EFFECTS OF BIOSTIMULATION ON BEHAVIOUR, GROWTH, AND REPRODUCTION OF RABBITS

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ZARIA, NIGERIA

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DEPARTMENT OF ANIMAL SCIENCE,

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DECEMBER, 2017

DECLARATION

I declare that the research work in this dissertation titled **Effects of biostimulation on behaviour, growth, and reproduction of rabbits** was conducted by me in the Department of Animal Science under the supervision of Dr. (Mrs) O.M Daudu and Dr. M. Abdulrashid. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this report was previously presented for the award of another degree or diploma in any other university.

EREKE, SAMUEL OBINNA   DATE

CERTIFICATION

This dissertation titled **Effects of biostimulation on behaviour, growth, and reproduction of rabbits** by Ereke Samuel Obinna meets the regulation governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to current scientific knowledge and literary presentation.

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DEDICATION

I dedicate this work to Almighty God for all His Merciful Grace in my life and to all Scientists working hard to solve the societal needs.

ABSTRACT

A 16-week study was conducted to evaluate the effect of biostimulation on behaviour, growth, and reproduction of rabbits. Seventy five (crossbreds) mature rabbits (2.6 -2.70 kg), comprising of 50 nulliparous does aged, 6-7 months old and 25 bucks, (8-10 months old), were used for this study. The rabbits were allocated into five treatment groups in a completely randomised design (CRD). Each treatment consisted of 10 nulliparous does and five bucks. The study plan involved an exposure ratio of one buck to two does, implying five replications per treatment and each replicate containing two does and one buck. However, the does in the control group were not exposed to any buck. The does in the treatment groups were placed in opposite cages separated by a distance of 2.5 cm to the bucks at a ratio of 2 does: 1 buck for 7,14,21 and 28 days of exposure. The results showed that the behavioural response of the does ranging from flattening at a corner, circling, mounting without lordosis and mounting with lordosis differed significantly ($P<0.05$) across treatments. The frequency of vulva colour occurrence varying from white, red to pink across treatments were observed. The reproductive performance of the biostimulated does also differed significantly ($P<0.05$) as gestation length, pregnancy rate and average litter weight at weaning were higher in the treatment groups. Reaction time, sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails of the exposed bucks differed significantly ($P<0.05$) from the control. There were evidence of linear increase in hormonal profiles (estradiol and testosterone), as duration of exposure advanced to 7 and 14 days, respectively. Therefore, this study suggests that biostimulation can be a veritable tool for improving reproductive performance in rabbits. Also, it was noted that exposing rabbit does to rabbit bucks longer than 7-14 days prior to mating may adversely affect their reproductive

performance. Hence, it is recommended that farmers should expose rabbit does to rabbit bucks 7 days prior to mating or artificial insemination for higher reproductive performance.

TABLE OF CONTENTS

Content **Pages**

CHAPTER FOUR

RESULTS

LIST OF TABLES

LIST OF FIGURES

Figures **Titles** **Pages**

CHAPTER ONE

1.0 INTRODUCTION

The increasing demand for animal protein has aroused so much interest in the production of animals with short generation intervals like rabbits (Obinne and Okorie, 2008). The expansion of the livestock industry in Nigeria can hold great promise to bridge the gap in the supply and demand of animal protein in the country (Apantaku *et al*.,1998) if attention is given to microlivestock such as rabbit. The composition and quality of rabbit meat is comparable to poultry and superior to meat of larger animals (ruminants), It is highly palatable with less fat, sodium and cholesterol (Atteh, 2004). Rabbits are commonly raised to provide tender meat for human consumption. Besides, high prolificacy is the most important economic factor in rabbit production (Belhadi, 2004; Nofal *et al*., 2005). In addition to this, rabbits have a number of other good characteristics that are advantageous to subsistence farming, such as their small body size and short gestation period of 30-31 days. The daily weight gain is directly proportional to the body weight which gives them rapid growth rate and early sexual maturity. These factors are responsible for rabbits attaining the weight of sexual maturity 30 % faster than other animals and makes them suitable as meat producing small livestock in developing countries (Arijeniwa *et al*., 2000, Ajayi *et al.*, 2005).

The profitability of rabbit production depends on the reproductive intensity and the number of kits weaned from a litter (Castellini *et al*., 2010). Although rabbits are reflex ovulators, the ovulation following instant mating sometimes does not result in successful conception. The inherent prolificacy in rabbits is currently facing biochemical, biophysical and environmental challenges which depresses their performance, therefore there is need for the application of some biological strategies to enhance their reproductive efficiency. Biostimulation methods are natural and cheaper alternatives to improve the reproductive performance of animals. Such methods have been found or tested in bulls (Bailey *et al*., 2005), bucks (Bonanno *et al*., 2011), hamsters (Cooke *et al*., 2001) and rams (Kasimanickam *et al*., 2007). Other approach to promote a steady reproductive performance in animals is the application of hormonal protocols (Rebollar *et al*., 2006). This however, increases labour cost and possible adverse effects on health due to residual effects.

The use of exogenous products such as hormones and antibiotics to improve livestock production goes against the perception of the consumers. Castellini *et al.* (2006) reported that the European Community Policy might impose a restriction on the use of hormones in relation to their residues in meat, animal welfare and the desire to preserve a natural meat quality. For these reasons, serious efforts have been made by the International Rabbit Reproduction Group (IRRG, 2005) to set up alternative methods which do not require the use of hormones in rabbit production in order to increase sexual receptivity at the time of insemination or natural mating. Several biostimulation approaches such as animal manipulation (doe-gathering) at the time of semen collection, dam-litter separation, evaluation of different reproductive rhythms or re-mating intervals, adoption of different feeding programs, buck effect and photoperiods have been tried in an attempt to obviate the use of hormones in animal reproduction (Ojeda and McCann, 2000). These biostimulation methods are believed to stimulate the release of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) through visual, olfactory (pheromonal) and tactile cues from the anterior pituitary gland under the influence of some releasing factors from the hypothalamus (Ojeda and McCann, 2000). The release of LH and FSH provides steroidal feedback to the hypothalamus which ensures the continuation of normal ovarian cyclicity (Frink, 1998). However, the exact effects and mechanism of biostimulation techniques and the subsequent behavioural responses on the reproductive performance of rabbit does and bucks have not been properly elucidated. Therefore, the present study was designed to evaluate the effect of biostimulation on the reproductive performance of nulliparous rabbit does as well as to ascertain its effects on semen characteristics of the bucks.

1.1 Justification

In order to contain the pressure of increased human population on the demand for meat and meat products, rabbit (*Oryctolagus cuniculus*) production is a viable option, because of their prolificacy, early maturity, fast growth rate, high genetic selection potential, high feed conversion efficiency and economic utilization of space (Hassan *et al*., 2012). To ensure a sustained rabbit production and development of the sector, rabbit breeds and breeding practices should be explored and challenges identified. However, poor response of rabbit does during service and subsequent decrease in ovulation and/or conception has necessitated the emergence of possible and cost effective techniques in improving reproduction in rabbits. Sexual responses of female rabbits to visual, acoustic/auditory and olfactory stimuli from the male have not been properly investigated, but ovulation is believed to be induced by mating through a neuro-endocrinological reflex which provokes an LH surge (Bakker and Baum, 2000).

Frank (1966) reported the possibility of certain pheromones secreted by the sebaceous glands of rabbit bucks to induce sexual receptivity in the does. It has also been suggested that rabbit does emit specific signals containing information on her sexual state that attracts male rabbits (Hudson and Distel, 1990). Till date, the precise nature of the pheromones or signals supposedly exchanged between the male and female rabbits remain obscure. However, it appears that male presence is beneficial to receptivity and fertility in nulliparous does (Berepudo *et al.*, 1993) but not in lactating does (Kustos *et al.,* 2000; Eiben *et al.,* 2001; Bonanno *et al.,* 2003). Biostimulation is one of the techniques which has been tested on other species and appears to be promising in enhancing fertility in animals (Theau-Clement, 2005). In addition, several evidences have shown that the use of exogenous gonadotropic hormones such as equine chorionic gonadotropin (eCG) to improve reproductive performance in rabbits has resulted in resistant effects, with subsequent decrease in conception rate and increase in mortality rate following frequent and/or high dosage treatment (Theau-Clément, 2007). Therefore, a practical and potent non-hormonal approach in improving fertility in rabbits remains imperative. This study aimed at evaluating the effect of different durations of exposure on the reproductive performance of rabbit does and bucks towards finding the best duration of exposure for optimum performance.

1.2. Objectives

The broad objective of this study was to evaluate the effect of different durations of exposure on the reproductive performance of rabbit does and bucks. The specific objectives were to determine the:

a. Behavioural responses of rabbit does exposed to rabbit bucks at the point of mating

b. Productive performance of rabbit does and bucks at different durations of biostimulation

c. Reproductive performance and hormonal profile of rabbit does and bucks at varied durations of biostimulation

1.3. Research hypotheses

$H_{0:}$ Biostimulation has no effect on the behavioural response of rabbit does and bucks

H_1: Biostimulation has an effect on the behavioural response of rabbit does and bucks

$H_{o:}$ Biostimulation has no effect on the productive performance of rabbit does and bucks

$H_{1:}$ Biostimulation has an effect on the productive performance of rabbit does and bucks

$H_{o:}$ Biostimulation no effect on the reproductive performance and hormonal profile of rabbit does and bucks

$H_{1:}$ Biostimulation has an effect on the reproductive performance and hormonal profile of rabbit does and bucks

CHAPTER TWO

LITERATURE REVIEW

2.1. Brief History and Origin of Domestic Rabbits

The genus *Oryctolagus*, which originated in Southern Spain 6-6.5 Million years ago (Lopez-Martinez, 1977), is now represented by only one species; European rabbit, *Oryctolagus cuniculus*. The European wild rabbit evolved around 4,000 years ago on the Iberian Pennisula. When the Romans arrived in Spain around 200BC, they began to farm native rabbits for their meat and fur. The Romans called this practice 'cuniculture' and kept the rabbits in fenced enclosures. Inevitably, the rabbits tried to escape and it is perhaps no surprise that the latin name '*Oryctolagus cuniculus*' which means 'hare-like digger of underground tunnels'. The spread of the Roman empire, along with increasing trade between countries and continents, helped to introduce the European rabbit into many more parts of Europe and Asia. Little has been written about traditional rabbit production in Africa, with the exception of Mathewman (1997) who reported that rabbit production in Africa dated back to around 400 BC when hunters tried to domesticate wild rabbits caught from their hunting expeditions. The European rabbits were introduced to new countries as they were explored or colonised by European adventurers and pioneers.

2.2 Breeds of Rabbit

Rabbit breeds are distinctively identified phenotypically by body size, shape and the coat color. Based on these classifications, American rabbit breeders association in 2010, recognised 47 distinct rabbit breeds. The most common rabbit breeds in the tropics include: New Zealand white, Californian, Chinchilla, French Lop, Dutch, Checkered giant, Flemish giant, Angora and Rex. Based on body size, rabbits have further been classified as: small sized rabbits weighing about 1.4 – 2kg at maturity, medium sized breeds weighing 4–5.4kg, and large breeds weighing 6.4 – 7.3kg (USDA, 1972). In this classification, New Zealand white and Californian white are medium sized breeds. They are the most popular for meat production due to their good growth characteristics (white fur preferred by processors) and a high meat: bone ratio (Oseni, 2008; Mailafia *et al*., 2010). The New Zealand white is also well recognised as a dam breed based on its outstanding maternal genetic merits for litter size, milking, and general mothering ability (McNitt *et al*., 2000). New Zealand rabbits are slightly larger than the Californian, 4.1-5.9 versus 3.6-4.5 kg. The New Zealand rabbit has a complete white, red or black body, whereas the Californian is white with colored nose, ears and feet. The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller (3.2 kg) than the American Chinchilla (4.5 kg). Smaller breeds, on the other hand, are mostly kept as pets and include Chinchilla, Dutch and the Angora (Moreki, 2007)

2.3. Physiology of the Rabbit Doe

2.3.1 Puberty and sexual maturity in rabbit Does

Rabbits are well known for their ability to reproduce quickly. Puberty occurs between 4-6 months, and in smaller breeds it occurs earlier than in larger breeds (Harcourt-Brown, 2002). In rabbits, sexual maturity varies with age (125-150 days), breed, lineage, food and environmental factors such as photoperiod, temperature and seasonality. Generally, the smaller breeds such as the Polish mature at about 4 months of age, while the medium weight breeds such as NewZealand, Chinchillas and Californians mature at 6 to 7 months and the heavy Flemish at 9 to 12 months. Different feeding systems (*ad libitum* vs restrictive) will affect reproductive performance in rabbits (Rommers *et al*., 2001), and some researchers recommended that breeding should begin when the female rabbit reaches about 75% of its adult weight. Better litter size was observed in rabbits with higher body weights fed *ad libitum* at first insemination (Rommers *et al*., 2002). In modern management schemes, however, young does are not inseminated at a fixed body weight but at a fixed age (Rommers *et al*., 2002).

2.3.2 Estrus and estrous cycle in rabbit does

In contrast to most other domestic livestock species, rabbit does do not show sexual cycles with regular heat periods during which ovulation occur spontaneously. It has long been assumed that the rabbit doe is in permanent estrus. However, it has been demonstrated that does alternate periods of acceptance (estrus) and of refusal to mating (diestrus), which varies highly between animals (Theau-Clément *et al.*, 2011). A female rabbit is defined as 'receptive' when it accepts mating, as indicated by its position of lordosis in the presence of a buck. Sexual receptivity can be evaluated more accurately by the colour of the vulva (IRRG, 2005). The relationships between female sexual behaviour, sex steroid hormones, lactation and colour and turgidity of the vulva have been investigated in rabbits throughout pregnancy, pseudo-pregnancy, and post-partum pregnancy**.** During pregnancy, no clear correlation was detected, moreover, the does were sexually receptive despite high blood progesterone and low estrogen concentrations. In contrast, during pseudo-pregnancy, females with a white vulva never accepted mating and the number of those with a red vulva, being sexually receptive, gradually rose toward the end of pseudo-pregnancy when progesterone declined to basal levels. In the post-partum period, does are highly receptive the first day after parturition and shortly (1-2 days) after weaning (Theau Clement *et al.*, 2000).

2.3.3 Mating in rabbits

A female rabbit is defined as receptive when it accepts mating, as indicated by its position of lordosis indicated by its position of lordosis in the presence of a buck. The productivity of receptive females is three to four times higher than that of lactating does and non-receptive does (Theau-Clement., 2005, 2007). This is due to the superiority in the frequency and intensity of ovulation, fertilization rate and embryo viability. Receptivity, measured by a test in the presence of a male or by observation of the color and turgescence of the vulva, reflects the state of oestrus or dioestrus of does (Theau-Clement., 2005, 2007). At the physiologic level, sexual receptivity is correlated with more pre-ovulatory follicles on rabbit ovaries (Kermabon *et al*., 1994) and consequently with higher concentration of plasma estradiol (Rebollar *et al*., 1992). The receptivity of rabbit does can also be determined by the use of three methods; (a) Colour of the vulva**;** when the colour of the vulva appears red or pink, it indicates that the doe is receptive at that point, and purple or white in a non-receptive rabbit doe. (b) Rectal temperature**;** when the rectal temperature >38°C, the doe is receptive or through (c) vagina cytology; when the percentage of keratinized cell >50 % (Najjar *et al*., 2013). However, if a doe is receptive and the buck is active, mating occurs immediately. In that, the doe is taken to the buck's cage and when mating is completed, the buck falls over its side. The doe should be returned to its cage and re-mated after five hours to ensure conception (Denis and Matheron, 1991).

2.3.4 Sexual receptivity in rabbits

Receptivity is an important parameter to predict fertility and production of the rabbit doe. But, this parameter is hard to be detected because of incompatibility with cycled production (Theau-Clément, 2007). Sexual receptivity, or the willingness of the female rabbit to allow mating (Tsiligianni *et al*., 2004), involves the adoption of a lordosis posture (Beyer *et al*., 2007). Economically, sexual receptivity at the moment of insemination highly influences reproductive performance and consequently the productivity. Thus, in industrial rabbit farm, oestrus synchronisation based on PMSG injection is compulsory and improves receptivity and increases the pregnancy rate (Alfonso and Pagés-Mantè, 2002). Receptivity of a rabbit doe is related to the ovarian status which contains at this stage a large number of follicles and a high concentration of plasma estradiol (Kermabon *et al*., 1994). Ovarian status affects the colour of the vulva which is the most method used in industrial rabbit farm to detect if the doe is receptive or not (Castellini.,1996). For some does, colour of the vulva is not usually indicative of oestrus stage (Boiti *et al*., 2005). Thus, finding other methods which can be more predictive for doe receptivity are alternatives to better estimate the oestrus stage of the doe.

2.3.5 Mechanism of ovulation in rabbit does

Mammalian species are classified as spontaneous or induced ovulators. Spontaneous ovulators (e.g., cattle, pigs, sheep, humans) display a cyclical increase of estradiol which triggers GnRH release from the hypothalamus, LH release from the pituitary, and ultimately ovulation regardless of mating activity. Induced ovulators (e.g., rabbits, camelids, cats), however, require stimulation associated with mating to provoke GnRH release and then the surge of LH which is necessary to elicit ovulation (Bakker and Baum, 2000). Female rabbits are classified as reflex ovulators because ovulation takes place after mating, hence, rabbits do not have a regular estrous cycle as in other domestic species such as cattle. It has been reported that the period of receptivity last about 5 to 6 days or 7 to 10 days in the absence of mating followed by 1 to 2 days (Harkness *et al*., 2010) or 4 days of non-receptivity. In female rabbits, lordosis is usually displayed in response to male pelvic thrusting (Moralí *et al*., 2003; cited in Beyer *et al*., 2007). In females that are willing to accept mating, it is more likely to observe an enlarged reddish-purple vulva as a result of high concentrations of estrogens, although, some females accepts mating when the vulva is small and pale (Beyer *et al*., 2007). However, the ovarian function in rabbits is not well understood. Unlike South American camelids (i.e. induced ovulating species in which follicular wave activity has been well documented), the pattern of follicle development in rabbits has not been established. Compared to most other mammals, the formation, activation and development of ovarian follicles occurs entirely postnatally in rabbits, with primordial follicle assembly presumably completed between 2 and 4 weeks of age (Hutt *et al*., 2006).

Follicular development is thought to be supported by the growth of 5 to 10 follicles on each ovary at any given time. Once follicles reach an ovulatory size, they secrete estrogens in increasing amounts and rabbits show sexual receptivity for a period of time. When those follicles degenerate, secretions of estrogen decline and female rabbits become non receptive (Harcout-Brown, 2002). It has been suggested that, the ability to ovulate in response to mating differ between receptive (accepted mating) and non-receptive rabbits (refused mating) and 12 h post-mating (Boumahdi-Merad *et al*., 2011). Failure to ovulate in rabbits may be caused by a lack of discharge of LH rather than to a lack of mature follicles in the ovaries (Harkness *et al*., 2010).

In studies involving direct and histological examinations of the ovaries at different times post-mating, ovulation was detected 10 to 13 hours after mating (Fee and Parkes, 1929). Evidence has shown that copulation in rabbits induces activation of GnRH cells in the hypothalamus and results in a GnRH surge and then in a LH surge (Blaustein and Ershine, 2002). In rabbits, ovulation can also be induced by exogenous administration of hormones (Harkness *et al*., 2010). In commercial rabbit production systems, GnRH, given intramuscularly or intravaginally added to the seminal dose has been used to stimulate ovulation (Ondruška *et al*., 2008; Quintela *et al*., 2008).

2.3.6 Gestation and pseudo-pregnancy in rabbit does

Gestation length in different breeds of rabbits range between 28 to 36 days (Morimoto, 2009). However, rabbit does normally kindle 31-33 days after the mating or artificial insemination (Rashwan *et al.*, 2003). When a doe is mated and the mating is not fertile, it happens that the ova are shed, the corpora lutea are formed, hormones are released by the corpora lutea, the mammary glands are stimulated to activity and may even secrete some milk, the uterus increases in size, the remaining reproductive system and the doe will behave as if pregnant, although she is not. This condition is known as pseudo-pregnancy, in which the doe acts as though she is about to give birth to young, i.e. she makes a nest at the end of pseudo-pregnancy, but the nest building may be very slight. Any of such signs observed as early as the 16th or 17th day after mating (or sometimes quite a few days earlier) may be taken as a fairly reliable signs that the doe is pseudo-pregnant (Harcourt-Brown, 2002).

2.3.7 Testing for pregnancy

There are two common methods used for pregnancy testing:

(1) Test breeding: As the term suggest, this entails taking the doe again to the buck's hutch 18 days after her first mating. If she refuses service by fighting and growling the buck, you assume that she is pregnant.

(2) Palpation: In this technique, the stomach of the doe is carefully felt and manipulated so as to feel the presence of the embryo in the doe's womb. By running the fingers along the abdomen between the back legs, a small bead-like lump (developing foetuses) can be felt if the doe is pregnant. By day 28, the mammary glands would have developed significantly and this can be regarded as the final confirmation of pregnancy. The pregnant doe removes fur from her abdomen to make nest from 29^{th} -30^{th} day in readiness for kindling (Beyer *et al.*, 2007).

2.4 Physiology of the Rabbit Buck

2.4.1 Anatomy of the rabbit bucks reproductive system

The reproductive system of the rabbit buck consists of the testes, epididymis, ampoules, vas deferens, urethra, penis, preputial glands and the accessory glands. It presents a peculiarity in the external genitalia which is common in marsupials and rabbits with a well-developed scrotum located cranial to the penis and the urogenital opening (Capello and Lennox, 2006). The scrotum has few hairs (Donnelly, 2004), and it is formed by the tunica vaginalis, tunica dartos and cremaster muscle. Its main function is to keep the testicles away from the abdominal cavity, so that the right testicular temperature is maintained between 0.5 and 4°C below body temperature, as required for normal spermatogenesis (Alvariño, 1993). The scrotum and abdomen are connected through the inguinal ring, which conveys the excretory duct (vas deferens) that comes from the epididymis.

During periods of sexual inactivity or stress the testicles return to the abdominal cavity through the inguinal ring and may go down again by the action of the cremaster muscle (Capello and Lennox, 2006). They are positioned cranially to the penis (Brewer, 2006; Capello and Lennox, 2006), located in the scrotum, each one on one side of the inguinal line, positioned almost horizontally. Rabbit's testicles are similar to those of cats, but can move freely from the scrotum to the abdomen through an opening in the inguinal canal (Brewer, 2006). Soft tissue herniation and strangulation of the bowel is prevented by a large fat mass associated with the epididymis, which lies in the inguinal canal when the testis is in the scrotum (Donnelly, 2004). The position of the testicles depends on many factors, including body position, body temperature, reproductive activity, repletion of the gastrointestinal tract, amount of abdominal fat (Capello and Lennox, 2006) and stress (Richardson, 2003). The appearance and testis weight depend on the location. For example, testes located in the scrotum are heavier, firm in texture and red in colour. Abdominal testes are light, reddish-brown and limp. Cryptorchid rabbits display sex drive, but fertility is impaired. The scrotum does not develop, but if there is the scrotum and the testicles are not palpable, it is likely that they are retracted into the inguinal canal or abdominal cavity (Paré and Paul-Murphy, 2004).

The functional part of the epididymis consists of a single duct. It originates in the efferent ducts, highly curled over the head, body and tail, and connects straight to the vas deferens with a U shaped tail. The rabbit is one of the species in which sperm stored in the cauda epididymis exhibit vigorous motility even in their own fluid. The vas deferens extends dorsocranial to the body of the epididymis through the inguinal canal and enters the abdominal cavity. The final portion of the vas deferens forms a loop around the ureter and at this point becomes fusiform. Although the thickness of the diameter does not differ from the rest of the vas deferens, this segment is generally called ampulla (ampulla vas deferens). The glands of the rabbit reproductive tract differ in number, location, size and proportion, among other aspects, like those in other mammals (Vasquez and Del Sol, 2009). This set of glands consists of a vesicular gland, bulbourethral gland and a complex formed by the prostate, pro prostate and paraprostate (Vásquez and Del Sol, 2009). The vesicular gland is located between the prostate gland complex (a very muscular bag with a glandular lining) and the two ampoules which are side by side. Thus, the vesicular gland is variable in length and sometimes becomes temporarily enlarged, depending on the amount of fluid therein. This fluid fluctuates from slightly viscous to gel consistency. This gland contributes 45.6% of the ejaculate volume of rabbits. Some authors have reported that the rabbit prostatic complex, which is located on the dorsal side near the urethra and the bladder consists of the seminal vesicle, vesicular gland and prostate gland. Others noted that the prostatic complex is formed by the vesicle gland, coagulation gland, dorsal ventral lobe of the prostate and bulbourethral glands. The coagulation gland is called the pro prostate, the dorsal lobe and ventral prostate paraprostate. The prostate complex consists of three lobes: pro prostate, prostate and paraprostate (two units) (Dimitrov, 2010). It is noteworthy that the different elements of the rabbit prostate gland have anatomical, histological and immunohistochemical varieties, suggesting that each part of the gland plays a specific role in reproduction (Dimitrov, 2010).

The prostate is located caudally to the vesicular gland and cranially to the prostate and the latter is located cranially to the bulbourethral glands (Vásquez and Del Sol, 2002). This gland is not derived from the wollfian duct, nor is the source gel mass (erroneously called the coagulation gland). Nor is it a lobe of the prostate, but an independent glandular unit that has a separated duct system. The accumulation of white granular secretion in the pro prostate gland gives a whitish appearance and makes the compartmentalization visible observed from the outside (Holtz and Foote, 1978a). The prostate gland is yellowish-white in color and is located between the pro prostate and bulbourethral glands (Vásquez and Del Sol, 2001). It shares the same connective tissue capsule as the pro prostate, and only a small layer of tissue separates these two glands (Holtz and Foote, 1978). The paraprostate glands are small and were named this way because they are located on both sides of the prostate. In other words, the right and left sides are located ventrally and sideways to the pro prostate (Dimitrov and Stamatova, 2011). They have an irregular embossed surface and are hammer-shaped (Vásquez and Del Sol, 2001).

The rabbit bulbourethral gland is a small mass of glandular tissue that is surrounded by a capsule and widely covered by skeletal bulb glandular muscle that separates it into lobules. This gland originates in the urethral wall, as distinct from other species. It is fairly small in the rabbit, but relatively larger than that of man (Vásquez and Del Sol, 2001). The penis is the copulatory organ. An unusual feature of the rabbit is the absence of the glans in the penis (Brewer, 2006), but the body of the penis is cylindrical, 40-50 mm long and the diameters decrease at its end. During rest from sex, it lies in the foreskin, which is located ventrally to the anus (Brewer, 2006) and caudally to the testicles (Capello and Lennox, 2006).

2.4.2 Puberty and sexual maturity in rabbit bucks

Rabbits are well known for their ability to reproduce quickly. Puberty occurs between 4-6 months, and earlier in smaller breeds than in larger breeds (Harcourt-Brown, 2002). In rabbits, sexual maturity varies with age (125-150 days), breed, lineage, food and environmental factors such as photoperiod, temperature and seasonality. Puberty in rabbits precedes the appearance of sperm in the ejaculate, so puberty and sexual maturity are different phases (Macari and Machado 1978). Skinner (1967) affirmed that at 63 days of age, rabbit testes descend into the scrotum. Other studies revealed that although the rabbit is pubertal in 4 months, the testes are not in the scrotum yet, the descent is observed into the scrotum only at six months of age (Fraser, 1988).

However, sexual maturity is defined as the moment at which the daily spermatozoa production ceases to increase, which is reached at 32 weeks in New Zealand White rabbits. Studies revealed that rabbits attain sexual maturity at 18 weeks of age. However, rabbits are pubertal when their testicles become androgenically active and accessory glands begin to produce fructose and citric acid, thus, the animal develop a characteristically male behaviour (Skinner,1967).

2.4.3 Spermatogenesis and sperm production in rabbit bucks

Spermatogenesis starts between 42 and 63 days of age, but sperm do not appear in the ejaculate before 119 days (Skinner, 1967). It is known that spermatogenesis is a process that depends on the low temperature of the scrotum. Thus, temperatures higher than that of the scrotum (e.g., abdominal temperature) may affect spermatogenesis adversely (Hua *et al.,* 2000). The total estimated duration of spermatogenesis in rabbits depends on the point chosen as the beginning of spermatogenesis. If it is judged that spermatogenesis begins with the first part of a series of division of spermatogonia leading to the production of primary spermatocytes, then about four cycles of the seminiferous epithelium (4 x 10.9 = 43.6 days) are required. However, if it is assumed that spermatogenesis begins with the formation of spermatogonia stem and that the lifetime of such stem cells is a cycle of seminiferous epithelium, then spermatogenesis extends 43.6 days (Swierstra and Foote, 1965).

Morton (1988), considering that one cycle lasts 10.8 days and not 10.9 as suggested by Swierstra and Foote (1963), stated that spermatogenesis in this specie lasts about 48 days. Swierstra and Foote (1963) demonstrated that the cycle of seminiferous epithelium of rabbits is divided into eight stages, using as criterion the shape of the spermatids core, the location of spermatids and spermatozoa in relation to the basement membrane, the presence of meiotic figures and release of spermatozoa in the lumen (Swierstra and Foote, 1963). The authors also reported that during spermatogenesis there is considerable loss of spermatogenic cells in the rabbit with about 20 % fewer spermatids than expected from the theoretical considerations. A lower number of spermatids were also observed by Morton (1988) and Zhang *et al.* (2002). Swierstra and Foote (1963) reported that most of this loss occurs during and immediately after the two maturation divisions.

However, recent studies have demonstrated the presence of round spermatids in the epididymis (sloughing of spermatids), in other words, they leave the testes before maturation (Zhang *et al.*, 2002). The authors also suggest that the age of the animal and season contribute to the sloughing of spermatids, which may occur more frequently after puberty or when spermatogenesis begins to occur in an active form. In this species, multinucleated spermatids are often found (giant spermatids), but this incidence may be increased by stress or environmental management (Barakat, 2007). Multinucleated spermatids are easily recognised because they are spherical in shape and have from two to four small, rounds, pyknotic cores with dense chromatin.

The testis is the main source of testosterone in rabbits (Castro *et al.,* 2002) which is the main androgen produced during sexual maturation (Chubb *et al.,* 1978). Although their essential function is the maintenance of normal spermatogenesis, serum testosterone levels above the baseline level do not appear to influence the efficiency of spermatogenesis (Castro *et al.,* 2002).

However, around the 6th week of spermatogenesis, there is a quick increase in FSH and LH concentrations in blood, which precede the onset of testosterone secretion and consequently the manifestation of puberty (Chubb *et al.,* 1978). This relationship between FSH and androgen suggests that FSH may play a regulatory role of steroidogenesis during rabbit puberty. The testicles continue to grow and increase sperm production until six months of age (Morton, 1988). Spermatozoa can already be present in the cauda epididymis at around 15 weeks of age (Chubb *et al.,* 1978).

The daily spermatozoa production increases from 15 to 52 weeks of age (Ewuola and Egbunike, 2010). Other studies reported that there is positive correlation between the gonadal reserve and testicular weight (Orgebin-Crist, 1968) with body of the rabbit (Ewuola and Egbunike, 2010). However, daily exposure to a continuous 14 hour light period negatively affects gonadal reserves. Under normal conditions, the average yield is 147.4 10^6/ day and 1g of testis produces 26.5 x 10^6 spermatozoa/ day (Orgebin-Crist, 1968). However, if the animal is subjected to sperm collections twice weekly, the daily release of spermatozoa in the ejaculate is consistently lower than the testicular production, indicating that approximately 50% of the spermatozoa produced are reabsorbed (Holtz and Foote, 1972). Different daily production of spermatozoa has been observed: 148 ± $11x10^6$ spermatozoa per day, 187×10^6 / day (Holtz and Foote, 1972) and $210x10^6$/day (Amann and Lambíase , 1969). It is noteworthy that the rhythm of semen collection does not affect daily spermatozoa production (Amann, 1966). A recent study showed that in this species, the spermatozoa reserve is smaller in the left testis and left epididymis than in the right ones (Ewuola and Egbunike, 2010).

2.5. Semen characteristics of rabbit bucks

The semen is a mixture of spermatozoa produced by the testes and seminal plasma secreted by the epididymis and different accessory glands, which are combined at the time of ejaculation (El-Azim and El-kamash, 2011). However, rabbit semen consists of two main parts, fluid and a gelatinous portion (Mukherjee *et al.*, 1951).

2.5.1 Gel plug

The gel plug or gelatinous mass from rabbit semen originates in the vesicular gland (Holtz and Foote, 1978) and it is androgen-dependent (Mitchell, 1984). It consists of a significant amount of estrogenic substances and citric acid, and small amount of fructose. When animals are subjected to two daily semen collections, the gelatinous mass can be found in 75.4% of first ejaculates and only 4.8% in the second ejaculate (Holtz and Foote, 1978). A large number of spermatozoa can be entrapped and the gelatinous mass semen inactive, but after dilution in saline solution and incubated at 37°C, the gelatinous mass dissolves releasing the spermatozoa, which in turn become highly active (Mukherjee *et al.,* 1951). It is believed that this occurs because during ejaculation the two semen fractions are in a fluid state (Mukherjee *et al.,* 1951). Later studies showed that the consistency of the fluid contained in the vesicular gland varies from slightly viscous to gel (Holtz and Foote, 1978). Although it is common in rabbit semen, no function was found for the gel apart from preventing the loss of retrograde spermatozoa in rodents (Quesenberry *et al.*, 2004). This function has also been suggested by Mukherjee *et al.* (1951), who speculated that after ejaculation, the gel fills the vaginal lumen as buffer coagulant. Thus, IRRG Guidelines (2005) recommends removing the gel immediately after collection before evaluating rabbit semen.

2.5.2 Seminal plasma

It represents the fluid portion of the semen and its presence positively affects the survival of spermatozoa motility in rabbits (Castellini *et al.,* 2000, Hagen *et al.,* 2002). Seminal plasma contains constituents such as carbohydrates, lipids, proteins and minerals (Zaniboni *et al.*, 2004, Castellini *et al.*, 2006), which are important for sperm metabolism. Fructose and glucose is one of the main energetic constituent in semen, as well as substrates for the sperm cell metabolism (Arruda-Alencar, 2011). Previous studies have found that the initial fructose concentration in the seminal plasma is usually higher in the summer (245.9mg/dl), while the testosterone level is high in the spring (137.0mg/dl) (Okab, 2007). Thus, the concentration of fructose in seminal plasma reflects the testosterone activity and semen quality (Okab, 2007). However, the sugar concentration in rabbit semen is well below that found in ruminants (Roca *et al.*, 1993). Another sugar identified in rabbit seminal plasma was glucose (Arruda-Alencar, 2011), that despite being present in low concentrations (13.8 to 22mg/dL) was considered an effective constituent of seminal plasma in this species.

Rabbit seminal plasma contains Na, K, Ca, Mg, Se and Zn (Castellini *et al.*, 2007), and some trace elements such as Cu, Fe, Mn, Cd, Pb and Ni (Lukáč *et al.*, 2009). The Na and K were found in similar concentrations (1:1), but the mineral concentrations cited were much lower than those found in other species (Holtz and Foote, 1978). It contributes little to maintaining the semen osmotic pressure, so it seems that the organic components are the main constituents responsible for this maintenance (Holtz and Foote, 1978). Seminal Na concentration reduces significantly in the case of removal of the seminal vesicle gland (Del Niño *et al.*, 1997). Rabbit seminal plasma also contains several drops and vesicles (prostatic secretory granules) of different sizes and origins, which play different roles that are partially unknown (Castellini *et al.*, 2006). Mourvaki *et al.* (2010) suggested that the prostatic secretory granules may represent a source of protection for spermatozoa against oxidative stress *in vitro* by supplying the spermatozoa with endogenous alpha-tocopherol.

2.5.3. Sperm motility

Motility is a common feature of spermatozoa in the entire animal kingdom, it shows the percentage of spermatozoa moving steadily in a straight line (Chrenek *et al.*, 2007). Moreover, for species with internal fertilization, motility is important for the transport of spermatozoa in the reproductive tract and oocyte penetration (Holt and Van Look, 2004). For the determination of motility, drops of semen suspension should be placed on a slide and covered with cover slide and observed on microscope (IRRG Guidelines, 2005). The subjective evaluation of motility and sperm morphology are the two most widely used laboratory tests to evaluate semen in rabbit insemination (Lavara *et al.*, 2008a). These characteristics result in potential spermatozoa fertility, because there are some correlations between seminal parameters with motility, indicating the relationship between morphometric parameters and semen quality of rabbits (Hagen *et al.* 2002; Lavara *et al.*, 2008b). Until recently, sperm quality was evaluated based on the subjective evaluation of parameters such as mass and individual motility, as well as concentration and morphology (Verstegen *et al.*, 2002). The authors affirm that subjective estimates of semen parameters are affected by many factors, including changes in the training and experience of the evaluators. Computer Assisted Semen Analysis (CASA) was developed for an objective assessment of spermatozoa motility. This system includes a phase contrast microscope equipped with a heating plate, connected to a high resolution video camera and a computer (IRRG Guidelines, 2005). In goats, the motility evaluation method was superior to the conventional computer method (Cavalcante *et al.*, 2005). Roca *et al.* (2000) rated the progressive motility of spermatozoa in rabbits using an arbitrary scale 0-5 (0, 1, 2, 3, 4 or 5, D 0-10, 10-25, 25-50, 50-70, 70 -90 or 90-100%, respectively, showing progressive spermatozoa motility). The age at which 50% of sperm cells in the ejaculate of rabbits are motile is 117 days (Bell and Mitchell,1984).

2.5.4. Sperm morphology

In rabbits, semen characteristics, particularly morphological features from medium to high heritability (Lavara *et al.*, 2008c) can be evaluated with optical microscopy procedures using different staining techniques (IRRG Guidelines, 2005). In resume, the rabbit sperm morphometric measurements were: total length 46 to 55µm (Eddy, 2006); head length 7.8 to 8.6µm (Bedford, 1963); middle piece 8.5µm with mitochondria arranged and about 41 turns (Eddy, 2006); the main piece measured about 38µm long (Cummins and Woodall, 1985). The head is shaped like a spatula and the acrosome does not extend beyond the core, and it also has a small equatorial region (Eddy, 2006). Kuzminsky *et al.* (1996) developed an illustrated guide to various abnormalities of rabbit semen, indicating that average values observed by quantitative optical microscopy (x400) were: 18.2 % total abnormalities, head abnormalities 2.9%, tail abnormalities 13.6% and 1.7% broken spermatozoa. The authors suggested counting only the curly tails to speed up the process of semen analysis, because these are the most representative of abnormalities in the tail and are easily observed under an optical microscope, even when viewed under low magnification. They also affirmed that for an acceptable ejaculate, the concentration of spermatozoa with curly tails should not exceed 17-18% of 200 cells observed. It is worth noting that rabbit spermatozoa are very sensitive to high ambient temperatures, and abnormal spermatozoa can indicate a heat stress condition suffered by the animal (Finzi *et al.,* 1995). The authors also asserted that this condition can be easily observed by the increase in the number of curly tailed spermatozoa, since this abnormality has 80% correlation with total morphological abnormalities. Among other abnormalities, Branham (1969) found that the presence of the intermediate piece of the protoplasmic drop is associated with low rabbit spermatozoa displacement speed. The rabbit spermatozoa acrosome is evident as a swelling in the anterior margin of the head (Gould *et al.,* 1971). The lines demarcating the equatorial segment are much closer together than in the hamster.

2.5.5 Semen volume and sperm concentration

The ejaculate volume and sperm concentration in rabbits may range from 0.3 to 0.6ml and 150-500x10^6 sperm/ml, respectively (Adams and Singh, 1981). However, seminal characteristics can vary among different breeds: Rex (0.54 ± 0.03ml, 415.10 ± 10.11x10^6 sptz/ml), New Zealand White (0.54 ± 0.04ml; 416.72 ± 9.16 x 10^6 sptz/ml), California (0.62 ± 0.03ml, 454.11 ±11.40 x10^6 sptz/ml) and Baladi Red (0.56 ± 0.04ml, 423.23 ± 12.11x10^6 sptz/ml) (Hassanien and Baiomy, 2011). Other factors that may affect these parameters include diet (Kamel and Attia, 2011), collection frequency (Castellini *et al.,* 2006), age (Theau-Clement *et al.,* 2007) and ambient temperature (Finzi *et al.*, 1994). It is noteworthy to state that the semen volume seems to be much more affected by temperature than the sperm concentration (Garcia-Tomás *et al.,* 2008, Roca *et al.,* 2005). Lebas *et al.* (1997) affirmed that when sexual stimulation was performed without copulation, 1-2 min before copulation, the sperm concentration increases. Previous studies have shown that this type of stimulation increases the number of spermatozoa in the vas deferens and consequently in the ejaculate (Prins and Zaneveld, 1979). According to the authors, this is because, in rabbits, during sexual stimulation sperm are moved from the epididymis into the vas deferens where they are quickly removed during ejaculation. Thus, the vas deferens is an active organ during sexual inactivity (Prins and Zaneveld, 1979).

2.5.6 Semen colour

Some studies have reported that rabbit semen is white and the intensity depends on sperm concentration (Alvarez *et al.*, 2006). There are some ratings for the colour of rabbit semen. For Bilbao (1996), the semen is often pearly white and ivory, but gray semen is considered of poor quality. Alvarez *et al.* (2006) reported that milky-white semen is the best and predominant in the rabbit and represents normal semen with good quality. Yellowish semen is often contaminated with urine that is normally obtained when the temperature is too high in the artificial vagina (Chang, 1959). Several studies have associated color and appearance as a single parameter (Matavelli, 2008; El-Azim and El-kamash, 2011). Normal semen is white, homogeneous and opalescent (IRRG Guidelines, 2005). However, according to Matavelli (2008), the ejaculate is mostly milky-white, but the best quality is found in creamy-white semen. Arrebola and Fernandez (2011) reported that pearly-white semen is good quality and other colours are classified as poor, likewise, a uniform appearance is the most desirable.

2.6 Behaviour of rabbits.

Rrabbit's behaviors and behavior problems are consequences of the way its wild ancestors and their captive descendants lived and survived (Cowan, 1987). The wild European rabbit is a highly social species that lives in complex social groups in warrens that sometimes become large. (Drescher,1992). Within a colony, multiple subgroups of two to eight rabbits spend most of their time together. The females in the subgroup are typically related, while the males maintain a rigid dominance hierarchy by ritualized signaling (Bigler and Oester, 1994). As young bucks mature, they are usually driven out of the subgroup by the older bucks and will seek to join another subgroup or even a different warren. The young bucks are most likely to integrate into a new subgroup or colony outside of the breeding season. Members of the subgroup and colony all watch for predators when outside feeding (Hansen and Berthelsen, 2000). In general, rabbits are probably better off if kept in groups of two or more animals.

However, as with all social species, appropriate social behavior is learned. A rabbit that is adopted as a kitten and subsequently kept alone will have poor social skills and is likely to be either extremely aggressive or extremely timid when it encounters a member of its own species. Nevertheless, some rabbits can be habituated, desensitized, and counter conditioned to other rabbits. How long this process takes varies; some rabbits are calm and adapt readily to new situations, while others are reactive and less tolerant of new situations (Baumans, 2005).

Mating behaviours in rabbits includes; allowing mounting with or without lordosis, circling within the cage, flattening at a corner and aggressiveness of the rabbit doe towards the buck. Flattening at a corner and aggressiveness of a rabbit doe towards the buck is counter-productive and shows the unwillingness of a rabbit doe to accept mating (Beyer *et al*., 2007).

2.7 Buck Effect

There has been an increasing interest in the use of safer, cheaper and non-hormonal methods in improving livestock productivity so as to reduce the amount of exogenous hormones employed in animal production worldwide because of the growing concerns in terms of residues in food products (meat and milk) (Martin *et al*., 2004). One of such methods is the use of the "male effect", otherwise known as biostimulation (Keskin, 2003). The male effect is described as the influence of a male on oestrus behaviour and ovarian activity in female co-species. This phenomenon is mediated via pheromones or cues, which are percieved by the females. The application of biostimulation in rabbit reproduction in the tropics require more attention as it offers a potentially useful, cheaper and practical ways to improve reproductive efficiency in rabbits. The response to the "male effect" is influenced by the aggressiveness of the buck, serving capacity of the buck, intensity of the stimulation, body condition and nutritional status of the buck and does (Martin *et al*., 2004). The stimulation of auditory, tactile, olfactory and visual sensations play essential roles in the overall response to the "male effect", although the olfactory stimulation is suggested to be the most important.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Experimental site

The study was conducted at the Rabbit Research Unit of the Swine and Rabbit Research Programme, National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika lies within the Northern guinea savannah Zone of Nigeria and located on latitude 11^0 12'N and longitude 7^0 33' E with an altitude of 691 meters above sea level. Annual rainfall range is between1100 and 1200 mm, while mean temperature is about 24.4 ^{0}C with the lowest temperature occurring during the early dry season (November-January), while, the highest temperatures are experienced during late dry season between February and April (Ovimaps, 2015).

3.2. Experimental Animals and Design

A total of 75 mature (crossbreds) rabbits, which comprised of 50 nulliparous rabbit does of about 6-7 months of age and 25 mature bucks age between 8-10 months were used for this study. The animals were divided into five treatment groups comprising of ten nulliparous rabbit does and five rabbit bucks per group in a Completely Randomized Design. The study was planned such that there was an exposure ratio of one buck to two does, implying five replications per treatment and each replicate containing two does and one buck. However, the does in the control group were not exposed to any buck.

3.2.1 Experimental diets and management

A standard basal rabbit diet (ME=2707 Kcal/Kg, CP=18.15 %, 100g/day) was provided to the experimental animals with water *ad libitum* for 16 weeks. During acclimatization, the animals were treated with oxytetracycline to eliminate any infection (Table 3.1). The does in treatment 1 served as the control (the non biostimulated) group, while does in treatments 2, 3, 4 and 5 were biostimulated by placing them in opposite cages 2.5 cm apart from the bucks and mated (breed to breed) at weekly intervals (7, 14, 21 and 28th day), respectively to determine the effect of duration of exposure on their reproductive performance. All does were naturally mated with the bucks 5 hours apart at a mating ratio of 1:2 (one buck to two does). All serviced does were individually housed in flat deck metal cages measuring 60 x 60 x 50 cm, placed in a ventilated house of 5ft walls. Abdominal palpation was conducted two weeks after mating of each group for pregnancy test as the case may be. Nest boxes were provided to the palpated and confirmed pregnant does one week before kindling.

3.3 Data Collection

3.3.1 Behavioural responses of the does

The behavioural signs monitored include: receptivity, vulva colour, mucus secretion, micturition, aggressiveness towards the male, circling within the cage, allowing mounting without lordosis, and allowing mounting with lordosis. The vulva colours were checked in all the does between 08:00 and 10:00 a.m throughout the period of exposure. A colour chart of seven shades of white, somewhat pink (pinkish), deep pink (pink), cherry red (red), dark red (blue red), purple with grey over tone and purple were used as reference points (Orire, 1993). Mucus secretion was determined by observing the vulva epithelial cells of each doe daily for any vaginal discharge. Flattening, aggressiveness and doe circling were determined by noting the temperament and docility of the does towards the bucks at the point of mating by visual appraisal. Lordotic posture was determined by noting the ease at which the bucks mounted the does. Sexual receptivity was determined by noting the successful copulatory thrust with ejaculation displayed by the bucks at the point of mating (Chibundu, 2005).

3.3.2 Productive performance of the does and bucks

The productive parameters for both the does and bucks measured included:
Initial live weight (g): This was determined by weighing the animals and recording their weights before commencing the experiment.

Feed intake (g): This was determined by subtracting the weight of the left over feed from the weight of the feed given daily (100g/day of concentrate).

Daily weight gain/day (g): This was determined by dividing the total body weight gain by the total number of days the experiment lasted.

Total body weight gain (g): This was determined by subtracting the initial weight from the final weight.

3.3.3 Blood sampling and hormonal assay

Blood samples (1ml) were collected using 2ml syringe between 08-09:30 am from the ear vein of five randomly selected rabbit does and five bucks on treatment basis into a sterile sample bottle without anti-coagulant. The Samples were collected once from the does and thrice from the bucks at 30 minutes interval on each day of collection. The blood samples were collected into test tube free of EDTA, properly labelled and analysed at the Laboratory of the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria. The blood samples were kept in a refrigerator at about 4^{o}C for about 3 hours to aid sedimentation after which they were spun in a centrifuge at 3,000 rpm for 10 minutes and the serum separated and stored frozen at -10 ^{o} C. The hormonal profiles (estradiol and testosterone) were conducted using Enzyme Linked Immunosorbent Assay (ELISA) Technique as described by Jemenez *et al.* (2005).

3.3.4 Testosterone assay procedure

Serum testosterone was determined using ELISA kits (AccuBind^{B} ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630,USA), intended for quantitative determination of testosterone concentration in serum using ELISA microplate reader (ELx800). The sensitivity of the assay was 0.0576ng/ml. Within assay precision, coefficients of variation for low, normal and high pooled controlled serum samples were 9.8%, 4.8% and 5.6%, respectively.

Before proceeding with the assay, serum references of testosterone, samples and reagents, were brought to room temperature (25.0°C). The procedure was carried out as follows:

10 µL (0.010 ml) of each serum reference for testosterone (0.0, 0.1, 0.5, 01.0, 2.5, 5 and 12 ng/ml) were added and coated in duplicate wells A1 through B12 (A1-A12, B1-B12).

10 µL (0.010 ml) of respective serum samples were pipettted into wells B3 through B12 (B3-B12, C1-C12, D1-D12, E1-E12, F1- F12, G1-G12, H1-H12)

50 µl (0.050 ml) of the working testosterone enzyme reagents were then added to all the wells.

The microplates were gently swirled for 30 seconds to mix.

50 µL of testosterone biotin reagents were added to all the wells.

The microplates were gently swirled again for 30 seconds to mix and then covered and incubated for 60 minutes at room temperature.

Contents were discarded following incubation in a sink by decanting and the plates were blot dried with absorbant tissue paper. These were washed 3 times by tapping and blot drying on a tissue paper following addition of 350 µL of wash buffer in each well before each wash.

100 µL of working substrate solutions were added to the entire well in the same order and then incubated at room temperature for 15 minutes.

50 L of stop solutions were finally added to all the wells and gently mixed for 15 seconds.

The absorbance of each well was read at 450 nm (wavelength) in ELx800 microplate reader within 20 minutes of adding the stop solution.

Absorbance from the micro reader was subjected to MasterPlex® ReaderFit ELISA analysis software from which absorbance for each duplicate serum reference versus corresponding testosterone concentration in ng/ml was plotted. A best-fit curve was automatically generated. Testosterone concentration in ng/ml for each unknown sample was interpolated from best-fit curve**.**

3.3.5 Estradiol assay procedure

Serum estradiol was determined using ELISA kits (AccuBind^{B} ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630,USA), intended for a quantitative determination of estradiol concentration in serum using ELISA microplate reader (ELx800). The sensitivity of the assay was 8.20pg/ml. Within assay precision, coefficients of variation for low, normal and high pooled controlled serum samples were 4.8%, 3.8% and 8.2%, respectively.

Before proceeding with the assay, serum references of estradiol, samples and reagents, were brought to room temperature (25.0°C). The procedure was carried out as follows:

25 µL (0.025ml) of each serum reference for estradiol (0.0, 0.5, 1.0, 1.5, 2.0, 2.50 and 3.0 pg/ml) were added and coated in duplicate wells A1 through B12 (A1-A12, B1-B12).

50 µL (0.050 ml) of respective serum samples were pipettted into wells B3 through B12 (B3-B12, C1-C12, D1-D12, E1-E12, F1- F12, G1-G12, H1-H12)

The microplates were gently swirled for 30 seconds to mix, covered and incubated for 30 minutes at room temperature.

50 µl (0.050 ml) of the estradiol enzyme reagents were then added to all the wells.The contents were discarded following incubation in a sink by decanting and the plates were blot

dried with absorbant tissue paper. These were washed 3 times by tapping and blot drying on a tissue paper following addition of 350 µL of wash buffer in each well before each wash.
100 µL of working substrate solutions were added to the entire well in the same order and then incubated at room temperature for twenty minutes.

50 µL of stop solutions were finally added to all the wells and gently mixed for 15-20 seconds.

The absorbance of each well was read at 450 nm (wavelength) in ELx800 microplate reader within 20 minutes of adding the stop solution.

Absorbance from the micro reader was subjected to MasterPlex® Reader Fit ELISA analysis software from which absorbance for each duplicate serum reference versus corresponding estradiol concentration in pg/ml was plotted. A best-fit curve was automatically generated. Estradiol concentration in pg/ml for each unknown sample was interpolated from best-fit curve.

3.3.6 Reproductive performance of the does

Pregnancy confirmation: Abdominal palpation was carried out two weeks after mating to confirm pregnancy. This was done by running fingers along the abdomen between the back legs, presence of small bead-like lumps (developing foetus) is usually an indication of pregnancy. Further confirmation was done by day 28 as the mammary gland would have developed significantly.

Pregnancy rate: This was determined by calculating the percentage of matings that resulted to pregnancy. The number of pregnant does were obtained after the abdominal palpation and thereafter expressed in percentage of the total rabbit does mated.

Gestation length (days): This was determined by recording the interval between the mating date and kindling date.

Litter size at birth (g): This was determined by counting the number of kits born per doe and recording the number of kits born alive or dead.

Litter birth weight and weekly weight gain (g): The litter birth weight was determined by weighing the kits at the time of birth, while the weekly weight gain was determined by weighing the kits weekly using a sensitive weighing balance.

3.3.7 Reproductive performance of the bucks

Reaction time(s): This was determined using a stop watch by recording the time interval (in seconds) between the introduction of a doe into the buck's cage to a successful copulatory thrust with intromission (Chibundu, 2005), and eventual ejaculation.

Semen collection and evaluation

Semen was collected from the exposed bucks once weekly between 08-10am using an artificial vagina and the ejaculates examined for:

Semen colour: This was determined by visual appraisal of the semen and coded as 5 Excellent, 4 very good, 3 good, 2 fair, 1 poor and 0 very poor (Rekwot *et al*.,1997).

Semen Volume: This was measured by collecting and observing the ejaculate in a calibrated test-tube and the volume of each ejaculate recorded.

Semen P^H: This was measured with a P^H meter immediately after the collection of the semen. The P^H of each individual sample collected was determined using Chemo craft P^H paper. In each case, one inch of the paper was inserted into the semen while in the tube for five seconds and removed and properly air dried. The colour change observed after drying was placed to the colour chart on the P^H paper. The corresponding P^H number for the colour on the chart was recorded as the pH of the sample (Rekwot *et al*.,1997).

Sperm Motility: This was determined by observing a drop of semen on a glass slide with cover slide under a microscope (type; electric, model; CX21FS1, manufacturer; Olympus Corporation,Tokyo, Japan) at x400 magnification, with a warm stage maintained at 37^0C. Gross motility was estimated as percentage scores according to the procedure outlined by Rekwot *et al*. (1997).

Sperm Concentration: This was determined using the red blood cell counting chamber of a haemocytometer (model; 1280) that was crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares placed on a microscope (type; electric, model; CX21FS1, manufacturer; Olympus Corporation, Tokyo, Japan) and viewed at x 400 magnification. The total number of small squares on the haemocytometer is 400. Sperm cells were counted diagonally from top left to the bottom right. Sperm cells counted were multiplied by a dilution factor (19mls of diluents /1ml of semen) and a multiplication factor (in thousands). The value obtained was recorded as sperm concentration (Rekwot *et al*., 1997).

Percent Live and Dead Spermatozoa: The percentage live and dead sperm was estimated by preparing a smear of individual semen sample using Eosin-nigrosin stain immediately after collection. A drop of semen was placed on a clean glass slide using a pipette. A drop of the Eosin-nigrosin solution was placed alongside the semen on the slide. The slide was gently turned to allow a uniform mixture of the two samples. One-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide and allowed to dry. The principle is that the dead sperm cells absorb the stain and appear pink or red, while the live sperm cells reject the stain and remain unstained (Hancock, 1951).

Percentage abnormalities: The percentage abnormalities (morphology) were determined by viewing the stained slide under a microscope and examined individual sperm cells for deformity.

3.4 Model and data analyses

All data generated were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2004). Dunnette's (1955 procedure) test was used to separate means between different treatment groups. Data on the behavioural attributes were transposed by scoring them in percentages.

The linear model used for statistical analysis of the data collected is as shown below;

$X_{ij} = U+D_i+E_{ij}$

Where:

X_{ij} = Observation, U = overall mean,

D_i = effect of buck exposure on does behavioural responses.

E_{ij} = random error.

Table 3.1: Percentage Composition of the Experimental Diet

Ingredients	(%)
Maize	55.00
Wheat Offal	25.00
Soya cake	19.00
Mineral & Vitamin Premix	0.25
Salt	0.25
Lysine	0.25
Methionine	0.25
Total	100.00
Proximate analysis	
Metabolisable Energy (Kcal/Kg)	2707.00
Crude Protein (%)	18.15
Ether Extract (%)	3.61
Crude Fibre (%)	11.70
Calcium (%)	1.08
Available Phosphorus (%)	0.66
Methionine (%)	0.52
Lysine (%)	1.13

***Biomix premix supplied per kg of diet: Vit.A, 10,000 iu; vit D_3, 2000 iu; vit E, 23 mg; vit.k, 2mg, vit B_1, 1.8; vit B_2, 5.5 mg; Niacin, 27.5mg; pantothenic acid,7.5mg; vit B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; chloride, 300mg; cobalt, 0.2; Copper, 3mg; Iodine 1mg; Iron, 20 mg; Manganese, 40 mg; selenium, 0.2 mg; Zinc, 30 mg; Antioxidant, 1.25mg.(Manafactured by: Bio-organics Nutrient System Limited, Ibafo Ogun State, Nigeria)*

CHAPTER FOUR

4.0 RESULTS

4.1 Behavioural Characteristics of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

The behavioural characteristics of rabbit does exposed to rabbit bucks at different durations is shown in Figure 4.1. There were significant ($P<0.05$) differences in the number of rabbit does that exhibited flattening at a corner, circling, mounting without lordosis and mounting with lordosis across the treatments. The percentage flattening (90%) and circling (100%) observed in the non biostimulated group differed significantly ($P<0.05$) higher from the biostimulated groups.

4.2 Productive Performance of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

The productive performance of rabbit does exposed to rabbit bucks at different durations is shown in Table 4.1 There were no significant ($P>0.05$) differences in feed intake, weekly weight gain and final weight gain in both the control and experimental groups.

4.3 Productive Performance of Rabbit Bucks Exposed to Rabbit Does at Different Durations of Biostimulation

The productive performance of rabbit bucks exposed to rabbit does at different durations is presented in Table 4.2 There were no significant ($P>0.05$) differences in feed intake, weekly weight gain and final live weight in both the control and experimental groups.

4.4. Vulva Colour of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

The frequency of occurrence of the four different vulva colours recorded in this study is shown in Figure 4.2. In the control, whitish vulva (90%) was dominant and differed significantly ($P<0.05$) from the treatment groups. The seven days biostimulated group recorded four distinct colours (whitish 20%, cherry red 40%, pinkish 20% and dark red 10%). However, in the 14 days biostimulated group, four different colours were also observed (pinkish 40%, cherry red 30%, whitish 20% and dark red 10%). The 21 days biostimulated group had two different vulva colours of 50% whitish and 30% cherry red, while the 28 days biostimulated group recorded 40% whitish and 30% cherry red.

4.5 Sexual Receptivity of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

The percentage of instantaneous mating of rabbit does exposed to rabbit bucks at different durations is shown in Figure 4.3. It was observed that the percentage of instantaneous mating differed significantly (P<0.05) across the treatments. While the rabbit does in the 7 days biostimulated group had the highest acceptance (70%) to mating, the 14, 21, 28 days biostimulated group and the control had 60%, 50%, 40% and 30% acceptance to mating, respectively. The highest mating success rates were obtained in the group of rabbit does with red and pink vulva compared to dose' with purple and whitish vulva.

4.6 Hormonal Profile of Rabbit Does and Bucks under Different Durations of Biostimulation

The estrogen concentration of rabbit does exposed to rabbit bucks at different durations is shown in Table 4.4. The estrogen concentrations of the biostimulated rabbit does differed significantly (P<0.05) from the control. This variation also existed within the treatment groups. The trend of this variation revealed a peak estrogen concentration of 67.4pg/ml after 7 days of exposure to the bucks, thereafter, it declined to 55.8pg/ml on the 14^{th} day, 38.6pg/ml on the 21^{st} day and 48.0pg/ml on the 28^{th} day.

However, Figure 4.5 shows the testosterone concentration of rabbit bucks exposed to rabbit does at different durations. The highest testosterone concentration of 13.0ng/ml was recorded on the

14th day followed by 11.0ng/ml on the 7th day of biostimulation. These were significantly ($P<0.05$) higher than the control (8.26ng/ml), the 21st (9.94ng/ml) and 28th (9.74ng/ml) day of biostimulation.

4.7 Reproductive Performance of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

The reproductive performance of rabbit does exposed to rabbit bucks at different durations of biostimulation is shown in Table 4.3. There were significant ($P<0.05$) differences in gestation length, pregnancy rate and average litter weaning weight. Rabbit does biostimulated for 7 days before mating had 80% pregnancy rate followed by rabbit does biostimulated for 14 days before mating with 70% pregnancy rate. However, in rabbit does biostimulated for 21 days before mating, pregnancy rate declined to 40%, while in rabbit does biostimulated for 28 days before mating, the pregnancy rate was 50%. In the control group, a pregnancy rate of 30 % was observed.

4.8 Reproductive Performance of Rabbit Bucks Exposed to Rabbit Does at Different Durations of Biostimulation

The reproductive performance of rabbit bucks exposed to rabbit does at different durations is shown in Table 4.4**.** The reaction time of the control group and the experimental groups differed significantly ($P<0.05$) with the shortest reaction time of 16 seconds recorded in rabbit bucks exposed to rabbit does 7 days prior to mating. The non exposed rabbit bucks had the longest reaction time of 37 seconds. Also, sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails of rabbit bucks exposed to rabbit does differed significantly ($P<0.05$) higher than the control and across treatments.

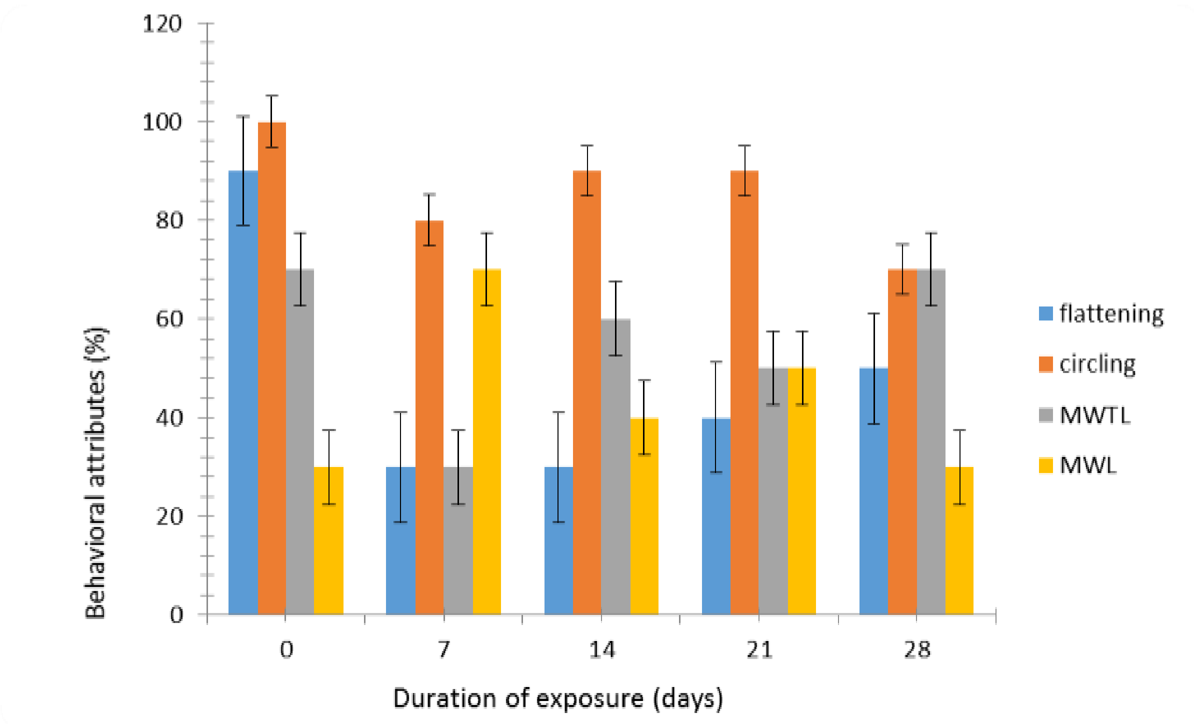

Figure 4.1: Behavioral attributes of rabbit does exposed to rabbit bucks at different durations of biostimulation. MWTL= mounting without lordosis, MWL= mounting with lordosis.

Table 4.1: Productive Performance of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

	Duration (days)						
Parameters	0	7	14	21	28	SEM	p-value
Initial live weight (kg)	2.80	2.75	2.85	2.80	2.70	0.088	1.00
Final live weight (kg)	2.60	2.45	2.80	2.70	2.55	0.19	1.00
Weight gain/day (g)	0.09	0.02	0.04	0.08	0.01	0.09	1.00
Feed intake/day (g)	87.00	86.50	85.00	87.50	89.5	1.20	0.99

Means with different superscripts differed significantly ($P < 0.05$) within rows, 00, 07 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively, N= sample size (10) per treatment. SEM=standard error of the mean

Table 4.2: Productive Performance of Rabbit Bucks Exposed to Rabbit Does at Different Durations (in Days) of Biostimulation

			Duration	(days)			
Parameters	0	7	14	21	28	SEM	p-value
Initial live weight (kg)	2.60	2.65	2.70	2.60	2.70	0.04	0.65
Final live weight (kg)	2.70	2.65	2.80	2.75	2.90	0.05	0.93
Weight gain/day (g)	0.83	0.00	0.83	0.12	0.01	0.03	0.51
Feed intake/day (g)	89.00	91.50	85.50	87.50	88.0	1.24	0.99

Means with different superscripts differed significantly ($P < 0.05$), 00, 07 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively, N = sample size (10) per treatment. SEM=standard error of the mean

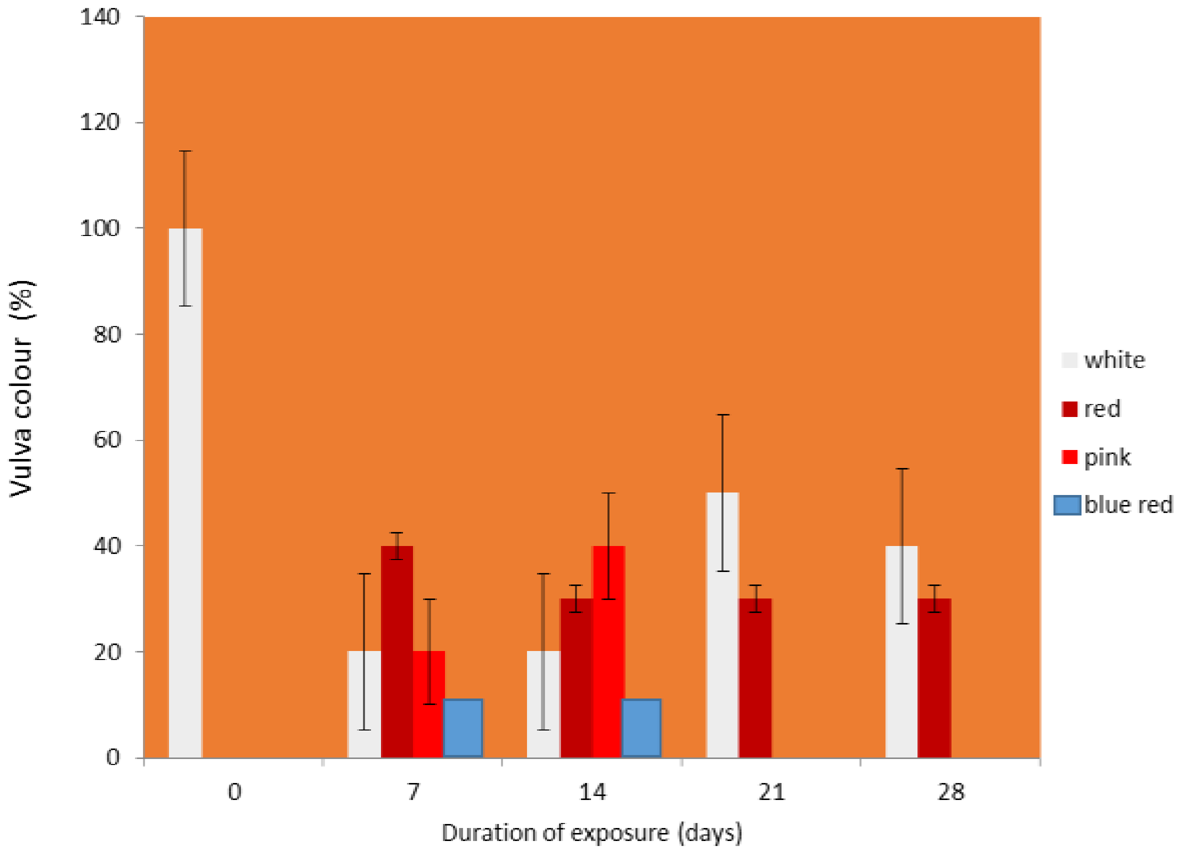

Figure 4.2: Vulva Colour of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation

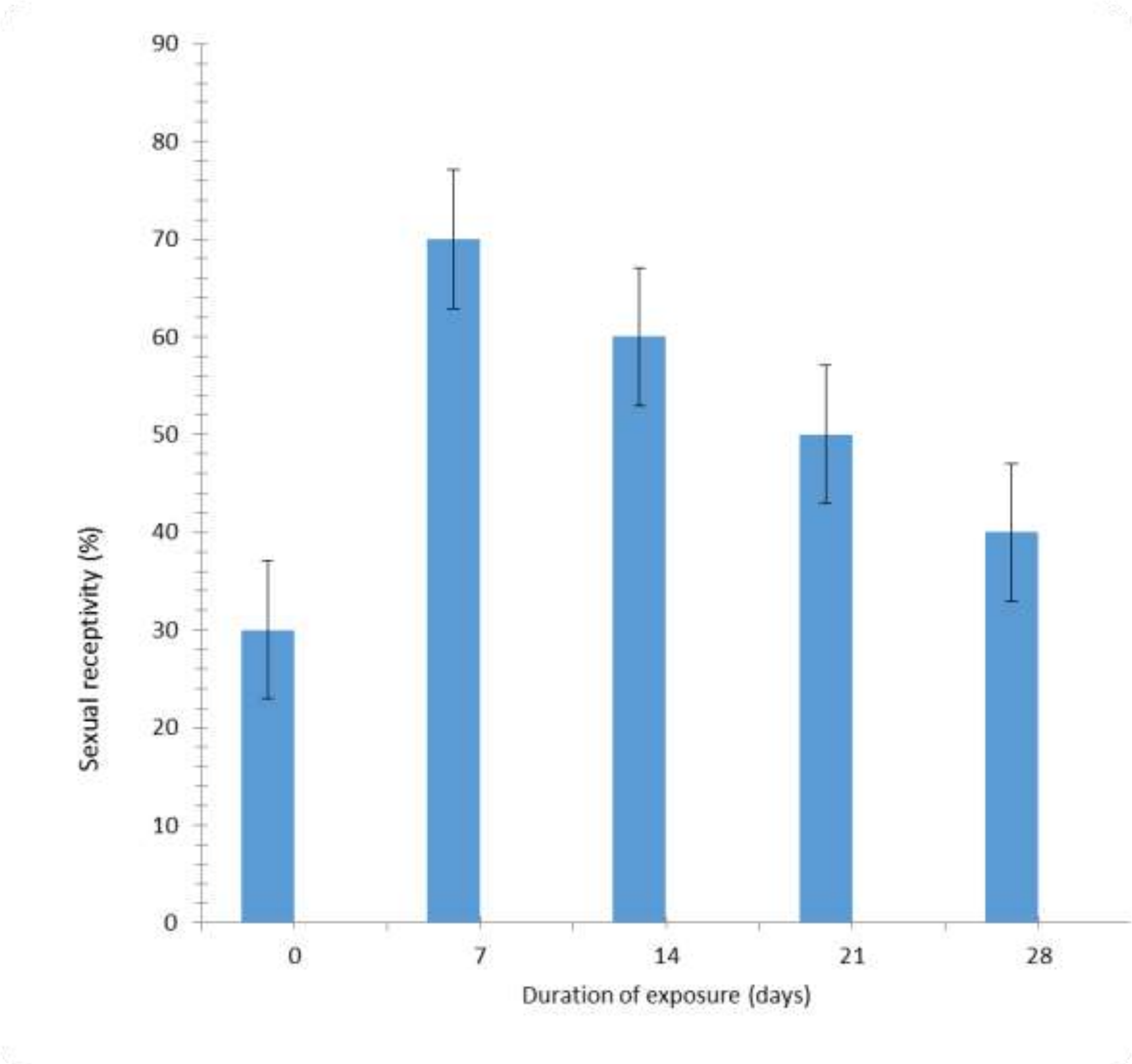

Figure 4.3: Sexual receptivity of rabbit does exposed to rabbit bucks at different durations of biostimulation

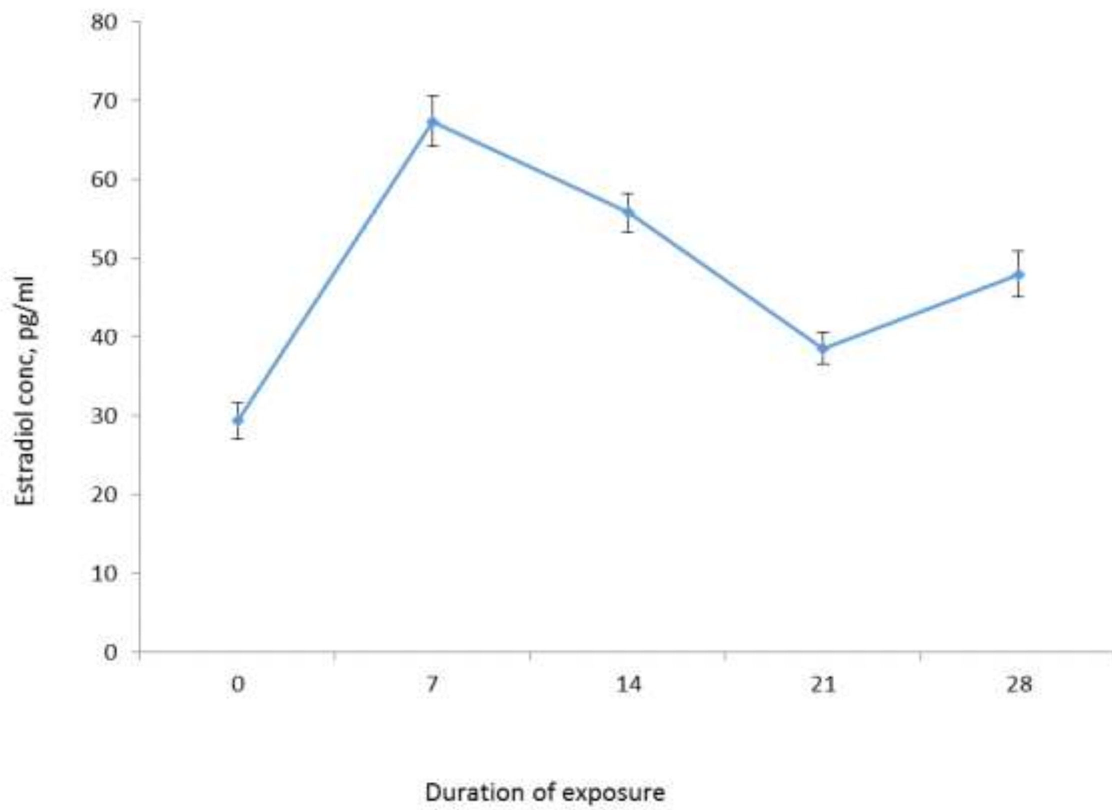

Figure 4.4: Estradiol concentration (pg/ml) of rabbit does under different durations of biostimulation

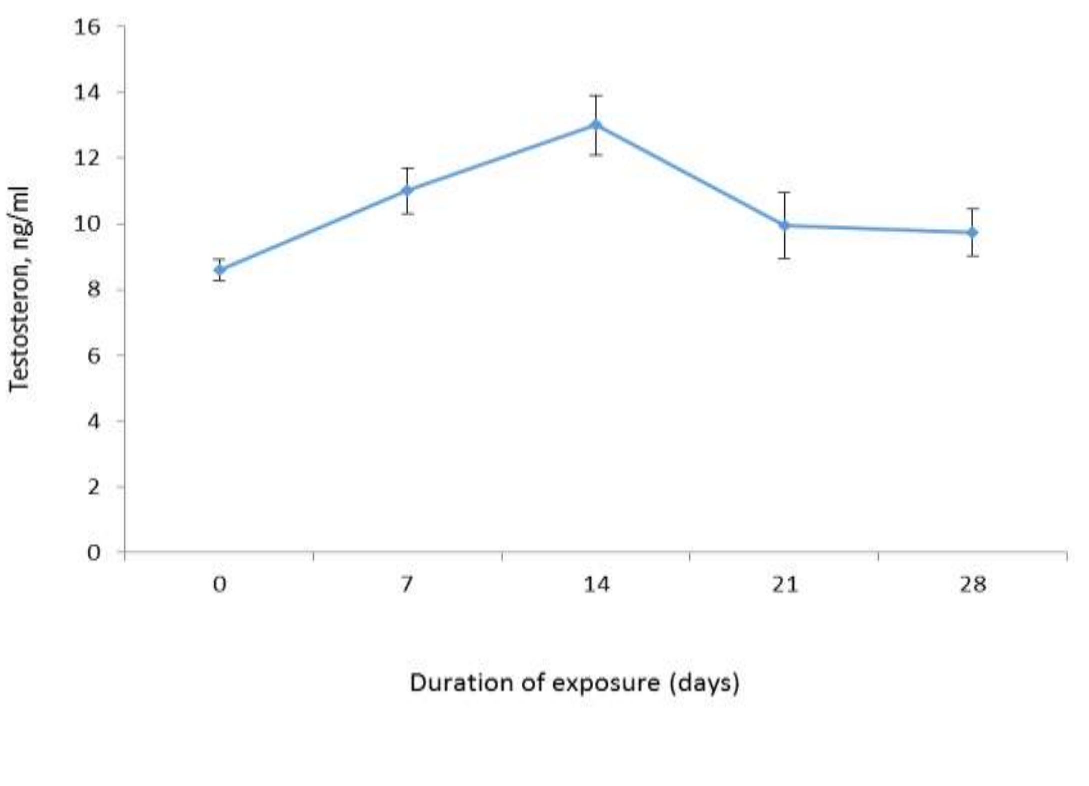

Figure 4.5: Testosterone concentration (ng/ml) of rabbit bucks under different durations of biostimulation

Table 4.3: Reproductive Performance of Rabbit Does Exposed to Rabbit Bucks at Different Durations of Biostimulation.

	Duration (days)						
Parameters	0	7	14	21	28	SEM	P value
Pregnancy rate (%)	30.00^{c}	80.00^{a}	70.00^{ab}	40.00^{b}	50.00^{b}	5.69	0.01
Gestation length (days)	31.00^{c}	30.20^{ab}	29.10^{a}	31.25^{c}	31.20^{c}	0.68	0.02
Kit mortality (%)	36.36	36.58	54.54	36.36	43.33	19.23	0.85
litter size at birth(n)	3.67	5.12	4.70	8.25	6.00	2.70	0.65
litter birth weight(g)	46.36	40.24	40.78	45.45	45.83	3.42	0.16
litter size at weaning(n)	3.00	3.25	2.14	3.50	1.00	1.13	0.44
litter weaning weight(kg)	0.99^{b}	0.98^{b}	1.09^{ab}	1.20^{a}	0.85^{c}	0.10	0.02

Means with different superscripts differed significantly ($P < 0.05$) within rows, 00, 07, 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively, N= sample size (10) per treatment. SEM=standard error of the mean

Table 4.4: Reproductive Performance of Rabbit Bucks Exposed to Rabbit Does at Different Durations of Biostimulation

Parameters	Duration (days) 0	7	14	21	28	SEM	P value
Reaction time (s)	37.00^{c}	16.00^{a}	23.40^{ab}	27.20^{b}	25.00^{b}	3.99	0.02
Semen volume (mls)	1.50	2.50	1.00	3.00	2.00	0.84	0.21
P^{H}	8.00	7.00	7.50	8.00	7.00	0.97	0.91
Sperm motility (%)	70.00^{c}	80.00^{ab}	85.00^{a}	80.00^{ab}	75.00^{c}	4.71	0.05
Sperm concentration ($x10^{6}$)	152.00	159.00	166.00	156.00	151.0	15.5	0.36
Live sperm (%)	75.00^{b}	80.00^{ab}	90.00^{a}	85.00^{ab}	80.0^{ab}	5.94	0.03
Dead sperm (%)	25.00^{b}	20.00^{ab}	10.00^{a}	15.00^{ab}	20.0^{ab}	5.90	0.02
Normal sperm cells (%)	69.00	73.00	89.00	73.00	79.0	8.03	0.12
Detached heads (%)	17.00	13.00	03.00	12.00	8.0	3.81	0.30
Coiled tails (%)	10.00^{b}	09.00^{b}	06.00^{a}	11.00^{b}	7.0^{ab}	2.52	0.05
Bent tails (%)	04.00	05.00	02.00	04.00	6.0	1.87	0.45

Means with different superscripts differed significantly ($P < 0.05$) within rows, 00, 07, 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively, N= sample size (10) per treatment. SEM=standard error of the mean

CHAPTER FIVE

5.0 DISCUSSION

5.1 Behavioural characteristics of rabbit does exposed to rabbit bucks at different durations of biostimulation

The instantaneous behaviour of rabbit does when presented to an intact buck could give a very good indication of her sexual receptivity. Aggressiveness, flattening at a corner and circling are behaviours indicative of a doe's unwillingness to mate. It was observed that the percentage of the lordotic posture varied across treatments. The rabbit does in the 7 days biostimulated group had the highest mounting with 70% lordotic posture. Thus, the rabbit does in this group may be described to have displayed a higher oestrus intensity as demonstrated by the higher degree of docility exhibited by the does at the point of mating. However, the rabbit does in the control group recorded 90% flattening at a corner, 100% circling, 70% mounting without lordosis and 30% mounting with lordosis which differed significantly compared to the 30%, 80%, 30% and 70% from the 7 days biostimulated does, 30%, 90%, 60%, and 40% from the 14 days biostimulated does, 40%, 90%, 50% and 50% from the 21 days biostimulated does, 50%, 70%, 70% and 30% from the 28 days biostimulated does, respectively. These differences may be due to a higher synchronization of the biostimulated does as a result of the pheromonal cues perceived from the bucks during the exposure period. However, this observation is in accordance with the report of Ola and Oyegbade (2012) who reported that mounting with lordosis was displayed by over 50% of rabbit does exposed to rabbit bucks during mating.

5.2 Vulva colour of rabbit does exposed to rabbit bucks at different durations of biostimulation.

The differences observed in the vulva colour of these does across the treatments suggest that cues from the bucks might have stimulated the endocrine systems of these does to different degrees of follicular growth, follicular wave recruitment and accumulation of anthral fluids, hence the disparities in their vulva colours and oestrus intensities at the point of mating. At oestrus (heat), the vulva colour of most mammals is red (Beyer *et al*., 2007), hence, the does with reddish vulva were assumed to be on heat, while does without reddish vulva were not. Therefore, it appears that biostimulation can lead to a higher oestrus response in rabbit does as was observed in rabbit does biostimulated for 7 days prior to mating. The vulva colour also affected the willingness of the does to mate, as most of the does with either reddish or pinkish vulva willingly accepted mating, while there was a delay in mating by the other does whose vulva colours were white, pale-pink, purple and purple with grey overtone. This observation is in agreement with the findings of Najjar *et al.* (2013) who reported 60% red and 40% pinkish vulva colour of rabbit does treated with PMSG prior to artificial insemination.

Based on this observation, it can be suggested that pheromone from rabbit bucks has similar pharmacodynamics as found with hormonal induction in rabbit does.

5.3 Sexual receptivity of rabbit does exposed to rabbit bucks at different durations of biostimulation

In this study, it was observed that receptivity by rabbit does at the point of mating differed across treatments. The trend of acceptance showed that rabbit does in the 7 days biostimulated group recorded 70 % successful mating, while the 14, 21, 28 days biostimulated groups and the control had 60%, 50%, 40% and 30% acceptance, respectively. The higher acceptance rate over the control is an indication that the pheromonal cues from the bucks led to a higher mating response in the biostimulated does. Kermabon *et al*. (1994) also reported that receptivity of a rabbit doe is related to her ovarian status which depends to a large extent on the number of ovarian follicles and a high concentration of plasma estradiol. Similarly, Theau-Clement (2007) noted that sexual receptivity in rabbit does have generally been based on the colour and turgidity of the vulva.

These findings have provided further evidence that exposing rabbit does to rabbit bucks prior to mating improves the does willingness to mate. Also, it was observed that the does' responsiveness to the treatment effect (pheromonal cues from the bucks) decreased with increase in the duration of exposure (in days), hence a decrease in the acceptance to mating across the treatments. This may be due to a constant exposure of the does to the bucks till the time of mating without any other stimulus variation.

5.4 hormonal profile of rabbit does and bucks under different durations of biostimulation

The does' estrogen profiles of 67.4pg/ml after 7 days of exposure, 55.8pg/ml on the 14th day, 38.6pg/ml on the 21st day and 48.0pg/ml on the 28th day, suggests a differential in the magnitude of the does' ovarian activities due to the treatment effect as estrogen supports the maturation of oocytes and stimulates the growth of uterine lining. This observation is similar with the findings of Ungerfeld *et al. (*2003) who observed a higher estradiol pulse frequency in ewes that responded to ram effect than in ewes that did not.

However, the bucks' testosterone peak of 13.0ng/ml on the 14th day suggests that this exposure might have exerted a higher stimulation on the hypothalamo-hypophyseal gonadal axes of the biostimulated bucks for an increased secretion of gonadotropins (FSH and LH) which accelerated a higher testosterone secretion with higher sperm liveability (higher motility, percentage live and percentage normal sperm cells) in the treatments than the control. The differences in the magnitude of testosterone secretions across the treatments is a measure of the different degrees of stimulation received by the bucks at different time intervals. Therefore, longer duration of biostimulation seems to improve reproductive traits of rabbit bucks. This finding agrees with the work of Kishk (2008) who reported higher testosterone secretion in rams exposed to odour from cycling ewes. This result showed that a close male-female proximity in rabbits prior to mating has a potent effect in increasing the androgenic activities in rabbit bucks.

5.5 Reproductive performance of rabbit does exposed to rabbit bucks at different durations of biostimulation

An intensive investigation on the reproductive performance of the rabbit does in this study revealed that biostimulation favoured the treatment group (does exposed to bucks' cues) as the fecundity and prolificacy of the treatment group differed from the control group. The pregnancy rate declined with increase in the duration of doe exposure to the bucks. Hence, it can be assumed that the sensation of the cues from the bucks diminished with increased duration of doe exposure.

However, the differences observed in pregnancy rate (7 days biostimulation versus control, 14, 21 and 28 days biostimulation, also, 14 days biostimulation compared with control, 21 and 28 days biostimulation) may be attributed to a super-ovulation which led to the shedding of larger anthral follicles due to a higher ovarian stimulation and pituitary responsiveness in the biostimulated does. This observation is in consonance with the result of Laborda *et al*. (2008), who reported that ovulation can be induced in rabbit does by the sight of a sexually active male in close proximity. This observation also agreed with the reports of Kustos *et al.* (2000); Eiben *et al.* (2001) and Bonanno *et al.* (2003), who reported that rabbit does placed adjacent to rabbit bucks before mating had larger litter size at birth over the control. Nevertheless, it was noted that the prolificacy of the rabbit does in the treatment group was negatively affected with increase in duration of exposure. This observation is in conjunction with the findings of Ola and Oyegbade (2012), who reported that exposing rabbit does to rabbit bucks for a longer period appears to be detrimental to their reproductive performance.

5. 6 Reproductive performance of rabbit bucks exposed to rabbit does at different durations of biostimulation

The treatment effect also improved some reproductive traits of the rabbit bucks exposed to rabbit does. The shortest reaction time of 16 seconds was recorded in rabbit bucks exposed to rabbit does for 7 days while the non exposed rabbit bucks had the longest reaction time of 37 seconds. The shortest reaction time of 16 seconds recorded in this study is lower than 27.9 s obtained by Brun *et al*. (2004) but higher than 14.2 s found by Marai *et al*. (2002). At the point of mating, the control group displayed a copulatory thrust with ejaculation in 37 seconds, while in 7, 14, 21 and 28 days biostimulated bucks, copulatory thrust with ejaculation occurred within 16, 23.4, 27 and 25 seconds respectively. Differences in the durations (days) of exposure might be responsible for these variations.

Also, there were significant differences in sperm motility, percentage live sperm, percentage dead sperm and coiled tails across treatments. The differences in the reproductive performance in the treatment groups may be due to a variation in the perception of the treatment effects by the bucks. This is in line with observation of Silvestre *et al*. (2004) who reported that controlled male exposure to estrous females, viewing and periodical change of females enhances male sexual performance in several mammalian species. However, Boehm *et al*. (2005), reported that pheromones detected by the olfactory system stimulates male and female sexual behaviours in mammals. The positive effect of the does' stimuli on the bucks reproductive parameters (increased sperm motility, sperm concentration, and percentage live sperm) agrees with responses found in bulls (Bailey *et al*., 2005), boars (Kunavongkrit *et al*., 2005), hamsters (Cooke *et al*., 2001) and rams (Kasimanickam *et al*., 2007).This finding is also in close agreement with the reports of Alvari˜no (2000) and Kishk (2008) which show reported a higher semen quantity and quality in rams exposed to odour and estrous manifestations from ewes.

Therefore, it seems that pheromones (olfactory), auditory and visual cues could mediate increased reproductive potentials of rabbit bucks in physical close proximity to rabbit does with limited contact.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The results showed that there were no significant difference in the productive performance of the rabbit does and bucks. However, there were significant differences in the behavioural attributes displayed by the rabbit does at the point of mating. Circling and flattening at a corner which are indicators of rabbit does unwilling to mate decreased in the biostimulated groups. This decrease in circling and flattening at a corner were more prounounced in the 7 and 14 days biostimulated groups. Mounting with lordotic posture was higher in the biostimulated groups but highest in the 7 days biostimulated group, which is an indication that the treatment effect positively affected the behavioural response of the rabbit does. Also, the vulva colour of the rabbit does varied across treatments.

Prior to exposure, the rabbit does had whitish vulva and upon exposure to the bucks, the vulva colours were graded; from whitish to pinkish, cherry red and dark red. This observation implies that the oestrus intensities of the biostimulated does were implicated by the treatment effect. The rabbit does with reddish and pinkish vulva willingly accepted mating and performed better than does whose vulva were whitish or pale.

The degree of acceptance to mating were significant across treatments. Rabbit does with reddish and pinkish vulva had highest acceptance to mating. Therefore, biostimulating rabbit does improves their acceptance to mating. Nevertheless, the plasma concentration of estradiol was improved in the biostimulated group. This differed significantly across treatments and may be responsible for higher expression of estrus (heat) in the biostimulated does. The higher estradiol profile of the rabbit does may be responsible for higher reproductive performance in the biostimulated does' as gestation length, pregnancy rate and average litter weaning weight were better than the control. In line with this, the testosterone concentration of the biostimulated rabbits bucks differed significantly from the control. It was observed that reaction time, sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails of the biostimulated rabbit bucks differed significantly from the control. Therefore, biostimulation seems to improve the reproductive performance of rabbit bucks and does kept under normal condition.

6.2 Conclusions

This study concludes as follows:

1. Flattening at a corner and circling by rabbit does during mating can be minimised by exposing the does to rabbit bucks prior to mating. This is illustrated in the 7 days (flattening 30 %,) and 28 days biostimulated does (circling 70 %) respectively.
2. Vulva colour can be used as a reliable tool for estimating rabbit does on heat as was observed in rabbit does biostimulated for 7 days prior to mating in this study.
3. To obtain higher pregnancy rate, liter size, weaning weight and shorter gestation period, rabbit does should be mated when their vulva colour is red or pink.
4. Sexual receptivity of rabbit does and libido in rabbit bucks can be enhanced by keeping rabbit does and bucks closer to each other without physical contact for about 7-14 days before mating .
5. The plasma estradiol and testosterone concentrations of rabbit does and bucks can be enhanced by 7-4 days controlled exposure.

6. Exposing rabbit does to bucks prior to mating has a potent androgenic activity in the bucks.
7. Biostimulation is a veritable tool for improving reproduction in rabbits. Meanwhile, exposing rabbit does to rabbit bucks longer than 7-14 days prior to mating may adversely affect reproductive performance.

6.3 Recommendations

1. Rabbit does should be mated when their vulva colour is reddish or pinkish.
2. For higher litter size, rabbit does should be exposed to rabbit bucks 7-14 days prior to Mating.
3. To improve serum testosterone concentration of rabbit bucks, 14 days exposure to rabbit does (biostimulation) is recommended.
4. Buck exposure to rabbit does prior to mating should be adopted and more research using different durations and breeds should be conducted to evaluate the effect of biostimulation on the attainment of puberty in rabbits.

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