 2007). For instance, outbreaks in Australia caused losses exceeding $ 40

million which reduced the national agricultural production by 3-4 % (Singleton, 1997). A study on feeding habits of *M. natalensis* shows that it consumes an average of 10 g of cereals per day. Therefore, 1000 animals during outbreaks can consume 10 kg of crops per day or 3650 kg in the same area per year (Leirs, 1995). These amounts are big enough to cause significant shortages of food supplies in the affected area. Thus rodent pests play a significant role in influencing food security and poverty alleviation programs for the rural poor (Singleton *et al.,* 2010).

2.5.2 Public health importance

Most rodents, including *M. natalensis* are responsible for transmitting about 60 diseases directly or through vector to other animals and humans, often resulting in high morbidity and mortality (Gratz, 1994). Rodent borne diseases such as plague and leptospirosis are important as the diseases lead to decreased labour productivity and increased health care costs (Begon*,* 2003; Durnez*et al.,* 2007;Makundi*et al.,* 2008; Katakweba*et al.,* 2012; Asenga*et al*., 2015).

2.5.3 Households and industrial materials

Rodents also destroy infrastructure such as electrical cables, pipes, furniture, paper products and clothing materials by gnawing (Lund, 1994; Fritzen*et al*., 2013). Some damages such as fire due to electric cable destruction can be quite disastrous or hazardous (Kilonzo, 2006). Despite these, negative effects to the economy, some rodent species are also beneficial in maintaining the ecosystem and are used for research and training (Kasso*et al*., 2010; Mgode*et al*., 2014).

2.6 Rodent Pest Management

2.6.1 Rodent control measures

Management of rodents is difficult and expensive, especially in developing countries such as Tanzania. Control methods can be physical, mechanical, chemical, biological and ecological (Sridhara and Babu, 2006; Desoky, 2013). Achieving satisfactory rodent mortalities is not feasible by physical killing, trapping and use of rodenticides (Makundi, *et al*., 2005;Sarker*et al*., 2013). Killing a large number of rats in the short term cannot solve rodent problems since the population may actually increase after the initial decline. Rodenticides can be destroyed by water during rainfall and increase the chances of poisoning to non-target organisms (Thakur *et al*., 2013). Moreover killing methods are neither the most effective nor economic in practice (Smith, 1994 Singleton *et al.,* 1999, Stenseth*et al.,* 2001, Mulungu*et al.,* 2014).

Preventing access to the vulnerable crops is the most effective method of reducing rodent damage but the method is difficult or has limitations because poor farmers cannot afford the cost of fencing off crops (Sridhara and Babu, 2006).

2.6.2 Fertility control

Fertility control is a technique that reduces the production of young and consequently induces a reduction in either fertility or fecundity (Bomford, 1990). Anti-fertility drugs can cause permanent or temporary sterility in either sex, reduce the number of offspring or impair the fertility of offspring produced (Marsh and Howard, 1977). Females can develop sterility by suppressing secretion of anterior pituitary, preventing follicular development and maturation, blocking the passage of ova in the oviduct, preventing fertilization and implantation and interfering with gestation. In males it acts by suppressing of gonadotrophin secretion, inhibition of spermatogenesis and interference during transport

and storage of sperm. Also it may block production and release of sexual pheromones and thus interfering with reproduction behaviour (Sridhara and Dubey, 2006). Control mechanisms by using anti-fertility compounds allow timing of reproduction with respect to age, time, season, and other periodic environmental conditions. Changes in breeding can occur with variations in temperature, rain fall, nutrition, and health status of animals (Ulysses, 1991).

Fertility control is a cost-effective biological control method (Murdoch, 1994; Fayrer-Hosken*et al.,* 2000). This method is more humane, species specific, leaves no toxic residues and no primary or secondary effects on non-target species (Tuytenns and Macdonald, 1998; Sridhara and Dubey, 2006). In addition, infertile animals can remain in the population and therefore sustaining density dependant feedback to recruitment and survival (Zhang, 2000). Also, fertility control may reduce population increase before it reaches levels that lead to destruction.

2.6.2.1 Fertility inhibitors for animals

Several anti-fertility compounds have been used in controlling reproduction in various animal species, through contraceptive or sterilization. Contraception precludes the birth of offspring but retain fecundity, while sterilization renders animals sterile (Kutzler and Wood, 2006; Fagerstone*et al.,* 2010). The most common approach to wildlife contraception was through the use of steroid hormones, particularly natural and synthetic estrogens, progestins, and androgens, similar to those found or used in humans (Massei*et al.*, 2014). However, some chemicals such as *Alpha-chlorohydrin* (sterilizant) and *bromocriptine*(enzyme inhibitor of prolactin) have been used (Sridhara and Dubey, 2006). Synthetic progestins such as norgestoment, melengestrol acetate (MGA), megestrol acetate (MA) and levonorgestrel have been widely used in zoo animals, livestock and wildlife

(Nave *et al.,* 2002; Massei*et al.*, 2014). These anti-fertility chemicals found to be 92 % effective at postponing estrus in bitches and very effective in many carnivore species, primates and ungulates (Massei and Miller, 2013).

Levonorgetrel is a progesterone analogue used as emergency contraceptive to prevent pregnancy by preventing or interrupting ovulation and egg implantation in females. It is effective only in first few days after mating, before the ovum is released from the ovary or before the sperm fertilize the ovum (Gemzell-Danielsson and Marions, 2004; Nokiva*et al*., 2007). It affects the cervical mucus or the ability of the sperm to bind to the egg (Asa and Porton, 2005). The mechanism of action of levonorgestrel is still unclear whether it has effects on fertilization or implantation (Nokiva*et al*., (2007).

Quinestrol is a synthetic estrogen homolog. It is the major component of long-term oral contraceptives used by women (Zhao*et al*., 2007). Little is known regarding the effect of quinestrol on male fertility (Zhao *et al*., 2007; Massei*et al*., 2014).

Other known fertility inhibitors are immunocontraceptive vaccines. These vaccines stimulate the immune system to produce antibodies against gamete proteins, reproductive hormones and other proteins essential for reproduction. These antibodies interfere with the normal physiological activity of the reproductive system consequently infertility occurs in targeted animal (Talwar and Gaur, 1987; Miller and Killian, 2002).

Synthetic hormones such as quinestrol and levonorgestrel have been used in controlling rodent species such as Brandt's voles (*Lasiopodomysbrandtii*), Mongolia gerbils (*Merionesunguiculatus*) and plateau pikas (*Ochotonacurzoniae*) found in China (Liu *et al*., 2012; Liu *et al*., 2013; Li *et al*., 2014). Anti- fertility effects of these compounds appear to

impair reproductive performance of rodents by reducing reproductive organs and interfering with spermatogenesis and therefore decrease sperm concentration, motility and increase the number of abnormal sperms in males and reduce pregnancy rate and litter size in females (Shen*et al.,* 2011; Wang *et al.,* 2011; Lv*et at el.,* 2012; Fu *et al.,* 2013).

2.6.2.2 Delivery methods

A proper delivery technique is very important in order to make fertility control humane, effective, species specific, cost-effective and limit exposure to non-target species (Fagerstone *et al.*, 2006).Currently, fertility control agents are administered by direct injection following capture, implant or are delivered remotely through biobullets (DeNicola*et al.,* 2000). Syringe-dart, used to anaesthetized wild animals has also been used to administer contraceptives to large ungulates (Delsink*et al.,* 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Study Area

The study was conducted at the Pest Management Centre (PMC), Sokoine University of Agriculture, located in Morogoro Tanzania.

3.2 Study Design

Laboratory experiments using single and mixed compounds of quinestrol (QE), levonorgestrel (LNG) and quinestrol/levonorgestrel combination at concentrations of 10, 50 and 100 parts per million(ppms).

3.3 Experimental Animals

A total of 316 sexually mature rodents; males (n=158) and females (n=158) *M. natalensis* captured in maize fallow fields of Sokoine University of Agriculture were used.

3.4 Maintenance of Animals and Sample Collection

Animals were captured using Sherman live traps using peanut butter as bait, then transported to SPMC laboratory. Animals were singly caged with wood-shavings as bedding material. Food and water was given *ad-libitum* for two weeks for acclimatization before the commencement of the experiment.

3.5 Bait Preparation

3.5.1 Preparation of solutions of compounds

i. A bait base was first prepared consisting of 2/3 crushed maize by weight, 1/3 maize flour by weight and 2.5% fish meal with a final bait weight of 10 kg.

Before mixing with the crushed maize, the maize flour and fish meal mixture were mixed in boiling water and cooked for about 15 minutes to form a stiff paste '*ugali*'. This was left to cool down to room temperature.

ii. The experimental compounds QE, LNG and QE/LNG were weighed carefully using an electronic balanceas follows: 0.1 g, 0.5 g and 1 g for preparing concentrations of 10 ppm, 50 ppm and 100 ppm respectively. For QE/LNG combination the ratios were 1/3 quinestrol and 2/3 levonorgestrel (Kejuan *et al*., 2007). Each of the contraceptive compounds was dissolved in 100 mls of absolute ethanol at 60-70°C in a pre-warmed water bath.

iii. The solution of contraceptives was mixed thoroughly in sugar solution containing 200 g of sucrose in 1000 mls of water. The mixture was stirred thoroughly to form a suspension of fertility compounds in sugar solution.

iv. The mixture of sugar solution and fertility compounds suspension was showered on the crushed maize and the mixture was added to the flour-fishmeal stiff paste'*ugali*'. This was thoroughly mixed to ensure a uniform distribution of the crushed maize in the stiff paste.

v. Using an electric household meat mincing machine the paste was made into pellets. The pellets were dried in shade at ambient temperature; each concentration of compounds was prepared separately and used within one month.

Vi. Plain bait (without anti- fertility compounds) was prepared in a similar way. This was used for feeding the control group of animals during the experiment.

3.5.2Estimation of bait acceptance and effects of contraceptives on male and female animals

Bait acceptance was evaluated using 50 male and 50 female *M. natalensis* to determine bait intake by weight. Each animal was kept in a separate animal cage and given 10 g of

bait containing contraceptive for seven consecutive days. Water was supplied for each animal. Control animals were given plain food without contraceptives. Each treatment contained five animals of each sex which were treated with varying concentrations of QE, LNG and QE/LNG as shown in the Table 1. A control group was fed with plain bait.

Table 1: No choice experimental set-up

Treatment	Number of animals	Sex	
		Male	Female
Control	10	5	5
QE 10 ppm	10	5	5
QE 50 ppm	10	5	5
QE 100 ppm	10	5	5
LNG 10 ppm	10	5	5
LNG 50 ppm	10	5	5
LNG 100 ppm	10	5	5
QE/LNG 10 ppm	10	5	5
QE/LNG 50 ppm	10	5	5
QE/LNG 100 ppm	10	5	5

QE=Quinestrol; LNG=Levonorgestrel

3.5.3 Pairing experiment for observing pregnancy and litter size

A total of 108 pairs of male and female adult animals were used for pairing. Treated rodents were fed 10 g contraceptive baits in three different concentrations and untreated rodents were fed 10 g plain bait. The bait was delivered for seven consecutive days in all treatments and water was given *ad libitum.* Each animal was kept in separate animal cage during bait delivery.A total of thirty six adult animals of either sex in each test set were treated with varying concentrations of QE, LNG and QE/LNG. Another 36 animals were given plain bait. Each concentration of the compound was given to 12 animals of either sex; another control 12 animals were given plain bait in each test set. The control group

wasincluded in each test set of the compounds; in total there were nine test sets. After feeding animals with different concentrations of contraceptive baits, they were paired in four categories as follows: untreated females paired with untreated males (pair 1) (control), untreated females paired with treated males (pair 2), treated females paired with untreated males (pair 3) and treated females paired with treated males (pair 4) for each concentration of compounds for 10 days (Fig.1). The male animals were removed and female animals were left for observation of pregnancy for 30 days.

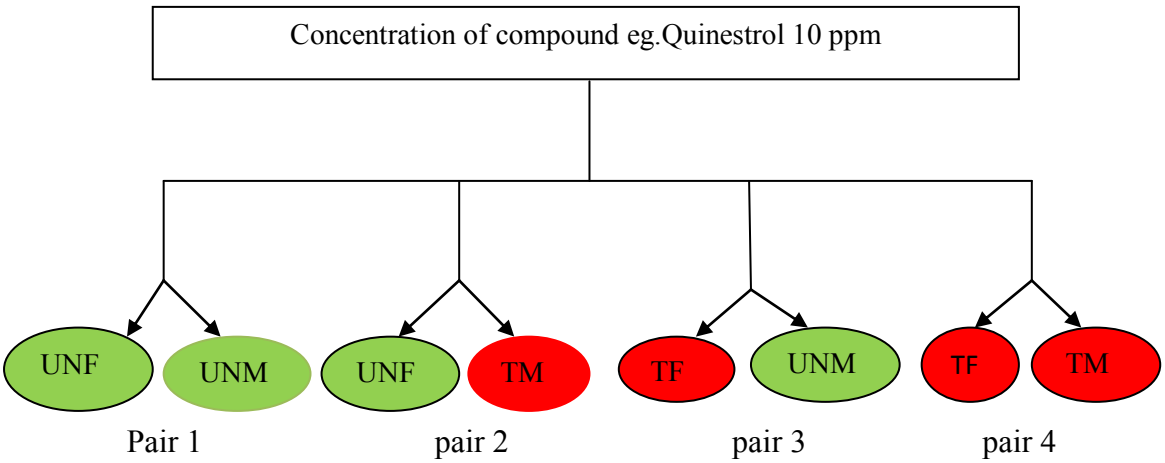


Figure 1: Pairing experimental set up

Pair 1= Untreated female (UNF) paired with untreated male (UNM)

Pair 2=Untreated female (UNF) paired with treated male (TM)

Pair 3=Treated female (TF) paired with untreated male (UNM)

Pair 4=Treated female (TF) paired with treated male (TM)

3.6 Data Collection

3.6.1 Bait acceptance and weight loss

A total of 100 adult male and female animals were fed with single and combination of the contraceptive compounds (quinestrol (QE) and levonorgestrel (LNG) at different

concentrations (10, 50 and 100 ppms) for seven days. Each animal was given 10 g/day. Before feeding with the contraceptive or plain bait the body weight of each animal was measured using an electronic balance while in a restraining cotton bag. The animals were weighed daily for seven days. The amount of bait consumed was determined by subtracting the weight of the left over feed from the weight of bait given in the preceding day (10 g each day). In each animal cage an animal feeder was used to prevent from mixing with bedding material, water or any other materials weights. Percentage weight loss was obtained by subtracting the final weight from initial weight x 100 divided by the initial weight.

(Final weight-Initial weight)*100 / initial weight = Percentage weight loss.

3.6.2 Effects of QE and LNG on male reproductive organs

After feeding the animals with contraceptive bait for seven days 47 males were anaesthetized using diethyl ether for histological observations. Male reproductive organs; testes, epididymis and seminal vesicles were removed and their weight measured.

3.6.2.1 Sperm count and motility

The epididymis was removed and sliced using scissors in a glass petridish containing 1 ml of 0.85% normal saline and drop of the suspension placed on a microscope slide under a cover slip for sperm motility observation, using an ordinary light microscope at x 20. Another drop of the same suspension was used to prepare a smear for sperm morphology analysis. The remaining samples were put in glass test tubes and kept at 4^0 C for about two hours in order to release the sperms. The samples were then diluted 1:10 by adding 9 mls of distilled water and placed in modified fuchs Rosenthal (B.S.748) counting chamber and followed the WHO (2010) protocol for sperm counting.

3.6.2.2 Sperm morphology analysis

The smears were air dried and fixed with equal parts of ether: ethanol (absolute) for 30 minutes then stained using 10% Giemsa for a maximum of 30 minutes, washed with running tap water, dried and examined under oil immersion x 100 to assess the morphology of sperm head, body and tail. A maximum of 200 sperms were observed per slide and sperm abnormalities were recorded as percentages.

3.6.3 Observation of pregnancy development and number of offspring / litter size

After pairing, female animals were left to feed on normal diet for 25 days. The newborns in each litter were counted, weighed and their mean weight compared to the control animals.

3.6.4 Evaluation of uteri and ovary

Female animals that were not pregnant from all treatments including controls (untreated females paired with untreated males) were sacrificed for uteri and ovary observation. The uteri and ovary were dissected, weighed and all abnormal features were noted.

3.7 Data Analysis

Data on the daily consumption, weight of the animals in all treatments were recorded in Excel spread sheet. Statistical analysis was performed using SAS package (Version 9.1) and STAT. Comparisons were made between the treatments using ANOVA. Mean separation was done using Tukey's or Duncan. All graphs were drawn using Microsoft Excel 2007.

CHAPTER FOUR

4.0 RESULTS

4.1 Bait Acceptance

4.1.1Consumption of bait

The mean consumption of bait per day was significant higher ($F_{(6,560)} = 5.7665, p = 0.00001$) on day one, and slightly lower on day two and day threecompared to the restof thefour days(Fig. 3). The overall bait consumption in treated groups of animals was significantly lower than that of the control group($p < 0.0001$). Mean consumption of bait from all treated groups was significantly reduced ($F_{(9,560)} = 39.092$, $p = 0.0000$) compared to that of control group (Fig. 2). Means separation of bait consumption for each treatment is shown in Table 2 (Tukey's HSD test). Generally, in all treatments it was observed that consumption rate decreased slightly with increasing concentrations of fertility control compounds (10, 50 and 100 ppms) from the second day to day six with slightly increase on day seven, except for LNG 10 which decreased from day four (Fig. 3).

There was an interaction effect between treatment and sex onbait acceptance ($F_{(16,392)} = 10.007$, $p < 0.001$) with higher acceptance of treated bait in female rats than male rats (Fig. 4). Tukey's HSD test Table 3.

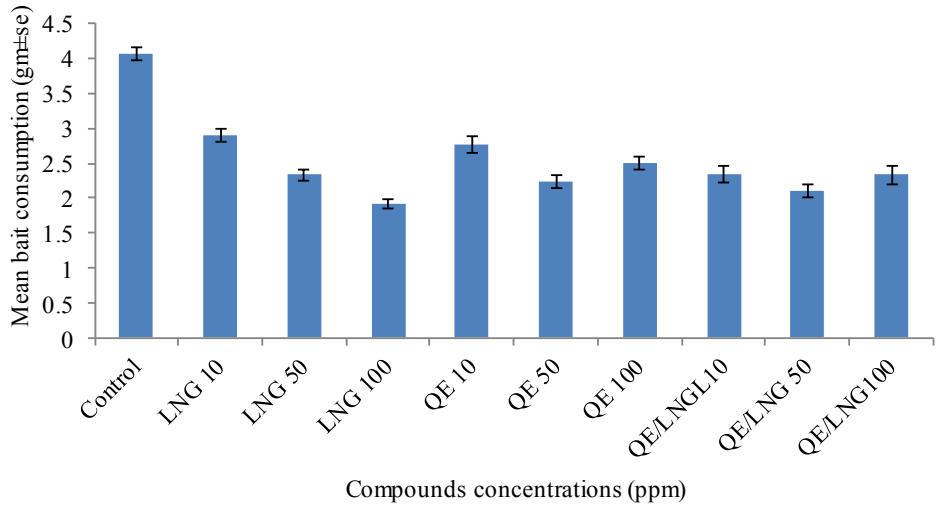


Figure 2: Mean consumption of bait containing levonorgestrel (LNG), quinestrol (QE) and quinestrol/ levonorgestrel (QE/LNG) combination by *M. natalensis*.

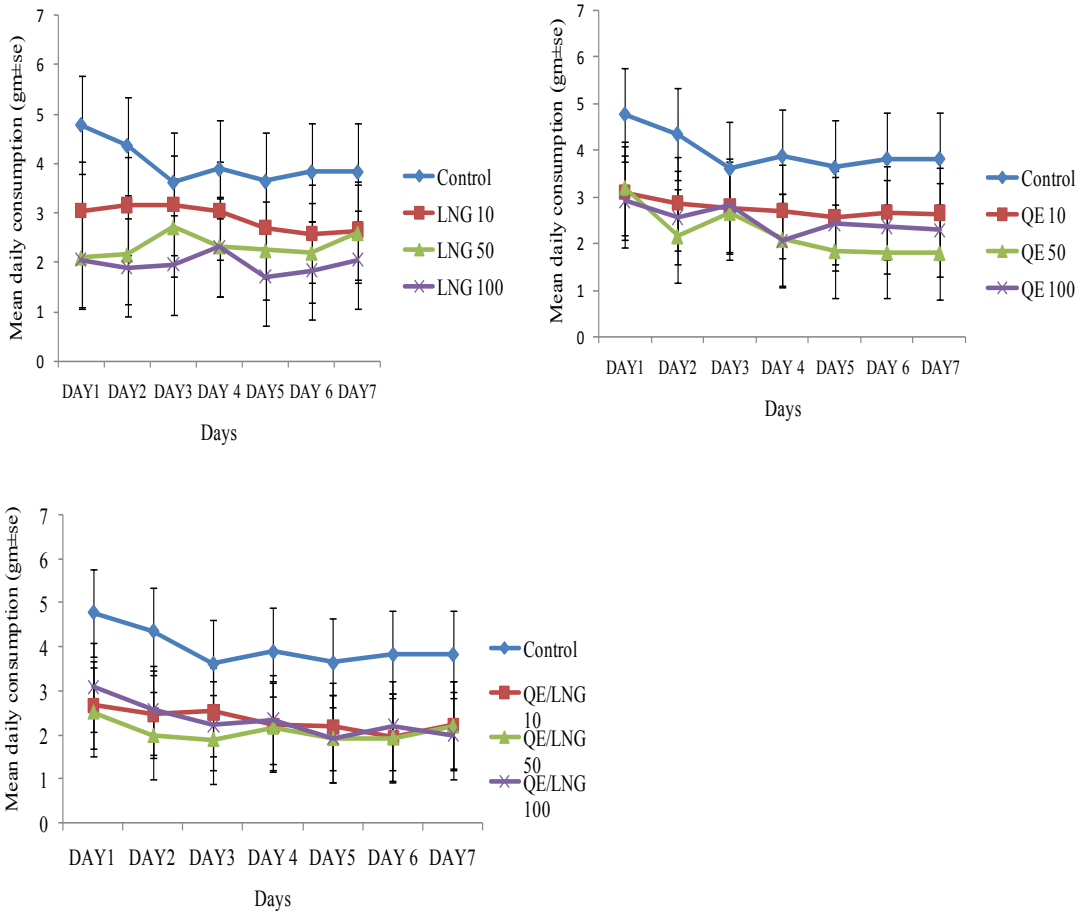


Figure 3: Mean daily bait consumption rate by *M. natalensis* of bait containing quinestrol (QE), levonorgestrel (LNG) and quinestrol/ levonorgestrel (QE/LNG) combination for seven days.

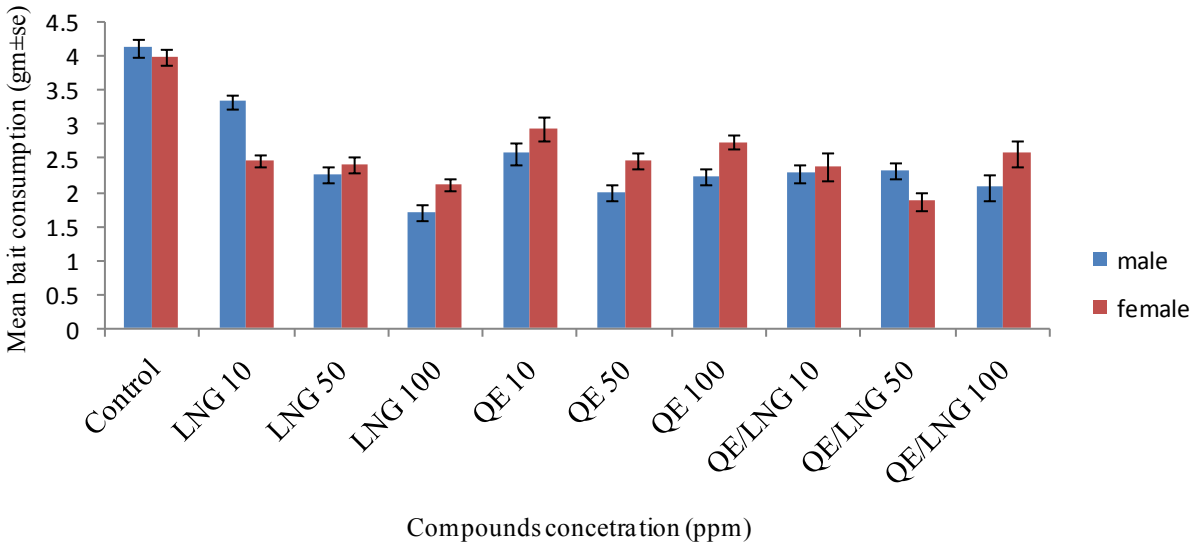


Figure 4: Interaction effects of treatment and sex on bait acceptance by *M. natalensis*

Table 2: Mean consumption of bait containing levonorgestrel (LNG), quinestrol (QE) and quinestrol/levonorgestrel (QE/LNG) combination by *M. natalensis*.

Treatment	**Mean Weight (gm)**
Control	4.062571[a]
LNG	
LNG 10	2.901571[b]
LNG 50	2.337714[cde]
LNG 100	1.917[e]
QE	
QE 10	2.757857[bc]
QE 50	2.230143[de]
QE 100	2.496714[bcd]
QE/LNG	
QE/LNG 10	2.335286[cde]
QE/LNG 50	2.097714[de]
QE/LNG 100	2.333143[cde]

Means with different superscripts a-e differ significantly at $p \leq 0.05$ (Tukey's HSD test)

Table 3: Interaction effects between treatment and sex on bait acceptance by *M. natalensis*

Treatments	mean weight (gm)	
	Male	Female
Control	4.13^{a}	3.99^{ab}
LNG 10	3.34^{b}	2.46^{cde}
LNG 50	2.26^{def}	2.41^{cde}
LNG 100	1.71^{h}	2.13^{def}
QE		
QE 10	2.58^{cd}	2.94^{bc}
QE 50	1.99^{fgh}	2.47^{cde}
QE 100	2.25^{ef}	2.75^{bcd}
QE/LNG		
QE/LNG 10	2.28^{def}	2.38^{def}
QE/LNG 50	2.33^{def}	1.87^{gh}
QE/LNG 100	2.09^{ef}	2.58^{cd}

Means with different superscripts a-e differ significantly at $p \leq 0.05$ (Tukey's HSD test)

4.1.2 Effects of QE and LNG on body weight

Animals in all treatments showed significant weight loss ($F_{(9, 101)} = 14.42$, $p < 0.0001$) compared to that of the control group. The body weight of treated animals decreased with increasing number of days of consumption of treated bait (Table 4 and Figure 5).

Table 4: Body weight loss on *M. natalensis* following the consumption of levonorgestrel (LNG), quinestrol (QE) and quinestrol/levonorgestrel combination

Treatment	Mean Initial wt (gm)	Mean final wt (gm)	Weight loss (gm±se)	Corrected % Weight Loss
Control	43.72	43.54	0.18±0.35[a]	0.41
LNG				
10 ppm	36.46	34.16	2.29±0.60[b]	6.28
50 ppm	38.71	34.70	4.0±0.60[bcd]	10.33
100 ppm	38.75	33.18	5.19±6.0[de]	13.39
QE				
10 ppm	47.11	41.49	5.62±0.60[de]	11.9
50 ppm	37.43	33.28	4.15±0.60[cde]	11.08
100 ppm	45.39	39.57	5.82±0.60[e]	12.82
QE/LNG				
10 ppm	34.00	31.35	2.66±0.60[bc]	7.82
50 ppm	32.48	29.56	2.92±0.60[bc]	8.98
100 ppm	34.39	31.98	2.33±0.60[b]	6.77

Means with different superscripts a-e differ significantly at $p \leq 0.05$ (Duncan multiple range test).

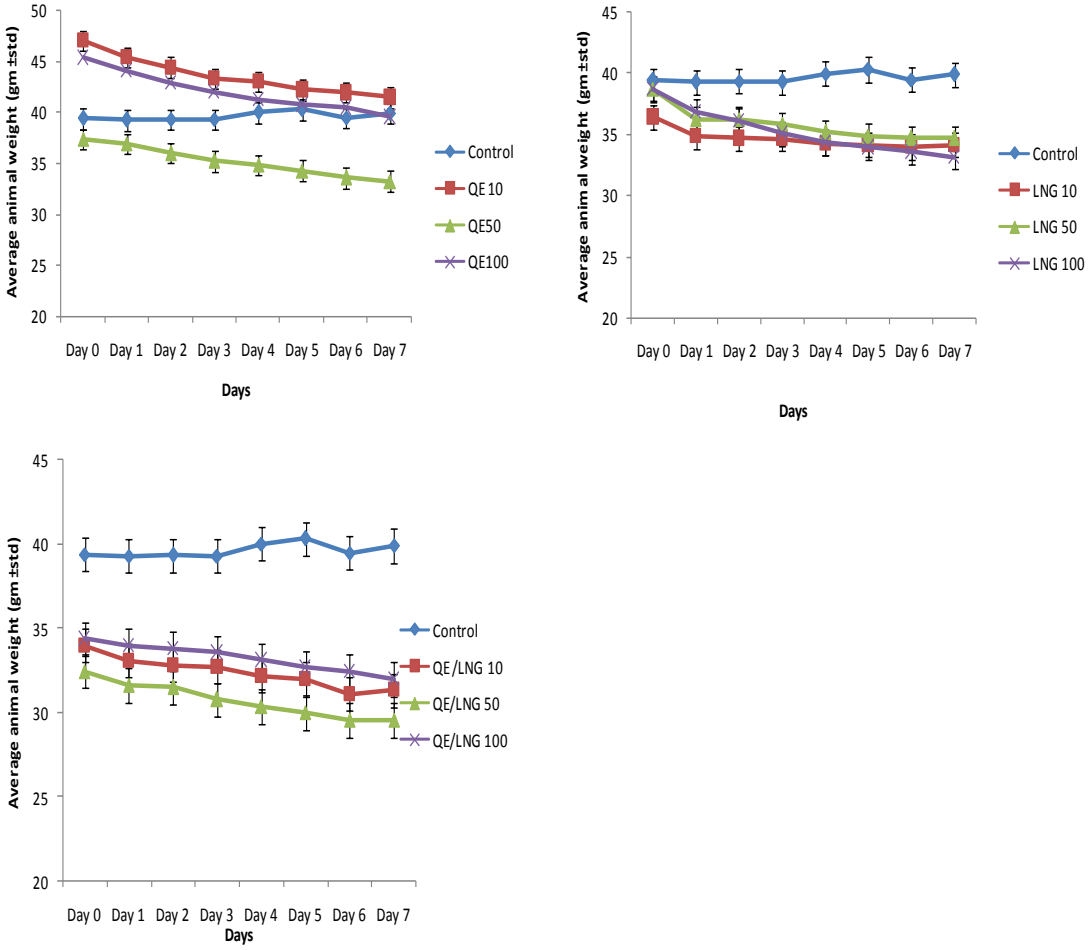


Figure 5: Effects of quinestrol (QE), levonorgestrel (LNG) and quinestrol/levonorgestrel (QE/LNG) combination on body weight of *M. natalensis*.

4.2 Effects of QE/LNG on Reproductive Organs

4.2.1Male reproductive organs

4.2.2Testis weight

The overall mean testis weight of QE, LNG and QE/LNG combination treated groups were significantly reduced compared to the control group ($F_{(9, 43)} = 4.77$, p=0.0002). However, QE/LNG combination treated animals showed significant lower testicle weight compared to QE or LNG separately (Table 5).

4.2.3 Epididymis weight

The mean epididymis weight in contraceptive treated animals was significantly lower ($F_{(9, 43)} = 2.17$, $p = 0.0434$) than that of control group (Table 5).

Table 5: Effects of Levonoegestrel (LNG), Quinestrol (QE) and quinestrol/levonorgestrel on male reproductive parameters

	Variables investigated		
Treatment	**Testes weight (mg)**	**Epid weight (mg)**	**Sv weight (mg)**
Control	840±0.06[a]	770±0.06[a]	370±0.02[a]
LNG			
10 ppm	740±0.09[ab]	530±0.11[ab]	210±0.04[bc]
50 ppm	740±0.09[ab]	640±0.11[ab]	130±0.04[bcd]
100 ppm	860±0.09[a]	720±0.11[ab]	260±0.04[ab]
QE			
10 ppm	530±0.09[bc]	600±0.12[ab]	50±0.05[d]
50 ppm	380±0.09[c]	420±0.11[ab]	30±0.04[d]
100 ppm	570±0.09[abc]	750±0.11[a]	50±0.04[d]
QELNG			
10 ppm	440±0.11[c]	390±0.12[b]	80±0.05[d]
50 ppm	380±0.09[c]	510±0.11[ab]	120±0.04[cd]
100 ppm	390±0.11[c]	430±0.12[ab]	80±0.05[d]

Means with different superscripts a-d within a treatment column differ significantly at $p \leq 0.05$ (Duncan multiple range test).

Epid=Epididymis, Sv= Seminal vesicles

4.2.4 Seminal vesicles weight

The seminal vesicles weights of all contraceptive treated groups were significantly lower ($F_{(9, 43)} = 11.55$, $p < 0.0001$) than that of the control group (Table 5). However, the lowest seminal vesicle weight was observed in QE and QE/LNG treated animals.

4.2.5 Sperm concentration, motility and morphology

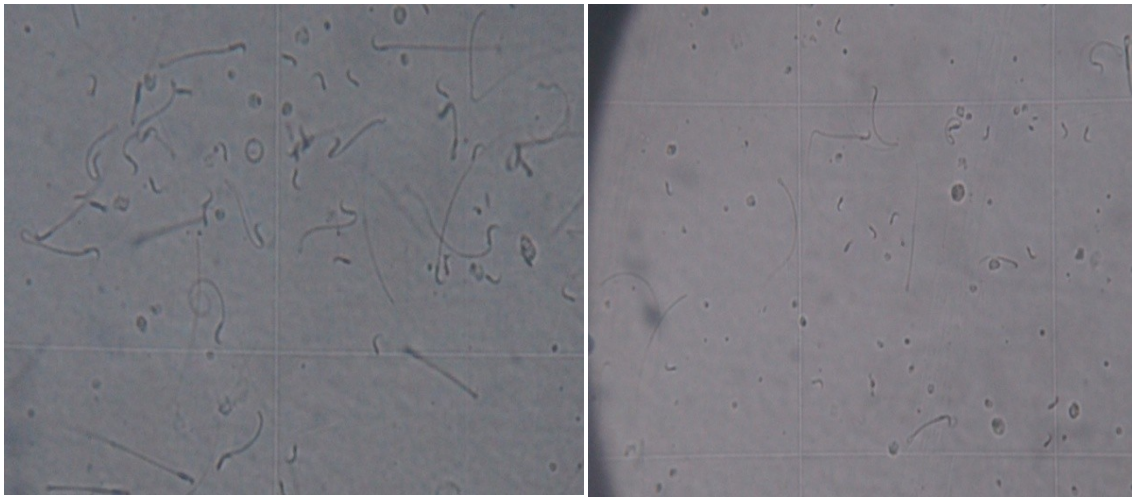
Sperm concentration and motility decreased significantly ($F_{(9, 43)}$ =13.18 p=0.0001, and $F_{(9, 43)}$ =25.34, p=0.0001) in treated animals. A significant increase ($F_{(8, 31)}$ =22.10, p=0.0001) of abnormal morphology of sperms occurred in treated animals compared to the control group (Table 6).

Table 6: Effects of Quinestrol and Levonorgestrel on sperm parameters

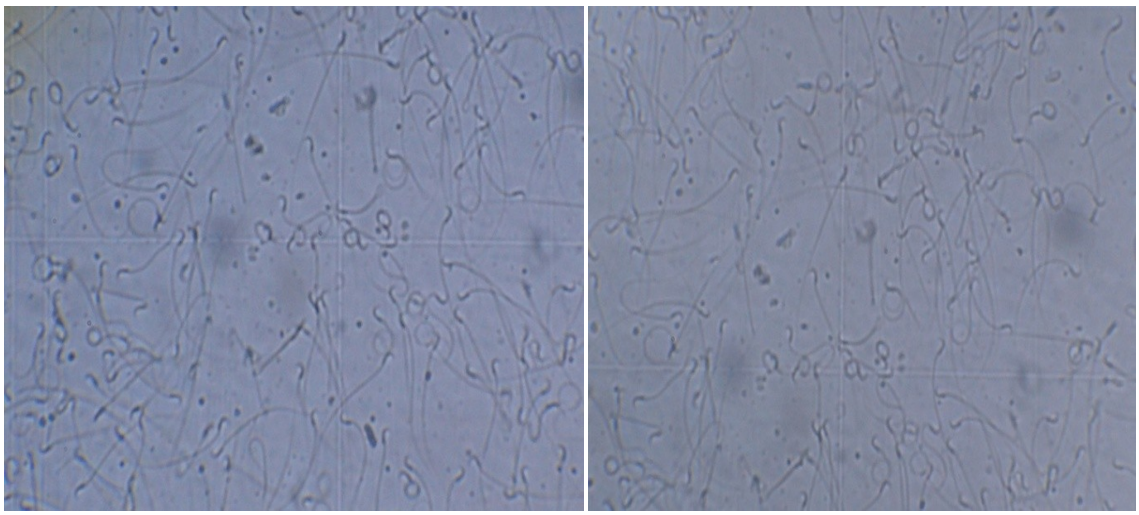
	Variables investigated		
Treatment	**SC (*10^5)**	**SM%**	**ASM%**
Control	2198.4±151.6^{a}	86.0±4.41^{a}	16.0±3.28^{d}
LNG			
10 ppm	719.0±262.6^{b}	60.0±7.64^{b}	26.0±5.69^{cd}
50 ppm	691.0±262.6^{b}	32.0±7.64^{cd}	24.78±6.43^{cd}
100 ppm	586.8±262.6^{b}	50.0±7.64^{cd}	30.0±5.69^{cd}
QE			
10 ppm	286.3±296.9^{b}	32.5±8.63^{cd}	77.19±6.45^{ab}
50 ppm	12.8±7.64^{de}	12.8±7.64^{de}	86.01±7.53^{ab}
100 ppm	181.3±262.6^{b}	15.0±7.64^{de}	69.93±6.43^{b}
QELNG			
10 ppm	234.1±296.9^{b}	1.2±8.63^{e}	50.63±7.53^{c}
50 ppm	22.0±262.6^{b}	1.4±7.63^{e}	95.24±13.44^{a}
100 ppm	61.3±296.9^{b}1.2±8.63^{e}	----------	

Means with different superscripts a-e within a treatment column differ significantly at$p$≤0.05 (Duncan multiple range test).

SC=Sperm count, SM= Sperm motility, ASM=Abnormal sperm morphology, (----- No sperm seen)



a b



c **d**

Plate 1: Sperm count in hemocytometer

a and b = sperms of male*M. natalensis* treated with contraceptive, c and d = sperms of untreated male *M. natalensis*

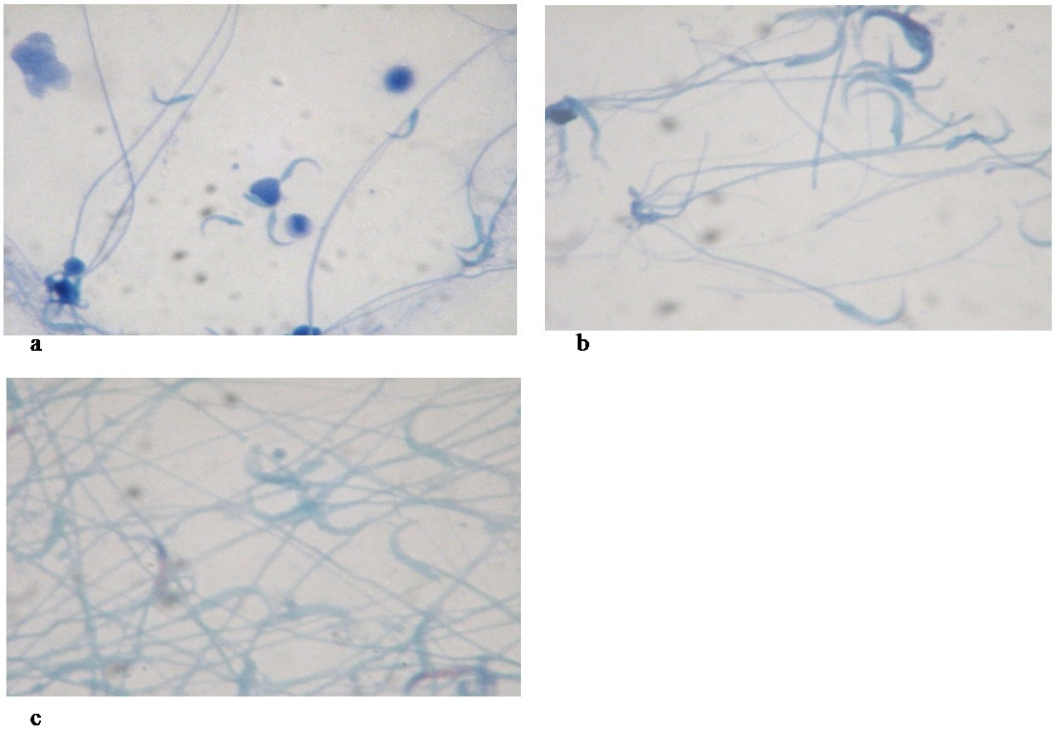


Plate 2:Abnormality of male *M. natalensis* sperms in treated animals.

a. = head –tail separated sperms from QE/LNG treated animal, b. = head – tail, separated sperms from QE treated animal, c.= Sperm stained from untreated animal.

4.3 Effects of QE and LNG on Female Reproductive Organs

4.3.1 Uteri and ovaries weight

The weights of the uteri and ovaries of animals treated with QE, LNG and their combination were not significantly different (p> 0.05) from those of control groups after seven days of bait delivery. Also, in all treated animals there were no observable changes in the uteri structure except for the marked uterus oedema in some QE treated animals.

4.3.2 Effects of QE and LNG on pregnancy and litter size of *M. natalensis*

Pregnancy and litter size were significantly reduced ($F_{(3, 12)}$=20.17, p=<0.0001) and ($F_{(3, 12)}$=16.22, p= 0.0002 respectively) in treated animals paired with untreated or treated of either sex compared to that of control pairs (untreated paired with untreated animals)

respectively (Table 7 and 8). However, with the three formulations there were no pregnancies in treated females paired with treated males (TFTM).

Table 7: Pregnant females and litter size at different concentrations of contraceptives and animal pairing

Activity	N	Pregnant females	Litter size
Concentration (ppm)			
100	36	0.222[a]	1.889[a]
50	36	0.167[a]	1.195[a]
10	36	0.139[a]	1.139[a]
Compounds			
QELNG	36	0.306[a]	2.444[a]
LNG	36	0.167[ba]	1.361[ba]
QE	36	0.0556[a]	0.417[b]
Pairing of Male&Female			
UNFM	27	0.444[a]	3.704[a]
TFUNM	27	0.222[b]	1.629[b]
UNFTM	27	0.037[cb]	0.296[cb]
TFTM	27	0.000[c]	0.000[c]

Means with different superscripts a-c within a treatment column differ significantly at $p \leq 0.05$ (Duncan multiple range test).

Table 8: Pairing of males and females exposed/non exposed to contraceptive compounds

	CONC (ppm)	No of females	Pregnant females		Litter size	
			Mean	std	Mean	Std
TFTM	10	9	0.00	0.00	0.00	0.00
TFUNM	10	9	0.22	0.44	0.89	2.03
UNFM	10	9	0.44	0.53	3.67	4.47
UNFTM	10	9	0.00	0.00	0.00	0.00
TFTM	50	9	0.00	0.00	0.00	0.00
TFUNM	50	9	0.22	0.44	1.89	3.76
UNFM	50	9	0.33	0.50	2.89	4.37
UNFTM	50	9	0.00	0.00	0.00	0.00
TFTM	100	9	0.00	0.00	0.00	0.00
TFUNM	100	9	0.22	0.44	2.11	4.26
UNFM	100	9	0.33	0.53	4.56	4.53
UNFTM	100	9	0.11	0.33	0.89	2.67

UNFM=Untreated female paired with untreated male

TFUNM=Treated female paired with untreated male

UNFTM=Untreated female paired with treated male

TFTM=Treated female paired with treated male

CHAPTER FIVE

5.0 DISCUSSION

In this study the effects of fertility control agents on multimammate rats were evaluated. Results showed that acceptability of bait containing quinestrol and levonorgestrel was lower compared to plain bait. From the current study bait consumption was affected by concentration of the compounds and varied with the sex of the animal. Contraceptive baitswere slightly more accepted by female than male animals. In the present study there was a reduced consumption of bait with increased concentration of the compounds. In QE/LNG combination the average bait consumed was variable with days. These findings are in contrast with other studies that showed no significant differences in bait consumption with the control groups. Liu *et al.* (2012) reported that there were no significant differences on the effects of QE and LNG on Plateau pikas in bait consumption at 0.005%. Chen *et al*. (2010) observed no significant differences between treated and control groups for baits formulation with oak, grain feed and carrot containing 10 ppm quinestrol, levonorgestrel and their combination. Quinestrol resulted to significant decline in consumption after three or four days under laboratory conditions.

Treated animals experienced weight loss from day one to–day seven of bait delivery. This could be caused by low feed intake but also due to unknown effects of contraceptives that could interfere with normal body physiology. Lv*et al.* (2012) reported that quinestrol decreases the body weight of Mongolian gerbils (*Merionesunguiculatus*) but not levonorgestrel. Lv and Shi (2011) reported similar results with increasing concentration of quinestrol. However, Liu *et al.* (2012) reported that quinestrol had no effects on the body weight of *Rattusnitidus* of either sex over seven days of treatment. In the current study animals treated with quinestrol and levonorgestrel alone experienced much lower weight

loss than those treated with quinestrol/levonorgestrel combination. This could be one of the effects of long acting contraceptive (quinestrol/levonorgestrel combination) on *M. natalensis.*

In the current study quinestrol and levonorgestrel had some effects on reproductive status of male and female of *M. natalensis*. The results demonstrated that after seven days of bait delivery the weight of male reproductive organs (testes, epididymis, and seminal vesicles) decreased. Sperm concentrations and motility of male *M. natalensis*also decreased and abnormal sperms morphology increased. This demonstrates that QE and LNG have anti-fertility effects on *M. natalensis*. However, among the three compounds (QE, LNG and QE/LNG combination) quinestrol and quinestrol/levonorgestrel at 10, 50 and 100 ppms were more effective than levonorgestrel alone. O'Donnell *et al*. (2001) argued that quinestrol slows down spermatogenesis in rats. According to *Li et al*. (2014) quinestrol adversely affects semen quality although some processes such as maturity in the epididymis and secretion of seminal vesicles can cause the same effects as demonstrated by Gonzales(2001). These findings are similar to those reported by Zhang *et al*. (2006), Wang *et al*. (2011), Liu *et al.*(2012) and Li *et al*. (2014) who studied the effects of different concentration of QE and LNG in male greater long tailed hamsters, Brandt's voles, Plateau pikas and white mice at different concentrations. However, Lv*et al*. (2012) investigated the effects of QE in the offspring of treated mothers and revealed that male and female offspring were infertile whereas all males and females from LNG treated mothers were fertile. In addition, Zhao *et al*. (2007) reported that male Brandt's voles were sensitive following QE treatment but not levonorgestrel or QE/LNG combination. Furthermore, QE alters testicular structure and decrease sperm count following 5 or 14 consecutive days treatment.

The findings from the current study further indicate that 10 ppm QE and QE/LNG combination was sufficient to induce infertility in male *M. natalensis*under laboratory conditions.Zhang *et al*. (2005) found that 10 and 30 ppm QE/LNG bait caused infertility effects of the greater long-tailed hamsters (*Tscherskia triton*). Also, Zhao *et al.* (2007) found that a QE dosage of 0.35mg/Kg body weight effective to control voles these rodent species in the field. Moreover, Lv and Shi (2011) reported that 0.1 to 2.7µg/g body weight quinestrol administered intragastrically for six days inhibited fertility of Mongolian gerbils under laboratory conditions. According to Huo *et al.* (2007), treatment with multiple dosages of 10 ppm QE/LNG combination within one week interval showed higher anti-fertility effects on female Mongolia gerbils than single dosage treatment. Conversely, high dosage of QE and QE/LNG combination administered for seven days impeded spermatogenesis in male *M. natalensis.*

In females of *M. natalensis* treated with QE, the uterine oedema observed may be due to hormonal changes. Previous study by Lv and Shi (2011) observed similar structural changes of the uterus in female Mongolian gerbils treated with QE and proposed that the changes may be related with abnormal amounts of oestrogen and progesterone. Zhao *et al*. (2007) reported no significant differences on the ovaries and uteri of Brandt's voles treated with QE, LNG and QE/LNG combination from those of control females at day 15, day 30 and day 75. Also, no structural changes were recorded in the uterine luminar and glandular epithelium, stroma, or the follicle in the ovaries of any of the groups. In contrast Liu *et al*. (2013) found reduced weight of ovaries but not uteri of *Rattusnitidus* treated with QE. Lvand Shi (2011) found increased gonadosomatic indices of uteri and reduced gonadosomatic indices of ovaries after QE treatment in Mongolian gerbils. Elsewhere, Lv*et al*. (2012) reported that QE increases the weight of the uterus while the ovary weight remained unchanged in female young from QE treated mothers, but not in LNG treated

mother in Mongolian gerbils. According to Lv and Shi (2011), inconsistent findings in female reproductive organs might be caused by interspecies differences of oestrogen and progesterone sensitivity in the reproductive organs of different species of rodents.

Pregnancy and litter size in *M. natalensis* were affected by the compounds. The most effective compound in reducing pregnancies of *M. natalensis* was QE, compared to LNG and QE/LNG combination. However, there were no significant differences between untreated females paired with treated males and treated females paired with treated males. In both males and females treated with the different concentrations of each compound no pregnancies were observed. This indicates that the fertility control is more effective if both sexes are treated. This is the kind of scenario expected in the course of application of the contraceptives in the field.

Wang *et al*. (2011) showed reduced pregnancy rate and litter size in female Brandt's vole paired with treated males at 0.001%, 0.003% and 0.006% QE and the anti- fertility effects of 0.006% were maintained for four weeks. Lv*et al*. (2012) found reduced litter size in females from QE treated mothers in contrast to LNG treated mothers. Furthermore, Huo*et al*. (2006) reported changes in uterine structure in more than 50% of female Mongolian gerbils treated with 1 mg/ Kg body weight of QE/LNG combination, and that the uteri were severely disrupted by higher dosages of QE/LNG combination. Liang *et al*. (2006) confirmed the effectiveness of QE/LNG combination in reducing fertility in male and female Mongolian gerbils. In a field study, Wan *et al*. (2006) found reduced pregnancy and litter size by 60% of Djungarian hamster (*Phodopuscampbelli*) treated with 0.01 % QE/ LNG.

This study is the first to report on the effects of anti-fertility compounds on Multimammate rats in East Africa. The anti-fertility effects of synthetic steroid hormones (quinestrol and levonorgestrel) in rats have been observed in other studies (Lv and Shi, 2011; Liu *et al*., 2013). Quinestrol and levonorgestrel have been confirmed to have anti-fertility effects that can delay rodent breeding in the treated area and therefore reduce rodent population before attaining the level that causes more problems (Fu *et al*., 2013). From the results of this study, it can be concluded that QE alone, or in combination with LNG can be included in integrated rodent pest management strategies for multimammate rats, *M. natalensis* in East Africa.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1Conclusions

The results of this study have shown that QE, LNG and QE/LNG have significant anti-fertility effects in *M. natalensis.* They affect consumption rate of bait containing these compounds hence reduce the body weight of both male and female *M. natalensis.* In male *M. natalensis* physiological effects observed were reduced weight of testes in QE and QE/LNG, reduced epidydimis weight in QE/LNG and reduced seminal vesicles weight at all concentrations of the compounds used.

These contraceptives also reduce sperm concentration, motility and increased abnormal sperm morphology. However, QE and QE/LNG have shown the highest anti-fertility effects compared to LNG alone at all concentrations.

Pregnancies and litter size were significantly reduced. Quinestrol was the most effective compound which stopped pregnancies at all concentrations. The effects of contraceptives on pregnancy and litter size reduction were stronger when both male and female fed on treated bait.

6.2 Recommendations

The following are the main recommendation from the current study:

i) Further studies of QE and LNG hormones are recommended to establish the duration of anti-fertility effects in *M. natalensis.*

ii) Evaluation of QE and LNG under field conditions should be done to assess the reproduction performances and population dynamics.

iii) Investigation of the effects of the contraceptives in off-springs of *M. natalensis* treated mothers is recommended to know their reproductive performance at maturity without treating them with the contraceptives.

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APPENDIX

1. Preparations of Giemsa stain stock solution

To make 250ml of stock solution

Giemsa powder 1.8 g was weighed on clean weighing boat (preweighed), and transferred to a dry brown bottle of 250 ml capacity which contained few glass beads.

Using a clean dry measuring cylinder 125 ml methanol was measured and added to the stain and mixed.

Using the same measuring cylinder 125 ml glycerol was measured and added to the stain and mixed

The bottle of stain was placed in a water bath at 50-60^{0}C for up to 2 hours to enable the stain to dissolve.

The bottle was labelled and stored at room temperature in the dark.

Working solution of Giemsa stain 10% was prepared using 10 ml of filtered Giemsa and 90 ml of distilled water (1:10) dilution

2. Normal saline 0.85%

Sodium chloride 4.25 g was weighed and transferred to a clean bottle to hold 500 ml

Distilled water was added to the 500 ml mark and mixed until the salt was fully dissolved.

The bottle was labelled and stored at room temperature.