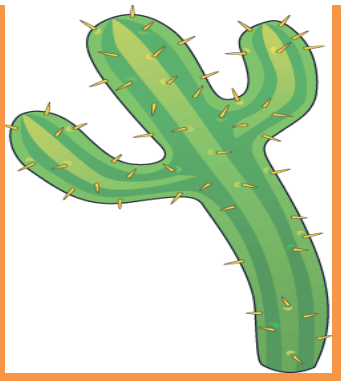

Fertility control of *Arvicanthis niloticus (Rüppel, 1842,* Rodentia: Murinae) a major agricultural rodent pest: Implication for sustainable rodent pest management in Tigray, Ethiopia

By:

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A thesis Submitted in Partial Fulfillment of the Requirements for the

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DECLARATION

I, Daniel Desta Welenchal, hereby present for consideration by the Department of Animal Rangeland and Wildlife Science, within the College of Dryland Agriculture and Natural Resources at Mekelle University, my dissertation in partial fulfillment of the requirement for the degree of Masters in **Fertility control of *Arvicanthis niloticus (Rüppel, 1842,* Rodentia: Murinae) a major agricultural rodent pest: Implication for sustainable rodent pest management in Tigray, Ethiopia**. I sincerely declare that this thesis is the product of my own efforts. No other person has published a similar study which I might have copied, and at no stage will this be published without my consent and that of the Department of Animal Rangeland and Wildlife Science.

Name of the student _____ Signature & date _____

Approval

Name of the main adviser_____ Signature & date _____

Name of the co-adviser _____ Signature & date _____

Name of external examiner _____Signature & date _____

Name of internal examiner _____Signature & date_____

Name of Postgraduate coordinator _____Signature and date _____

Name of Department head _____ Signature & date_____

ABSTRACT

Rodent pests, including the African grass rat (*Arvicanthis. niloticus*), cause significant agricultural damage in Tigray. This study was carried out to evaluate the effects of Quinestrol (QE), Levonorgestrol (LV), and their combination (QL) on reproduction fertility of African grass rat *A. niloticus*. A total of 160 *A. niloticus* (80 males and 80 females) were used to evaluate bait acceptance and reproductive performance of *A. niloticus* at concentrations of 10, 50, and 100 ppm. After ten days of acclimatization, the animals were fed the contraceptive baits for seven days for the females and fourteen days for the males before being paired and sacrificed for histological observation. The examination involved dissecting female animals over eight days and male animals over fifteen days, examined and weighted the reproductive organs of *A. niloticus* (ovaries, uterus, testes, seminal vesicles, and epididymis) to assess their reproductive health and fertility. Fertility control compounds significantly ($p < 0.0001$) reduced bait acceptance and body weight compared to control except LV at 10 ppm. Treatment and sex had significant interaction effects ($p < 0.001$) on bait acceptance and body weight. Treated females showing slightly higher bait consumption than males. Quinestrol (QE) and its combination (QL) significantly ($p < 0.001$) reduced reproductive organ in *A. niloticus* compared to untreated animals, while levonorgestrel (LV) had no such effect. Quinestrol also significantly ($p < 0.001$) decreased the weight of male reproductive organs and negatively impacted on sperm concentration and motility with increased sperm abnormalities, which contributed to its antifertility effect. There was significant difference ($p < 0.001$) in the weight of female reproductive organs (ovary, uterus) between treated and untreated rodents. Quinestrol and its combination (QL) treatment at higher concentrations were caused mild edema, increasing uterine and ovarian weight which indicates a physiological response to estrogen stimulation involving tissue explosion and fluid retention. Quinestrol at 50 ppm was evaluated for its impact on reproductive success and had significant effect on pregnancy rate and litter size when both male and female *A. niloticus* were treated. These results demonstrated that the reproduction rate of *A. niloticus* can be suppressed by Quinestrol which proved to have antifertility effect.

Key words: Rodent pest, fertility control, Quinestrol, Levonorgestrol, *A. niloticus*

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ACRONYMS AND ABBREVIATIONS

ANOVA.....Analysis of variance

EBPM…….. Ecologically-based pest management

EBRPM…… Ecologically-based rodent pest management

FSH ……..Follicle-stimulating hormone

GnRH…… Gonadotrophin releasing hormone

IPM …….Integrated pest management

LH …….. Luteinizing hormone

LV…….. Levonorgestrol

Ppm…… Parts per million

QE…… Quinestrol

QL…… Quinestrol and Levonorgestrol

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CHAPTER ONE

1. INTRODUCTION

1.1 Background and Justification

Rodent pests cause significant global issues that are responsible for considerable damage to agricultural production (Ruscoe et al. 2022). Globally, rodents are among the major agricultural pests which damaging and destroying over 30% of crops in both pre-harvest and post-harvest conditions as well as in storage areas that compromise food security, human health, and biodiversity (Singleton, 2001; Swanepoel et al. 2017). They pose a serious threat to agriculture with nearly all crops being potential targets for rodent attacks. In addition to crop damage rodent pests are known to transmit various infectious agents and serve as reservoirs for diseases (Battersby, 2015). In sub-Saharan Africa, rodent pests are the most destructive pests of cereal crops that leading to significant agricultural losses and high reproductive rate are a key factor contributing to the outbreaks of rodent populations (Andreassen et al. 2020; Mdangi et al. 2013). Remarkably, they cause major losses to cereal crops in Uganda, Tanzania, Nigeria, and other East African countries (Mayamba et al. 2019).

Population outbreaks of rodents in Africa occur frequently and during these outbreak years it is estimated that over 80% of potential harvests may be lost in East Africa (Mwanjabe et al. 2002). Earlier reports from Kenya indicated economic losses due to rodents with 20 – 30% damage to maize crops, 34 – 100% loss of young wheat and 34% loss of barley during rodent outbreaks (Gadisa et al. 2016). In Ethiopia, rodents cause significant agricultural damage. They are a major, though often overlooked, threat to food systems, typically resulting in 10-20% losses both in fields and during storage, (Yonas et al. 2021).

Similar to other African countries rodent pests cause damage during both pre-harvest and post-harvest periods. Various studies estimate losses of 15 – 40% in pulses and oilseeds, 13 – 29% in root crops, 9 – 48% in coffee, and 21 – 60% in cotton due to rodent infestations (Amera and Abate, 2008). Reports from Tigray and central Ethiopia indicate that maize crops suffer damage of approximately from 9 - 44% and 26.4%, with cereal crop losses ranging (Bekele et al. 2003; Meheretu et al. 2010). Among the rodent pests, the African grass rat *Arvicanthis niloticus* (Ruppel, 1842) is one of the most widespread and well-known genera recognized as a major agricultural pest. Its reproductive success particularly after the annual dry-season depletion gives it a competitive edge over other rodent species (Meheretu et al. 2014). In Ethiopia, it is one of the most significant rodent pests that causing substantial crop damage (Meheretu and Leris 2019, Welegerima et al. 2020), particularly in the highlands of Tigray (Meheretu et al. 2014; Welegerima et al. 2020). The species is also playing a crucial role in the transmission of zoonotic disease pathogens (Mgode et al. 2014). Therefore, there is a pressing need to implement effective rodent management strategies to prevent ongoing losses in agricultural production.

Rodent pest control can be achieved through both lethal and non-lethal methods (Witmer, 2018). Non-lethal control methods include barriers, repellents, habitat management, frightening devices, and fertility control. Lethal methods encompass kill traps, shooting, flooding, and the use of rodenticides. Rodenticides are the most commonly used and dominant method for controlling rodent pests worldwide (Capizzi et al. 2014). These substances include both acute and anticoagulant rodenticides. While effective rodenticides pose significant risks as they not only kill targeted rodent pests but also endanger non-target species that may ingest the toxicants or consume poisoned rodents (Elliott et al. 2016). Furthermore, these control methods are not rodent-specific and can lead to resistance as rodents may develop bait shyness and avoid familiar poisoned baits (Saxena, 2014). The adverse effects on non-target species and the environmental impact caused to the drawbacks of rodenticide use.

Consequently, there is a critical need to develop innovative rodent control methods that are both effective and environmentally friendly such as fertility control. Understanding the fertility control mechanisms and population dynamics of *A. niloticus* is essential for implementing sustainable pest management approaches. Fertility control for *A. niloticus* is a promising alternative that warrants further exploration. Research on hormonal reproductive disruption in *A. niloticus* has not been

tested, yet it has the potential to yield significant insights. Therefore, this study aims to address the gap in knowledge regarding the effects of the hormones Quinestrol and Levonorgestrol on the reproductive performance of *A. niloticus* rather than to use toxicant rodenticides.

1.2 Statement of the Problem

Rodent pests pose a significant threat to agricultural lands worldwide that causing substantial damage to crops, stored food, and human property which leading to considerable economic losses (Singleton et al. 2021). Rodent impacts are escalating in developed countries, due to changes in farming practices, abandonment of farmlands, and increased diversity and availability of crop types (Massawe et al. 2007; Ruscoe et al. 2022). These pests are particularly detrimental to farmers as they can severely diminish crop yields. For instance, studies have shown that rodent pests result in an estimated yield loss of 5% to 15% in maize production in Tanzania translating to approximately 412,500 tons of maize lost annually (Makundi et al. 2010). In Tigray farmers have reported pre-harvest yield losses ranging from 9-44% in cereal crops due to rodent infestations (Meheretu et al. 2010) with an overall annual crop yield loss of around 20% attributed to these pests (Bekele and Leirs, 1997).

In Africa, *Arvicanthis* is widespread well-known genus and is as one of the major important agricultural pests (Meheretu et al. 2014). Its reproductive success, especially after the annual dry-season depletion, makes it more successful than other competing rodents. In Ethiopia, *Arvicanthis* (Geoffrey, 1803) is one of the important pest rodents causing significant damage to crops (Swanepoel et al. 2017). Thus; there is a clear need to implement rodent management strategies to prevent continuous loss of agricultural products. Current rodent control practices are often based on the killing of rodents using poison, trapping and hunting. The most commonly used control measure for rodent pests is rodenticides (Buckle and Smith 2015). However, such control methods have serious drawbacks such as persistence of the chemicals led to poisoning of predators and scavengers as well as environmental pollution (Buckle and Smith, 2015), development of rodenticide resistance and heavy reliance on chemicals (Endepols et al. 2012; Witmer, 2019). Yet farmers largely relied on rodenticides because the rodenticides have economic benefit on a short-term basis and often use excessive quantities of rodenticides. The shortcomings of lethal methods for rodents with high breeding potential are apparent as the rodent population can rapidly recover (Hein and Jacob, 2015). These rodents may learn to avoid rodenticides in the future, enabling them

to thrive and reproduce without being affected by control method and develop avoidance behaviors and learning to steer clear of baits and traps in the future. Despite the availability of alternative methods for controlling rodent populations, rodenticides have been the dominant choice for many years, as reported by (Witmer et al. 2007).

However, there is growing public concern about the risks associated with rodenticides, particularly their potential dangers to humans, non-target animals, and the environment (van den Brink et al. 2018). In Africa, small-holder farmers consider rodents as difficult pest to control due to lack of effective management strategies (Makundi et al. 2010; Welegerima et al. 2020). Moreover, farmers have no alternative control methods available to them, such as non-lethal and non-chemical control methods.

It is therefore essential to focus on environmentally-friendly control methods such as fertility inhibitors, which are one of the strategies of ecological based rodent management that lead to a more sustainable control of pest rodent populations. Fertility control is considered cost effective a long-term approach (Miller et al. 1998), prevention to the rapid rebound of rodent populations, environmentally friendly and more humane (Tang et al. 2012). It regulates rodent pest population by decreasing the negative effects of chemical rodent control strategies on the ecosystem and has been gradually accepted (Massei et al. 2024).

These fertility limiting agents cause inhibitory effects directly on a specific process such as development of oocytes and ovarian follicles, implantation or indirectly affect a number of sites such as oviduct, uterus, and vagina within the reproductive tract (Liu et al. 2012). Steroid hormones, such as Levonorgestrol (LV), a synthetic form of progesterone, and Quinestrol (QE), a synthetic estrogen, are often used as anti-fertility compounds in rodents and proven to show contraceptive effects (Liu et al. 2012; Massawe et al. 2018). These agents are less bio accumulate along the food web and typically they are designed to have short-term effects on the target species and are metabolized or excreted by the organisms that consume them.Trials from China and Tanzania using these hormones Levonorgestrol and Quinestrol confirm that different species respond to the hormones in different ways. Hence, the study was aimed to investigate the effects of Levonorgestrol and Quinestrol on bait consumption and reproductive performance in *Arvicanthis niloticus* under laboratory condition.

1.3 Objectives of the Study

1.3.1 General Objective

The main objective of the study is to investigate the effect of fertility control agent's Levonorgestrol, Quinestrol, and a combination of the two on reproduction performance of *A.niloticus.*

1.3.2 Specific Objectives

Specifically this study aimed to

- ✓ Evaluate the effect of Levonorgestrol, Quinestrol and their combination on bait acceptability,
- ✓ Evaluate the effect of Levonorgestrol, Quinestrol and their combination on bodyweight,
- ✓ Evaluate the effects of Levonorgestrol, Quinestrol and their combination on the reproduction organs, and
- ✓ Evaluate the effects of fertility control compounds on pregnancy and liter size

1.4 Significance of the Study

Fertility control is increasingly recognized as a humane and effective approach to managing overabundant wildlife populations including rodents by prioritizing animal welfare and reducing reliance on lethal methods. This strategy aims to curb rapid rodent population growth which can lead to significant agricultural damage and ecological imbalances. By managing fertility farmers can protect their crops thereby enhancing agricultural productivity and contributing to food security particularly in regions where rodent infestations threaten local economies.

Additionally, effective rodent management through fertility control can diminish the risk of zoonotic disease transmission, safeguarding public health. Moreover, hormonal contraceptive methods used in fertility control typically have lower environmental impacts as they are designed to break down quickly and minimize harm to non-target species. Overall, fertility control represents a progressive step in sustainable agriculture and wildlife management addressing the complexities of rodent control while promoting ethical treatment of animals and environmental health. There is a growing interest in finding alternative methods of rodent control that are environmentally sustainable, efficient, and safe, aligning with public health interests. The use of

fertility control as an alternative to chemical rodenticides is gaining attention due to its ecological soundness, humaneness, safety, and cost-effectiveness compared to traditional mortality control methods. Research on the contraceptive hormones Levonorgestrol and Quinestrol has demonstrated that their environmental accumulation and non-target impacts are minimal. These products break down quickly when exposed to ultra violet light, water, or soil, reducing the risk of adverse environmental effects (Tang et al. 2012; Zhang et al. 2014). These fertility control agents have proven effective for various rodent species, including *Rattus nitidus* (Liu et al. 2013), *R. rattus* (Selemani et al. 2022), *Bandicota bengalensis* (Sidhu et al. 2020), *Ochotona curzoniae* (Liu et al. 2012), *Meriones unguiculatus* (Fu et al. 2013), *Tscherskia triton* (Zhibin et al. 2005), and *Mastomys* natalensis (Massawe et al. 2018). Given that *Arvicanthus niloticus* is a significant agricultural pest in Tigray effectively managing its fertility could have substantial implications for reducing its impact on agricultural production in the region by mitigating crop damage and the associated economic losses. Therefore, this study is significant to provide a viable alternative rodent management that supports sustainable agricultural practices and benefits the environment.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Biology of African grass rat (*Arvicanthis niloticus*)

The African grass rat (*Arvicanthis niloticus*) is a medium-sized, herbivorous rodent. The African grass rat has a stocky, rounded body, with a coat of brown-gray fur that has a rough, spiny appearance. The African grass rat is an herbivore that mainly eats vegetative plants and grass seeds and they are distributed in the Tigray Region and along the eastern slope of the Abyssinian massif (Bryja et al. 2019). The African grass rat breeds every 23 to 25 days during the cold dry season, and the gestation period is about 23 days. The African grass rat usually lives for about 2 years in captivity. This adaptability to diverse habitats underscores its ecological versatility and dependence on certain landscape features for shelter and nesting (Meheretu et al. 2015). The ability to thrive in various types of vegetation makes *A. niloticus* a successful inhabitant of both natural and agricultural landscapes (Refinetti, 2004).

2.2 Taxonomy and Ecology of *Arvicanthis niloticus*

Among small mammalian species rodents exhibit considerable diversity in morphology, habitat utilization, behavior, life history strategies, and distribution. *Arvicanthis niloticus* is commonly known as African grass rat which is one of such rodent species that has garnered attention particularly due to its status as an agricultural pest. The systematic classification of the genus *Arvicanthis* has been contentious. Currently, the genus is recognized to include up to six species like *A. blicki, A. abyssinicus, A. dembeensis, A. somalicus, A. nairobae,* and *A. testicularis* (Corti and Fadda, 1996). It is generally accepted that populations from the Nile Delta and West Africa belong to *A. niloticus*, while those from East Africa are classified under the other species. Many researchers have consolidated the genus into a single species, *A. niloticus* (Yalden et al. 1976; Bryja et al. 2019) reflecting a taxonomic revision that focuses predominantly on this species. Studies indicate that also different populations studied across various regions are distinctly separate groups. The taxonomic classification and genetic distinction of *A. niloticus* was previously identified as *A. dembeensis* in earlier studies. It notes that this species corresponds to the *A. niloticus* clade C1 as identified by (Dobigny et al. 2013). However, it is genetically distinct from the other clades of *A. niloticus* (C2-C4) and is not closely related to them, as evidenced by

both mitochondrial and nuclear genetic markers (Bryja et al. 2019). Furthermore, it states that samples from Egypt, which is the type locality for *A. niloticus*, fall within the mitochondrial clade C1, indicating that the name "*niloticus*" should specifically refer to the species found along the Nile River, in northern Ethiopia, and Yemen. This suggests a need for clarification in the nomenclature of these species based on their genetic relationships and geographic distribution

In Ethiopia, four species of this genus have been identified which includes *A. somalicus, A. dembeensis, A. abyssinicus,* and *A. blicki (*Yalden et al. 1976). *Arvicanthis dembeensis* corresponds to *A. niloticus* (Bryja et al. 2019; Dobigny et al. 2013). Particularly, *A. abyssinicus* and *A. blicki* share a closer genetic relationship while all species within the genus exhibit remarkable morphological differences indicative of their adaptation to varying altitudes. *Arvicanthis niloticus* has also been identified in the Omo River Valley in southwestern Ethiopia (Volobouev et al. 1988). *Arvicanthis niloticus* possesses a keen sense of smell, aiding in food foraging, and a developed sense of touch that facilitates locomotion mainly at night. The genus is primarily diurnal, feeding on fruits, seeds, and insects. This species demonstrates a high reproductive potential reaching sexual maturity within 36 to 50 days (Delany and Monro, 1985; Sicard et al. 1994).

2.3 Distribution and Geographical Range of *Arvicanthis niloticus*

The distribution of *A. niloticus* like other rodent species is influenced by various physiological and environmental factors. Key aspects include altitude, climate, and the availability of food resources. In Eastern Africa, rodents are mostly abundant accounting for approximately 28% of the total recognized mammal species (Bekele, 1996). Their adaptability to different environments allows them to thrive in diverse habitats. *Arvicanthis niloticus* is distributed across a wide swath of Africa south of the Sahara extending from Senegal in the west to Somalia in the east and along the Nile basin from Egypt down to Tanzania (Delany and Monro, 1986), along to the Nile River, in northern Ethiopia and Yemen (Bryja et al. 2019). This extensive range highlights the species adaptability and resilience in various ecological contexts

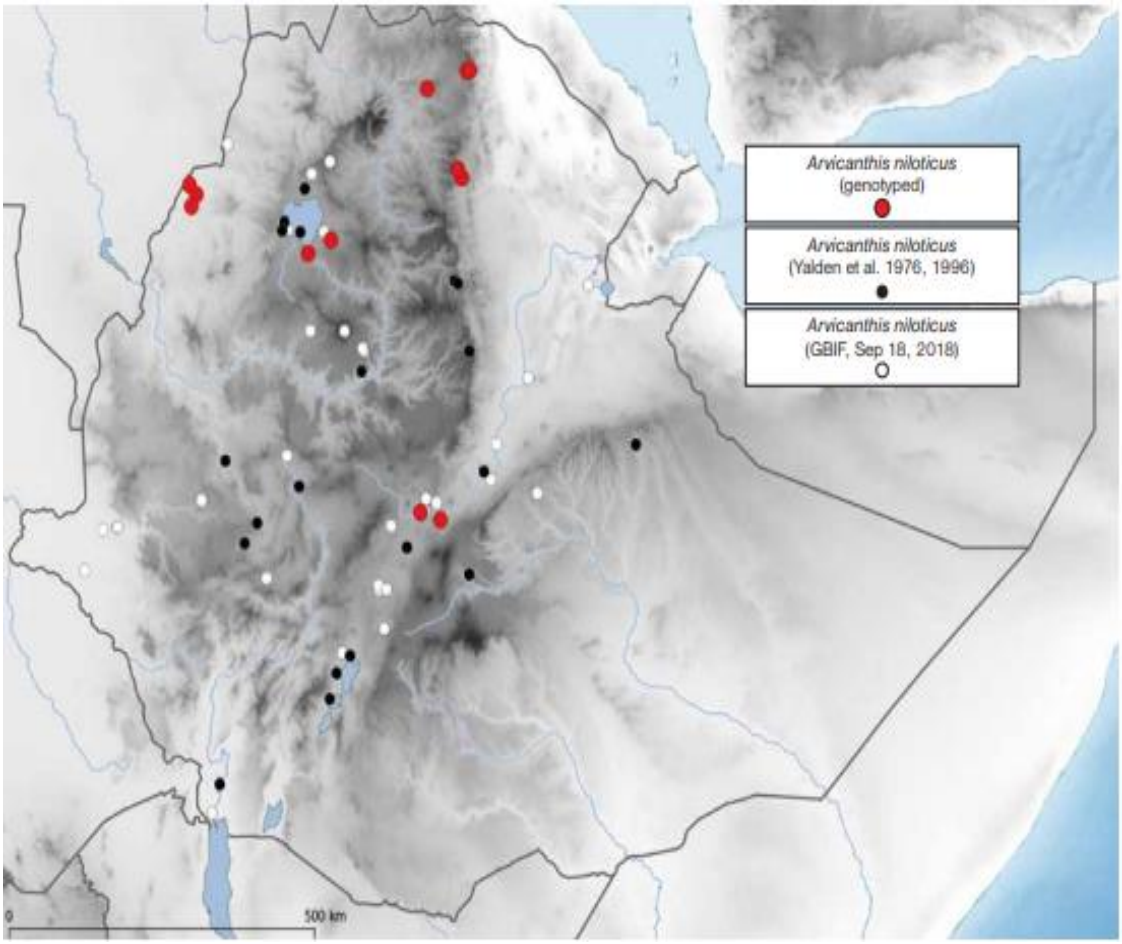


Figure 1; Distribution of Arvicanthis niloticus**.** Distribution of *Arvicanthis niloticus*. The localities from Yalden et al. (1976) were reported under the name *A. dembeensis*, while those from GBIF as *A. niloticus*. In both cases, some of these are likely to represent other taxa; i.e., *A. niloticus* "C2-C4" (in south-western part of the country) (Bryja et al. 2019).

2.4 Adaptation and Habitat Preferences of *Arvicanthis niloticus*

African grass rat (*Arvicanthis niloticus*) is primarily associated with savannas and grassland habitats. These environments provide essential resources. The diet of *A. niloticus* is diverse, consisting of grasses, seeds, insects, and fruits (Sicard et al. 1994). When near agricultural areas they may also consume cereals further contributing to their pest status. The structure of vegetation plays a crucial role in habitat selection. *A. niloticus* is a species that thrives in colonial burrows, requiring ground cover such as short bushes, trees, rocks, or termite mounds for nesting. It inhabits a variety of African environments, including dry savannas, sub-deserts, coastal scrublands, open woodlands, grasslands, and cultivated areas, all of which provide essential shelter (Refinetti, 2004). Areas with rich plant diversity can support larger populations by offering ample food and

shelter. *A. niloticus* exhibits a high reproductive potential, capable of producing large litters within a short time frame. This trait allows the species to rapidly adjust to environmental changes and increases its resilience to disturbances. Such adaptability is crucial in unstable habitats where food availability may fluctuate. *A. niloticus* has been respond quickly to instabilities in their habitats. They are adept at dispersing from unsuitable locations which facilitates population recovery and expansion into new areas (Leis et al. 2008).

2.5 Economic Importance of Rodents

2.5.1. The impacts and economic consequences of rodent Pests

Rodent pests are a widespread issue posing significant threats to agricultural productivity worldwide. The rural small-holder farmers encounter a variety of challenges related to both social and environmental factors in their efforts to produce food (Swanepoel et al. 2017). Together, these challenges can significantly impact the farmer's ability to sustain their livelihoods and ensure food security for their communities (Swanepoel et al. 2017). They are responsible for considerable damage to cereal crops with research indicating that they can cause direct damage through feeding, gnawing, and contaminating stored food (Witmer and Shiels, 2018). This damage occurs during both pre-harvest and post-harvest stages leading to spoilage and increased susceptibility to fungal and bacterial infestations (Singleton et al. 2021).

Globally, rodents are estimated to destroy about 30% of crops resulting in economic losses amounting to approximately $30 billion annually (Feldhamer et al. 2020; Singleton, 2010). This impact is particularly pronounced in sub-Saharan Africa where the prevalence of rodent pests significantly affects food security and agricultural viability (Htwe et al. 2016).

In sub-Saharan Africa, the most damaging rodent species are African Grass Rat (*Arvicanthis niloticus*) and Multimammate Rat (*Mastomys natalensis*). They are belonging to the family Muridae with extraordinary species that frequently cause severe crop damage. African Grass Rat has been known for its high reproductive capacity and adaptability and can rapidly increase in numbers particularly in agricultural areas. In addition to this Multimammate Rat (*Mastomys natalensis*) also another significant pest that plays a significant contribution to substantial agricultural losses. Recurrent outbreaks of these rodent species indicate their ability to thrive in suitable conditions leading to mass appearances that can devastate crops (Capizzi et al. 2014).

Their population dynamics are influenced by environmental factors, food availability, and habitat changes. In Africa, out of 381 recorded rodent species approximately 77 are considered pests with 12 to 20 species identified as significant agricultural threats (Singleton et al. 2007). For example in Tanzania it is estimated that rodents cause annual yield losses of 5-15% in maize production (Leirs, 2003). Earlier, in Ethiopia a diverse rodent population of approximately 84 species includes 11 species classified as agricultural pests impacting both major and minor crops (Bekele and Leirs, 1997). However, recently a total of 104 rodent species have been identified in Ethiopia, which belong to 40 different genera and 10 families (Bryja et al. 2019).

The classification into multiple genera and families implies varied adaptations and ecological roles among the species, which could be important for understanding the ecosystem and potential conservation efforts in Ethiopia. Essentially, in cultivated lands show higher rodent pest densities with estimates suggesting that rodents destroy 20-26% of cereal crops (Bekele et al. 2007). The major rodent pests identified in Ethiopia are *Mastomys natalensis* (Smith), *Arvicanthis niloticus* (Rüppel, 1842), *Mus musculus* (Linnaeus) (Demeke et al. 2007). They adversely affect rural communities by damaging their agricultural crop in the field (Meheretu et al. 2010).

2.5.2 The effect rodent pest in agricultural and ecological consequences

Almost all crops grown around the world can be damaged by rodents, including cereal grains, vegetables, cotton, alfalfa, sugar cane, potatoes, tree fruits, and many others (Smales et al. 2012). Rodents are commonly found in agricultural settings where farm structures provide shelter and abundant food sources. Key areas such as corn cribs, hay lofts, and grain storage facilities are particularly attractive to rodent pests (Kasso, 2013). Their presence in these environments leads to significant challenges for farmers (Singleton et al. 2021). Rodents are major contributors to crop damage worldwide, causing an estimated 30% loss in agricultural yields. In Ethiopia, rodent-related losses exceed 25%. The destruction of crops by rodents results in substantial economic losses and food shortages (Makundi et al. 2005).

Rodents prey on the eggs or young of birds and compete with native species for food and habitat and they serve as reservoirs for various zoonotic diseases that can affect humans and livestock. Rodents are critical vectors for numerous diseases, posing significant public health risks. They are known to carry pathogens responsible for illnesses such as Lassa fever, Plague, Leptospirosis, Toxoplasmosis, Murine Typhus, Salmonellosis (Rao, 2003). World Health Organization has estimated that 400 million human cases of rodent-related zoonosis occur every year (Colombe et al. 2019). These diseases can have severe implications for human health, agricultural practices, and livestock management.

2.6 Significance of Public Health

African Grass Rat (*Arvicanthis niloticus*) serves multiple roles in the transmission of zoonotic diseases, acting as vectors, reservoirs, and intermediate hosts for various disease-causing agents, including viruses and bacteria. This means they can carry and spread pathogens that can infect humans and other animals (Loiseau et al. 2008). Most rodents do comprise important reservoir hosts of zoonotic disease in Africa. Rodent borne diseases are important as the diseases lead to decreased labor productivity and increased health care costs (Asenga et al. 2015). *A. niloticus* serves as host and/or carrying vectors of various pathogenic organisms, such as acarines, insects, helminths, protozoans and viruses (Fagir and Rayah, 2009). It has been also shown to carry a number of pathogens, such as Leishmania major (Githure et al. 1986), Borrelia spp. (Trape et al., 1996), Leptospira spp. (Sebek et al. 1989), Rickettsia spp. (Julvez et al., 1997), Schistosoma spp (Duplantie et al. 2006) or Toxoplasma gondii (Mercier, 2013). This rodent is an herbivorous murine species inhabiting dry savannah, woodlands and grasslands across tropical Africa (Fagir and Rayah, 2009). Rodent species serve as reservoirs for a variety of diseases, which can have significant implications for public health. This means that these rodents can harbor pathogens that may be transmitted to humans and other animals, potentially leading to outbreaks of diseases (Monadjem et al. 2015).

2.7 Rodent Pest Management

2.7.1 Ecologically-Based Rodent Pest Management

The extent of losses and food insecurity in many countries is significant due to the failure of traditional and conventional methods to effectively management of rodent pests (Meheretu et al. 2010). Consequently, ecologically based rodent management (EBRM) is being considered as a viable alternative to mitigate these losses and improve food security. Ecologically-based rodent pest management (EBRPM) is a strategy that emphasizes the ecological principles necessary for effective rodent control in agricultural systems and in Africa is currently viewed as cost beneficial in the long-term approach (Belmain et al. 2015). Understanding the ecology and population dynamics of rodent species is crucial for developing these management strategies (Singleton et al. 2007). Historically, rodent management has faced stagnation due to insufficient research into their biology and behavior. There is a growing demand especially in developing countries for control methods that rely less on chemical rodenticides. Innovative formulations such as oil-based and encapsulated baits may help combat rodent resistance (Demeke et al., 2007).

Rodents are considered intelligent pests prompting the development of ecologically-based pest management (EBPM) techniques which build on integrated pest management (IPM) principles. EBRPM has been shown to effectively reduce rodent damage to crops in both fields and storage (Brown et al., 2019). Its core principles focus on safety, profitability, and durability while emphasizing biological understanding. IPM integrates various management practices to control pest populations below economically damaging levels (Palis et al. 2007). Integrated rodent pest management specifically employs multiple control methods tailored to specific situations prioritizing environmental controls and continuous evaluation to minimize disruption to agricultural ecosystems (Singleton et al., 1999). This approach aims to manage pest species and related diseases in an ecologically sustainable manner.

2.7.2 Biological control of rodents

Biological control of rodents is a key component of an integrated, ecologically-based approach to pest management. This technique utilizes natural enemies to manage rodent populations, effectively reducing pest abundance and in some cases preventing damage (Chambers et al. 1999). Natural predation has been suggested as an attractive, yet under-utilized component in EBRM studies (Makundi and Massawe, 2011). Numerous predators such as skunks, weasels, cats, dogs, foxes, coyotes, owls, hawks, and snakes, contribute to biological control by feeding on rodent pests. Several avian predators (T*yto alba*, *Elanus axillaris*, *Falco tinnunculus*, *Falco cenchroides*, *Bubo bengalensis*, *Buteo rufinus*) were commonly cited in the biological control of rodents (Labuschagne et al. 2016).

In Tigray Dogu'a Tembien these raptor birds such Augur buzzard (*Buteo augur*), Common Buzzard (*Buteo buteo*), Steppe Eagle (*Aquila nipalensis*), Lanner falcon (*Falco biarmicus*), Black kite (*Milvus migrans*), Yellow-billed kite (*Milvus aegyptius*) and Barn owl (*Tyto alba*) (Yonas and Leirs, 2019). Most studies indicate that avian predators (birds of prey) have a positive impact on controlling agricultural rodent pests. As a major aspect of integrated pest management (IPM) biological control is cost-effective, environmentally friendly, and minimally disruptive to ecosystems. It relies on the deliberate use of natural enemy's parasites, predators, and pathogens to control pest populations (Kaboodvandpour and Leung, 2010).

2.7.3 Chemical control of rodents

Chemical control remains the primary method for managing rodent populations (Buckle and Smith, 2015). This approach involves the use of rodenticides which are chemicals designed to kill rodents and are the most common pest control measure. When properly formulated and applied rodenticides require minimal manpower and can effectively reduce rodent populations (Brown et al., 2008). The effectiveness of rodenticide treatments varies significantly based on the type of toxicant, bait formulation, application method, and timing. Highly toxic, fast-acting rodenticides can achieve rapid population knockdown (Watt et al. 2005). However, these baits also present a high risk of poisoning non-target animals that may consume the bait or eat poisoned rodents (Jacob and Buckle, 2018). Rodenticides can be classified into two acute toxins and anticoagulants. Acute rodenticides are designed for in-field use and cause death within minutes to 24 hours after

ingestion. Common acute rodenticides include zinc phosphide, strychnine, and thallium sulfate (Buckle and Eason, 2015). Anticoagulant rodenticides are work by inhibiting the recycling of vitamin K which is essential for blood clotting, leading to uncontrolled bleeding after several days of ingestion. The first anticoagulants such as warfarin and diphacinone were developed in the late 1940s and act more slowly than acute toxins. They have a lower inherent toxicity and an antidote (vitamin K1) is available for accidental poisoning. More recent anticoagulants like brodifacoum and bromadiolone can cause death with a single feeding (Huckle et al. 1988).

Table 1; some diverse types of presently available rodenticides

Rodenticides		
Slow Acting/Chronic Rodenticides	**Fast acting/acute rodenticides**	**Respiratory**
Warfarin	Barium carbonate	Alluminium oxide
Coumatetralyl	Zinc phosphide	
Coumafuryl		
Bromadiolone		

(Sources: Demeke, 2007)

In most cases rodenticides broadly used. But current use of pest rodent's management seems to be inappropriate. Therefore the unwise and inappropriate use of rodenticides on rodent pest is results in genetic resistance, behavioral avoidance, non-target animal poisoning and environmental pollution (Singleton, 1999).

2.7.4 Fertility control of rodents

Rodent fertility control is being as an effective alternative for managing rodent populations by addressing both damage control and ecosystem conservation. This method can significantly decrease their population density by targeting the birth rates of rodents and Research, including findings from (Jacoblinnert et al. 2022). Fertility control focuses on reducing birth rates without causing immediate harm to existing populations. Fertility control is gaining attention as a non-lethal alternative for managing rodent pests. This method has the potential to significantly contribute to rodent control strategies (Jacob et al. 2008) by disrupting reproduction and leading to a temporary or permanent reduction in population (Fagerstone et al. 2002).

Effective implementation of fertility control relies on a thorough understanding of the reproductive biology of the target rodent species. Certain hormones can induce infertility in animals by disrupting the normal operation of the hypothalamic-pituitary-gonadal (HPG) axis. This axis is crucial for regulating reproductive functions, and when it is interfered with, it can lead to hormonal imbalances that affect fertility (Chen et al. 2022).Various approaches have been explored like hormonal agents, immune-contraceptive, and natural extract. Fertility control is seen as a complementary tool within integrated pest management and offers a long-term solution to suppressing rodent populations. Fertility-limiting compounds can induce either permanent or temporary sterility in both males and females, reduce the number of offspring, or impair the fertility of the offspring produced (Humphrys and Lapidge, 2008). Various antifertility compounds have been utilized to control reproduction in different animal species, employing methods such as contraception or sterilization (Massei and Cowan, 2014). The predominant approach oral delivery involves in wildlife contraception include a variety of substances such as synthetic hormones of estrogens, progestins, and androgens., plant compounds, and chemicals, as well as immune-contraceptive vaccines. This indicates a diverse approach to managing reproduction in animal populations, utilizing both synthetic and natural options (Massei and Cowan, 2014).

Levonorgestrol (LV) is a synthetic form of progesterone, and Quinestrol (QE), also a synthetic estrogen, and combination (QL) have been shown to induce varying levels of reproductive inhibition in different rodent species that cause infertility (Massawe et al., 2018). Synthetic progestins such as norgestomet melengestrol acetate, megestrol acetate and Levonorgestrol have been used in various animal populations (Zhibin, 2015). Levonorgestrol (LV) is a similarity to progesterone that used for an emergency contraceptive to prevent pregnancy by preventing or interrupting ovulation and egg implantation in humans and other animals as well as it affects the cervical mucus or the ability of the sperm to bind to the egg (Novikova et al. 2007). Quinestrol (QE) is a synthetic long-acting estrogen that, after oral administration, can be stored in rodent fat and gradually released; leading to long-lasting infertility (Zhao et al. 2007).

Fertility control represents a promising avenue for sustainable rodent management in agricultural systems. The anti-fertility agents Levonorgestrol (LV), Quinestrol (QE) and a combination (QL-1) for a range of doses 1–10 mg/kg, 10–50 ppm was delivered by oral baits that have been reported for numerous rodent species (Chen et al. 2022; Liu et al. 2012; Massawe et al. 2018; Selemani et al. 2022; Stuart et al. 2021; Wang et al. 2011; Zhang et al. 2004; Zhao et al. 2007).

2.8 Mechanism of Action of Fertility Control Agents

Fertility control is an efficient and economical approach for managing populations of certain species particularly pests and often heralded as a humane and effective technique for management of overabundant wildlife, including rodents (Massei et al. 2024). The effective delivery of fertility control agents is crucial for achieving population-level impacts. Early studies demonstrated that orally active estrogens, progestagens, and androgens can significantly disrupt reproductive processes in rodents affecting the uterus, ovulation, implantation, and spermatogenesis (Marsh, 1988).

Research indicates that a single baiting of Quinestrol or Levonorgestrol at doses of 10–50 µg/ml (0.001–0.005%) can effectively limit rodent breeding when administered at the start of the breeding season (Zhibin, 2015). Both compounds impair reproductive performance by reducing the size and function of male reproductive organs, disrupting spermatogenesis and decreasing sperm concentration and motility. In females also they can lower pregnancy rates and litter sizes by inducing uterine edema (Lv and Shi, 2012).

These fertility control agents decompose quickly with half-lives of 5–16 days in soil and less than 3 days in water under field conditions (Tang et al. 2012; Zhang et al. 2014). This rapid breakdown helps mitigate environmental impacts while effectively managing rodent populations.

Levonorgestrol and Quinestrol are contraceptives that prevent ovulation through distinct mechanisms. Levonorgestrol inhibits the release of follicle stimulating hormone (FSH) from the pituitary gland, blocking ovulation and disrupting endometrial cell growth to prevent embryo implantation (Zhao et al. 2007). In addition to this Quinestrol has strong estrogenic effects and inhibits gonadotropin-releasing hormone (GnRH) from the hypothalamus, preventing follicle maturation and ovulation (Luv and Shi, 2011).

Levonorgestrol and Quinestrol have been shown to affect the structure of reproductive organs and exert anti-fertility effects in several rodent species, including the greater long-tailed hamster (*Tscherskia triton,* Zhibin, et al. 2005), Djungarian hamster (*Phodopus campbelli*, Wan et al. 2006), Mongolian gerbils (*Meriones unguiculatus*, Huo et al. 2006, Fu et al. 2013), Brandt's voles (*Lasiopodomys brandtii*, Zhao et al. 2007), plateau pikas (*Ochotona curzoniae*, Liu et al. 2012), Rattus norvegicus (Sprague-Dawley rats, Liu et al. 2012) and The Natal multimammate mouse (*Mastomys natalensis*, Massawe et al. 2018).

A mixture of the contraceptive compound Levonorgestrol-Quinestrol has been used as a sterilant in reproductive endocrinology studies in several rodents (Fu et al. 2013; Liu et al. 2012, Massawe et al. 2018). The Levonorgestrol and Quinestrol and its combination effectively lowers follicle-stimulating hormone (FSH) levels and increases luteinizing hormone (LH) levels, inhibiting ovarian follicle maturation and ovulation, which reduces fertility. This combination is also noted for its good palatability, strong sustainability, and rapid degradation, making it an effective fertility control agent (Lv and Shi, 2012; Tang et al. 2012).

CHAPTER THREE

3. Materials and Methods

3.1 Study Site Description

The study was conducted in the Rodent Research Unit laboratory, Department of Biology, Mekelle University. The geographic position was situated between 13°28'45"N and 39°29'24"E, at an altitude of 2200 m above sea level.

3.2 Study Design

The experiment was conducted in laboratory-based experiments using single and varied compounds of Levonorgestrol (LV), Quinestrol (QE), and a mixture (QL) at concentrations of 10, 50, and 100 parts per million.

3.3 Set up and Experimental Animals

The experiment involved 160 *A. niloticus* with equal proportion between females and males. This implies that the study aimed to ensure equal representation of both sexes which can help understanding any potential differences in physiology and reproductive outcomes between the sexes. Before animal collection, experimental room was cleaned and fulfills the material that has been used in the laboratories. Animal was trapped and collected via Sherman live trap using peanut butter as bait from fields in and out of main campus Mekelle University, transported to Rodent Research Unit laboratory. Animals were transferred by using Zipped-Plastic Bag from the Sherman live trapping in to individual cages. All animals were fed 10% of their body weight and water was given *ad libitum*.

3.4 Handling of Collected Animals

Animals were separated into cages based on sex and allowed to acclimatize to the laboratory conditions for ten days. Adult male and female *A. niloticus* weighing more than sixty five grams with the same weights were used for experimental treatment otherwise they were released. The animals were required to be healthy and active. Daily checks of the animals were conducted and they were provided with 10 g of bait provided per weight, along with water provided *ad libitum* during the entire acclimatization period. All animals were checked for their healthy and no females were pregnant. During this period, any animals that appeared unhealthy and pregnant were

removed from the cage. Rodents were examined for behavioral, morphological conditions and an observation protocol was added especially for those of involving behavioral changes. At the end of the acclimatization period the animals were weighed and prepared for experimental treatment.

3.5 Bait Preparation and Solutions of Compounds

Powdered Levonorgestrol (LV) and Quinestrol (QE) (Beijing Zizhutiangong Science and Technology Ltd, China) were weighed to prepare rodent bait formulations at different concentrations (10, 50,100 ppm) in10% baits of their body weight. Rodent bait formulations were prepared for each of the two hormones and combinations of the two contraceptive bait treatments QE, LV and QE + LV. For the QE + LV combination, the compounds mixed equally at a ratio of 1:1. QE and LE were weighed carefully and each quantity of contraceptive was dissolved in 100ml of absolute ethanol within a 60 - 70ºC heated water bath. The ethanol-contraceptive solution was mixed with a sugar solution made from 200 g sucrose in 1000 ml water. The combined solution was turn milk white because QE/LV will form a suspension in water. The milk white suspension should be kept in stirring.

The suspension with QE/LV was showered on the standard baits and thoroughly mixed together was 1:1 (Levonorgestrol-Quinestrol). The contraceptive bait preparation was followed the protocols from Massawe et al. (2018). The control bait (0 ppm) was similarly prepared without contraceptives.

3.6 Experimental Design and Treatments

Because of the same age and weight the experimental design was selected complete random design. In order to run the experiment different experiments were performed. The first experiment was to select the effective dose acceptance of the hormone from those of three concentration and treatment (10, 50,100 ppm of Levonorgestrol (LV), Quinestrol (QE), and in combination of QL. This was aimed to collect data on body weight, bait acceptance, and reproductive physiological effects of both in male and female. The second experiment was to assess the effect of selected effective dose that identified in experiment one on the breeding outcomes pregnancies and litter sizes of *A. niloticus*. This was helped to assess on the number of pregnancies and birth sizes of treated and untreated animals.

3.7 Evaluations on Bait Acceptance

In this experiment three treatment with concentrations of contraceptive baits (10, 50, 100 ppm) using three consecutive experimental treatments were conducted. The experiments have been done at 10 ppm of Quinestrol (QE), Levonorgestrol (LV) and mixture of the two concentrations (QL), at 50 ppm of Quinestrol (QE), Levonorgestrol (LV) and mixture of the two concentrations (QL) and at 100 ppm of Quinestrol (QE), Levonorgestrol (LV) and mixture of the two concentrations (QL). Females for seven days and males for fourteen days were treated with contraceptive baits individually in their own cages. The response of different concentration level and treatments on the bait acceptance, body weight and reproductive organ of animals were assessed. In each experiment animals were providing daily with 10% of their body weight of bait containing a respective dose of contraceptive hormone and compare with untreated animals which were feed standard pellets. A total of 120 animals were used to evaluate bait acceptance and contraceptive effects. The same number of animals was used for all treatments with adjusted doses of contraceptives (Table 2). The experimental treatment was arranged in different hormones with different concentration of 0, 10, and 50, 100ppm as T1, T2, T3 and T4.

Table 2; Experimental arrangements of treatment

Treatment	**Experiments**			**Number of Animals**	
Group	Rates applications	Rates applications	Rates applications	Females	Males
Control	0 Ppm	0 Ppm	0 Ppm	15	15
Quinestrol (QE)	10 Ppm	50 Ppm	100 Ppm	15	15
Levonorgestrol (LV)	10 Ppm	50 Ppm	100 Ppm	15	15
Mix = QE+LV	10 Ppm	50 Ppm	100 Ppm	15	15
Total Rats				60	60
Dissection Date				Day 8	Day 15

3.8 Evaluation of Pregnancy and Litter Size

After the most effective dose has been defined in experiment, the effects of this dose on the reproductive success of animals were determined. About a total of 40 sexually mature *A. niloticus* were used for evaluating the reproductive performance of the treatment with equal proportions of the animal. The optimal concentration of contraceptive hormone defined in experiment, 50 ppm, was then used in this experiment. Females for seven days and males for fourteen days were treated with the selected contraceptive baits individually in their own cages. The same to experiment one, treatment group of animals were provided with their respective contraceptive bait 10% of their body weight (g) daily. Basically, for assessment of the pregnancy and liter size of the treated and untreated animals the following procedure has been conducted (see Table 3).

Table 3; Pairing Experimental Set up

Treatment and its concentration	Paired animal Display
QE 50ppm	Untreated Female - Untreated Male
QE 50ppm	Treated Female - Treated Male
QE 50ppm	Untreated Female - Treated Male
QE 50ppm	Treated Female -Untreated Male

Description

Pair 1 = Untreated female (UF) paired with untreated male (UM)

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

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

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

3.9 Data Collection

3.9.1 Bait acceptance by the animal

Acceptance of contraceptive baits has been done on both males and females of adult *A. niloticus*. To evaluate the bait acceptance equal proportion of male and female was used. Each of animals were separated in individual of their own cage and provided with 10 g of standard pellet or/and baits containing different concentration of the contraceptive level (10, 50, 100 ppm of for each of the treatments of QE, LV, QE+LV) with five animals of each sex per treatment that provides for 7consecutive days for female and 14 days for male. Water was provided *ad libitum* for each animal. For calculating the amount of bait acceptance by animals was obtained by subtracting the weight of remaining feed from the total weight of the bait provided in the first day (which is 10% of their body weights). In each animal cage an animal feeder was used to prevent from mixing with bedding material, water or any other materials weights. This calculation helps determine how much bait was actually eaten by the animals and allowed to assess their bait acceptance rates accurately.

3.9.2 Weight change of the animal

Before and after feeding with the contraceptive bait the body weight of each animal was measured using an electronic balance. Then, the body weight change of each animal was recorded daily. The Body weight change was determined by the difference between the initial and final body weight of the animal. During this time, assessment of any changing weights of the animal was recorded. Percentage body weight change was calculated by subtracting the final weight from initial weight multiply 100 divided by the initial weight.

3.9.3 The effects of treatments on male and female reproductive organs

Animal feeding with bait animals were killed by anesthesia of ethanol on day 8 for females and on day 15 for males for identification of any abnormality of the animal reproductive organs. The uterus, ovaries, testes, epididymis and seminal vesicles were observed and comparisons of organ weights were recorded with the control group following the method described by Zhang et al. (2006).

3.9.3.1 Sperm count and motility

Sperm counting parameters were evaluated following the method described by Massawe et al. (2018). Sperm was collected from the caudal epididymis of treated and untreated males that were dissected and placed in 1 ml of 0.9% normal saline. The caudal (tail) epididymis was cut to release sperm, and a drop of the solution was placed on a slide for evaluation under an Optika compound microscope that manufactured from Italy. A smear for sperm count and morphology was prepared from the suspension. The remaining samples were placed in glass test tubes with 9 ml of distilled water and refrigerated for 2 hours at 4ºC. Afterward, 9 ml of 0.9% saline was added, the solution was shaken. A drop of the solution was placed on a hem cytometer and sperm were counted under an Optika compound microscope that was measured using established methods (WHO 2010). Another drop was used to prepare smears, which were air-dried and fixed in nigrosin and eosin for 30 minutes. The smears were examined under oil immersion at 100x magnification to evaluate sperm morphology.

3.9.3.2 Reproductive performance of treatment on pregnancy and birth size

About a total of 40 animals (20 male and 20 female) adult *A. niloticus* were used to determine the potential effect of the contraceptives hormones on pregnancy rate and litter size. Accordingly daily checkups during animal pairing were done by observing behaviors indicative of effective copulation, monitoring the health and well-being of the animals, and identifying any potential issues that could hinder successful mating.

Animals provided with contraceptive bait of Quinestrol at 50 ppm treatment were paired with treated and untreated males for 10 days. After pairing, the observation of pregnancy development and the number of birth animals were monitored while the female animals were left to feed on a normal diet for 25 days. The newborns in each treated and untreated groups were counted and compared to the control animals. This information was then used to compare the pregnancy outcomes and litter sizes of the treated animals against those in the control group.

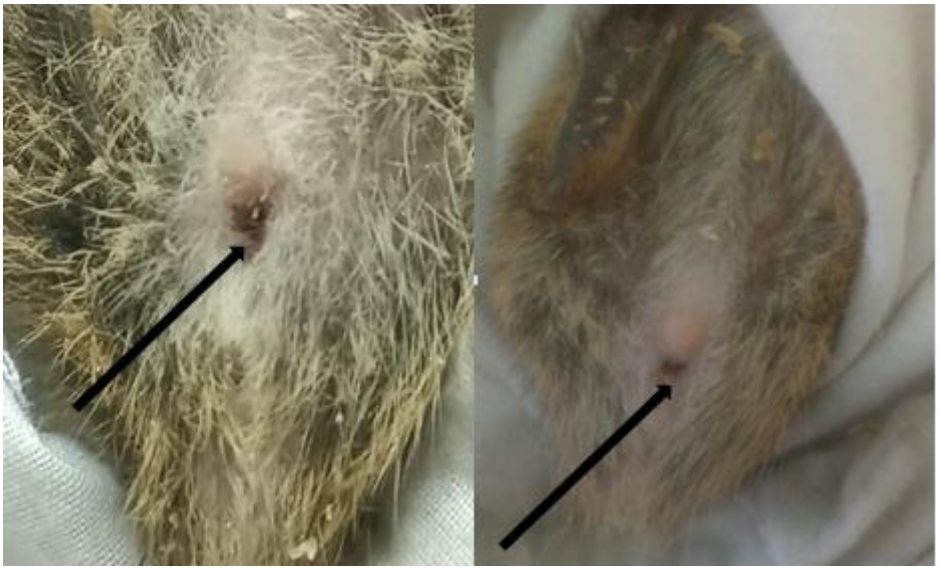


Figure 2; perforated female vagina an indication for recent mating

3.10 Data Analysis

Data on the bait acceptance, weight of the animals in all treatments were recorded in Excel spread sheet. Statistical analysis was performed using SPSS package (Version 26). Comparison of between treatments were made using analysis of variance (ANOVA). Graphs were drawn using Microsoft Excel software. Comparison of means values for significant differences was done by mean separation test using t test Least Significant Difference.

3.11 Animal Ethical Consideration

The research was carried out in Rodent Research Unit laboratory at the Department of Biology, Mekelle University. It emphasizes that all experimental procedures received approval from the university's ethics committee which ensuring that the study adhered to ethical standards. The research were followed the guidelines established by the American Society of Mammalogists (Sikes and Gannon, 2011). This was best practices for the humane treatment of mammals in research settings.

CHAPTER FOUR

4. Results

4.1 The effect of fertility control compounds on Bait acceptance

Bait intake was significantly lower in both sexes of animals treated with various concentrations of contraceptive hormones compared to the control group (Table 4). The overall bait intake of the treated male and female animals was significantly lower than the control group ($P < 0.001$) except with LV at 10 ppm. The mean bait intake from all treated groups was significantly reduced compared to the control group (female $F = 152.57$, $df = 9$, $p < 0.0001$ and male $F = 156.95$, $df = 9$, $p < 0.0001$) (Figure 3 and 4). Generally, the amount of bait consumed per day showed a slight increase over time (Figures 5 and 6). Quinestrol and the combination of Levonorgestrel and Quinestrol treatments showed a highly significant difference ($p < 0.0001$) at concentrations of 50 and 100 ppm.

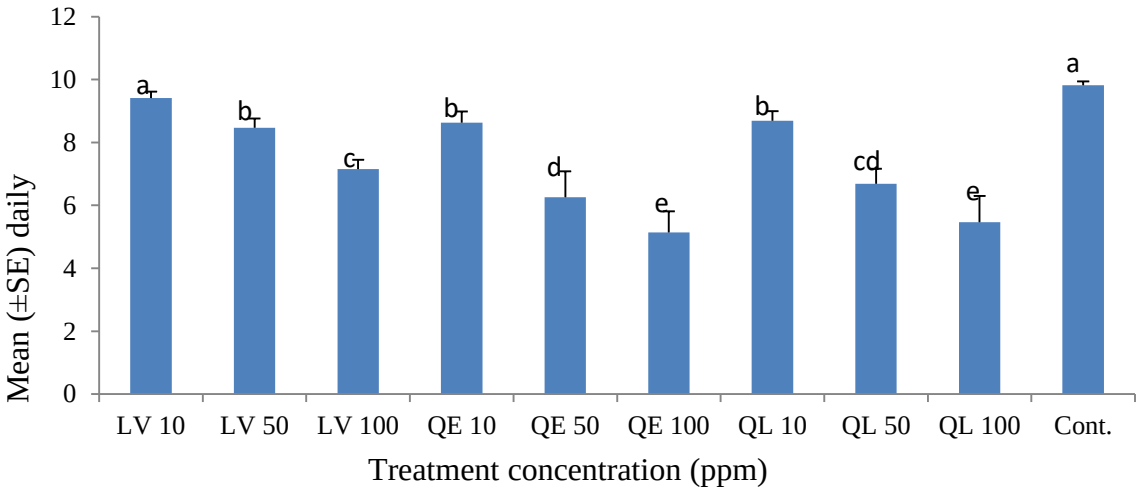


Figure 3; The mean (±SE) daily consumption over a 7-day feeding period of female adult *Arvicanthis niloticus* consuming bait containing contraceptive hormones at three concentrations (10, 50, and 100 ppm). Letters indicate significant differences between treatments as determined by the post-hoc Tukey test at the 95% confidence interval.

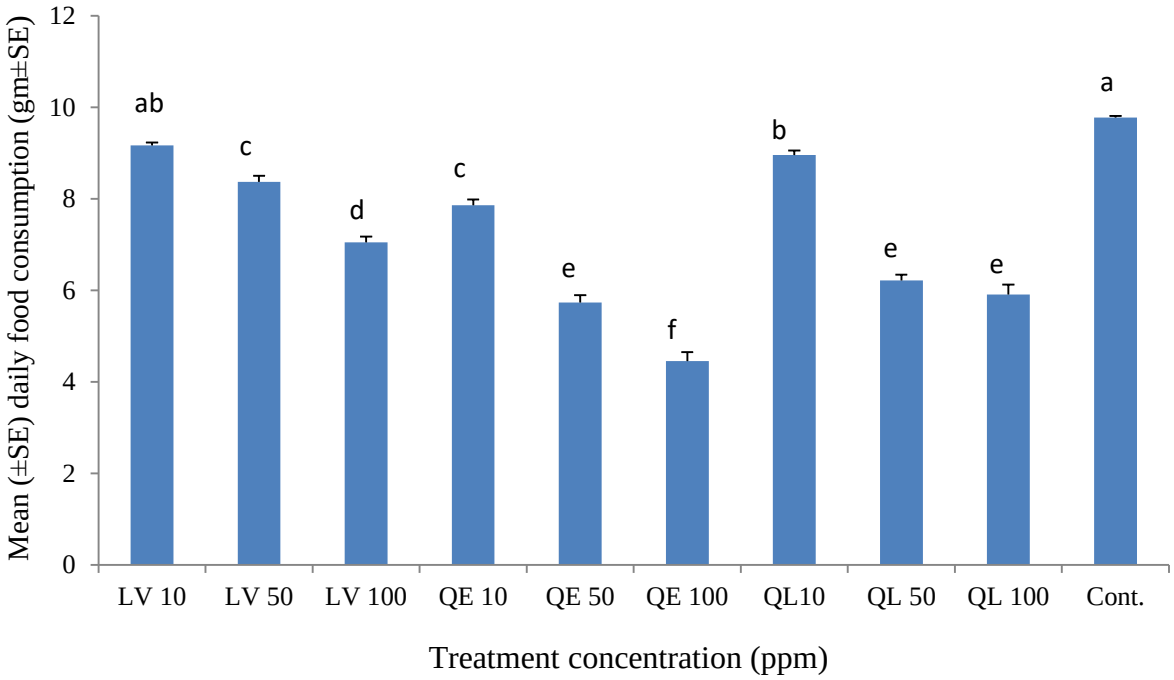
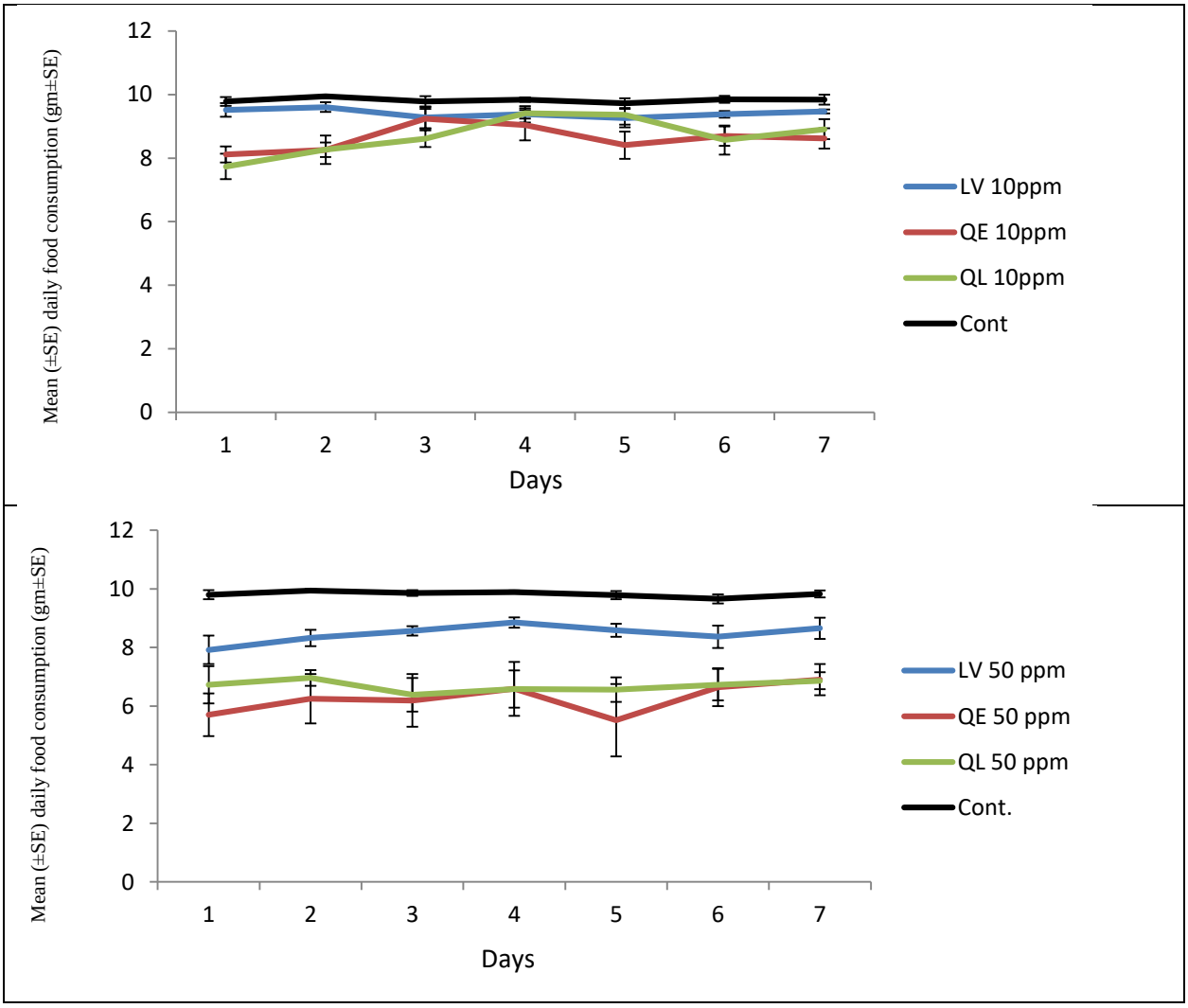


Figure 4; Mean (±SE) daily consumption over a 14 day feeding period of male adult *Arvicanthis niloticus* consuming bait containing contraceptive hormones at three concentrations (10, 50 and 100 ppm). Letters show significant differences between treatments by the post-hoc Tuckey test at the 95% confidence interval.

The mean daily bait consumption clearly indicate the difference of the three treatments with LV more consumed followed by QL and QE. This is clearly evident at higher concentration (50 and 100 ppm) (Figure 5 and 6).



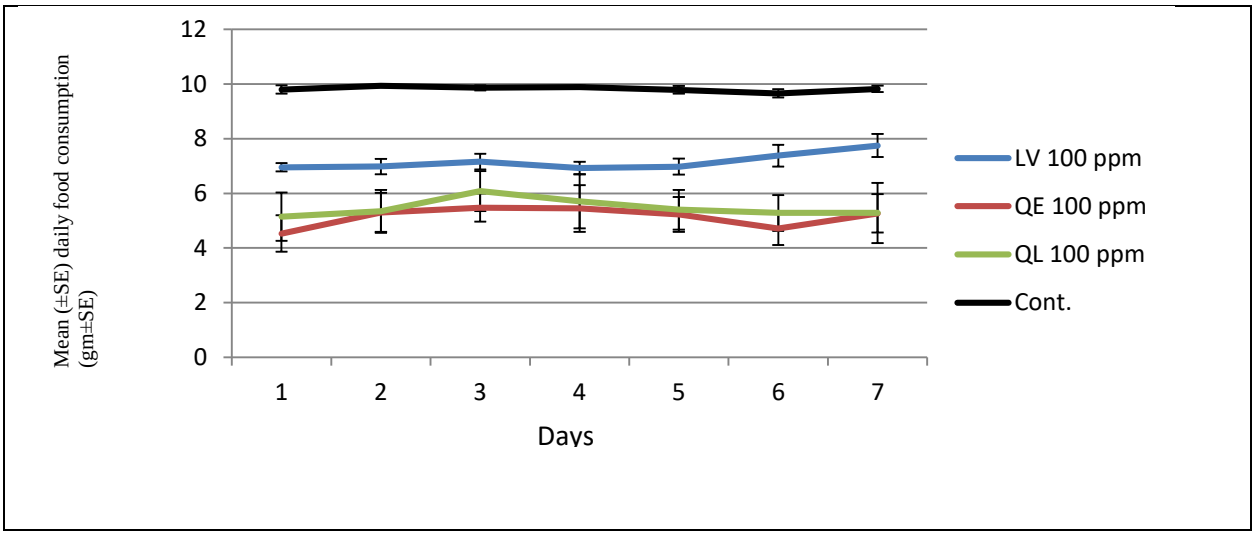
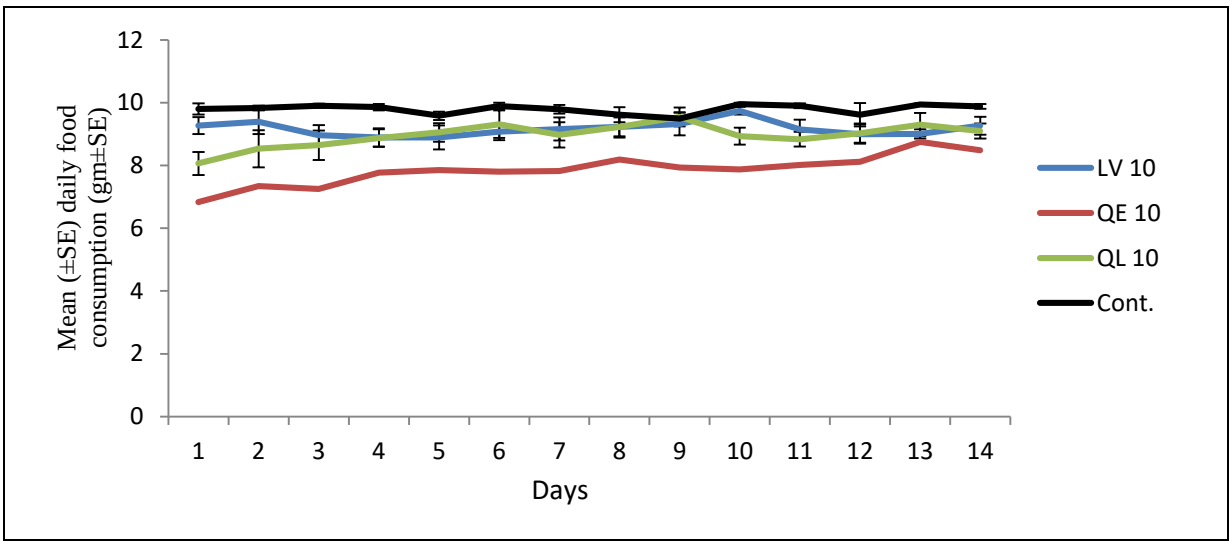


Figure 5; Mean daily bait consumption rate by female *Arvicanthis niloticus* of bait containing levonorgestrel (LV), quinestrol (QE), and quinestrol/ levonorgestrel (QL) combination for 7 days**.**



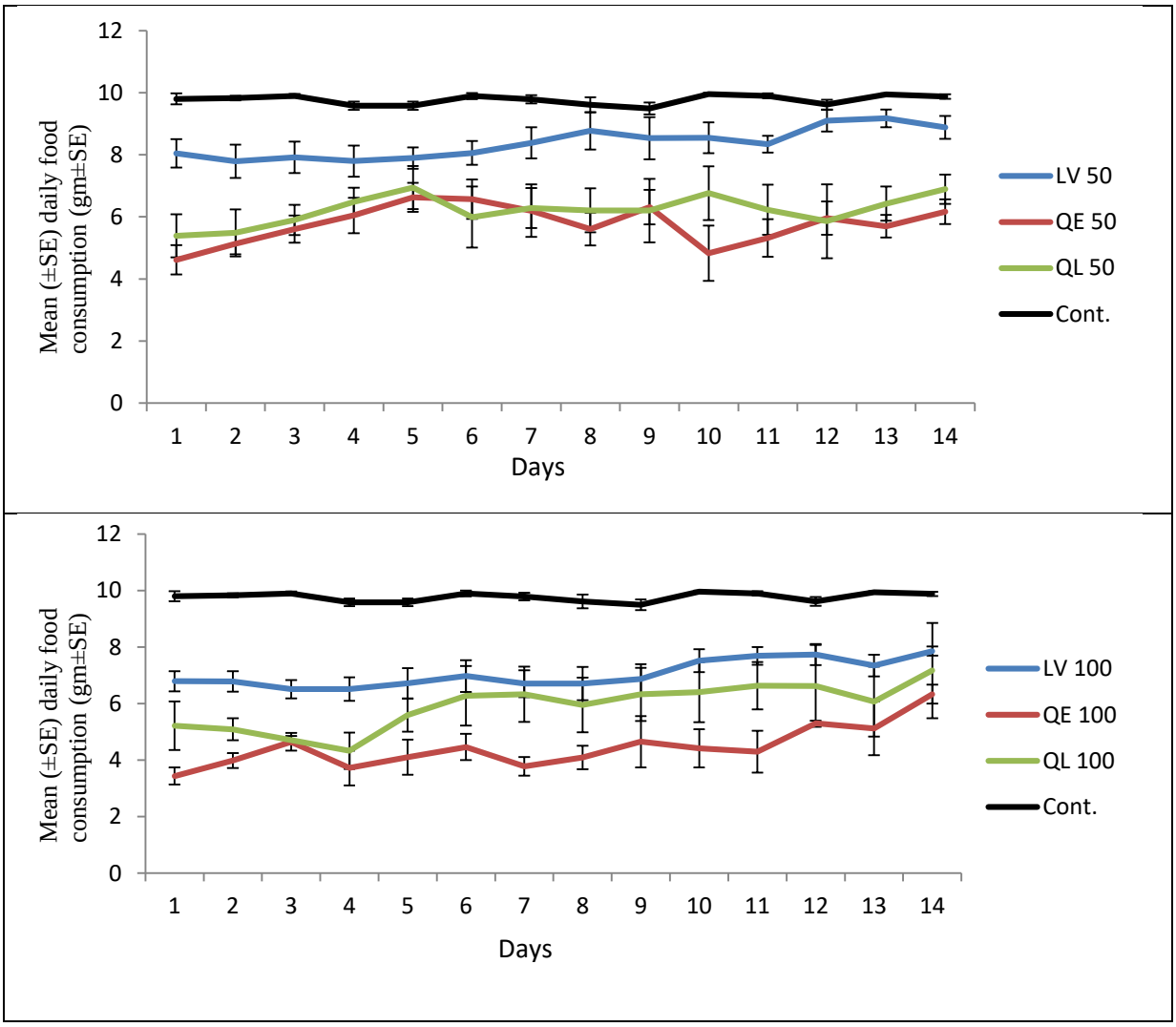


Figure 6; Mean daily bait consumption rate by male *Arvicanthis niloticus* of bait containing levonorgestrel (LV), quinestrol (QE), and quinestrol/ levonorgestrel (QL) combination for 14 days.

Most treatments showed significant mean differences in bait consumption compared to control animals, with consumption rates decreasing as the concentration of fertility control hormones increased. Quinestrol at 100 ppm ($t = -17.22$, $p < 0.0001$) and the combined treatment at 100 ppm ($t = -15.43$, $p < 0.0001$) demonstrated strong statistical differences. All treatments at 10ppm were showed minimal significance. Quinestrol at 50 ppm and the combination treatment at the same concentration showed significant reductions in mean differences (-3.02, $t = -8.09$ and -2.33, $t = -9.34$, respectively, $p < 0.001$) compared to the control group.

Table 4; the effect of fertility control compound displaying mean difference on bait acceptance of *A. niloticus*

TG	MBC	CMD	t<value
Control	9.83		
LV10	9.29	-0.54	-1.25
LV50	8.42	-1.41	-1.71*
LV100	7.20	-2.63	-2.09**
QE10	8.24	-1.59	-2.35*
QE50	6.15	-3.68	-8.09***
QE100	4.79	-5.04	-17.22***
QL10	8.33	-1.50	-1.53*
QL50	6.49	-3.34	-9.34***
QL100	5.63	-4.20	-15.43***

Note; "", "**" and *** indicates significance level at 10%, 5% and 1% respectively; TG= treatment group, MBC= mean of bait consumption, CMD= comparison of mean difference*

4.1.1 Interaction effect between treatment and sex on bait acceptance

Fertility control compounds showed negative mean differences in bait intake compared to the control group of both in male and female except with LV at 10 ppm, indicating decreased consumption among treated animals. Generally, in all treatments it was observed that bait acceptance by the female showed a slight increment compared to males.

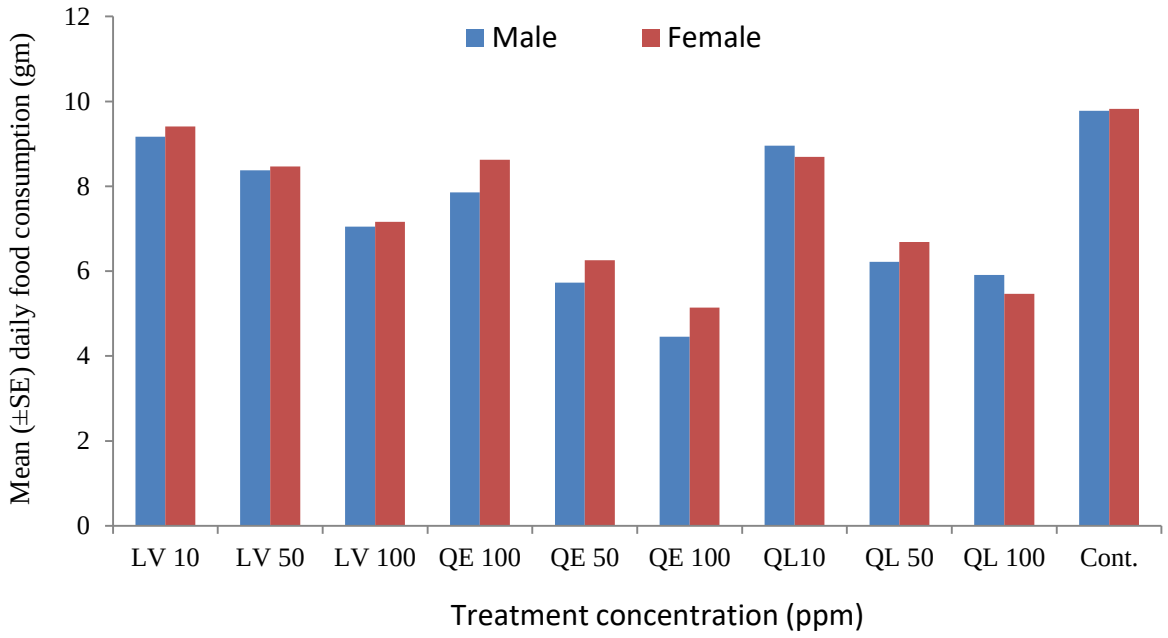


Figure 7; Interaction effects of treatment and sex on bait acceptance by *Arvicanthis niloticus*

Both male and female rats exhibited reduced bait acceptance as treatment intensity increased (Table 5). Notably, treated females consumed slightly more bait than males, especially in the LV50, LV100, QE10, QE50, and QE100 treatment groups, where the differences were statistically significant ($p < 0.05$). Females showed greater bait acceptance, especially under higher stress from Levonorgestrol and Quinestrol treatments, indicating they are more responsive than males. For example, males had mean differences of -3.02 ($t = -10.26$) in QE50 and -4.63 ($t = -12.85$) in QE100, both significantly lower ($p < 0.0001$) than females. Overall, males consistently exhibited lower bait consumption across most treatments. In the LV10 treatment, males had a mean of 9.17 compared to 9.41 for females, with no significance ($t = -2.10$).

Table 5; Interaction effect between treatment and sex on bait consumption male and female of *A. niloticus* in LV), QE and QL Combination

TG	Male *A. niloticus*			Female *A. niloticus*		
	MBC	CMD	**t-value**	MBC	CMD	**t-value**
Control	9.79			9.87		
LV10	9.17	-0.62	-1.40	9.41	-0.45	-1.32
LV50	8.37	-1.41	-1.81*	8.47	-1.45	-2.93**
LV100	7.05	-2.74	-2.62**	7.16	-2.71	-7.20**
QE10	7.86	-1.93	-2.51**	8.63	-1.24	-2.78**
QE50	5.76	-4.03	-10.26***	6.26	-3.61	-7.44***
QE100	4.44	-4.63	-12.85***	5.14	-4.72	-11.71***
QL10	8.96	-0.83	-2.82**	8.69	-1.17	-1.90*
QL50	6.21	-3.58	-7.39***	6.68	-3.19	-6.14**
QL100	5.93	-3.85	-10.53***	5.45	-4.42	-10.95***

Note; "", "**" and *** indicates significance level at 10%, 5% and 1% respectively; TG= treatment group, MBC= mean of bait consumption, CMD= comparison of mean difference*

4.2 The effect of different treatment on body weight change of *A. niloticus*

The results showed significant weight loss ($p < 0.001$) in animals across most treatments compared to the control group. Treatments such as QE50, QE100, QL50, and QL100 resulted in substantial weight reductions. Quinestrol (QE) at 100 ppm had the largest mean difference of - 47.8gm (t = -10.48), followed by QL100 at -39.01gm (t = -8.95). In contrast, treatments like LV10 and QL10 did not significantly ($p > 0.05$) change body weight with LV10 showing mean difference of -13.01 (t = -1.10) and QL10 at -10.51 (t = -2.51), suggesting a slight impact on fertility.

Table 6; the effect of different treatment on body weight change of *A. niloticus*

TG	BWC	CMD	t-value
Control	106.52		
LV10	98.51	-8.01	-1.10
LV50	89.89	-16.63	-2.2**
LV100	79.47	-27.05	-2.58**
QE10	82.22	-24.29	-2.39**
QE50	69.49	-37.03	-8.84***
QE100	57.10	-49.42	-10.48***
QL10	91.10	-15.41	-2.51**
QL50	74.03	-32.49	-7.60***
QL100	62.16	-44.36	-8.95***

Note; "", "**" and *** indicates significance level at 10%, 5% and 1% respectively; TG = treatment group, BWC= mean of body weight change, CMD= comparison of mean difference*

4.2.1 Interaction effect between treatment and sex on body weight change of *A. niloticus*

All treated animals showed significant weight loss ($p < 0.001$) compared to the control except LV and QL at lower concentration. The body weight of treated animals decreased with increasing time of consumption to the contraceptive hormones (Table 7). Treatments like QE50 ppm were showing a mean weight of 70.94 gm. and a *t* - value of -8.19 with high statistical significance ($p < 0.001$) in weight reduction. In contrast, LV10 and QL10 had no significant ($p > 0.05$) impact on body weight changes in both male and female animals. However, QE100, with a mean weight of 57.51 gm ($t = -11.56$), led to excessive bait reduction due to a higher dose, resulting in strong statistical significance ($p < 0.001$) in weight loss.

Table 7; the effect of treatments in male and female on body weight change of *A. niloticus*

TG	Male *A.niloticus*			Female *A.niloticus*		
	BWC	**CMD**	**t-value**	**BWC**	**CMD**	**t-value**
Control	115.15			97.88		
LV10	101.13	-14.02	-1.49*	90.89	-6.99	-1.43*
LV50	90.33	-24.82	-4.59**	81.46	-16.42	-2.576**
LV100	84.77	-30.38	-2.13**	72.17	-25.71	-2.91**
QE10	96.84	-18.31	-1.59*	77.60	-20.28	-2.80**
QE50	72.94	-42.21	-6.19***	61.03	-36.85	-9.55***
QE100	56.69	-58.46	-8.23***	50.51	-47.37	-13.56***
QL10	98.11	-17.04	-1.55*	84.89	-12.99	-2.12**
QL50	79.74	-35.41	-5.15***	68.31	-29.57	-8.04***
QL100	61.84	-53.31	-7.81***	54.49	-43.39	-12.43***

Note; "", "**" and *** indicates significance level at 10%, 5% and 1% respectively; TG= treatment group, BWC= mean of body weight change, CMD= comparison of mean difference*

4.3 Body Weight Changes in *A. niloticus*: Initial and Final Measurements during Experimental Treatments

The study on body weight loss in *A. niloticus* using Levonorgestrel, Quinestrol, and their combinations exposed significant weight changes across treatment groups (Figure 8). The control group showed weight gain, with initial (Iw Mean) and final weights (Fw Mean) of 101.5 and 110.33, respectively, resulting in an increase of 8.83 (8.69%). The study indicates decreasing the weight of animal with increased the concentration of treatments.

Body weight changes in *A. niloticus* during experimental treatments

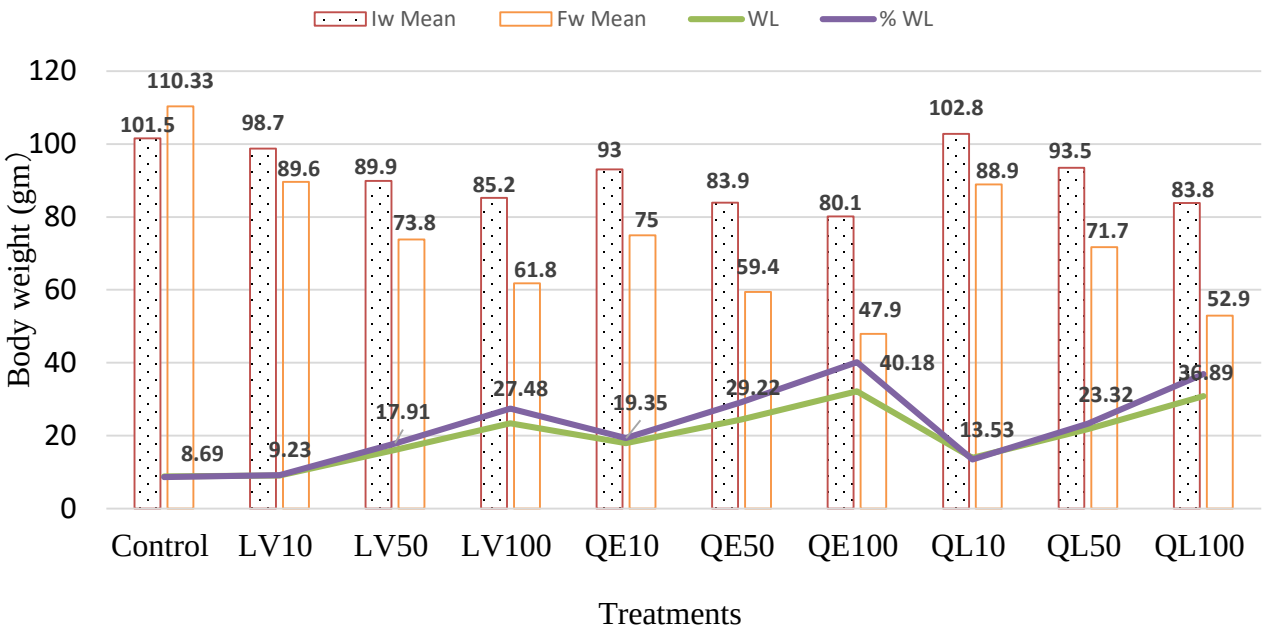


Figure 8; Body weight change of the animal both male and female in the experimental treatment

Note: *IW= initial weight, FW = final weight, WL = weight loss, %WL = percentage of weight loss*

4.4 The Effects of fertility control compound on Reproductive Organs of *A. niloticus*

4.4.1 Evaluation the effect of hormones on male reproductive organs (testis, epididymis and seminal vesicles weight)

The results indicated a significant effect of the contraceptive hormones on the mean weight of male reproductive organs compared to the control group ($F_{9, 40} = 21.952$, $p < 0.001$) (Figure 9). Higher weight loss was observed at concentration of QE 100, QL 100 and QE 50 ppm.

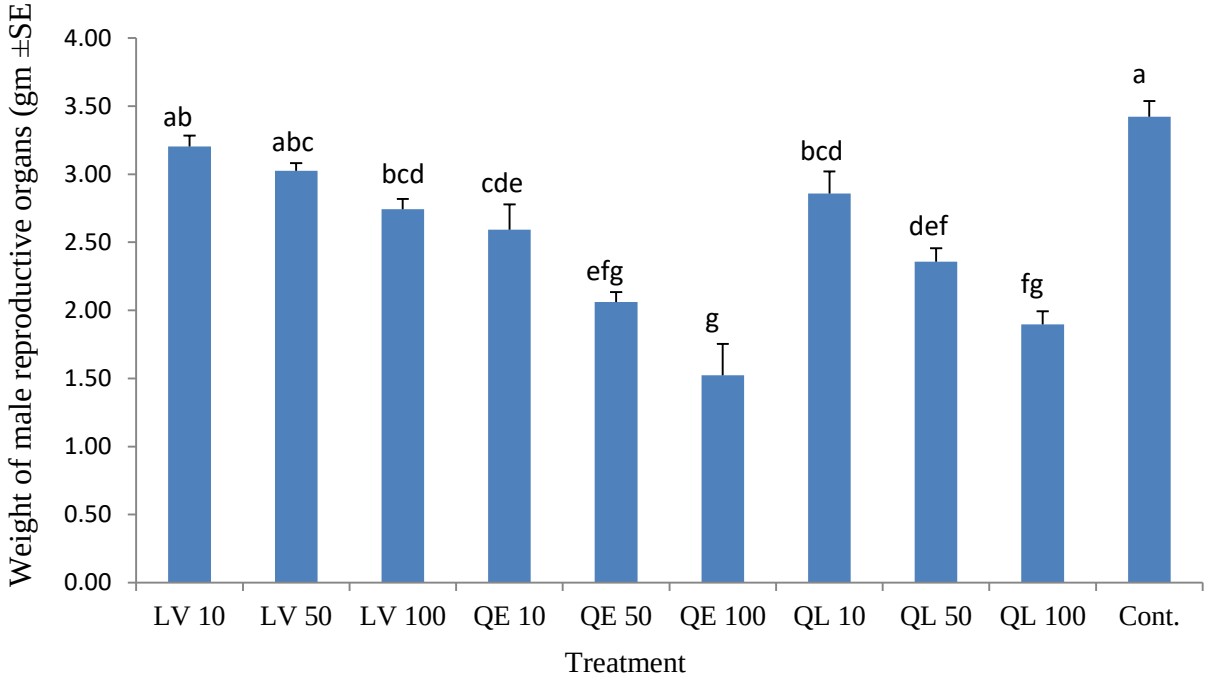


Figure 9; Mean weight (± SE) of reproductive organ (testis, epididymis, and seminal vesicle) of treated and control male Arvicanthis niloticus. Letters show significant differences between treatments by the post-hoc Tuckey test at the 95% confidence interval

4.4.2 Sperm count and motility

Sperm counts of treated animals decreased significantly ($F_{9, 40} = 21.448$, $p < 0.001$) compared to control group (Table 8). Lower sperm counts were observed in QE at 50 and 100 ppm as well as QL 100 ppm. Similarly sperm motility of treated group decreased significantly ($F_{9, 40} = 48.238$, $p < 0.001$) compared to control group with lower motility being observed in QE 100 ppm (36.80%), and QE 500 ppm (43.60%).

Table 8; Mean sperm concentration/count of *Arvicanthis niloticus* treated with contraceptive hormones (LV, QE and QL) at 10. 50 and 100 ppm.

Treatment	Sperm count (* 10^6)	Sperm mobility (%)
LV 10	65.28 ± 6.48b	75.50 ± 1.75b
LV 50	54.56 ± 3.90bcd	67.50 ± 1.72bc
LV 100	46.70 ± 0.99bcd	64.00 ± 1.37cd
QE 10	29.98 ± 5.35bcd	56.2 ±2.34d
QE 50	18.72 ± 0.62cd	43.6 ± 3.08e
QE 100	17.36 ± 0.48cd	36.8 ± 2.40e
QL 10	38.70 ± 4.13bcd	62.5 ± 1.78cd
QL 50	26.16 ± 4.99bcd	54.5 ± 2.42d
QL 100	19.50 ± 3.13cd	55.2 ± 2.00d
Cont.	1619.933 ± 270.73a	87.6 ± 1.84a

Letters show significant differences between treatments by the post-hoc Tuckey test at the 95% confidence interval.

These findings indicated that there was a detrimental impact of the treatments on sperm quality with Quinestrol showing the most significant negative effects on both sperm count and motility (Figure 11 and 12).

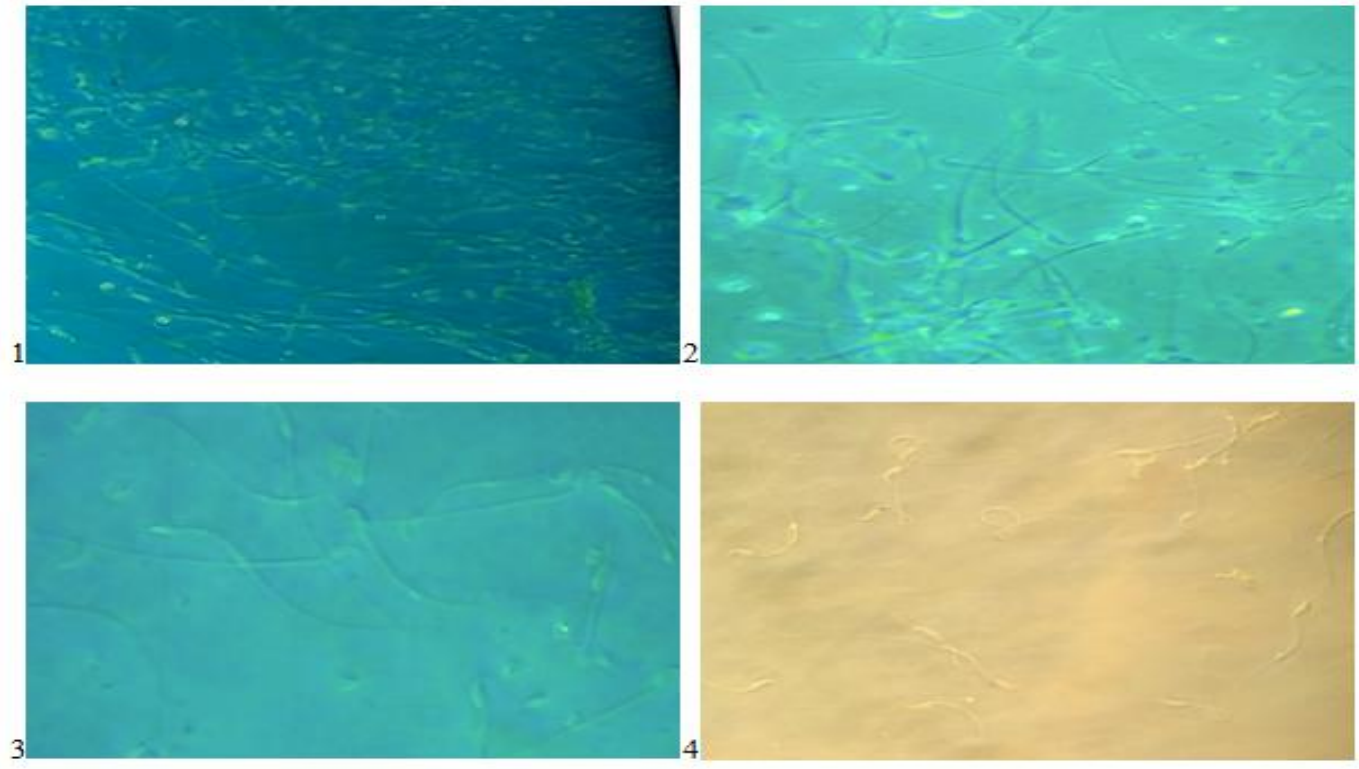
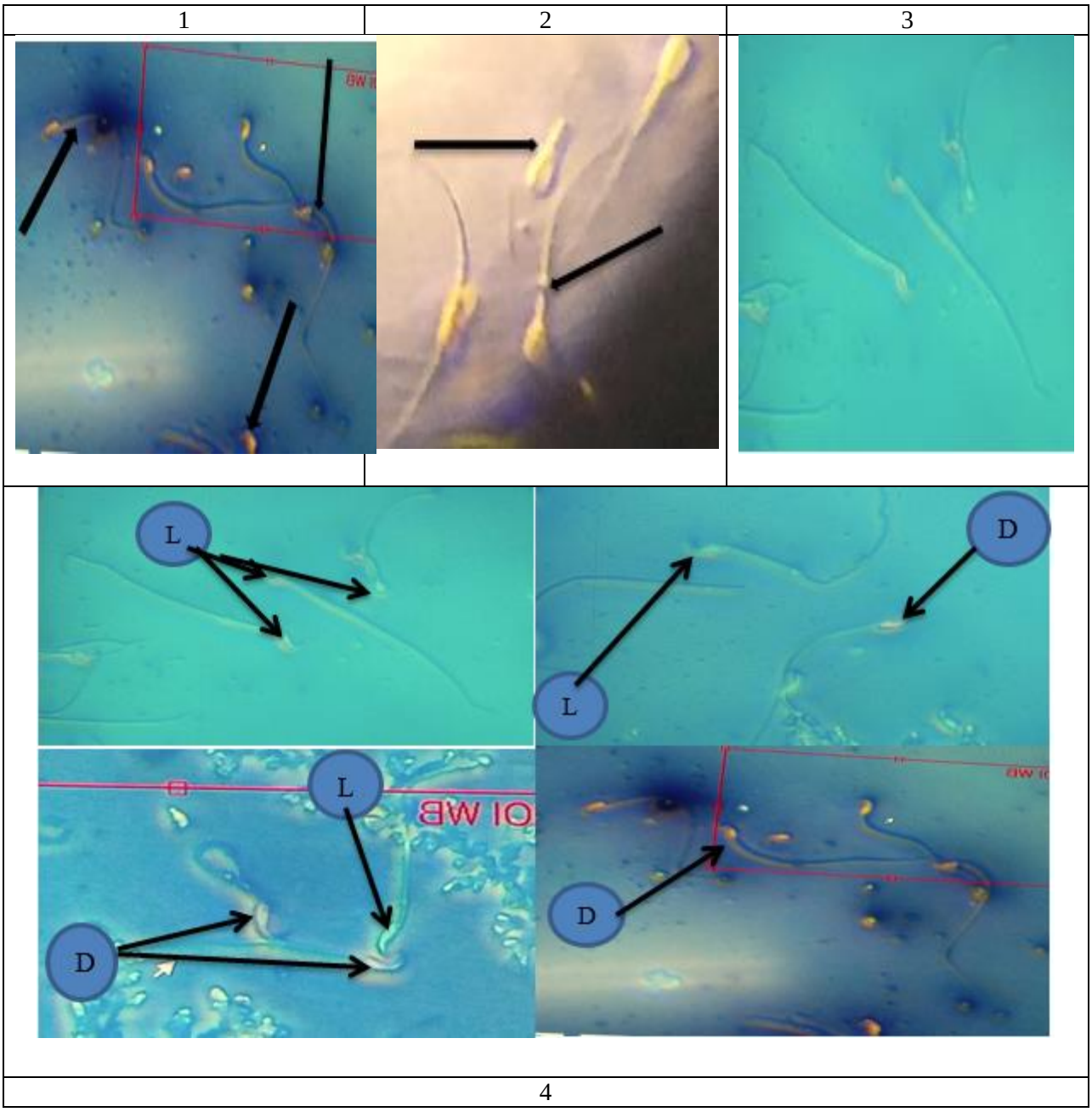


Figure 10; Sperm under microscope in treated and untreated of male *A. niloticus*

1and 2 = sperm of untreated animal, 3 and 4 = sperm of treated animal



Figure; 11 the effect of contraceptive hormones on sperm morphology of male *Arvicanthis niloticus***.** 1 detached head and belt tail; 2 detached head and irregular neck; 3. Sperm of untreated animal 4. Live (L) and dead (D) sperm of male *A. niloticus,*

4.4.3 Effect of hormones on female reproductive organs (uterus and ovaries) of *A. niloticus*

The mean weight of the female reproductive organ (uterus and ovaries) of animals showed significant difference among treatments ($F_{9, 40} = 7.92$, $p < 0.001$). Overall, the weight of the uteri and ovaries of treated animals increased with increasing hormone concentration. The weight of uteri and ovaries in lower concentration of treatments were not significantly different ($p > 0.05$) with the control group (Figure 13). The slight changes in weight, particularly due to edema in the female reproductive organs were most obvious in the group treated with the higher concentration of Quinestrol.

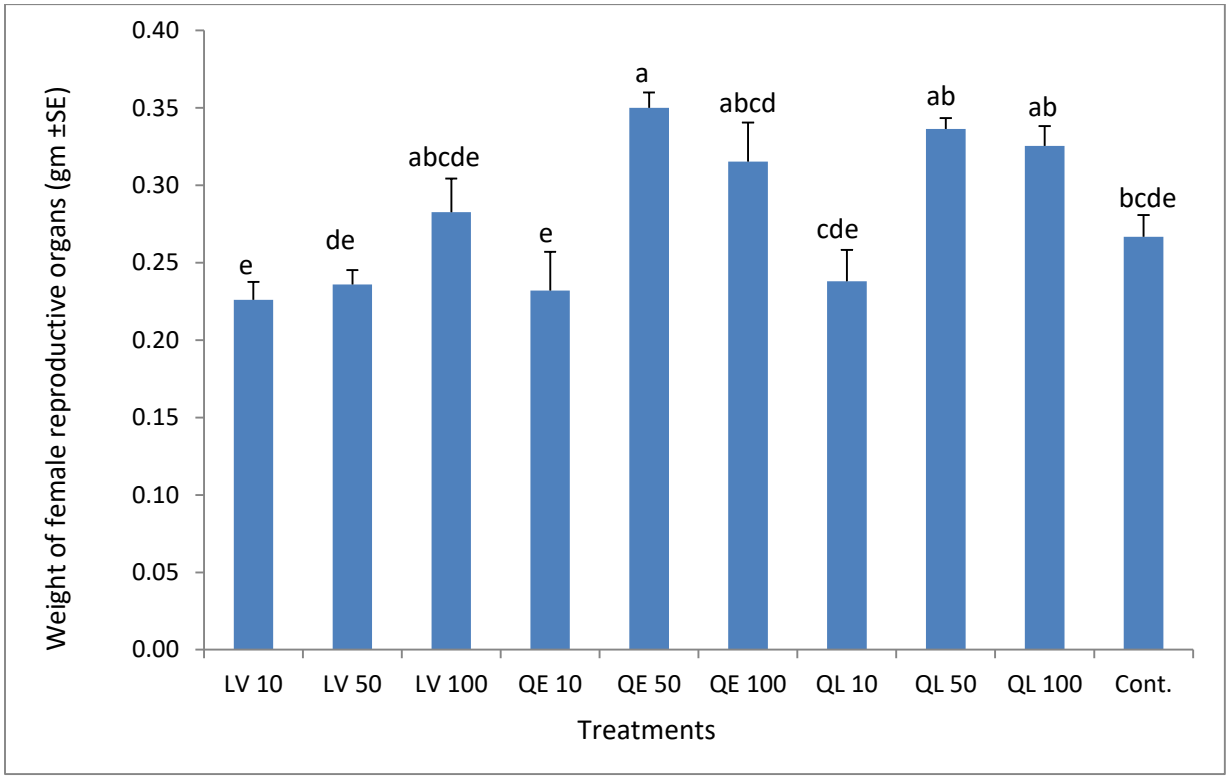


Figure 12; Mean weight (± SE) of reproductive organ (uterus and ovaries) of treated and control female *Arvicanthis niloticus***.** Letters show significant differences between treatments by the post-hoc Tuckey test at the 95% confidence interval**.**

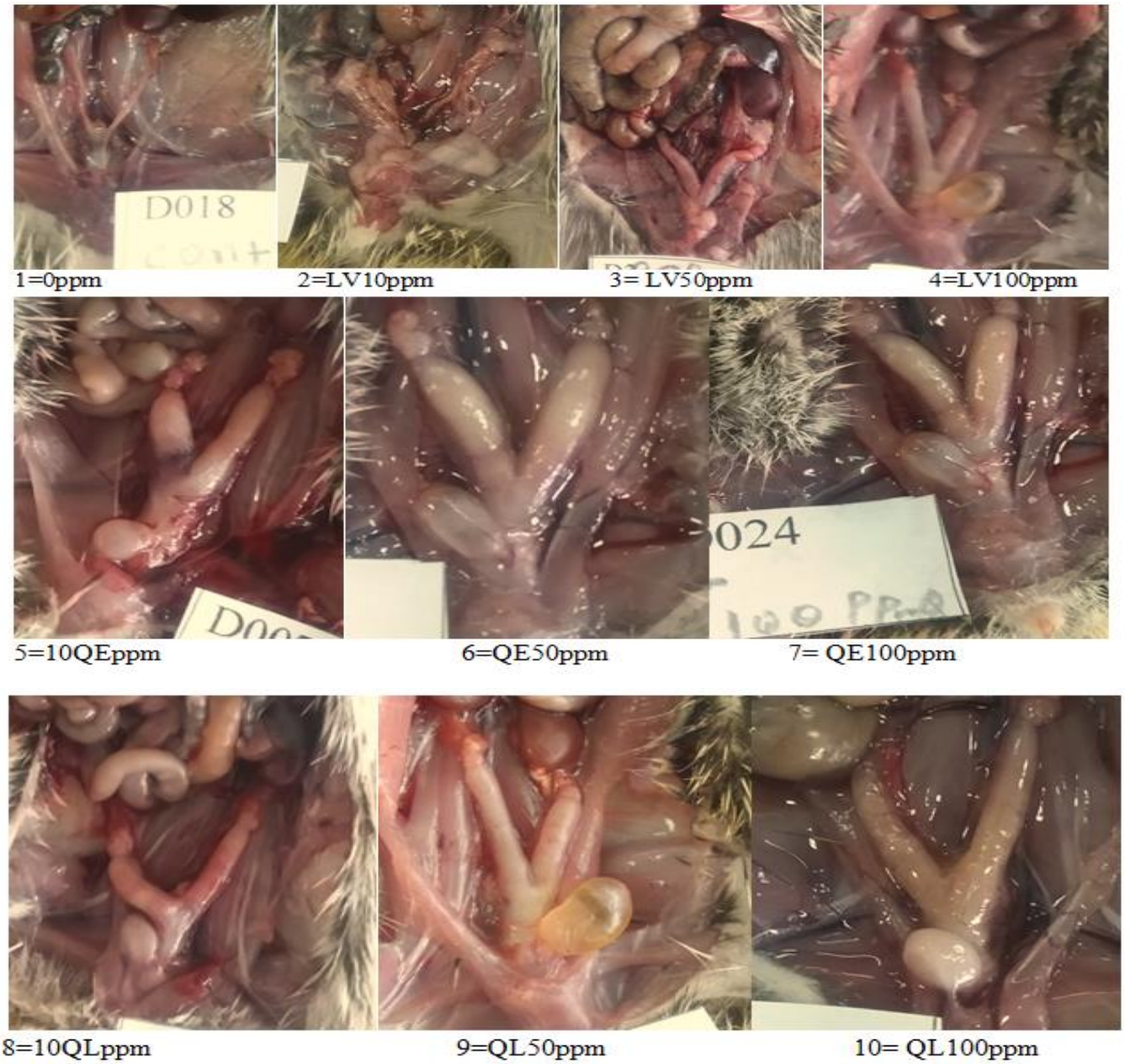


Figure 13; the effect of each treatment in reproductive organs of female *A. niloticus*

4.4.4 Effects of QE treatment at concentration of 50 ppm in pregnancy and Birth size of *Arvicanthis niloticus*

There were significant differences in reproductive results based on the treatment conditions. The control group consisting of both control females and males, achieved pregnancy rate with an average litter size of seven. In contrast, the pairing of untreated females with treated males resulted in an average litter size of three. Particularly, treated females paired with either control or treated males did not achieve any pregnancies. In both treated animals and treated female with untreated male animals were not achieve any pregnancy. However in other paired animals 40% number of pregnancies were observed (Table 9).

Table 9; the effects of QE treatment at concentration of 50 ppm in pregnancy and birth size of *A. niloticus*

QE 50ppm	No Females	Number of Pregnancy	% number of Pregnancy	Mean birth size
UF with UM	**5**	4	80	7
UF with TM	**5**	2	40	3
TF with UM	**5**	0	0	-
TF with TM	**5**	0	0	-

Note; UF= Untreated female; TF = Treated female; UM = Untreated male; TM = Treated male

CHAPTER FIVE

5. DISCUSSION

Fertility control containing quinestrol and/or levonorgestrel with different concentration on *Arvicanthis niloticus* was evaluated. The bait consumption was significantly lower in animals fed on bait containing the quinestrol and/or levonorgestrel. The study showed that the increment of contraceptive hormones significantly decreased bait consumption, with some extent differences between sexes in bait acceptance. Female animals consumed more bait than males which indicated that a potential variation in response to hormonal treatment. This study showed that there was decreased bait consumption with increased the level of the contraceptive compounds and the number of the days. Similar studies showed that lower consumption rate with increased concentration for *M. natalensis* (Massawe et al., 2018) and *Rattus rattus* (Selemani et al., 2022).
The consumption of the bait in levonorgestrel (LV) treated animals indicated that there was higher bait acceptance than the other hormones of Quinestrol (QE) and its combination of Levonorgestrol and Quinestrol (QL) particularly at lower concentration 10ppm. The female animals consumed more bait than males which indicated that a potential variation in response to hormonal treatment. For example Mhamphi (2016) and Massawe et al. (2018) showed *M. natalensis* females consume higher than males that fed on quinestrol and/or levonorgestrel. In levonorgestrel there was high consumption of baits with comparing to other fertility control agents and its control groups. In Quienestrol as well as its combination of Levonorgestrol and Quienestrol there was low bait acceptance compared with levonorgestrel level of compounds and with control group of animals. This result is compared to Selemani (2022) which shows the acceptance of bait treated with Quinestrol at 10 ppm, 50 ppm, and 100 ppm was significantly lower than that of control groups and Levonorgestrol treatments on *Rattus rattus*. Similar results is also reported on *Rattus argentiventer* by Alexander et al. (2022). However, Wang et al. (2011) and Shi et al. (2020) reported that Quinestrol had no effects on the overall bait consumption of male *Lasiopodomys brandtii.* The reduced palatability of Quinestrol is attributed to its unpleasant odor which may deter animals from consuming it. Despite this, the use of Quinestrol could potentially enhance fertility control in animals, especially when considering its effects on bait acceptance changes and reproductive organs compared to other contraceptive options. This finding is consistent with the study of Massawe et al. (2018) which reported that the increased concentrations of contraceptive

hormones become in reduced bait consumption in treated animals. This indicates that the presence of these hormones may make the bait less appealing that leading to decreased intake among the animals. In their activities of animal when the concentration of fertility control agents was increased the consumption of baits were decreased and becomes to die. However, this result is contrast to the Liu et al. (2012) that founds no significant differences in bait consumption between Plateau pikas given Quinestrol (QE) and Levonorgestrol (LV). This shows that both hormones are equally accepted by the pikas, making them similarly viable options for baiting acceptance.

In the present study, animals treated with Quinestrol, levonorgestrel and their mixture were observed to show lower body weight compared to the control animals. The body weight loss of treated animal was higher in Quienestrol and mixed of QL than the animal that treated with levonorgestrel and control animals in both male and female. Several studies were consistent with this finding such as Selemani, (2021) on commensal Rat *(Rattus rattus)*, Massawe et al. (2018) on *M. natalensis* which indicates the weight loss was higher in Quinestrol and combination of Levonorgestrel and Quinestrol treated animals than in levonorgestrel treated animals alone. This is because related to low consumption of Quienestrol and its combination of Levonorgestrol and Quienestrol (QL) during delivering of the contraceptive bait and due to unidentified physiological consequence of the fertility control agents. The results are in line with findings of Alexander et al. (2022) on rice field rat (*Rathhus argentiventer*), Selemani, (2021) on Commensal Rat *(Rattus rattus*), Massawe et al. (2018) on *M. natalensis* and Lv et al. (2012) on Mongolian gerbils (*Meriones unguiculatus*), indicating that contraceptive baits treated with Quinestrol, either alone or in combination with levonorgestrel (QL), resulted in greater body weight loss compared to control animals and baits containing levonorgestrel alone. This indicates that Quinestrol has a more pronounced effect on body weight reduction in these species, potentially affecting their overall health and reproductive dynamics. Treated animals with high concentration of these hormones became extremely ill and eventually died from malnutrition. Some studies have also shown that weight loss occurs related to lower feeding rates (Lv and Shi 2011, Massawe *et al.* 2018). In this study treated animal with Quienestrol and combination of Levonorgestrol and Quienestrol (QL) at concentration of 100ppm significantly reduced their weight than from those of untreated animals. The effect on body weight loss of treated animal with Quienestrol was extremely higher than animals treated with levonorgestrel and combination of the two fertility compounds. In contrast,

other research indicated that the body weight of *R. nitidus* was not affected by consuming a quinestrol bait (Liu *et al.* 2013) nor was the weight of male *B. indica* reduced by quinestrol (Sidhu *et al.* 2020). Omar et al. (2023) also reported no significant changes in the body weights of treated males and females at lower concentration. This highlights the importance of assessing how different rodent species responses to these contraceptive hormones. These variations might be related to differences to their sensitivity to tasting bitter compounds such as quinestrol, which emphasized the importance of evaluating different rodent species behavior in relation to contraceptive bait formulation and concentration of hormones employed.

The reproductive organs of both male and female of *A. niloticus* were negatively affected by the contraceptive hormones. The finding of this study demonstrated reproductive organs of males, sperm concentration/count and sperm motility were negatively affected by the hormones. The results of this study showed that hormones had a negative impact on male reproductive organs, sperm concentration/count, and sperm motility. The consumption of quinestrol at all concentrations, that is 10, 50 and 100 ppm reduced the weight of the male reproductive organ (testis, epididymis, and seminal vesicle) while such reduction is achieved only at higher concentration for LV 100 pm and QL at 50 and 100 ppm. Quinestrol at 50 and 100 ppm reduced the weight of male rats by 39.73 – 55.46%, the combined hormones quinestrol and levonogestrel also showed to reduce the weight by 31.08 – 44.58%. On the other hand levonorgestrel had less impact. These findings are similar to the results reported by Omar et al. (2023) which showed quinestrol reduced the weight of the reproductive organ of African grass rat (*A. niloticus*), Selemani et al. (2021) who indicated quinestrol reduced the reproductive organ of commensal rat (*Rattus rattus*), Massawe et al. (2018) which reported reduction of multimammate rat (*M. natalensis*) as well as Zhang *et al*. (2006), Wang *et al*. (2011), Liu *et al.*(2012) and Li *et al*. (2014) who studied the effects of quinestrol in male greater long tailed hamsters (*Tscherskia triton*), Brandt's voles (*Lasiopodomys brandtii*), Plateau pikas (*Ochotona curzoniae*) and white mice at different concentrations.

Sperm count and motility are routinely used as indicators of the reproductive potential of male. The findings from the current study further indicate levonorgestrel and/or quinestrol at 10, 50 and 100 ppm decreased the sperm count and sperm motility. The sperm count of male *A. niloticus* fed on quinestrol at 50 and 100 ppm and combination of levonorgestrel and quinestrol at 100 ppm were highly reduced than the other treatments at different concentration including untreated *A. niloticus*. In Brandt's voles, quinestrol had lowered the sperm count (Zhao *et al.*, 2007). In Sprague-Dawley rats, quinestrol and combination levonorgestrel and quinestrol had decreased the sperm count (Liu *et al.,* 2013). Moreover, Massawe et al. (20180 reported that quinestrol and combination of quinestrol and levonorgestrel reduced the sperm count and motility of male *M. natalensis* by more than 95%. The finding of this study also indicate the male *A. niloticus* fed on quinestrol and combination of quinestrol and levonorgestrel at 50 and 100 ppm reduced the sperm count by more than 85%. Selemani et al. (2022) found that quinestrol and combination of quinestrol and levonorgestrel at concentrations of 10 ppm and 50 ppm reduced the sperm count and sperm motility of *R. rattus*. Basically, Quinestrol and combination of Levonorgestrol and Quinestrol has been depressed the sperm motility as well as reduced the weight of the reproductive organs of *A. niloticus*. This was similar with the study of Liu et al. (2013) that determined that Quinestrol increases estrogen level consequential in a reduction in spermatogenesis and suppression the production of sperm.

The same to male reproductive organs in female *A. niloticus* treated with these hormones were a significant effect on their reproductive organs (uterus and ovaries) than control groups. The current study indicates that the weight of uterus and ovaries treated with Quienestrol and combination of Levonorgestrol and Quienestrol was increased. The female treated with quienestrol at 10 ppm, 50 ppm, 100 ppm concentration levels were an obvious uterine edema and changes morphology of the organ. The consequence of the hormone on their reproductive organs has been greater effect at 50 ppm and 100 ppm than the other fertility control treatment baits. Comparable results were described in Selemani, (2021) on Commensal Rat *(Rattus rattus),* Lv and Shi, (2011) on female of Mongolian Gerbils (*Meriones unguiculatus*) and in Massawe et al. (2018) on multimammate mouse (*M. natalensis)* treated with quienestrol and supplied baits in some way combination of Levonorgestrol and Quienestrol that have been observed on structural changes of the uterus and ovaries. Sickness, death and unwanted physiological activities were observed with increased the level and concentration of the fertility compounds. However, the results from this study contrast

with those of Liu et al. (2013) on female *Rattus nitidus* and Zhao et al. (2007) on female *Brandt's voles*, which conveyed that Quinestrol did not goes to a reduction in uterus weight or changes in morphology of the reproductive organs of female animals. This discrepancy indicates that the effects of Quinestrol may vary significantly among different species or experimental conditions. There was also higher sickness as well as death at Quienestrol and its combination of Levonorgestrol and Quienestrol at 100 ppm levels. Different studies like Su et al. (2017) determined the structural changes and edemas uterus and ovaries of the treated animal that a result of increased abnormal amount of estradiol and progesterone level that becomes bulge of the uterus. The fertility compound of Quienestrol at 50 ppm concentration was selected for observation of its effectiveness on pregnancy and litter size of *A. niloticus.* Pregnancy and litter size of *A. niloticus* was affected by the contraceptive baits of Quienestrol at 50 ppm concentration. Treated males paired with untreated females had smaller litters and lower pregnancy success compared to control pairs that indicates a reduced reproductive health in the treated males. In this study there was no significance difference observed between treated females paired with treated males and treated females paired with untreated males rather than untreated female met with treated male of *A. niloticus*. This indicates that the hormone is more effective in treated female *A. niloticus* than male or if both animals were treated. These results were consistent with the study Selemani, (2021) on Commensal Rat *(Rattus rattus*), Massawe et al. (2018) on *M. natalensis* which shows no pregnancy was observed in treated females paired with treated males and treated females paired with untreated males. This shows the fertility compound of Quienestrol at concentration of 50 ppm were more effective when either both animals were treated or either female animals were treated. There was no pregnancy in treated females due to the development of edema in the uterus. This was also comparable with Zhao et al. (2007) studies on Brandi's vole (*Lasiopodomys brandtii*) who reported no pregnancy in treated females mated with untreated male as well as reduced litter size from untreated females Brandi's vole mated with treated males. The result on studies of Massawe et al. (2018) on *M. natalensis* also indicates that the pregnancy and litter size was reduced when both sexes were treated with contraceptive baits of fertility compounds. This due to both male and female animals treated with contraceptive baits exhibited low reproductive rates and significant suppression of sperm cells in males. However, this study was opposing from Mhamphi (2016) in multimammate rats (*M. atalensis)* which showed that there were no significant differences between untreated females paired with treated males and treated females paired with treated males. Based

on this, the present studies encouraged the Quienestrol at concentration of 50 ppm would be used as per fertility limiting hormone for *A. niloticus* rodent pests.The results of the present study have demonstrated that quienestrol and combination of quienestrol and levonorgestrel have antifertility effect on *A. niloticus*. Quienestrol at 50 ppm were prominent to show antifertility effect on both sexes. This indicating a potential detrimental effect of Quinestrol treatment on reproductive success in these groups.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

In conclusion, the fertility compound of contraceptive hormones has a considerable fertility limiting effect on reproduction of *A. niloticus* rodent pest species. In both male and female, they have an effect on their consumption level and body weight change of the animals that comprising these fertility limiting compounds. As the results have shown that the weight of reproductive organs and sperm motility of male were decreased with increased abnormal sperm morphologies. In female also the contraceptive bait triggered that edema of the uterus that makes antifertility and difficult for implantation. From those of three contraceptive baits Quienestrol at 50 ppm of concentration were effective on reducing the physiological effect and greater antifertility effects on the *A. niloticus* rodent pest species than the other hormones. Hence, Quienestrol at the level of 50 ppm was effective in reproduction interruption and reducing on pregnancy and liter size of the animal. Pregnancy and liter size have been reduced in animals that treated with Quienestrol at the level of 50 ppm. However, the effect of the contraceptive bait in pregnancy and liter size of the animal were strong if both male and female animal were treated. In general, in this concern these fertility limiting hormones were a significant effect on reproduction and antifertility effects of *A. niloticus* rodent pest species.

6.2 RECOMMENDATION

- The effect of the contraceptive hormones on non-target species as well as its degradability in field condition should be studied.
- Evaluation of these contraceptive hormones should be carried under field conditions to assess the reproductive effect.
- Further studies should be carried out to evaluate the performance of all concentration fertility limiting compounds on pregnancy and birth size of *A. niloticus* rodent pest species.

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Appendix

Figure 14 *Arvicanthis niloticus*

Figure15; Materials used in the experiment and collection of animals

Figure 16; Equipment's for bait preparation in the **laboratories**

Figure 17; preparing a smear of sperm in a slide for identifying live and dead sperm

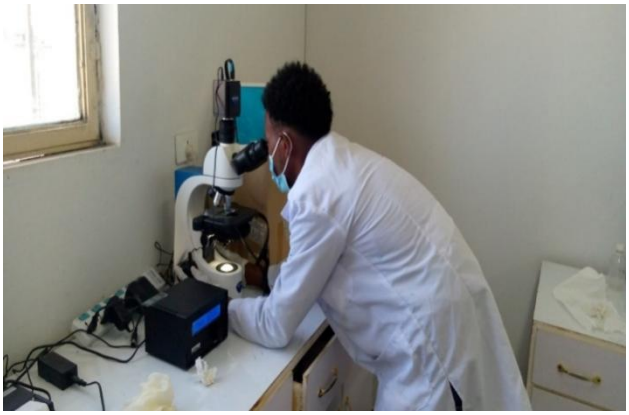

Figure 18; observing sperm parameters under microscope

Figure 19; dissecting of animals to evaluate various treatments under microscope

Daily bait acceptance of male in different concentration of treatment

ANOVA

Bait Acceptance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.241	3	8.747	14.119	.000
Within Groups	9.912	16	.620		
Total	36.154	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.020	3	6.007	5.673	.008
Within Groups	16.942	16	1.059		
Total	34.962	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.110	3	6.037	9.911	.001
Within Groups	9.746	16	.609		
Total	27.855	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.860	3	3.620	11.627	.000
Within Groups	4.982	16	.311		
Total	15.842	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.906	3	2.635	5.319	.010
Within Groups	7.927	16	.495		
Total	15.833	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.188	3	3.729	8.808	.001
Within Groups	6.774	16	.423		
Total	17.962	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.673	3	3.558	6.151	.006
Within Groups	9.254	16	.578		
Total	19.928	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.596	3	1.865	3.765	.032
Within Groups	7.928	16	.496		
Total	13.525	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.785	3	2.928	5.011	.012
Within Groups	9.349	16	.584		
Total	18.134	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.418	3	4.473	18.801	.000
Within Groups	3.806	16	.238		
Total	17.224	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.113	3	3.038	8.216	.002
Within Groups	5.915	16	.370		
Total	15.028	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.752	3	1.917	3.512	.040
Within Groups	8.735	16	.546		
Total	14.487	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.752	3	1.917	3.512	.040
Within Groups	8.735	16	.546		
Total	14.487	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.944	3	1.648	4.125	.024
Within Groups	6.392	16	.399		
Total	11.336	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	86.099	3	28.700	24.201	.000
Within Groups	18.974	16	1.186		
Total	105.073	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	71.918	3	23.973	19.461	.000
Within Groups	19.709	16	1.232		
Total	91.627	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.787	3	19.929	23.045	.000
Within Groups	13.837	16	.865		
Total	73.624	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37.804	3	12.601	12.575	.000
Within Groups	16.034	16	1.002		
Total	53.839	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.402	3	8.801	8.416	.001
Within Groups	16.731	16	1.046		
Total	43.133	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.631	3	15.210	7.973	.002
Within Groups	30.525	16	1.908		
Total	76.157	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.415	3	15.138	8.684	.001
Within Groups	27.891	16	1.743		
Total	73.306	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.614	3	18.871	12.602	.000
Within Groups	23.960	16	1.498		
Total	80.574	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40.276	3	13.425	5.800	.007
Within Groups	37.038	16	2.315		
Total	77.314	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	74.082	3	24.694	10.990	.000
Within Groups	35.951	16	2.247		
Total	110.033	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	64.014	3	21.338	15.656	.000
Within Groups	21.807	16	1.363		
Total	85.821	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.166	3	20.055	8.698	.001
Within Groups	36.891	16	2.306		
Total	97.056	19			

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	63.937	3	21.312	32.964	.000
Within Groups	10.345	16	.647		
Total	74.281	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44.498	3	14.833	22.755	.000
Within Groups	10.430	16	.652		
Total	54.928	19			

Daily bait acceptance of female in different concentration of treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.312	3	5.104	14.297	.000
Within Groups	5.712	16	.357		
Total	21.023	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.668	3	3.889	11.122	.000
Within Groups	5.595	16	.350		
Total	17.263	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.445	3	1.148	2.975	.063
Within Groups	6.175	16	.386		
Total	9.620	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.621	3	.540	1.345	.295
Within Groups	6.428	16	.402		
Total	8.048	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.691	3	1.564	3.182	.053
Within Groups	7.863	16	.491		
Total	12.555	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.393	3	1.798	4.427	.019
Within Groups	6.498	16	.406		
Total	11.891	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.507	3	1.502	5.177	.011
Within Groups	4.643	16	.290		
Total	9.150	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46.428	3	15.476	10.422	.000
Within Groups	23.760	16	1.485		
Total	70.188	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.718	3	13.239	12.204	.000
Within Groups	17.357	16	1.085		
Total	57.075	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46.998	3	15.666	10.649	.000
Within Groups	23.537	16	1.471		
Total	70.535	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.475	3	13.825	8.623	.001
Within Groups	25.653	16	1.603		
Total	67.128	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	55.918	3	18.639	8.437	.001
Within Groups	35.349	16	2.209		
Total	91.267	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.144	3	10.381	16.208	.000
Within Groups	10.248	16	.641		
Total	41.392	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	83.826	3	27.942	17.578	.000
Within Groups	25.434	16	1.590		
Total	109.260	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	71.175	3	23.725	15.705	.000
Within Groups	24.170	16	1.511		
Total	95.345	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.401	3	18.800	16.774	.000
Within Groups	17.933	16	1.121		
Total	74.334	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62.154	3	20.718	9.373	.001
Within Groups	35.365	16	2.210		
Total	97.519	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.846	3	22.282	17.187	.000
Within Groups	20.743	16	1.296		
Total	87.589	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.714	3	25.238	20.637	.000
Within Groups	19.567	16	1.223		
Total	95.280	19			

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.079	3	22.026	4.356	.023
Within Groups	70.789	14	5.056		
Total	136.868	17			

The Effects of hormones on Reproductive Organs of male *A. niloticus*

ANOVA

Reproductive organs Weight of male

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.486	9	1.832	21.952	.000
Within Groups	3.338	40	.083		
Total	19.824	49			

The Effects of hormones on Reproductive Organs of female *A. niloticus*

ANOVA

Weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.102	9	.011	7.917	.000
Within Groups	.058	40	.001		
Total	.160	49			

The effect of hormones on sperm count and motility of male *A. niloticus*

ANOVA

Sperm Count

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8417133.247	9	935237.027	21.448	.000
Within Groups	1744227.644	40	43605.691		
Total	10161360.892	49			

ANOVA

Sperm Motility

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39085.680	9	4342.853	48.238	.000
Within Groups	3601.200	40	90.030		
Total	42686.880	49			