TITLE: EFFECT OF HORMONAL BAITS (LEVONORGESTREL AND QUINESTROL) ON FERTILITY OF COMMENSAL RAT (*RATTUS RATTUS* Linnaeus, 1758)

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN WILDLIFE MANAGEMENT AND CONSERVATION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

This laboratory-based study examined the effects of quinestrol and levonorgestrel (singly and in combination) incorporated in bait on body mass and reproduction of the roof rat (*Rattus rattus*). A total of 140 *R. rattus,* (70 males and 70 females) were provided with 10 g of bait containing quinestrol (QU) or levonorgestrel (LE) or a combination of levonorgestrel and quinestrol (EP-1) at concentrations of (10 ppm and 50 ppm) for seven consecutive days consecutively. After 7 days, animals were dissected and the ovary, uterus, testis, seminal vesicles, and epididymis were weighed and examined. Bait consumption and body weight decreased significantly ($p = 0.0001$) in treated compared to control animals, with a minor difference between sexes. Quinestrol and EP-1 at 10 ppm and 50 ppm increased the mass of the uterus and ovary of females ($p = 0.0001$), which was associated with edema in the uterus. The mass of epididymis, testis, seminal vesicles, were reduced and sperm counts and motility were significantly reduced ($p = 0.0001$) particularly in the animals with QU and EP-1 at the higher concentrations. To determine the effects of contraceptive hormones on reproduction, 50 ppm of QU and EP-1 were used. A total of 160 animals of equal numbers and sexes were paired, keeping the ratio of one female to one male. Pregnancy and litter production was significantly reduced ($p = 0.0249$) in the treated pairs when compared to controls. It is concluded that quinestrol and EP-1 have a significant impact on both males and females *R. rattus* reproduction compared to levonorgestrel alone. These hormones will be very valuable when used as a fundamental method in controlling *R. rattu* reproduction.

DECLARATION

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

_____ _____

Mwajabu Selemani Date

(MSc. Candidate)

The above declaration is confirmed by

_____ _____

Prof. Rhodes Makundi Date

(Supervisor)

_____ _____

Prof. Apia Massawe Date

(Supervisor)

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Above all, peace and love of God be with you always.

DEDICATION

To my parents Mr. and Mrs. Bondogela from Shinyanga who laid the foundation of my life.

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LIST OF ABBREVIATIONS AND SYMBOLS

Fig	Figure
QU	Quinestrol
LE	Levonorgestrel
EP-1	Combination of Quinestrol and Levonorgestrel
SUA	Sokoine University of Agriculture
g	Gram
Ppm	Parts per million
>	Greater than or equal
<	Less than or equal
Kg	Kilogram
^{0}C	Degree Centigrade
ml	Milliliter
TM	Treated Male
UTF	Untreated Female
TF	Treated Female
UTM	Untreated Male
SD	Standard Deviation

CHAPTER ONE

1.0 INTRODUCTION

Rattus rattus is a rodent species of worldwide distribution (Meshkekar *et al.*, 2014). Some studies have indicated that the species originated from Asia and was distributed to other countries around the world at different periods, particularly during the barter trade (Aplin *et al.*, 2011; Bastos *et al.*, 2011; Rackham, 1979). *R. rattus* is an aggressive rodent, generalist feeders, and adaptive to different weather conditions and locations. This made it a successful invasive commensal rodent species (Pryde *et al.*, 2005). These rats are most abundant in urban areas and agricultural fields (Kuiti, 1995).

The home range of *R. rattus* depends much on the habitat they occupy whether urban, agricultural land or forest (Desley *et al.*, 2019). Generally, their home range varies from 0.2 ha - 0.8 ha (Desley *et al.*, 2019; Zealand and Society, 2019). During the breeding season, males have a longer home range than females (Pryde *et al.*, 2005). *R. rattus* are social animals, they reproduce 2 to 6 times in a year with a maximum of 10 pups per pregnancy (Tamarin and Malecha, 1972; Brooks *et al*., 1994). The population size varies among places and their high density in different places is facilitated by food availability, climatic condition, and high reproduction potential (Makundi and Mwanjabe, 1999).

A high density of *R. rattus* has contributed much to social-economic loss to humans because they are responsible for damaging stored and field crops (Kuiti, 1995), gnaw communication cables, timber, and other home materials (Harrington, 2004). Also, they are a very important vertebrate pest that facilitates the transmission of various zoonotic infectious diseases which are a threat to humans, domestic and wild animal health (Meerburg *et al*., 2009). For example, *R. rattus* has been identified as one among other

species to be the main reservoir host of *Yersinia pestis* causing bubonic plague in Lushoto, Tanzania (Kilonzo, 1982), and Madagascar (Rahelinirina *et al.*, 2010). Also, *R. rattus* involved in the transmission of toxoplasmosis (Mosallanejad *et al.*, 2012), trichinosis (Onyenwe, 2009), and typhus (Kumar *et al.*, 2004) among others. Just like other pest rodents, the management of this species relies on the use of mortality-based control such as the use of rodenticides and kill traps (Pitt *et al.*, 2011). Mortality control has only some short-term effects on a population as rodents reproduce fast enough to cover up for the lost population (Singleton *et al.*, 2007). The method is costly, and conservation unfriendly (Kilonzo, 2006). Biological control using for example domestic cat and its derivatives like cat urine has also been used in controlling *R. rattus* population especially in urban and village areas (Mulungu *et al*., 2017), but their effectiveness are low because *R. rattus* are fast runners and are very active (Newsome, 1990).

Due to this, the management of *R. rattus* and other rodent species has shifted from the traditional use of mortality-based control to ecologically-based, biological, and fertility-based control methods (Makundi and Massawe, 2011). Among the methods, fertility control involves the use of synthetic contraceptive hormones such as Levonorgestrel and quinestrol (Ming *et al.*, 2012). These hormones have long been used in preventing unplanned pregnancy in humans by interfering with the ovulation and the fertilization process (Gemzell-Danielsson and Marions, 2004). These hormones have also been proven to be effective in controlling some Equidae reproductive potential (Kirkpatrick and John 2019) and rodent's reproduction potential (Zhang, 2000). For example, quinestrol and levonorgestrel reduced the litter size and pregnancy in *Mastomys natalensis* (Massawe *et al.*, 2018). Quinestrol reduced the number of sperms and mass of testes of *Lasiopodomys brandtii* (Zhao *et al.*, 2007) and lowered the mass of epididymis and ovaries of *Rattus nitidus* (Liu *et al.*, 2013).

Other than the study by Massawe *et al.* (2018) on the effectiveness of fertility control on *M. natalensis* in Africa, there is no work done on other rodent species in Africa. The roof rat commonly inhabits houses in Tanzania as well as East Africa and causes losses of postharvest storage including food commodities in houses (Mdangi *et al*., 2013). As an alternative to the use of rodenticides and snap traps, this laboratory-based study intends to evaluate in the laboratory the ability of levonorgestrel and quinestrol in controlling the fertility of *R. Rattus.* This study will provide evidence on the potential use of hormones to control rodent populations in domestic areas as a way of reducing economic losses associated with rodent destruction on stored crops and other properties

1.2 Objectives

1.2.1 Overall objective

The overall objective of this study is to investigate the effects of contraceptive hormones on the fertility of *R. rattus.*

1.2.2 Specific objectives

i. To evaluate the effect of different dosages of levonorgestrel, quinestrol and their combination on bait acceptance and the bodyweight of *R. rattus*
ii. To evaluate the effect of levonorgestrel, and quinestrol on the reproductive physiology of males and females *R. rattus*
iii. To evaluate the effect of levonorgestrel, and quinestrol on the pregnancy and litter size of *R. rattus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of *R. rattus*

Rattus rattus have different common names; they have been addressed as black rats, ship rats, domestic rats, roof rats, or house rats. Their color ranges from blackish to brownish with white underneath from the neck to the tail (Shiels *et al*., 2014). Just like other rodents' species, *R. rattus* have a good sense of smell which helps them in finding food and escaping predation, in addition to a well-developed sense of touch that helps them in locomotion during the night (Shiels *et al*., 2014). Different studies including Shiels *et al.* (2013) and Abe (2007) have shown that *R. rattus* are omnivores and feed on varieties of food stuff including plants, fruits, other animals, and insects.

The same as other rodent species, *R. rattus* have high reproduction potential. They have a gestation period that lasts for 20 to 22 days and they take almost 20 to 29 days of weaning (Innes, 2005a). *Rattus rattus* can have a litter size of 3 to 8 pups at each pregnancy and in a year, they can have a maximum of four to six litters (Tobin *et al*., 1994). Other studies indicated the species to have up to 10 litter sizes per pregnancy (Tamarin & Malecha, 1972; Brooks *et al*., 1994). *Rattus rattus* have multiple partners and female mates with males which are available (Miller *et al*., 2010).

2.2 Ecology of *R. rattus*

Rattus rattus are invasive commensal rodents in many places. They tend to inhabit an area that was previously utilized or not well utilized previously, and they can occupy a large range of habitats (Michelle, 2000). *Rattus rattus* can even out-compete other species or native species away and inhabit an area. For example in Tanzania, *M. natalensis* used to

live in domestic places but way aggressively replaced by *R. rattus* (Kilonzo, 2006). Infield habitats, *R. rattus* prefer an area with profound cover and areas having dense leaf litter for cover (Michelle, 2000).

2.3 Distribution of *R. rattus*

Rattus rattus belong to the subfamily Murinae and they are distributed worldwide in almost all continents including Africa (Meshkekar *et al.*, 2014). Unlike other rodent species that occupy the same distribution area e.g. *M. natalensis*, and *R. rattus* are adopted to different kind of habitats. For example, they are distributed in forest habitats and in buildings in New Zealand (Pryde *et al*., 2005). They are distributed in coastal habitats as free animals and inhuman premises in the Western Mediterranean (Gilles *et al.*, 2008). In many places in Africa including Tanzania, *R. rattus* are mostly found in domestic premises (Kilonzo, 2006).

2.4 Economic Importance of *R. rattus*

2.4.1 Agricultural importance

Being a commensal rat, *R. rattus* are non-selective adaptive feeders. They feed on a variety of foods depending on the environment they occupy. Due to their feeding modes often being herbivorous, omnivorous, and predatory, *R. rattus* are a serious rodent pest causing high loss of crops both in storages and in fields (Makundi *et al.*, 1999). *R. rattus* also destroy livestock feed (Parshad, 1999).

2.4 2 Public health importance

R. rattus are vectors, reservoir, and intermediate hosts of various zoonotic disease-causing agents like viruses, helminths, and bacteria (Loiseau *et al.*, 2008). They are known to harbor fleas that carry *Yersinia pestis* which are the bacteria responsible for causing

plague (Kilonzo, 1982). In India, *R. rattus* was found to be infected with *Hymenolepis nana* and *Capillaria annulosa* which are helminths that infect children (Meshkekar *et al.*, 2014). In public health, *R. rattus* are involved in the transmission of serious diseases in societies that are less studied especially in developing countries. Those diseases include toxoplasmosis (Mosallanejad *et al.*, 2012), trichinosis (Onyenwe, 2009), and typhus *(*Kumar *et al.*, 2004).

2.4.3 Ecological importance

The massive invasion of *R. rattus* causes disturbance in the ecosystem, for example *R. rattus* caused a decline of land snail in the West Pacific due to predation (Chiba, 2010). They also had some impact on small local vertebrates and plants in Australia (Banks and Hughes, 2012). King (1985) has related bird extinction with the invasion of rodents. *R. rattus* often compete for resources with the native species that use the same resources (Banks and Smith, 2015). Apart from that *R. rattus* plays an important role in the ecosystem as they act as predators, and facilitates spore dispersal in Australian forests (Vernes, and McGrath, 2009).

2.5 Management of Rodents

Managing rodents in agricultural fields and human premises varies among people and places, but generally, chemical control methods such as the use of rodenticides and mechanical control like the use of kill traps are the most used methods (Aldakhil, 2009). Rodenticides have different kill mechanisms. For example, some of them prevent blood clotting causing an animal to bleed internally then die (Buckle *et al*., 1982). Other chemicals such as brodifacoum reduce the serum glucose level of rodents (Buckle *et al*., 1982). The uses of rodenticides and kill traps have shown

some positive results in controlling rodents but their limitations are that they kill non-targeted species. For animals with a good sense of smell and adaptive feeders like rodents, they may avoid eating bait containing rodenticide (Hansen *et al*., 2015). During the rains, the use of rodenticides may increase health risks as rodenticides may be carried away by water and contaminate the environment (Singleton *et al.*, 2007).

Due to the side effects of rodenticides, other methods that have relatively minimal effects on the environment such as fertility-control methods have been tested to control rodent populations. For example, the use of contraceptive chemicals such as quinestrol and levonorgestrel, surgical/chemical sterilization, and endocrine perturbation seems to cause temporary and/or permanent fertility control of some rodent species (Zhang, 2000). Apart from that, injection of steroid contraceptives has been observed to increase the high level of fat in blood and inhibit ovulation in rodents particularly *R. rattus* (Kumar *et al.,* 2019).

2.6 Effects of Contraceptive Hormones on Rodent's Reproduction

The success of different studies that have assessed the effects of fertility control by using synthetic contraceptive hormones is quite remarkable (Zhang, 2000). For example, a combination of Levonorgestrel, and quinestrol had reduced the fertility in both male and females of *M. natalensis* (Massawe *et al.*, 2018), *Lasiopodomys brandtii* (Zhao *et al.*, 2007), males of greater long-tailed hamsters (Zhang *et al.*, 2006), *Djungarian hamster* (Wan *et al.,* 2006) and *Mongolian gerbils* (Huo *et al*., 2006). Quinestrol alone had reduced the fertility of *R. nitidus* (Liu *et al.*, 2013). A contraceptive synthetic hormone reduced the reproductive success of rodents by causing uterus edema in female rodents and reduced litter size of rodents (Wan *et al.*, 2006; Massawe *et al*., 2018). Similarly, the hormone decreased the sperm count and weight of testis and epididymis (Zhao *et al.*, 2007; Massawe *et al.*, 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

Rodents were trapped in different housing premises in Morogoro Municipal (6°50’42.66”S, 37°39’29.14”E). The places include Chamwino, Mbuyuni, Mafiga, Manzese Sabasaba and Mlimani. The experiment was conducted in the laboratory at the Pest Management Centre (SPMC), the Sokoine University of Agriculture in Morogoro Municipal., Tanzania.

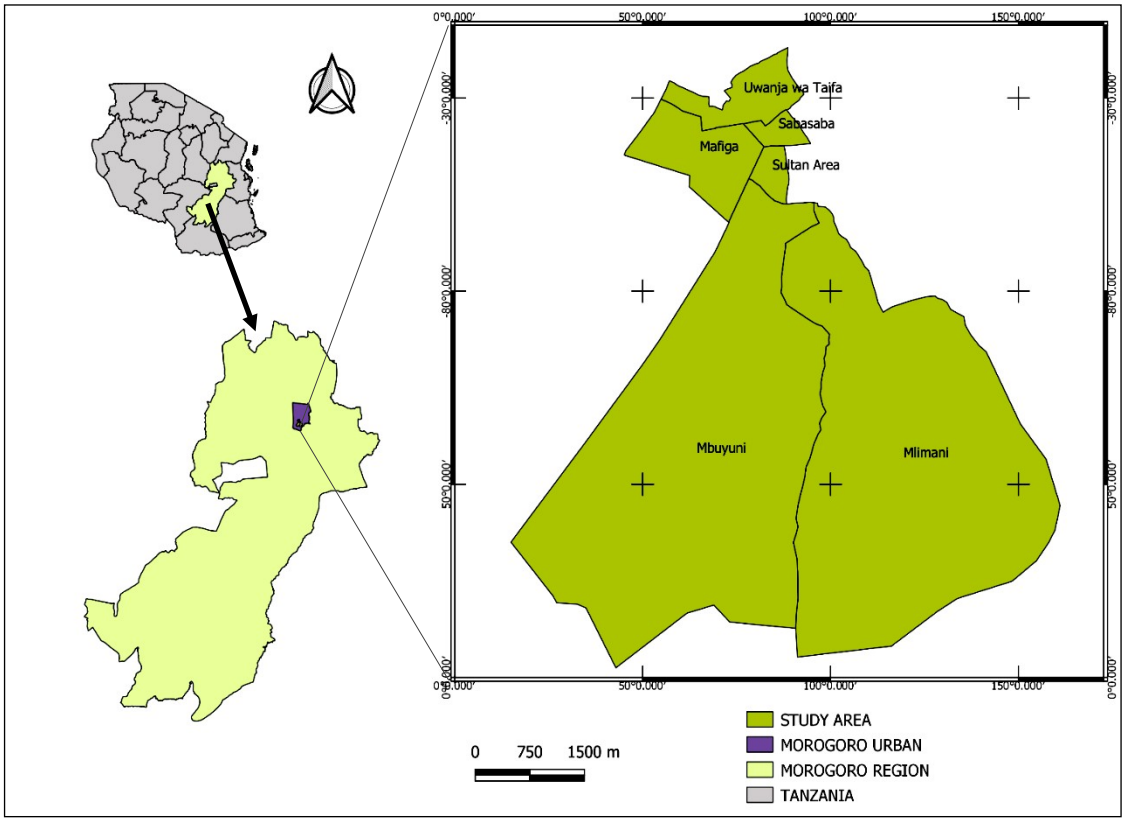


Figure 1: Map showing different wards in Morogoro Urban where rodents were collected

3.2 Sample Size

A total of 300 rodents of relatively equal proportion between females and males were used for the experiment. The selection of the sample size was based on the optimum sample from previous studies in different rodent species e.g. 316 *M. natalensis* in Tanzania (Massawe *et al.*, 2018; 173 *Lasiopodomys brandtii* in China (Zhao *et al.*, 2007). The selected sample size was large enough to give precise information regarding the parameters that were tested in the entire experiment.

3.3 Trapping and Handling of Rodents

3.3.1 Trapping of *R. rattus*

Rattus rattus were captured by using modified box traps baited with a mixture of peanut butter and maize flour, tomatoes, and sardines to increase trapping. Captured individuals were transported to the Pest Management Centre Laboratory for the experiments.

3.3.2 Handling of captured individuals

Adult animals with the weight of >50 g and < 180 g were used for the experiments. The weight range was inclusive of sexually mature animals (Aaron *et al*., 2014). After weighing, animals were separated into cages, based on sex, and allowed to acclimatize for 10 days before being transferred to individual cages for additional 20 days for them to get used to the laboratory conditions (Zhang *et al.,* 2006). The total acclimatization period was 30 days. This period was enough to ensure that all animals were healthy and that no females were pregnant. Daily checks of the animals were conducted and plain bait and water were provided at ad-libitum during the entire acclimatization period. During that period, an animal that appeared unhealthy (e g. with thin, poor coat condition, injury, or not feeding) was removed from the cages (Massawe *et al.,* 2018; Zhao *et al.,* 2007). At the end of the acclimatization period, animals were weighed ready for the experiment.

3.4 Bait Preparation

3.4.1 Plain bait preparation

The contraceptive bait preparation followed the protocols from Massawe *et al*. (2018). Briefly, the bait food was prepared by mixing maize flour, ground sardines pulp, and crushed maize. Ten kilograms (10 kg) of maize flour was mixed with 250 g of ground sardines to form a maize-sardines mixture. The maize-sardine mixture was further mixed with 10l of boiling water and cooked for 15min to obtain a stiff paste then left to cool at room temperature. One-third (3.3 kg) of the cooked stiff paste was mixed with two-thirds (6.7 kg) of crushed maize and the mixture was then passed through a mechanical pelletizer to provide 10kg of standard bait (see Plate 1).

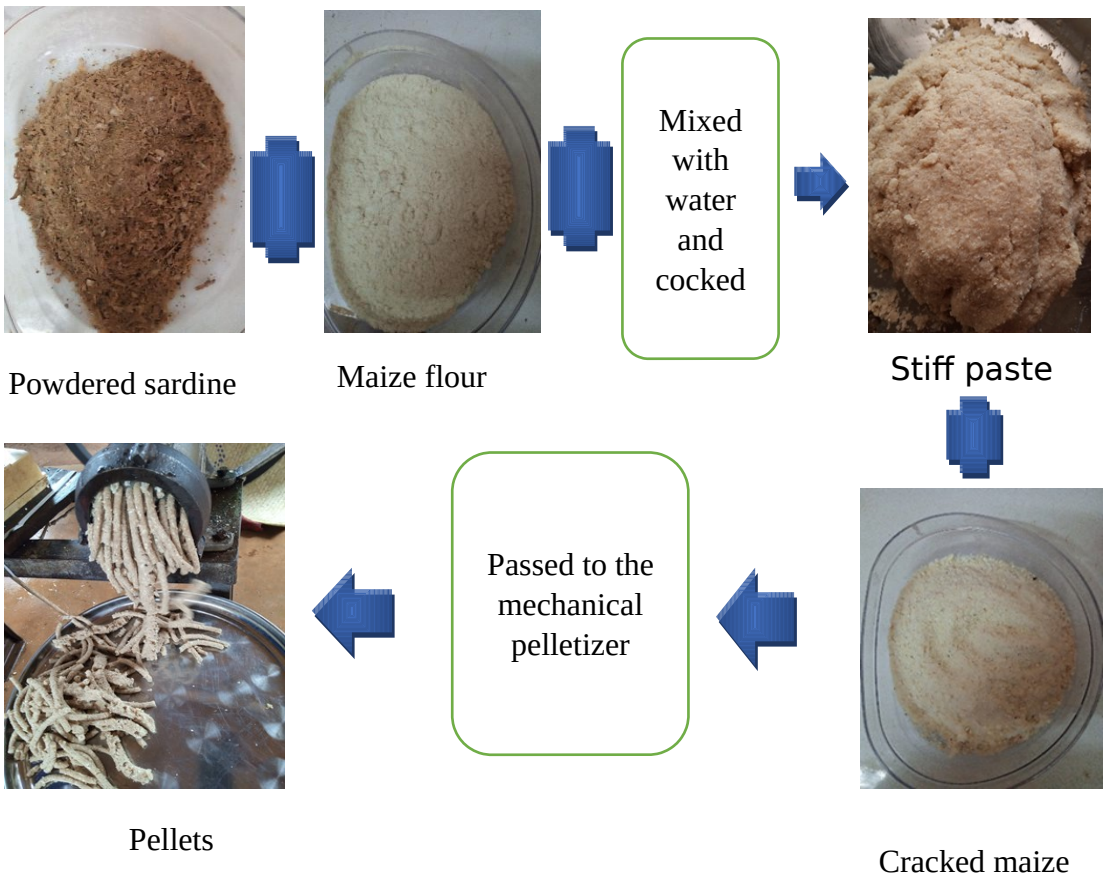


Plate 1: Pellets preparation

3.4.2 Preparation of 10 ppm of contraceptive bait

Powdered quinestrol and levonorgestrel (supplied by Beijing Zizhutiangong Science and Technology Ltd, China) were weighed at 0.1 g. This was dissolved in 100 ml of ethanol in 60-70ºC heated water in a suitable container. The ethanol-contraceptive solution was then mixed with a sugar solution containing 200 g sucrose in 1000 ml of water and shook vigorously. This solution was mixed with a mixture of 6.7 kg of cracked maize and 3.3 kg of a cooked mixture of maize flour and ground sardines (Massawe *et al.,* 2018). The mixture was then passed through a mechanical pelletizer to provide 10 kg of standard hormone bait. For the mixture of quinestrol and levonorgestrel (EP-1), the ratio was 1:3 (quinestrol: levonorgestrel).

3.4.3 Preparation of 50 ppm of contraceptive bait

A total of 0.5 g of powdered quinestrol and levonorgestrel were weighed and preceded as described by Massawe *et al.* (2018) in 3.4.2 above. The mixture was then passed through a mechanical pelletizer to provide 10 kg of standard bait. Similarly, for the mixture of quinestrol and levonorgestrel (EP-1), the ratio was 1:3 (quinestrol: levonorgestrel) were used.

3.5 Experimental Design

The layout of the experiment was factorial design with two factors (level i.e concentration and treatment i.e hormones). Two experiments were conducted in the laboratory. The first experiment assessed the effect of 10ppm and 50 ppm of quinestrol (QU), levonorgestrel (LE), and in combination (EP-1) on body weight, food acceptance, reproductive tracts of both male and female. This experiment also determined the optimal dose needed for maximum effects on *R. rattus*. The second experiment assessed the effect of optimal dose identified in experiment one on the pregnancy of *R. rattus.*

3.6 Experimental One

3.6.1 Bait uptake, weight, and assessment of short term immediate reproductive physiological effects

This experiment assessed the response of *R. rattus* to two concentrations of contraceptive baits (i.e 10 ppm and 50 ppm) using two successive trials. The first trial assessed 10 ppm of Quinestrol (QU), Levonorgestrel (LE), and Combination (EP-1). The animal's response to this dose determined whether it was effective or whether a higher or lower dose had to be used. The second trial assessed the effect of 50 ppm of Quinestrol (QU), Levonorgestrel (LE), and Combination (EP-1). During all these trials, body weight, bait acceptance, and reproductive tracts of animals were assessed and compared with untreated animals which were fed plain food. In each trial, animals were provided daily with 10 g of food containing a respective dose of contraceptive hormone(s). A total number of 140 animals consisting of 70 males and 70 females were used (Tables 1, 2).

Table 1: Treatment group, number of animals, and time for the first trial

Treatment Group (7days bait consumption)	Females	Males
Control (untreated), 0 ppm; 10 g	10	10
Quinestrol (QU), 10 ppm; 10 g	10	10
Levonorgestrel (LE) 10 ppm; 10 g	10	10
(EP-1), 10 ppm; 10 g	10	10
Total animals	**40**	**40**

Table 2: Treatment group, number of animals, and time for the second trial

Treatment Group (7days bait consumption)	Females	Males
Control (untreated), 0 ppm; 10 g	10	10
Quinestrol (QU), 50 ppm; 10 g	10	10
Levonorgestrel (LE), 50 ppm; 10 g	10	10
(EP-1), 50 ppm; 10 g	10	10
Total animals	**40**	**40**

3.7 Experimental Two

3.7.1 Assessment of pregnancy and liter size

Quinestrol and EP-1 at 50 ppm which was determined as the optimal dose in Experiment one were used in this experiment. A total of 160 *R. rattus* (i.e 80 males and 80 females) were fed with the selected contraceptive bait and control bait separately for seven days. After bait delivery, animals were paired at the ratio of one female: one male as observed in Fig 2. There were ten replicates for each treatment concentration. The selected paired females and males were inactive sexual condition (i.e perforated vagina and scrotal visibility for females and males respectively). After paring, animals were returned to individual cages and left for 30 days for observation of pregnancy and offspring. Pregnancy and litter size were compared between treated with the control animals.

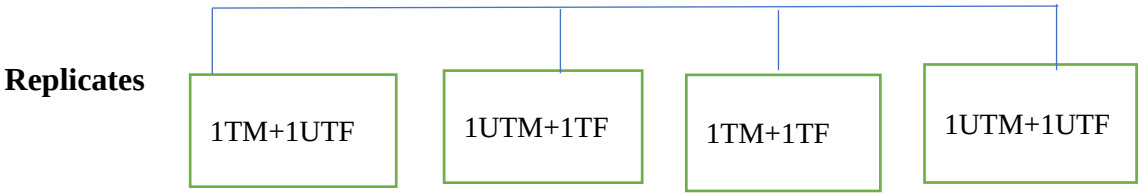


Figure 2: Pairing of animals treated with 50 ppm of quinestrol bait (For EP-1, same replicate and the same number of animals was used).

TM - Treated male

UTM - Untreated male

TF - Treated female

UTF-Untreated female

3.8 Data Collection

3.8.1 Bait acceptance and weight of animals

Bait acceptance was evaluated for each animal in an individual cage which was provided with 10 g of bait daily. The amount of bait consumed was obtained by subtracting the remaining unconsumed bait of the 10 g of provided bait. Animals were weighed before being supplied with the bait and after being given bait for seven consecutive days.

3.8.2 Reproduction physiology of male and females

After feeding with bait for seven consecutive days, animals were anesthetized using halothane then dissected for identification of any abnormalities in the female and male reproductive organs. Females were killed on day eight and the uterus was weighed and checked for any abnormalities and compared with the control animals. Males were killed on day 14 and the epididymis, seminal vesicles, and testes were weighed and compared with control animals for any weight differences (Zhang *et al*., 2006; Zhao *et al.,* 2007; Massawe *et al.,* 2018).

3.8.3 Sperm motility

The procedure for sperm motility was adopted from the study of Massawe *et al*. (2018). Epididymis of treated and untreated males was dissected and put in a glass petri-dish and 1ml of 0.85% normal saline was added into the petri-dish and then each epididymis cut into small pieces to release sperms. A drop of the solution from the petri-dish was placed on a slide glass and examined under a compound microscope for sperm motility. The sperm motility was assessed and expressed in a percentage of mobile sperms of the total count.

3.8.4 Sperm count

The remaining sample from the petri-dish from the above section was placed in a test tube with 10 ml of distilled water and refrigerated for 2 hours at 4ºC to release sperm. After 2 hours of refrigeration, 9 ml of 0.9% of saline was added to the solution and shaken. A drop of the solution was placed in a hemocytometer and sperms were counted under a compound microscope and sperm counter (WHO, 2010).

3.8.5 Birth and litter size

At the end of bait delivery, males and females' animals were paired until a vaginal plug or vaginal blood (an indication of successful copulating) was observed (see Plate 2), generally, it took 4 to 7days after pairing for copulation to take place. The observation of vaginal plug or vaginal blood was done early in the morning with the assumption that rodent's mate at night. Also, a piece of plain white paper was placed on the bottom of the pairing cage for observation of any vaginal blood droplets or vaginal plug droplets from copulated females. The copulated females were separated from males and returned to individual cages and left for 30 days for observation of birth. The young pups were counted and weighed individually on the day they were found and the pregnancy and litter size of the treated animals were compared with the controls. During this period, animals were provided with plain bait without a contraceptive. Additional food of fresh vegetables, sardine, and shrimps were provided and water *ad libitum* (Massawe *et al.*, 2018). The temperature was monitored and maintained at 29 to 30ºC. The room was kept dark and materials for hideout were provided in each cage.

Plate 2: Vaginal blood

3.9 Data Analysis

The main interest of this study was to investigate the effect of hormonal baits on reproductive physiology and litter size of *R. rattus*. Collected data were checked for normality by using a shapiro-wilk test for normality in R statistical software. The data collected were subjected to analysis of variance (ANOVA) procedure using SAS version 9.5 programs.

The General linear model $y_{ijk} = \mu + R_i + L_j + T_k + (LT)_{jk} + \varepsilon_{ijk}$

Where by y_{ijk}= Dependant variables or response,

μ = general mean,

R_i = Replication effect,

L_j = Treatment effect for j^{th} factor A (concentration level),

T_k = Treatment effect for k^{th} factor B (hormones types),

LT_{jk} = effect due to j^{th} factor A and k^{th} factor B,

$Ɛ_{ijk}$ = effect due to i^{th} replication, j^{th} factor A, and k^{th} factor B (i.e experimental error) were performed. Comparison of means values for significant differences was done by mean separation test using Least Significant Difference ($LSD_{0.05}$).

3.10 Animal Ethical Consideration

The study was conducted in laboratories of the Pest Management Centre, Sokoine University of Agriculture, Morogoro, Tanzania. All trials were approved by the university's ethics committee and followed the guidelines of the American Society of Mammalogists (Sikes and Gannon 2011).

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Quinestrol (QU), Levonorgestrel (LE) and Combination (EP-1) on-Bait Consumption

The overall bait consumption of animals treated with different concentrations of contraceptive hormones was significantly lower than that of untreated (control) animals for all sexes (Table 3, 4; Fig.3, 4). Similarly, the mean bait consumption was higher for LE than QU and EP-1treated animals in all study days except day one and day seven for males (Table 3; Fig. 6)and day six for females (Table 4; Fig. 5). The interaction between concentration and hormones for male had significant effect in all day of observation except day one ($F_{4, 72} = 1.95$, $p = 0.112$) and day seven ($F_{4, 72} = 0.70$, $p = 0.596$) (Table 3) while for females, significant interaction effect was observed in all days except day six ($F_{4, 72} = 1.58$, $p = 0.189$) (Table 4).

Table 3: ANOVA Table showing difference mean sum square of daily bait consumption of male *R.rattus*treated with Levonorgestrel (LE), Quinestrol (QU), and combination (EP-1)

SV	df	Mean sum square						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Replicates	9	11.04	5.78	7.93^{x}	13.9^{xx}	10.49^{xx}	6.04	9.62
Concentration	2	136.88^{xxx}	43.15^{xxx}	92.60^{xxx}	84.55^{xxx}	44.87^{xxx}	91.06^{xxx}	70.6^{xxx}
Treatment/ hormones	2	6.50	56.69^{xxx}	53.59^{xxx}	32.09^{xxx}	38.02^{xxx}	24.23^{xx}	8.33
Concentration + treatment	4	12.76	17.32^{xx}	16.7^{xxx}	16.09^{xx}	16.99^{xx}	5.93	4.07
Error	72	472.24^{xx}	6.55^{xxx}	4.57^{xxx}	3.94^{xxx}	3.04^{xxx}	3.76^{xxx}	5.84^{xxx}

Note =^{x} means $p<0.05$, ^{xx} means $p<0.01$, ^{xxx} means $p<0.001$

Table 4: ANOVA table showing difference mean sum square of daily bait consumption of female *R.rattus*treated with Levonorgestrel (LE), Quinestrol (QU), and combination (EP-1)

	df	Mean Sum Square						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Replicates	9	5.595	10.35^{xx}	6.39^{x}	4.23	12.63^{xx}	18.57^{xx}	17.38^{xxx}
Concentration	2	64.595^{xxx}	56.78^{xxx}	80.15^{xxx}	109.8^{xxx}	89.07^{xxx}	82.25^{xxx}	20.28^{x}
Treatment	2	72.986^{xxx}	58.76^{xxx}	49.24^{xxx}	46.05^{xxx}	37.94^{xxx}	13.88	15.41^{x}
Concentration + treatment	4	33.552^{xxx}	20.91^{xxx}	21.79^{xxx}	12.04^{xxx}	13.11^{x}	4.44	18.25^{xx}
Error	72	302.4	4.20^{xxx}	3.32^{xxx}	4.9^{xxx}	4.14^{xxx}	6.94^{xxx}	4.48^{xxx}

Note =^{x} means $p<0.05$, ^{xx} means $p<0.01$, ^{xxx} means $p<0.001$

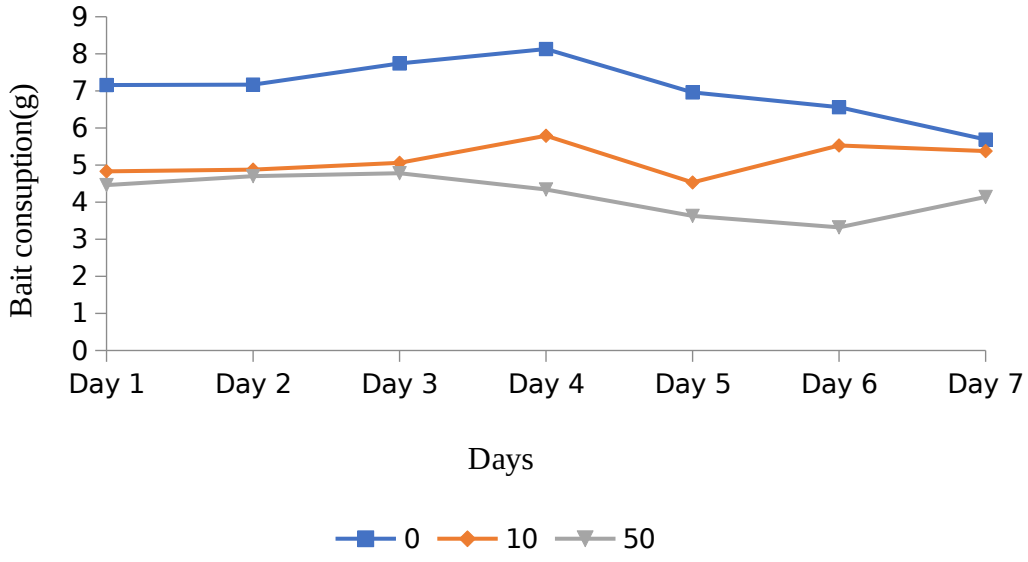


Figure 3: Mean bait consumption (± SD) of female *R. rattus* treated with different concentration of contraceptive bait

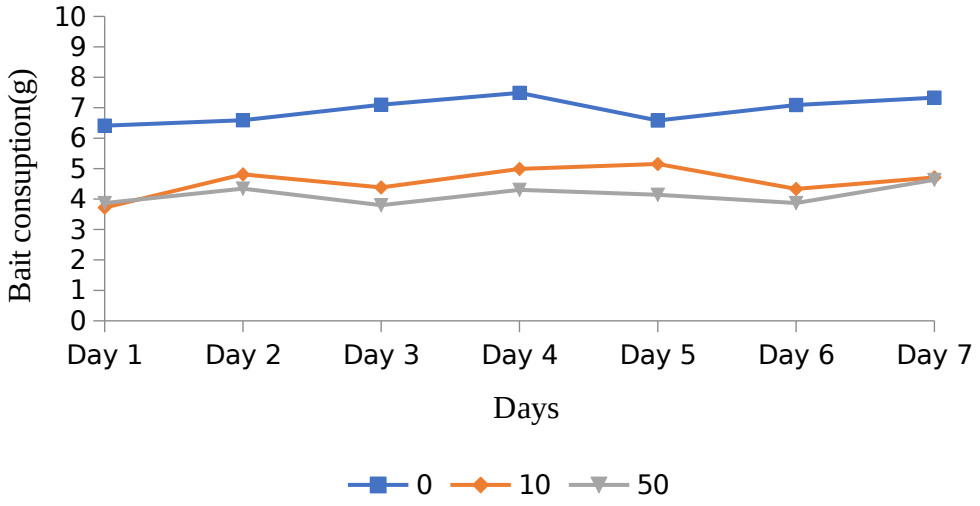


Figure 4: Mean bait consumption ± SD of female *R. rattus* treated with different concentrations of contraceptive bait.

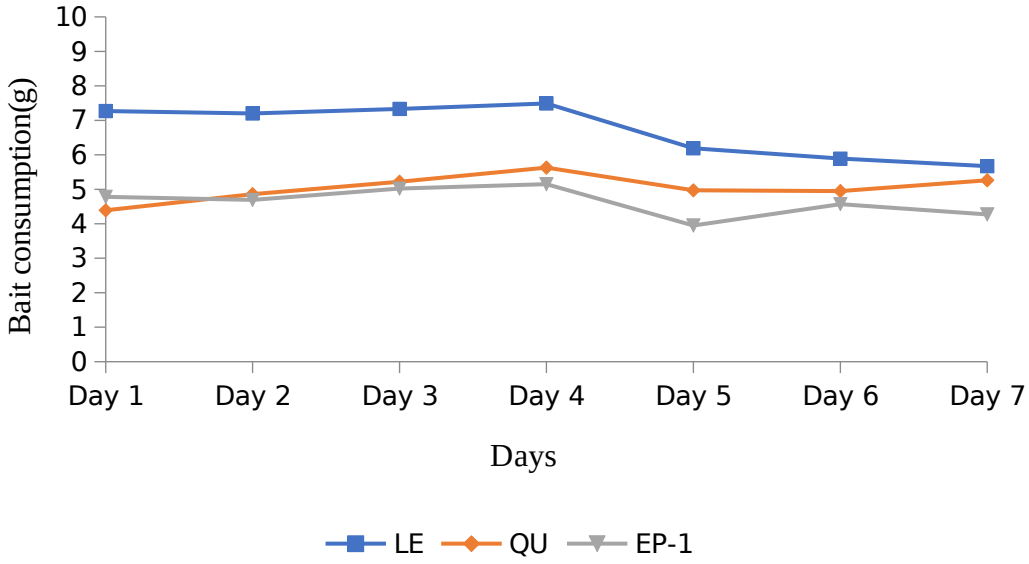


Figure 5: Mean bait consumption (± SD) of female *R. rattus* treated with the difference of contraceptive hormonal bait.

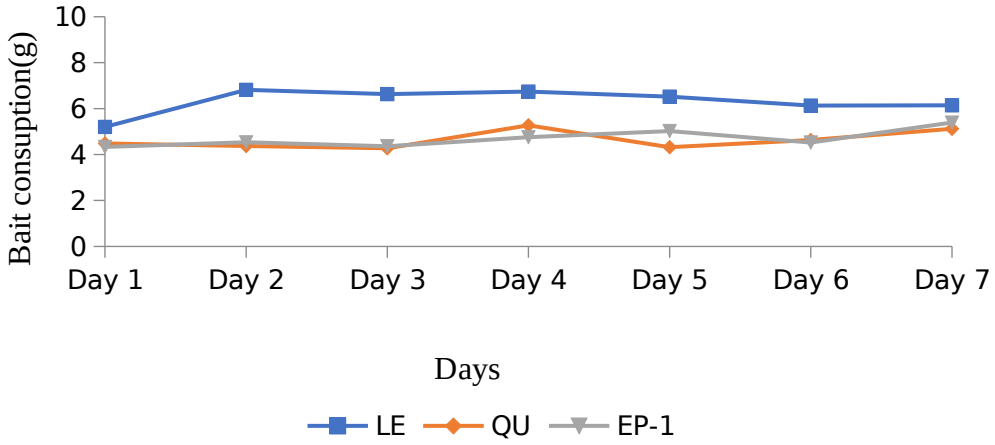


Figure 6: Mean bait consumption (± SD) of male *R. rattus* treated with different contraceptive hormonal bait

4.2 Effects of Contraceptive Bait on Animals' Weight

The mean weight of animals consuming hormone-treated bait decreased significantly ($F_{2,72}$ = 5.29, p = 0.0072) but increased in the plain bait fed animals (Fig. 7). The differences between concentrations in weight reduction were not significance differ ($F_{2,72}$ = 2.91, p = 0.0612). There were no interaction between hormones and concentrations in weight reduction ($F_{4,72}$ = 0.81,p = 0.5245).

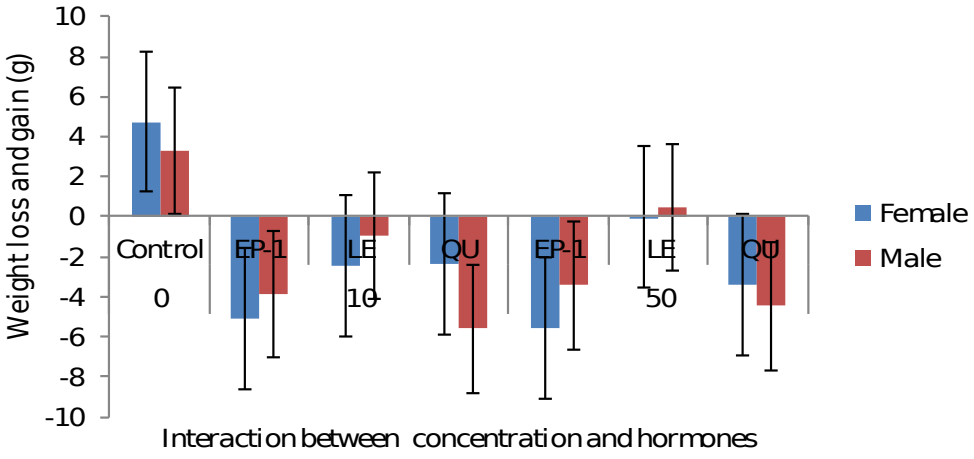


Figure 7: Mean weight loss and gain (± SD) of contraceptive hormones on body weight of *Rattus rattus*

4.3 The Effects of Contraceptive Hormones on Reproductive Physiology of Animals

4.3.1 Female reproductive organs

4.3.1.1 Uteri and ovary weight

The weights of uteri and ovaries of animals treated with 10ppm and 50ppm of contraceptive hormones were significantly higher ($F_{2, 72}$ = 102.84,p = 0.0001) than in the control animals. High weight gain was observed at concentration of 50 ppm (mean = 0.52 g) followed by 10 ppm (mean = 0.26 g).Similarly, highly significant effect ($F_{2, 72}$ = 15.38, p = 0.0001) of different hormones were observed in QU (mean = 0.38 g) and EP-1(mean = 0.36 g) followed by control (mean = 0.21 g). The interaction between hormones and concentrations were significantly observed ($F_{4, 72}$= 4.71, p = 0.002) (Fig.8). There were observable uterine edemas in treated animals with higher edema at 50 ppm compared to 10 ppm (Plate 3).

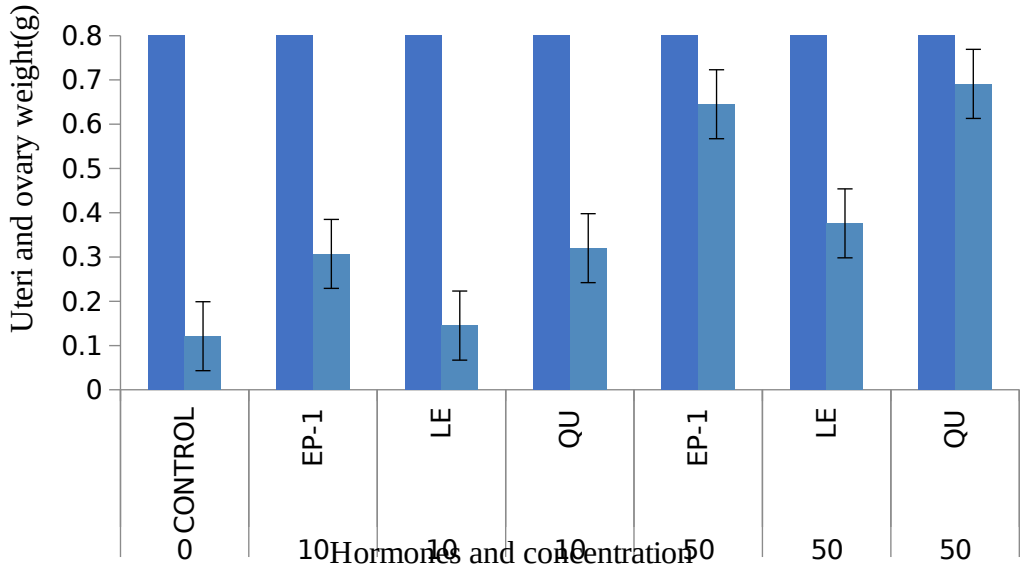
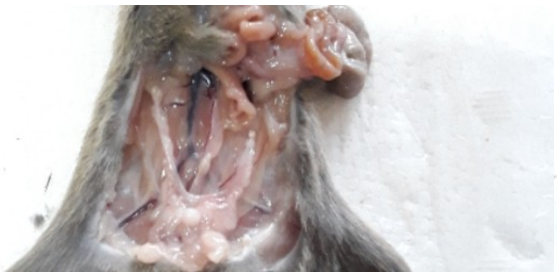
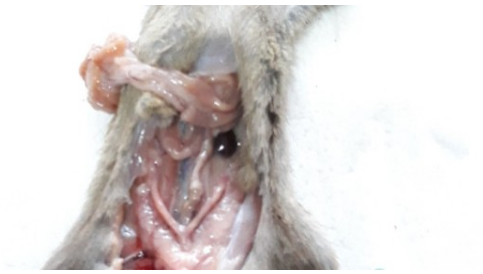


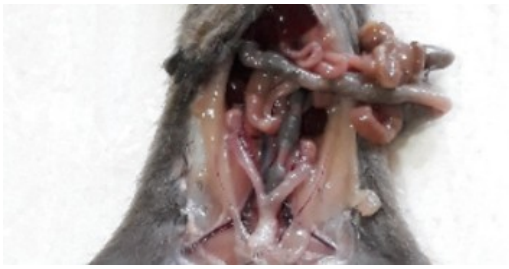
Figure 8: Mean weight (± SD) of ovary and uterus of treated and untreated female *Rattus rattus*



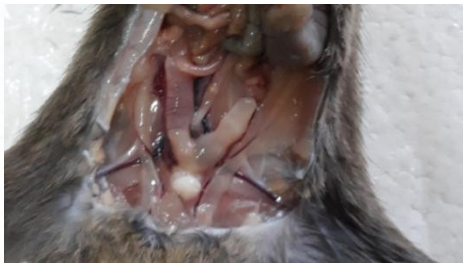
A = Control/ 0 ppm



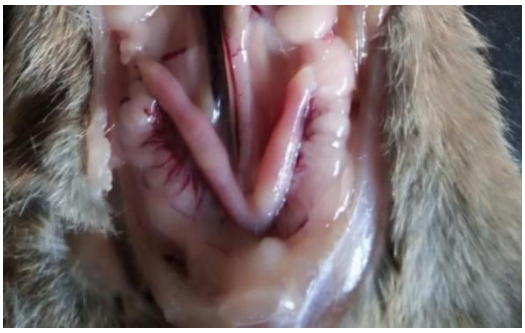
B = 10 ppm of Levonorgestrel (LE)



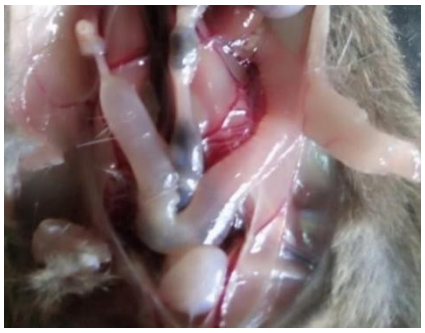
C = 10 ppm of combination (EP-1)



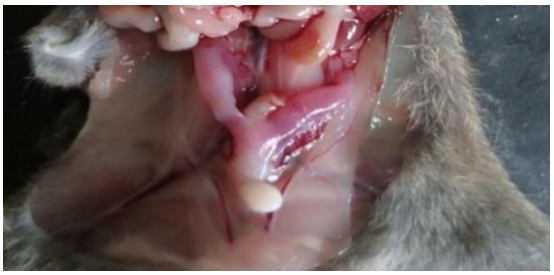
D = 10 ppm of Quinestrol (QU)



E = 50 ppm of combination (EP-1)



F = 50 ppm of Quinestrol (QU)



G = 50 ppm of Levonorgestrel (LE)

Plate 3: Effect of hormonal treatment on the uterus of *R. rattus* at 10 ppm and 50 ppm

4.3.2 Male reproductive organs

4.3.2.1 Mass of testis

Highly significant effect ($F_{2, 72}$ = 9.28, p = 0.0003) of concentrations was observed on male *R.rattus* testis weight, where high weight loss was observed at concentration of 50 ppm (mean = 0.65 g) followed by 10 ppm (mean = 0.87 g) and lastly a control (mean = 0.93 g). Likewise, highly significant effect ($F_{2, 72}$ = 4.01, p = 0.0223) of different hormones was observed, whereby QU (mean = 0.74 g) and EP-1 (mean = 0.77 g) while LE (mean = 0.92 g). The interaction between hormones and concentrations were not significantly differ ($F_{4, 72 =}$ 1.94, p = 0.1127).

4.3.2.2 Epididymis weight

The mean epididymis weight was significantly lower ($F_{2, 72}$ = 3.26, p = 0.0441) in QU (mean = 0.33 g) and EP-1 (mean = 0.37 g) compared to that of LE (mean = 0.47 g) and control animals. However, there was no significance difference among concentrations ($F_{2, 72}$ = 2.78, p = 0.0688). The interaction between hormones and concentrations were significantly observed ($F_{4, 72}$= 5.00, p = 0.0013) (Fig. 9).

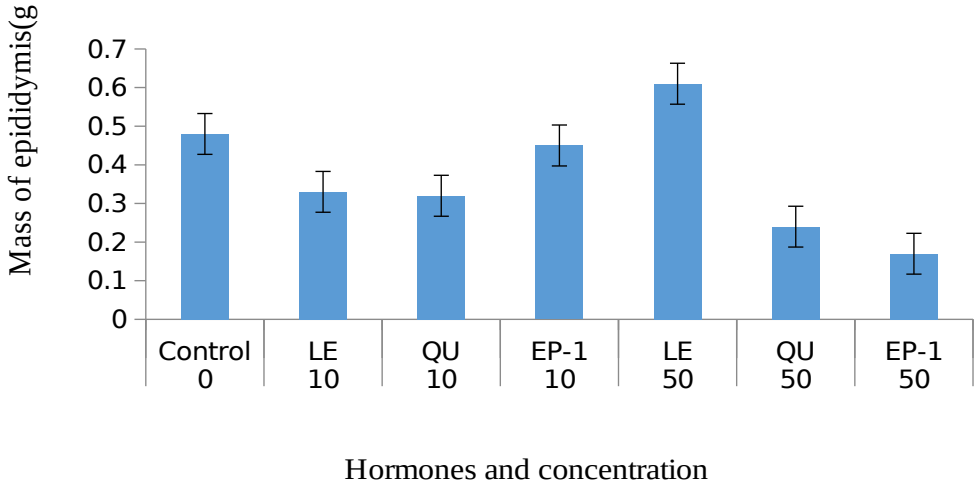


Figure 9: Mean mass of epididymis of treated and untreated male *R. rattus*

4.3.2.3 Seminal vesicles weight

Highly significant effect ($F_{2, 72}$ = 3.87, p = 0.0254) of concentrations was observed on male *R. rattus* seminal vesicles weight, whereby high weight loss of seminal vesicles was observed at concentration of 50ppm (mean = 0.14 g) followed by control (mean = 0.24 g) then 10ppm (mean = 0.22 g). The difference in hormones on seminal vesicles weight were not statistically observed ($F_{2, 72}$ = 0.19, p = 0.8236). The interaction between hormones and concentrations were observed to be significance ($F_{4, 72}$= 3.89, p = 0.0068) (Fig.10).

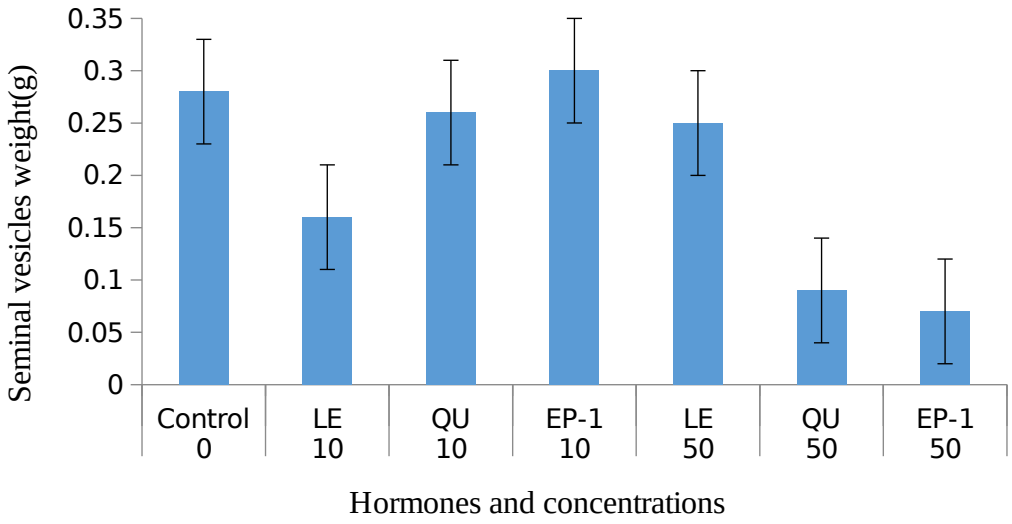


Figure 10: Mean seminal vesicles mass (± SD) of contraceptive hormones treated and untreated male *R. rattus*

4.3.2.4 Sperm motility

Sperm motility of treated animals decreased significantly ($F_{2,72}$= 29.63 p = 0.0001) compared to control animals with lower motility being observed in QU (47%), and EP-1 (52%) and then LE (70%). Similarly, highly significant effect ($F_{2,\ 72}$ = 15.38, p = 0.0001) of hormones concentrations was observed, where 50 ppm (mean = 36%), followed by 10 ppm (mean = 56%) while control (mean = 76%). The interaction between hormones and concentrations were also observed to be significance ($F_{4,72}$ = 8.44, p = 0.0001) (Fig. 11).

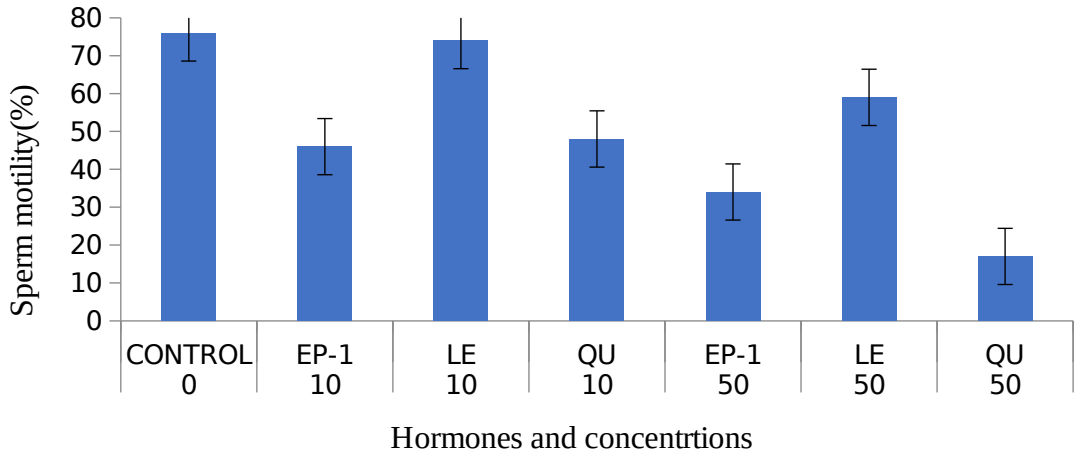


Figure 11: Mean percentage sperm motility (± SD) of control and fertility hormone-treated animals

4.3.2.5 Sperm concentration/counts

Sperm counts of treated animals decreased significantly ($F_{2,72}$ = 9.15, p = 0.0003) compared to control animals. However, lower sperm counts were observed in QU (mean = 28e6) and EP-1 (mean = 33e6) than in LE treated animals (mean = 44e6). The higher concentration (50 ppm) significantly ($F_{2,72}$ = 49.93, p = 0.0001) decreased sperm numbers compared to 10 ppm treated animals and 0 ppm. The interaction between hormones and concentrations were statistically observed ($F_{4,\ 72}$ = 2.79, p = 0.0326).

4.4 Effects of 50 pm of Quinestrol and EP-1 on Pregnancy and Litter Size of *Rattus Rattus*

The contraceptive baits were effective in reducing the number of pregnancies, particularly when the female consumed the bait, regardless of whether the male had also consumed a bait containing contraceptive or the untreated bait. In the untreated pairing, where both males and females did not receive contraceptives, the pregnancy success rate was 70% (n=10). The total number of offspring produced in the untreated pairing was 54 where most pregnant females had 8 pups each (one of the 7 females had only 6 pups).In cases where the pairing involved an untreated female with a treated male, the pregnancy rate was reduced to 30%. Here, the median litter size was 4 pups. Where the female had consumed either QU or EP-1 at 50 ppm, there were zero pregnancies. Results were shown to be significant through an analysis of variance (ANOVA pregnancy $F = 7.14$, $df = 6$, $p < 0.0001$; ANOVA offspring $F = 11.25$, $df = 6$, $p < 0.0001$).

CHAPTER FIVE

5.0 DISCUSSION

The bait consumption was significantly lower in treated animals than in untreated animals. However, levonorgestrel treated animals showed a higher consumption rate at 10 ppm and 50 ppm when compared to animals fed with 10 pm and 50 ppm of quinestrol and a combination of levonorgestrel and quinestrol (EP-1). Likewise, the bait consumption of levonorgestrel treated animals and the control group did not differ. The results also indicated that as the concentration of contraceptive hormones increases from 10 ppm to 50 ppm, the consumption remained the same. The low bait intake for quinestrol and EP-1 was caused by the bitterness of quinestrol. These results are compared to those reported by Massawe *et al.* (2018) which indicated that the same concentrations of contraceptive hormones lowered the bait consumption in treated animals. However, other studies e.g. (Wang *et al.*, 2011) indicated that quinestrol had no effects on the overall bait consumption of male Brandt's vole in China.

In the same contraceptive hormone's treatment, the bodyweight of animals decreased remarkably from day one to day seven of bait delivery. The weight loss was even higher in quinestrol and EP-1 treated animals than in levonorgestrel treated animals. This could be associated with low bait intake of quinestrol and EP-1 treated animals due to unknown physiological effects of these hormones. Similar results on the effects of contraceptive hormones on body weight were observed in *M. natalensi*s (Massawe *et al.*, 2018) and *Meriones unguiculatus* (Lv and Shi, 2011) after animals were fed with bait containing a high concentration of quinestrol and a combination of quinestrol and levonorgestrel. The results contrast with the study of Liu *et al.* (2013) which showed that quinestrol did not affect the bodyweight of *Rattus nitidus*.

Quinestrol and levonorgestrel had remarkable effects on the reproductive biology of both female and male *R. rattus.* The current results show the weight of the uterus of quinestrol and EP-1 treated females increased, and there was observable uterus edema in animals treated with 10 ppm and 50 ppm with greater effects at 50 ppm. Similar findings were reported in female *M. natalensis* (Massawe *et al.*, 2018), Mongolian Gerbils (Lv and Shi, 2011) and Djungarian hamsters (Wan *et al.*, 2006) after the animals been provided with EP-1. The findings from this study are in contrast with the studies by Liu *et al.* (2013) on female *Rattus nitidius* and Zhao *et al*. (2007) on female Brandt's voles which showed that quinestrol did not reduce the weight of the uterus or change the morphology. Edema in the uterus of treated animals has been suggested to be due to increases in estradiol and progesterone level and prolongation of estrogenic activity which results in swelling of the uterus (Su *et al*, 2017). However, the uterus of levonorgestrel treated animals increased only for 50 ppm treated animals but there was no effect at 10 ppm.

In male *R. rattus,* the consumption of quinestrol and EP-1 at concentrations of 10 ppm and 50 ppm reduced the weight of epididymis, testis, seminal vesicles, sperm count and decreased the sperm motility. Between the two concentrations 50 ppm seemed to have more significant impacts in lowering the weight of the male reproductive organ than 10 ppm. The effects of levonorgestrel alone on male reproductive biology at each concentration were not significantly different from that of the control group. Also, there were no differences between hormones in reducing the weight of the seminal vesicle of treated animals. The findings in the current study are similar to those reported by Liu *et al*. (2013) which showed that quinestrol reduced the wet mass of epididymis but not testis of *R. nitidius* after animals were fed with bait for 7 days. In *M. natalensis*, quinestrol reduced the mass of seminal vesicles and testis but not epididymis and levonorgestrel alone did have effects on the mass of seminal vesicles but not on testis and epididymis

(Massawe *et al.,* 2018). In Brandt's voles, levonorgestrel alone, and levonorgestrel+quinestrol did not affect sperm density but quinestrol had lowered the sperm count, mass of testis, and epididymis (Zhao *et al.*, 2007). In Sprague-Dawley rats, quinestrol and EP-1 (levonorgestrel+quinestrol) had decreased the weight of caudal epididymis and the number of spermatozoa (Liu *et al.,* 2013). In Djungarian hamsters, EP-1 (levonorgestrel+quinestrol) did not affect male testis (Wan *et al.,* 2006). The variations in the effects of contraceptive hormones on male reproductive organs as observed in the current and other studies are attributed to differences in species and differences in concentration of contraceptive hormones used. Quinestrol and EP-1 (levonorgestrel+quinestrol) lowered the sperm count and motility and reduced the mass of testis, and epididymis in *R. rattus*. It has been suggested that quinestrol increases estrogen level resulting in a reduction in spermatogenesis and suppression of testosterone production (Liu *et al.,* 2013).

Pregnancy and litter size of *R. rattus* were affected by contraceptive consumption. There were no statistical differences between the effects of the quinestrol and EP-1 on pregnancy or litter size. The litter size and pregnancy were reduced in treated males paired with untreated females when compared with the control pairs. There were no pregnancy or litter differences in treated females paired with untreated males likewise in treated females paired with treated males. These results indicate that contraceptive hormones are more effective when females are treated or when both sexes are treated. The absence of pregnancy in treated females could be attributed to the formation of edema in the uterus making it difficult for implantation. Studies by Huo *et al*. (2006) showed that the uteri of Mongolian gerbils treated with Quinestrol and levonorgestrel combination was disrupted causing an effect in female Mongolian gerbils' reproduction.

The results from (Zhao *et al.,* 2007) showed reduced litter size from untreated females Brandi’s vole mated with treated males but for the treated female's results showed no antifertility effect. In *M.natalensis,* the litter size was reduced when both sexes were treated with contraceptive hormones (Massawe *et al.,* 2018). Wan *et al*. (2006) approved that EP-1 lowered the pregnancy rate and litter size of Djungarian hamsters in field studies.

The results from the current study have indicated that quinestrol alone and EP-1 have antifertility effects on *R. rattus* than levonorgestrel alone. The effects were more obvious for 50 ppm of quinestrol and EP-1 compared to 10 ppm. The effects between quinestrol and EP-1 on the fertility of *R. rattus* did not differ. Animals were observed to consume EP-1 more than QU. Due to this, the current study suggests that EP-1 at 50 ppm may be used as hormones for the management of *R. rattus.*

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The findings from the current study indicated that contraceptive hormones (QU, LE, and EP-1) have significant fertility effects on the reproduction of *R. rattus*. In females, these contraceptive hormones caused uteri edema. In males, the contraceptive hormones reduced the mass of epididymis, the mass of seminal vesicles and testis, reduced the motility and concentration of sperms. Quinestrol (QU) and combination (EP-1) had significantly higher fertility effects on both males and females of *R. rattus* especially at 50 ppm when compared to Levonorgestrel alone.

Animals treated with 50 ppm of Quinestrol and 50 ppm of EP-1 had significantly reduced pregnancy and litter size than control animals with higher litter size reduction being observed in EP-1.

6.2 Recommendation

The study findings show how contraceptive baits affect the reproduction of *R. rattus* in laboratory conditions. The study recommends the decision-makers to consider the application of these contraceptive baits in the management of the rodent population because these baits are reasoned to be more humane, less costly, and conservation-friendly compared with rodenticides and rodent traps. Also, the study recommends other follow-up studies to assess the effects of the contraceptive hormones on the environments (e.g. non-target species, persistence, and breakdown of molecules), also the study to establishes how these hormones interfere with the reproductive performance of *R. rattus* in domestic premises.

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