AMELIORATIVE EFFECT OF BICARBONATE BUFFER, VITAMIN C AND BAOBAB FRUIT PULP MEAL ON GROWTH AND REPRODUCTIVE PERFORMANCE OF RABBITS UNDER TROPICAL ENVIRONMENT

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**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
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ANIMAL SCIENCE**

DEPARTMENT OF ANIMAL SCIENCE

FACULTY OF AGRICULTURE

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APRIL, 2016

DECLARATION

I hereby declare that the work in the thesis titled '**AMELIORATIVE EFFECT OF BICARBONATE BUFFER, VITAMIN C AND BAOBAB FRUIT PULP MEAL ON GROWTH AND REPRODUCTIVE PERFORMANCE OF RABBITS UNDER TROPICAL ENVIRONMENT'** has been performed by me in the Department of Animal Science under the supervision of Prof. P. P. Barje, Prof. G. T. Iyeghe-Erakpotobor and Prof. G. N. Akpa. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any University.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This thesis titled **"AMELIORATIVE EFFECT OF BICARBONATE BUFFER, VITAMIN C AND BAOBAB FRUIT PULP MEAL ON GROWTH AND REPRODUCTIVE PERFORMANCE OF RABBITS UNDER TROPICAL ENVIRONMENT",** by Kevin Usman ANOH meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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Prof. P. P. Barje — Date
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Dedication

This study is dedicated to God Almighty and to my dear daughter, Adela Nte-tey (Grace) Anoh

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LIST OF ABBREVIATIONS

BFPM- Baobab Fruit Pulp Meal

BPM- Breath per Minute

CL- Copora Lutea

CPM- Count per Minute

ET- Ear Temperature

FSH- Follicle Stimulating Hormone

GnRH- Gonadotropin Releasing Hormone

HR- Heart Rate

LH- Luteinizing Hormone

^{o}C – Degree Celsius

PDIFF- Pair-Wise Difference

ROS- Reactive Oxygen Species

RR- Rectal Temperature

RT- Rectal Temperature

T4- Thyroxine Hormone

THI- Temperature Humidity Index

ABSTRACT

The aim of the study was to evaluate the ameliorative effects of sodium bi-carbonate buffer, vitamin C and Baobab fruit pulp meal (BFPM) on growth and reproductive performance of rabbits under tropical environment. Four experiments were conducted. In Experiment 1, a total of thirty (30) weaned rabbits were used. The rabbits were allotted into the treatment groups with six (6) rabbits per treatment in a completely randomized design. Rabbits in the first group (T_1) were the control, animals in the treatment 2 and treatment 3 (T_2) and (T_3) were fed with diets as in the controls and given potassium bicarbonate ($KHCO_3$) and sodium bicarbonate ($NaHCO_3$) buffer respectively. Rabbits in treatment four (T_4) were fed diet containing synthetic vitamin C and the fifth group (T_5) was fed diet containing BFPM. Rabbits were given access to feed and water *ad libitum*. All recommended managerial practices were dully observed. Microclimate parameters of ambient temperature (AT) and relative humidity (RH) of the rabbitry were taken daily from February through June. The values were used to calculate temperature-humidity index (THI). Parameters monitored and measured on weekly basis included weight gain, feed intake and thermoregulatory parameters such as respiratory rate (RR), heart rate (HR), rectal temperature (RT) and ear temperature (ET). Blood samples (5 ml) were collected from the ear vein at 10.00 h from four animals chosen randomly from each group of rabbits respectively before, and at the end of the experiment and serum metabolite and thyroxine hormone levels were evaluated. In experiment two, a total of 50 adult rabbits were used comprising 25 males and 25 females. The rabbits were randomly allotted into five experimental treatment groups, with ten (10) rabbits per treatment in a completely randomized design. Rabbits were arranged in treatment groups as stated above. Parameters monitored were: thermoregulatory parameters, serum metabolites, thyrosine, testosterone and progesterone hormonal assay, semen quality characteristic, sperm morphology, epididymal and gonadal sperm reserve and reproductive performance of the female rabbits. In experiment 3, a total of thirty (30) weaned rabbits were used. The rabbits were randomly allotted into five experimental treatment groups, with six (6) rabbits per treatment in a completely randomize design. The animals were fed diets containing graded levels [0.0% (Control), 2.5%, 3.5%, 4.5% and 5.5%] of BFPM. Parameters monitored were the same as in experiment one. In experiment four, 50 adult rabbits were used, comprising 25 males and 25 females. The rabbits were randomly allotted into five experimental treatment groups, with ten (10) rabbits per treatment in a completely randomized design. Rabbits were arranged in treatment groups as in experiment three and parameters evaluated were the same as in experiment two. It was found that the THI values of year A and B were similar. THI of March to May showed that environmental conditions were stressful in these months to the animals. Vitamin C and BFPM significantly ($P<0.05$) reduced thermoregulatory parameters and enhanced feed intake compared to the treatments buffers. BFPM significantly ($P<0.05$) increased hormonal concentrations, semen quality of bucks and reproductive performance of does compared to other treatments. When graded levels of BFPM were used, it was found that the treatments with 4.5% and 5.5% significantly ($P<0.05$) reduced thermoregulatory parameters and enhanced feed intake compared to other treatments. While 2.5% and 3.5%BFPM, increased weight gain and final weight of growing rabbits, the treatments with 3.5 – 5.5% BFPM significantly ($P<0.05$) increased hormonal levels, semen quality of the rabbits and reproductive performance of does. It was concluded that BFPM is more effective in ameliorating heat stress in rabbit production and reproduction and can be used up to 5.5% inclusion level.

CHAPTER ONE

1.0 INTRODUCTION

One of the greatest challenges to successful livestock improvement and production in the tropics is heat stress. Reproduction in farm animals is highly affected by environmental factors and when environmental conditions are favorable, reproductive activity expresses its full potential. Favorable conditions must include adequate photoperiod, thermo-neutral conditions, food availability in quantity and quality, and a low stress environment (Marai *et al.,* 2001). Inadequate conditions may lead to a decrease in growth and reproductive capacity, varying from sub-fertility to infertility. Therefore, the expression of growth and reproductive potentials is only possible when animals develop and establish a homeostatic equilibrium with their external environment (Marai *et al.,* 2001)

Almost annually heat waves kill thousands of different rabbit breeds in tropical countries, which cause economic losses to both rabbit breeders and consumers (Daader *et al*., 2003). The thermoneutral zone of growing rabbit (6-12 weeks of age) is 15-18^{o}C. In tropical and sub-tropical countries, climatic heat is a major constraint on animal productivity; production and reproduction are impaired as a result of the drastic change in biological function caused by heat stress (Daader *et al*., 1997).

Feed intake and digestibility have been shown to reduce with increase in environmental conditions (Ayyat *et al*., 1996). Rabbits that were treated with 1.2% of potassium bicarbonate ($KHCO_3$) buffer were reported to have improved performance (Fayes *et al.* 2011) during hot summer conditions in Egypt. Vitamin C (ascorbic acid) is one of the most widely studied vitamins used to alleviate heat stress in rabbits. It promots growth and improves reproduction in

rabbits (Zeweil *et al*, 2009; Afifi and Makled 1995). Vitamin C is a good scavenger of free radicals hence serves as a good anti-oxidant (Zeweil *et al*, 2009). Baobab fruit bulb has been reported to contain high vitamin C (Odetokun, 1996; Adejuyitan *et al.* 2012). The use of Baobab fruit pulp in ameliorating heat stress in rabbits is yet to be explored.

The aim of this study is to ameliorate the deleterious effect of heat stress with bi-carbonate buffer, vitamin C and baobab on growth and reproductive performance of rabbits reared under tropical environment.

1.1 Hypothesis:

This study was designed to evaluate the following hypotheses:

Ha: Bicarbonate buffer and vitamin C have an effect in ameliorating heat stress in rabbits during growth and reproductive phases under tropical condition.

Ho: Bicarbonate buffer and vitamin C do not have an effect in ameliorating heat stress in rabbits during growth and reproductive phases under tropical condition.

1.2 Objectives of the Study

The objectives of the study were to evaluate the effect of buffer, vitamin C and baobab:

1. on growth and reproductive performance of rabbits reared under tropical condition.
2. on thermoregulatory response and semen quality of rabbits reared under tropical condition.
3. on serum metabolites and hormonal profile of rabbits reared under tropical condition.
4. To determine the optimum level of baobab in ameliorating heat stress in rabbits under tropical condition.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 General Aspect of Reproduction in Rabbits

Reproduction in rabbits is a complex process, is controlled by many substances called hormones. Rabbits like camelids and cats are induced ovulators. They require stimulation associated with mating to trigger the release of gonadotropin-releasing hormone (GnRH) and then the surge of Leutinizing hormone (LH), which is necessary to elicit ovulation (Bakker and Baum, 2000). The aspect of reproduction in rabbits can be seen from the male and female perspective.

2.1.1 The male

2.1.1.1 Gonad development and puberty

The gonads in rabbits have been reported to begin to differentiate on the 16th day after fertilisation (Theau-Clement *et al*., 1995). After birth, the testes develop less quickly than the rest of the body. From the age of five weeks, they begin to grow very rapidly. Accessory glands undergo a similar development, but at a more even rate and are less precocious. Spermatogenesis begins between days 40 and 50 (Nakarishi *et al.,* 2004). The testicular tubes become active at about 84 days. The first spermatozoa are present in the ejaculate at about 110 days (Nakarishi *et al.,* 2004).

Sexual maturity, defined as the moment when daily sperm production ceases to increase, is reached at 32 weeks by New Zealand White rabbits in temperate climates. However, a young buck can be used for reproduction from the age of 20 weeks. Indeed, the first manifestations of sexual behaviour appear at days 60 to 70, when the rabbit makes it first attempts at riding

(Theau-Clement *et al*., 1995; Roca *et al* 2005). Coitus may occur for the first time at about 100 days, but the sperm have either very weak or completely lack viability cells in the first ejaculates. So first mating should be timed for age 135 to 140 days (Roca *et al* 2005).

2.1.1.2 Sperm production

Under ideal condition, the volume of semen ejaculated by rabbits is reported to be about 0.3 to 0.6 ml. Concentration is evaluated at 150 to 500 × 10^6 spermatozoa per ml, but both volume and concentration are liable to vary (Theau-Clement *et al*., 1995; Roca *et al* 2005). False mountings, one or two minutes before copulation, increase the concentration of the ejaculate. In two successive servicing the first acts as a preparation for the second, which is less voluminous but more concentrated. During subsequent mating the volume of the ejaculate decreases, while concentration increases between the first and the second ejaculate and then diminishes (Theau-Clement *et al*., 1995). The total number of spermatozoa per ejaculate follows the same trend.

Maximum spermatozoa production is obtained by using the buck regularly once a day. Daily spermatozoa production is roughly 150 to 300 million, independent of the rate of ejaculation. The maximum epididymis reserve is only one to two billion spermatozoa, only partially mobilizable for repeated ejaculations (Nakarishi *et al.*, 2004).

2.1.2 The female

2.1.2.1Gonad development, puberty and sexual maturity

As in the male foetus, female sexual differentiation takes place on the 16th day after fertilisation. Ovogonial division begins on the 21st day of foetal life and continues until birth (Harkness *et al*., 2010). The first follicles appear on the 13th day after birth and the first antrum follicles at about

65 to 70 days. Does are able to mate first at 10 to 12 weeks, but as a rule this will not produce ovulation (Harkness *et al*., 2010).

It has been reported that female rabbits reach puberty that is the onset of sexual receptivity and ovulation at around 14 weeks of age (Hulot *et al*., 1982; Diaz *et al*, 1988, Rommers *et al*., 2001). Other factors that affect attainment of puberty are breed (Harkness *et al*., 2010) and feeds and feeding system (Rommers *et al*., 2001). Some researchers recommended that breeding begin when the female rabbit reaches about 75% of its adult weight (Gosálvez *et al*., 1994). Body weight is known to affect litter size in rabbits. Better litter size is observed in rabbits with higher body weights (that is those fed *ad libitum*) at first insemination (Rommers *et al*., 2002).

The pattern of follicle development in rabbits has not been established. Unlike most other mammals, the formation, activation and development of ovarian follicles occurr entirely postnatally in rabbits, with primordial follicle assembly completed presumably between 2 and 4 weeks of age (Hutt *et al*., 2006). Some authors have indicated that follicles with a diameter >1.8 mm are present only in receptive females (Lefèvre and Caillol, 1979). However, there is no clear understanding of the relationship between follicle diameter and ovulatory capability in rabbits. Preovulatory follicles are also referred as those > 800 to 900 um in diameter (Kranzfelder *et al*., 1984). Others have reported that preovulatory follicles are those that are >1.5 mm or >2 mm in diameter (Parkes, 1931; Hunzicker-Dunn *et al*., 1979; Marongiu and Gulinati, 2008).

2.1.2.2 The Oestrus Cycle

Female rabbits are classified as induced or reflex ovulators because ovulation takes place after mating (Heape, 1905; Friedman, 1929; Spies *et al*., 1997; Harkness *et al*., 2010); hence, this phenomenon has made rabbits not to have a regular oestrous cycle (Harkness *et al*., 2010) as in

other domestic species for example in the Bovine. Stein and Walshaw, (1996), however reported that rabbits display periods of sexual receptivity and non-receptivity. They found that period of receptivity lasted about 5 to 6 days or 7 to 10 days (Harkness *et al*., 2010) in the absence of mating. This period was followed by 1 to 2 days of non receptivity (Harkness *et al*., 2010) or 4 days (Alvariño and Ubilla 1993).

It has been noted, however, that 90 percent of the time when a doe has a red vulva she will accept mating and ovulate (Beyer *et al*., 2007), whereas when the vulva is not red in colour, the doe will accept service but chances of her getting pregnant is only 10 percent. A red vulva is therefore, a strong indication, though not a proof, of oestrus (Abdel-Ghaffar and Agag, 1994; Morura *et al*., 2003; Vicente *et al*., 2008). However there are no sufficient reports on the relationship between this external sign and subsequent occurrence of ovulation or pregnancy (Adams, 1983). Hoffman *et al*. (2010) reported that post-mating administration of estradiol prevented the decline in chinning and receptivity.

Studies on sexual behavior in female rabbits during pregnancy, pseudopregnancy, and post partum reveals that some pregnant rabbits accept mating, but the release of gonadotropins and ovulation did not occur (Mills and Gerardot, 1984). Non-pregnant rabbits are more sexually active than pregnant rabbits (Beyer and Rivaud, 1969; Stoufflet and Caillol, 1988). Likewise, sexual behavior or receptivity was reduced in pseudopregnant rabbits, but increased again by the end of the pseudopregnant period, when progesterone concentration returned to basal levels (Caillol *et al*., 1983). Rabbits are highly receptive during the post partum period, the following day of parturition and after weaning (Day 28 of lactation), when they are able to ovulate (Beyer and Rivaud, 1969). Primiparid rabbits are less receptive than nulliparid rabbits. Receptivity also

has been found to vary depending on stage of lactation (that is, lactating are less receptive than non-lactating) and receptivity may also influence reproductive performance (Theau-Clément, (2007).

2.1.2.3 Ovulation

Ovulation is normally induced by the stimuli associated with coitus, and occurs 10 – 12 hours after mating. Investigators in an early study suggested that follicles which are able to ovulate remain for about 7 to 10 days and then regress (Hill and White, 1933). Follicular development is thought to be supported by the growth of 5 to 10 follicles on each ovary at any one time. Once follicles reach an ovulatory size, they secrete oestrogens in increasing amounts, and rabbits show sexual receptivity for a period of time. When those follicles degenerate, secretions of oestrogen decline and females rabbits become non receptive (Harcout-Brown, 2002). Likewise, results of Lefèvre and Caillol, (1978) have shown that receptive rabbits have more large follicles and a higher concentration of estradiol in the follicular fluid than those of non receptive rabbits. It has been reported that 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 (Hulot *et al*., 1988). Pseudopregnancy occurs after a sterile mating or after hormonal induction of ovulation in rabbits and lasts about 16-18 days (Dharmarajan *et al*., 1988; Harcourt-Brown, 2002).

2.1.2.4 Fertilisation, Gestation and Kindling

When sperm is deposited by the male in the upper part of the vagina, the spermatozoa make their way upwards rapidly. They can reach the fertilization area (in the distal ampulla, near the isthmus) 30 minutes after coitus (Harper 1973). During their journey, the spermatozoa undergo a maturation process which enables them to fertilize the oocytes. Of the 150 to 200 million spermatozoa ejaculated, only two million (1 percent) will reach the uterus (Abdel-Ghaffar and

Agag, 1994; Morura *et al*., 2003; Vicente *et al*., 2008). The rest are defeated by obstacles at the cervix and uterotubal junction (Rashwan *et al*., 2003). The egg reaches the uterus 72 hours after ovulation the egg divides on its way through the oviduct (Holt 1989). The uterine wall differentiates, but the uterine dentellus appears only five to eight days after coitus. It is the synchronisation of these phenomena that makes possible the implantation of the egg. Implantation proper takes place seven days after mating, at the blastocyst stage (Harcout-Brown, 2002). Distribution of the blastocysts is roughly equidistant in each horn, but the blastocysts never move from one uterine horn to the other. From the third to the 15th day after mating the progesterone rate continues to increase, then remains stationary and finally drops rapidly before parturition (Morura *et al*., 2003). The maternal placenta develops along with the foetus, reaching its maximum weight towards the 16th day of pregnancy. The foetal placenta is visible about the tenth day and becomes larger until birth (Morura *et al*., 2003).

The end of pseudopregnancy is marked by the maternal behaviour of the doe and nest-making, linked to the swift drop in blood progesterone (Beyer and Rivaud, 1969). While such pseudopregnancy is much used in research laboratories on the physiology of reproduction, it is very uncommon in natural-mating rabbitries. When a doe is serviced under unfavourable conditions, she does not ovulate (Harcourt-Brown, 2002; Marai, *et al*., 2004), and it is exceptional for ovulation to occur without fertilisation (as in mating with a sterile but sexually active buck). Unfertilised ovulation can occur in 20 to 30 percent of artificially inseminated does injected with GnRH. In this case, an injection of prostaglandin $PGF_{2\alpha}$ on the 10th or 11th day will halt the pseudopregnancy and the doe can be fertilized just 14 days after an earlier infertile insemination. Without $PGF_{2\alpha}$ treatment, the doe cannot be fertilized again until aftre week (Hoffman *et al*., 2010).

The mechanism of parturition is not very well known. It seems that the secretion of corticosteroids by the supra-renals of the young plays a part, as in other animal species, in giving the signal for parturition. The $PGF_{2\alpha}$ may also be instrumental in intiating the process. At the end of gestation, the doe makes a nest for the litter with her own fur and materials she has available such as straw and shavings. This behaviour is linked with an increase in the oestrogen/progesterone ratio and with the secretion of prolactin. The doe does not always make a nest, or she may kindle outside the nesting box (Hoffman *et al*., 2010).

2.2 Reproductive Endocrinology in Rabbits

A major share of the control of sexual functions in both the male and the female begins with secretion of GnRH by the hypothalamus. This hormone in turn stimulates the anterior pituitary gland to secrete two other hormones called gonadotropic hormones LH and FSH (Dharmarajan *et al*., 1988). In turn, LH is the primary stimulus for the secretion of testosterone by the testes, and FSH mainly stimulates spermatogenesis. The male hormone, testosterone produced by the testicles, stimulates the development of the vas deferens, and associated glands. It is also responsible for the extra muscle growth in males as compared to females, and for the male odour which helps to stimulate the female to stand still for mating (Labib *et al*., 1978).

In females, FSH stimulates the growth of follicles on the surface of both ovaries. Each follicle contains an ovum (Dharmarajan *et al*., 1988). In rabbits, the follicle remains mature for about 10 days and if mating does not take place, they die and disappear; at the same time, new ones are developed. As follicles grow, they produce oestrogen which has a feed-back effect on FSH. Estrogen also stimulates signs of oestrus and oestrus behavior. LH, on the other hand, stimulates the shedding of the eggs, and together with FSH, they stimulate the uterine for attachments to the uterine walls. A hormonal signal is sent to the ovaries which produce progesterone from the site

of the shed ova. This site is called *corpora lutea.* Together with other hormones, this progesterone acts on the uterus to prevent its contracting and also to supply the needed nutrients for the maintenance of the foetus (Labib *et al*., 1978).

At the end of pregnancy, changes in the levels of estrogen and progesterone signals the uterine to secret oxytocine from the pituitary gland (Guyton and Hall, 2006). This hormone helps in the contraction of the uterus and expulsion of the young. Also expelled are the membranes in which the fetuses have been growing are also expelled. Oestrogen from the tissue surrounding the foetus stimulates the duct development in the mammary gland. Progesterone and prolactine from the source stimulates the aveoli and the milk gland for milk-let down (Guyton and Hall, 2006).

During development of the corpora lutea, the theca interna takes part in the active corpus luteum (CL), while the theca externa takes part in the vascularisation and forms the sheath of the fully-grown CL (Deanesly, 1930). In addition, LH causes the differentiation of granulosa cells to luteal cells (Labib *et al*., 1978). The growth and subsequent regression of CL result primarily from changes in the volume, rather than changes in the number of individual luteal cells (Dharmarajan *et al*., 1988). Kelley and Brinkley, (1971) reported that corpora lutea reached maximum size by Day 9 (Day 0 = Day of mating), started to regress by Day 15, and were significantly reduced in size by Day 21. In another study, Dharmarajan *et al*. (1988) reported that CL volume decreased after Day 11. The progesterone concentrations were found to be high (13.4 ng/ml) on Day 10 and low (1.5 ng/ml) by Day 18 (Miller and Keyes, 1976). Browning *et al*. (1980) reported a similar result; they documented high progesterone concentrations on Days 10-12, followed by a steady decline till less than 1 ng/ml on Days 16-18. By using an ovarian *in vivo* perfusion method, Dharmarajan *et al*. (1988) reported a significant rise in progesterone secretion

from Day 1 to Day 11, a decline by Day 18. Interestingly, when pseudopregnant rabbits were hysterectomized on Day 1 and treated with estradiol, the progesterone concentrations remained elevated (around 11 ng/ml) at least until Day 28 (last day of observation) (Miller and Keyes, 1976). In addition, when pseudopregnant rabbits were hysterectomized on Day 4 and treated with LH on Day 9, life-span of corpora lutea was prolonged to Day 24 (Kelley and Brinkley, 1971). It was suggested in some studies that the uterus has a role in limiting the life-span of the CL in pseudopregnant rabbits (Kelley and Brinkley, 1971; Miller and Keyes, 1976). The secretion $PGF_{2\alpha}$ by the uterine endometrium has been suggested to have a direct luteolytic effect on the rabbit CL (Scott and Rennie, 1970; Koering, 1974).

In pregnant rabbits, the maximum mass of CL was reached on Days 16 to 20 and then declined gradually to Day 28, near term (Abdul-Karim and Bruce, 1973). The progressive reduction in luteal cell numbers was related to the gradual decline in mass of CL, but a clear structural regression of CL, that is, reduction in surface area of capillaries per CL and decline in CL blood flow was observed by Day 28-30 (Dharmarajan *et al*., 1988). In an early study Hilliard *et al*. (1968) reported that serum progesterone concentrations from CL declined after Day 16 in pregnant rabbits. Results of later studies showed that highest values of serum progesterone concentrations were reached on Days 10-12 and were similar to those found during pseudopregnancy, then progesterone declined slightly but remained elevated through Day 28 (Browning *et al*., 1980). Similar results were described by Egea del Prado *et al*. (1984), who found maximum progesterone concentrations by Days 11 to 13 (10.8-11.8 ng/ml), followed by a slightly decreased, but elevated progesterone until Day 25 to 28 (7.15 ng/ml). Maintenance of pregnancy (30 to 32 days) in rabbits requires progesterone production by CL (Greewald and Rothchild, 1968; Rashwan *et al*., 2003), and the maintenance of the CL function is dependent on

the presence of the conceptus and estradiol (Greep, 1941; Holt, 1989). A critical period for the CL appeared to be days 5 to 6 after mating, during which the presence of estradiol is essential for continued luteal development and secretion of progesterone (Miller and Keyes, 1978).

2.3 Rabbit Reproduction and Environment

2.3.1 Season

In Europe the season is usually analyzed in terms of the combined effects of light and ambient temperature (Lebas *et al.,* 1986*).* In tropical climates, the ambient temperature effect seems to be dominant, but an effect due to variations in the length of day light cannot be excluded (Marai et al 2004). The reproduction cycles of the European wild rabbit are strongly influenced by the season. The reproduction period can be longer or shorter, at either end, according to both ambient temperature and availability of feed (Lebas *et al.,* 1986; Marai *et al*., 2004).

Marai *et al*. (2004) stated that the season of the year exert significantly effect on litter weight at 21 days of age and kit body weight at birth. The effect of the season on reproductive performance of rabbits could be different in does and in growing rabbits. Choudhary *et al.* (2001) described the highly influential effect of the season on gestation period, kindling interval and litter weight at weaning. Bhatt *et al*. (2002) found that litter size and weight at birth, litter size at weaning as well as litter weight at weaning were all higher during winter as compared to those during summer and the rainy season. Similar results were reported in Angora rabbits by Kumar *et al*. (2005).

The effect of the season has also been revealed in weight gain. McNitt and Lukefahr (1993) reported a significant impact of the season on the growth of rabbits, with the lowest gain in

summer. In concurrence with these findings, Bhatt et al. (2002) obtained the most successful results for rabbit reproduction during the winter season.

2.3.2 Lighting

Data in literature are conflicting on the effect of daylight length on reproductive activities of the rabbit. Some studies showed that the reproductive traits increase (Lebas *et al.*, 1986; Theau-Clement *et al.*, 1990; Uzcategui and Johnson, 1987), others showed either no effect or even negative effects (Hassanien, 1980), with exposure to long daylight, Ibrahim (1985) and Mady *et al.* (1990) however obtained favourable effects with decreasing length of day light.

Exposure of the does to long daylight (16L:8D) showed negative effects on most of the traits studied, when compared to exposure to short daylight (8L: 16D) (Marai *et al*., 2007). Such effects may be mainly due to disturbance of the physiological activities of the animals as a function of elevated ambient temperature, since exposure of the animals to lamps radiation in the warm sub-tropical conditions increases the perception of warmth and such feeling aggravates during the hot climate conditions in summer. Marai *et al*. (2007) also reported in their studies that the group exposed to 8L:16D consumed more feed, when compared to the increase in eating frequency, since rabbits are nocturnal and tend to increase feed intake during darkness (Lebas *et al*., 1986). Marai *et al*. (2007) reported that light stimulus synchronizes the circadian cortisol rhythm. The remarkably increase in cortisol hormone in does exposed to natural daylight may be due to activation of the hypothalamic-pituitary- adrenal axis and the consequent increase of plasma glucocorticoid concentrations (Uzcategui and Johnson, 1987). The negative effects which were more remarkably observed on doe rabbit traits exposed to long daylight (16L: 8D) when compared to those exposed to short daylight (8L:16D), and its aggravation during the hot period

of the year may be due to the increase in the heat load. The heat load is caused by exposure to lamps light radiation, under the warm and bright days of the subtropical environment of Egypt. Such effects may be mainly due to disturbance of the physiological activities of the animals as a function of elevated ambient temperatures (Marai *et al*., 2002a). The above mentioned detrimental effects were reflected in the studies conducted by Marai *et al.,* (2004) where they reported a significant decrease in litter size, litter weight, milk yield, milk efficiency (kits' weight gain/milk intake per kit) and mortality at birth and pre-weaning.

In males exposed to artificial lighting for only eight out of 24 hours, significantly more spermatozoa were present in the gonads than in those exposed to light for 16 hours, although a slightly larger amount is usually collected in ejaculates from the latter (Elena *et al*., 2012). Does, however, are far more opposed to mating with only eight hours of light than they are with 16. For both males and females 12 hours of light a day produce average results (Marai *et al*., 2004).

2.3.3 Ambient Temperature

In tropical and subtropical regions, rabbits suffered from many problems related to hot climate, particularly heat stress. Most of the sweat glands in rabbits are not functional and perspiration (evacuation of water via the skin) is never great because of the fur (Gonzalez *et al*., 1971). The comfort zone for rabbits is 15 to 20 ^{o}C, and this zone makes rabbits to be able to withstand cold weather than warmer one. The metabolic rate increased by about 20% in rabbits, when exposed to high air temperature ranged from 30 to 35 ^{o}C (Gonzalez *et al*., 1971).

Marai *et al*. (2004) reported that hot period conditions negatively affected thermoregulatory parameters (respiration and body temperatures of ear, rectum and skin), gestation period, daily

feed intake and water consumption during pregnancy. They also affected serum total proteins, glucose, total lipids, urea-N, creatinine, SGPT and SGOT at mating and 1st and 2nd halves of pregnancy, albumin at mating, globulin during 1st half of pregnancy, cholesterol at mating and 1st half of pregnancy other parameters affected negatively by hot conditions are: alkaline phosphatase at mating and 1st half of pregnancy, acid phosphatase at mating and 2nd half of pregnancy and T3 and cortisol hormone during 2nd half of pregnancy. Conception rate was also affected adversely by exposure to the environmental hot conditions Marai *et al*. (2004).

The impact of temperature on spermatogenesis has been studied by various authors, but usually for short periods ranging from just a few hours to a few weeks at most. In a prolonged five-week trial, Oloufa *et al*. (1951) noted actual falls in the volume and concentration of ejaculates at a high temperature (33°C). A high temperature also affects sperm motility even after such short periods of exposure as eight hours at 36°C, or medium periods such as 14 days at 30°C. Furthermore, temperatures in excess of 30°C reduce the bucks' sexual urge and this seems to be the worst effect (Lebas *et al*., 1986).

2.4 Causes and Symptoms of Heat Stress in Rabbits

Heat stress is the state at which mechanisms of the body are activated to maintain an animal's body thermal balance, when exposed to intolerable (uncomfortable) elevated temperature (Lebas *et al*., 1986). Heat stress can also be defined as a stress inflicted by a wide range of environmental conditions that induce a state of physiological strain within an animal’s body, and means that animals are not able to regulate their heat homeostasis passively.

It occurs mainly when animals are exposed to high ambient temperatures, high humidity, low wind speed, and high direct and indirect solar radiation (Willmer *et al*., 2000). Rabbits, as homoeothermic animals, can regulate the heat input and output of their bodies using physical, morphological, biochemical, and behavioural processes to maintain a constant body temperature (Marai and Habeeb, 1994). Exposure of rabbits to heat stress evokes a series of remarkable changes in their biological functions which end with impairment of production and reproduction (Marai *et al.*, 2002, 2004).

2.4.1 Effects of heat stress on growth performance

Marai *et al*. (2001) found that daily weight gain and feed intake of growing rabbits declined with heat stress. Reductions in feed intake, growth rate, egg production and feed efficiency have been reported as immediate consequences of heat stress in poultry production (Zeweil *et al*. 2009).

Zeweil *et al*. (2009) reported that daily feed intake decreased by 13.69 and 10.15 % in the group reared under high temperature when compared to those reared under normal or medium temperature, respectively. The negative effect of ambient temperature increase was also noted by Kotby *et al*. (1977) who concluded that heat stress could inhibit the hypothalamic appetite center and thyroid function that reduce feed intake leading to a decrease of the metabolic rate, and Boiti *et al*. (1992), Afify and Makled, (1995) and Ashor (2001) found that ambient temperature was considerably lower in the group exposed to high air temperature, which positively correlated with feed intake and feed conversion ratio. The decline in feed intake of rabbits under high temperature was also reported by Ayyat *et al*. (2004)

2.4.2 Effects of heat stress on thermoregulatory parameters

The main thermoregulatory mechanism in rabbits is by heat exchange in the ears that have a large arteriovenous anastomotic system. In the nose, the nasal glands moisten inspired air, which also has a role in thermoregulation (Marai *et al.*, 2002). Anoh *et al.* (2014) reported a negative effect of heat stress on thermoregulatory parameters (heart rate, respiratory rate, rectal temperature and respiratory rate), when rabbits were transported for three hours under hot conditions in the tropics. The effect was more significant in adult rabbits than the young and growing rabbits. Marai and Habeeb (2004) indicated that between 0 – 30^{o}C, latent heat evacuation is only controlled by altering the breathing rate. Farghly (2011) reported that heat stress was apparent in the rabbits during the hot period due to failure of thermoregulatory mechanism.

Rectal temperature increases with exposure to high ambient temperature (Tharwat *et al.*, 1994). The increased value in rectal temperature was estimated to be about 1.0^{o}C, when the ambient temperature rose from 18.8^{o}C in winter to 30.7^{o}C in summer (Gad, 1998). The increase in rectal temperature of the heat-stressed rabbits may be due to either poor ability of the animals to prevent the rise in rectal temperature or failure of the physiological mechanisms of animals to balance the excessive heat load, caused by exposure to high ambient temperature (Habeeb *et al.*, 1992).

2.4.3 Effects of heat stress on Reproductive performance

2.4.3.1 Semen quality

It is worthy to note that semen characteristics are not immediately affected by heat. It depends on the duration of spermatogenesis, when damaged spermatogenic cells do not enter ejaculates for sometime after heat stress. In general, alteration in semen as a result of heat stress occurs about two weeks after heat stress and do not return to normal value until up to eight weeks following the end of the stress (Daader *et al*., 1997).

Sperm quality is better during cold season than during warm season, with high percentage of live sperm in rabbits (Borg *et al*., 1993; Daader *et al*., 1997). Low levels of testosterone induced by high temperature altered the normal epididymal function, producing an increase in number of dead spermatozoa (Mieusset *et al*., 1992b; Marai *et al*., 2002; Banks *et al*., 2005). As a consequence male fertility decreases. The exposure of high (THI) had immediate negative effects on sperm viability (Theau-Clement *et al*., 1995; Marai *et al.,* 2002; Roca *et al.,* 2005), although in general the increase number of dead spermatozoa show a great rise above two weeks after the highest THI (Garcia-Tomas *et al.,* 2008) and the effect tend to persist between 40 and 60 days after the end of heating (Casady *et al*; 1953).

Semen quality varies according to the temperature within the year. Hence, increased incidence of sperm abnormality has been reported during the warmer season of the year (Ax *et al*., 1987; Finizi *et al*., 1995; Daader *et al*., 1997; Safaa *et al*., 2008), which are immediately evidenced after active exposure to high THI (Theau-Clement et al 1995; Marai *et al*., 2002; Roca *et al.,* 2005) showing a great drop two weeks later of the highest THI (Roca *et al*, 2005; Garcia-Tomas

et al., 2008). The reduction of sperm does not return back to the physiological level until 6-8 weeks after the end of heat stress (Malmgren and Larsson, 1984; Meyer-hopper *et al*., 1985).

Season has been reported to affect sperm motility index, and the lowest motility was recorded during summer (Ax *et al*., 1987, El-Sharbiny, 1987). Motility is the main factor that allows sperm to reach the oviduct through the cervix and the utero tubal junction (Nakarishi *et al.,* 2004). The reduction in progressive motility in presence of high temperature (Jannes *et al.,* 1998; Armstrong *et al.,* 1999; Roca *et al*., 2005) is related with the production of Reactive oxygen species (ROS) (Aitken *et al*., 1989). However, the exact mechanism through which ROS causes a reduction in motility is not understood. One hypothesis shows that hydrogen peroxide (H_2O_2) can diffuse across the membrane into the cells and inhibit the activity of some vital enzymes that controls glucose flux and the intracellular availability of NADPH. This is used as a source of electrons by spermatozoa to fuel the generation of ROS (Aitken et al 1989).

Motility is one of the most sensitive indicators of heat stress. The negative effects on semen quality have been reported for mice (Ren *et al*., 2005; Perz-Crespo *et al*., 2008), rabbits (Theau-Clement *et al*., 1995; Marai *et al*., 2002; Roca *et al*., 2005), boars (McNih and First, 1970; Stone 1981; Malmgren and Larsson 1984), returning to physiological levels 6-8 weeks after the end of heat stress (Heitman and Cockrell, 1984; Meyer-hoeffer *et al*; 1985).

2.4.3.2 Conception rate and litter size

Rabbit does are very sensitive to heat stress, which could be an important factor influencing their fertility. Marai and Rashwan (2004) revealed that the doe is capable of producing 10 litters a year, but in a hot climate it has only 4 or 5 litters. Choudhary *et al*. (2001) have described season

to be highly influential on gestation period, kindling interval and litter weight at weaning. Bhatt *et al*. (2002) found that litter size and weight at birth, litter size at weaning as well as litter weight at weaning were all higher during winter as compared to those during summer and the rainy season. Similar results were reported by Kumar *et al*. (2005) in Angora rabbits.

2.4.4 Effects of heat stress on hormonal levels and serum metabolite

Ondruska *et al.* (2011) reported that high ambient temperature caused some changes in various blood plasma parameters in growing as well as in adult rabbits. The impact was much on growing individuals, which might be due to the instability of regulatory mechanisms for growth and metabolism in growing rabbits (Habeeb *et al*., 1993). Total protein (TP) levels in the plasma of heat-stressed NZW rabbits was reported to be significantly lower only in adult females (Ondruska *et al.* 2011). However, a comparison of both groups showed that TP levels were actually lower in the heat-stressed group. Ondruska *et al.* (2011) also reported a decline in plasma TP with rising temperature, which was attributed to a dilution of plasma TP caused by the increase in water consumption (Okab *et al*., 2008), and/ or wincreases in protein utilization and amino acid transamination in the heat-stressed rabbits (Habeeb *et al*., 1993). Moreover, increases in serum cortisol concentrations during hot temperature exposure might inhibit protein synthesis in tissues and may promote protein catabolism (Ayyat and Marai, 1997; Okab and El-Banna, 2003). Significant changes in the concentration of plasma TP either due to species differences or seasonal variations have been thought to be related to changes in albumin or globulin concentrations (El-Masry and Marai, 1991; Okab and El-Banna, 2003; Okab *et al*., 2008). Increase in plasma glucose levels in growing rabbits and decrease glucose levels in the heat-stressed adult rabbits have been reported (Ondruska *et al.* 2011). The incrased glucose level

attributed was due to a decrease in glucose utilisation in order to preserve energy during heat stressed condition in growing rabbits (Habeeb *et al*., 1997), while the decrease in glucose levels in the heat-stressed adult rabbits was said to be due to increase in glucose utilisation during muscular movements required for high respiratory activity (Habeeb *et al*., 1992; Okab *et al*., 2008), or due to increases in corticosteroid concentrations (Habeeb *et al*., 1997). Nevertheless, other researchers have demonstrated that decreases in energy metabolism (gluconeogenesis and glycogenolysis) during heat were exposure correlated with decreases in plasma insulin and thyroxin concentrations (Herbein *et al*., 1985; Habeeb *et al*., 1996). Plasma cholesterol concentrations have been reported to rise with increase in environmental temperature; which may be due to an increased activity of HMG-CoA reductase – the limiting enzyme in cholesterol synthesis (Akira, 1992). However, some studies have reported falls in cholesterol concentrations due to increases in total body water resulting from exposure to elevated ambient temperature (Marai *et al*., 1995; Habeeb *et al*., 1996).

2.5 Methods of Alleviating or Ameliorating Heat Stress in Rabbits

2.5.1 Physical and Mechanical Techniques

The rabbit is cooled by three primary factors: Respiration, ears and nasal mucosa (Marai *et al*., 1995; Habeeb *et al*., 1996). Eighty percent of heat dissipation in rabbits occurs through the evaporation of moisture during respiration (breathing) (Marai *et al*., 2001). Fans have been used to cool rabbits during heat stress. Fans help this cooling process by speeding evaporation. However, fans are expensive and they are also the least safe as they are not designed for agricultural use. The fan motors may overheat and malfunction, resulting in a possible fire hazard. Poor maintenance can reduce a fan's efficiency by 40% or more. When rabbits are molting, fans may need to be cleaned as often as daily for full efficiency.

Bottle with cool water placed in the cage is another method of cooling the rabbit and reducing heat stress in the rabbit. The bottles may be of only limited help without the proper air movement and evaporation, but can aid in cooling surrounding air. The bottles also require regular changing.

2.6 The use of additives and supplements in alleviating heat stress

2.6.1 Role of buffers in ameliorating heat stress

Some good buffers are efficient scavengers of hydroxyl radicals with rate constants of ~109 /M s (Hicks and Gebicki, 1986). Tris, tricine, and HEPES (in that order) were shown to inhibit the loss of a competitive solute, thymine, in radiolyzed water (Hicks and Gebicki, 1986). HEPES and Tris but not phosphate inhibited the rate of auto-oxidation of hemoglobins A (pI_6.9) and S (Harrington, 1998). The mechanism was not specifically attributed to free-radical scavenging, but rather by the binding of the phosphate anion to the 2, 3-(BPG) binding site at pH 7.0. This binding favored a shift to the deoxy state that was linked to more rapid methemoglobin formation (Schmidt *et al*., 1998). In contrast, HEPES and Tris, being positively charged at pH 7.0, were not bound as readily as phosphate to the 2, 3-BPG electropositive region in the hemoglobin molecule. HEPES and MOPS also accelerated the decomposition rate of the oxidant, peroxynitrite ($ONOO^-$) (Volkin 1993) Results indicated that phosphate buffer could chelate with Fe (II), thereby promoting its oxidation, even in the absence of free hydroxyl radicals (Welch *et al.*, 2002).

In addition, another study was conducted to compare the hydroxyl radical quenching ability of phosphate, carbonate, and bicarbonate buffers. Results showed that phosphate buffer quenched hydroxyl radicals less efficiently than did carbonate or bicarbonate buffers (Kochany and Kochany, 1992; Hasegawa, 1993). The ability of buffers to scavenge free radicals assumes

increased importance with the emergence of depot protein formulations administered by intramuscular or subcutaneous injection in conjunction with the absence of glycosylation in recombinantly produced proteins. Non-glycosylated proteins are more prone to denaturation by free radical attack than Olinked glycosylated proteins (Hasegawa 1993; Querol, 1999). However, studies have not recognized that other factors such as protection against free radical– mediated denaturation and/or a decrease in the amide proton exchange rate may have been partly responsible for the sustained activity of recombinant bovine granulocyte colony stimulating factor (rbG-CSF, pI _6.6), when formulated in organic buffers, rather than in acetate buffer (Encinar and Fernandez 1998; Kasraian, 2001).

Adding Sodium bicarbonate buffer (NaHCO3) (10g/Lwater) induced slightly, improvement for live body weight (LBW) of rabbits at 49 days of age. The same result was observed in final LBW (at 84 days of age). On the other hand, adding NaHCo3 in drinking water when feed was restricted significantly decreased performance when compared to control group. Fayes *et al.* (2011) reported that adding of 1.2% of potassium bicarbonate ($KHCO_3$) to the diet of rabbits improved feed intake and weight gain but was not able to reduce rectal temperature and respiratory rate during hot summer conditions in Egypt.

2.6.2 Role of Vitamin C in ameliorating heat stress in livestock

Vitamin C, which is present in most animal cells, has numerous biochemical functions. This metabolic factor is essential for growth and counteracting infections, caused by pathogenic bacteria and viruses. Verde and Piquer (1986) noted that the plasma vitamin C concentration was significantly reduced in animals exposed to stress. In other species, supplementary vitamin C has been shown to be beneficial in reducing the effects of stress. This means that the metabolic need for vitamin C increase at certain conditions. Therefore, the improvement of animal's

performance by the effect of vitamin C may be the alleviation of retardation in the thyroid function, (Coates, 1984). Abdel –Monem (2001) studied the effect of supplementation vitamin C at 250 and 500 mg / kg of doe rabbit's diet, on litter size, litter weight, pups gain, pre weaning mortality and gestation period, and reported improvements in these parameters. Vitamin C synthesiz in rabbit liver has been demonstrated to protect the animal from heat stress and improve disease resistance in rabbits by optimizing the function of the immune system (1994; Bain, 1996) During stress, vitamin C produced is however rapidly consumed and the amount synthesized falls below animal requirements.

Puron *et al.,* (1994) reported that addition of vitamin C, (200-600 mg) in diets improves growths, feed efficiency, and livability in heat stress. Bain (1996) revealed that vitamin C aids conversion of protein and fat into energy for production and survivability through increase corticosteroid secretion. Vitamin C supplementation also increases performance, yield better carcass trait in broilers, reared under heat stress conditions (32^{o}C) (Sahin, and Kucuk, 2001).

2.6.3 Baobab and it role in ameliorating heat stress in livestock

Baobab *Adansonia digitata* is a massive deciduous tree, up to 20-30 m tall with a diameter up to 2-10 m at adult age (Sidibé and Williams, 2002). The trunk is often of vast girth. The bark is smooth, reddish brown to grey, soft and possesses longitudinal fibres (Sidibé and Williams 2002). *Adansonia digitata* is highly branched (plate 1). The stout branches are near the trunk, and the young branches are often tomentose (Bosch *et al*., 2004). In general, *A. digitata* is deciduous tree, completely bare during the dry season and turns green during the rainy season (from June in the Sahel zone). In high humidity areas, some trees keep their leaves almost the year round. The fruits, called "monkey bread" measure between 10 and 45 cm long and have often different forms (ovoid, spherical, fusiform, elongated). Their weight varies significantly among

individuals and provenances. De Smedt *et al*. (2011) reported 165 to 305 grams in a study in Mali, while Assogbadjo *et al*. (2005) reported 204 to 276 grams in Benin. The endocarp consists of a white mealy pulp, completely dry at maturity. The white, powdery pulp is reported to have a high content of vitamin C. One source reports that the content of vitamin C in the baobab fruit is 1690 mg/kg, compared with 1060 mg/kg for fresh hot pepper (Agbessi Dos-Santos, 1987).

Sauberlich (1994) summarized the state-of-the-art information on the nutritional and clinical uses of vitamin C, also known as ascorbic acid that is a powerful antioxidant and extremely important in human nutrition. Vitamin C consumption has been shown to be related to low blood pressure, enhanced immunity against many tropical maladies, lower incidence of cataract development and lower incidence of coronary disease. Due to the high vitamin C content of baobab it can be used as a nutritional supplement (Agbessi Dos-Santos, 1987).

Fig. 2.1 Baobab tree with leaves and unripe pulps

Fig. 2.2 Baobab tree trunk

It has been reported that broiler chickens fed BFPM diets during hot season performed better than those fed the vitamin C supplemented diets as well as the control diet (Adeosun, 2012). The researcher further demonstrated that weight gains were improved by 21.38% during hot-dry season. BFPM supplementation in diets up to 3.5% gave better performance and egg quality characteristics of layers than the control diet during the hot dry season (Adeosun, 2012). At 12:00 h and 15:00 h, both vitamin C and BFPM diets resulted in lower rectal temperature of the birds than the value obtained in birds in the control diet (Adeosun, 2012).

Oladunjoye *et al.* (2014) did not record significant changes in the final weight, total weight gain, average daily gain, feed intake, feed conversion ratio and mortality of the rabbits fed graded levels of baobab fruit pulp and seed meal. Dietary treatments also had no effect on serum composition as the values obtained for total protein, albumin, glucose; serum creatinine, ALT, and AST were not significantly different across the treatments (Oladunjoye *et al.,* 2014). Growing rabbits can tolerate up to 15% of baobab fruit pulp and seed meal in their diet (Oladunjoye *et al.,* 2014). Anene *et al.* (2012) found that weight gained by juvenile *Clarias gariepinus* decreased with increased level of baobab seed meal in replacement for soybean meal in their diet. Mwale *et al.* (2008) also observed a decrease in the weight gain of guinea fowl keets at 10% and 15% inclusion level of baobab seed meal.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

This study was carried out at the Rabbit Unit of the National Animal Production Research Institute (NAPRI) Shika, Zaria. Shika lies between 11^{o} 12' 42" N and 7^{o} 33' 14" E at an altitude of 691 m above sea level (Ovimaps, 2014). Zaria has an average rain fall of 1100mm which starts from late April and early May to mid-October and an average temperature of 37^{o}C and average relative humidity of 75%.

3.2 Preparation of Buffer

Potassium bicarbonate, sodium bicarbonate and carbonate anhydrous salts were purchased from a laboratory equipments and chemicals vendor in Samaru-Zaria. Distilled water was prepared in the Multiuser Laboratory of the Department of Chemistry, Ahmadu Bello University Zaria. The buffer solution was prepared in the Department of Biochemistry, Ahmadu Bello University Zaria. The solution was prepared according to the methods of Chandra (2006) at a pH of 7.5.

3.3. Experiment 1: Effects of Bi-Carbonate Buffer, Vitamins C and Baobab on the Growth Performance of Weaned Rabbit Reared under Hot Tropical Condition

3.3.1 Experimental Animals and Diet

A total of thirty (30) weaned rabbits of mixed sex were used in this study. The study was carried out during the hot period in Zaria (March – June). The rabbits were divided into 5 groups of 6 rabbits per group. The groups were randomly allotted to 1 of 5 experimental treatment- groups in a completely randomized design. The treatment groups consisted of: Control (Water without anti-stress), Potassium bicarbonate ($KHCO_3$), Sodium Bicarbonate ($NaHCO_3$), solution with feed

respectively, Feed- Vitamin C, and BFPM as additives respectively (designated T1, T2, T3, T4 and T5 respectively). The water was offered *ad libitum* but changed daily in morning. All rabbits were fed the same concentrate feed. All recommended managerial practices were dully observed.

3.3. 2 Data Collection

3.3.2.1 Meteorological Data of Rabbit Microclimate

The microclimate (ambient temperature and relative humidity values) within the rabbit house were recorded twice daily at 08:00 h and 15.00 h during the study period using a digital thermometer (Cocet, Shenzhen-Guangdong, China). The data collected was used to compute the temperature humidity index (THI), an indicator of the thermal comfort level of the rabbits. The THI was calculated using the modified formula for the rabbit by Marai *et al*. (2001) as follows:

$$THI = t - [(0.31 – 0.31 \times RH) (t – 14.4)]$$

Where RH = relative humidity /100.

t = ambient temperature.

The values of THI obtained were compared to that classified for tropical regions as shown below:

a. < 27.8 = Absence of heat stress,

b. 2). 27.8 - 28.9 = Moderate heat stress,

c. 28.9 – 30 = Severe heat stress

d. above 30 = Very severe heat stress.

3.3.2.2 Growth Performance Parameters

Parameters monitored and measured on weekly basis included weight gain and feed intake, and mortality were recorded as they occurred.

3.3.2.3 Measurement of Thermoregulatory Parameters

Parameters measured included rectal temperature (RT) and ear temperature (ET), respiratory rate (RR) and heart rate (HR). Measurements were taken at 14.00 h to 15.00 h of the day. Rectal and ear temperature were measured with a digital thermometer. The ear temperature was measured by placing the digital thermometer in direct contact with the central area of the auricle. RR was measured by visually counting the flank movements for one minute with the help of a stop clock. HR was measured by counting the heart beat for one minute with the help of a stethoscope.

3.3.2.4 Serum Metabolite

Blood sampling was carried at the beginning and at the end of the experimental period. On each occasion, the blood sampling was done at 10.00 h. Four rabbits were randomly selected from each treatment group and 5 ml of blood was collected from ear veins into sample bottles without anticoagulant. The blood sample was allowed to clot and the serum harvested after centrifuging the samples at 3000 rounds/minute for 15 minutes. The harvested serum was analyzed for serum glucose total protein, albumin and cholesterol concentration. The blood analsis was done in the Haematological Laboratory of the Ahmadu Bello University Teaching Hospital, Shika-Zaria, using an auto analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc. San Antonio, Texas, USA

3.3.2.5 Determination of Thyroxine (T4) Concentration

Another 5 ml of blood samples were collected through the ear vein, randomly selected from the treatment groups, from each group of rabbits, respectively into a bottle without anticoagulant and allowed to clot. The blood samples were centrifuged at 3000 rounds/minute for 15 minutes. The serum harvested was stored at -10^{o}C until analyzed for T_4. T_4 concentrations was determined using ELISA kits (Liaison® T4 Byk-Sangtec Diagnostica, Dietzenbach, Germany).

3.4 Experiment 2: Effects of Bicarbonate Buffer, Vitamin C and Baobab on the Reproductive Performance of Rabbit Reared under Hot Tropical Condition

3.4.1 Experimental Animals and Diets

A total of 50 adult rabbits consisting of 25 males and 25 females were used in this study. The rabbits were divided into five experimental groups with 10 rabbits (5 males and5 females) per group. Each group was randomly assigned to one of five experimental treatments in a completely randomized design. Rabbits were given access to feed and water *ad libitum*. All recommended managerial practices were dully observed.

3.4. 2 Data Collection

3.4.3 Thermoregulatory Parameters

Parameters measured included RR, Heart Rate HR, RT and ET. Measurements were taken at 14.00 h to 15.00 h of the day. The parameters were measured as stated above.

3.4.4 Serum Metabolites

Blood samples (5 ml) and serum were collected as stated above. Metabolites analyzed were total protein, albumin and cholesterol concentrations. There were determined as stated above.

3.4.5 Thyroxine and testosterone hormonal assay

Blood samples were collected from bucks before and after the experiment and from the does before mating, during and after pregnancy as stated in fig. 3.3.2.5. Serum was collected and stored as stated in 3.3.2.5. The concentrations of T_4 and testosterone in blood serum were determined using commercially available ELISA kits (Diagnostic Procedure Corp., Los Angeles, CA, USA) according to the manufacturer's instructions.

3.4.6. Semen quality evaluation:

Adult bucks were trained for semen released and collection via an artificial vagina, while attempting to mount a teaser doe over a two-week period prior to experiment period. During this period the bucks were tested in order to be sure that they were reproductively normal based on their libido and semen characteristics, and were allowed to adjust to the twice-weekly schedule of experimental semen collection. Ejaculate volume (ml), semen pH, semen colour, sperm motility (%), sperm concentration (x 10^6 /ml) and morphology (%) as well as live to dead ratio were determined at the Fertility Laboratory of the Artificial Insemination Unit, of NAPRI, Ahmadu Bello University, Zaria.

3.4.7 Evaluation of Epididymal and Gonadal Sperm Reserves

Three bucks were selected from each treatment group and slaughtered. The two testes of each of the rabbits were carefully removed and evaluated for their gonadal sperm and/or spermatid reserves. The weight, length and volume of each testis were determined. The determination of sperm and spermatid reserves was done according to the standard method of Igboeli and Rakha (1971) and Rekwot *et al*. (1994). Briefly, each testis was homogenized in 20 ml of saline with antibiotic and centrifuged for about two minutes. After rinsing the blender container with 50 ml of saline and adding this to the effluent, the volume of the homogenate was measured and 5 ml of the homogenate was transferred to a conical flask and further diluted with 30 ml of saline. The homogenate was then stored overnight at $5^{o}C$ to allow sperm cells to ooze out of the tissues. Gonadal sperm/spermatid concentration was determined with a haemocytometer using the erythrocyte counting chamber of a haemocytometer that was crossed with microscopic grids containing small squares (Cole, 1974; Rekwot *et al.*, 1994).

3.4.8 Reproductive Performance of Rabbit Does

A total of 25 adult female rabbits were used in this phase. The animals were arranged in treatment groups as stated above. The rabbits were allowed to adjust to the treatment for four weeks before mating. Does were brought individually to be serviced by the buck (1 buck:1doe / treatment). The does were tested for pregnancy two weeks after mating. Does that were not pregnant were re-mated. Parameters monitored included, date of kindle, litter size, weight of litter, weight of kitten, survivability (%) of kitten at weaning and kitten weight at weaning.

3.4.9 Hormonal Assay

Blood (5 ml) samples was collected before, during and after pregnancy from does through their ear vein into a bottle without anticoagulant, and allowed to clot. Serum was harvested from the clotted blood and stored as stated in 3.3.2.5. T_4 and serum progesterone concentrations were determined for does using commercially-available ELISA Kits (Diagnostic Procedure Corp., Los Angeles, CA, USA) according to the manufacturer's instructions.

3.5 Experiment 3: Effect of Supplementing Graded levels of Baobab Fruit Pulp Meal on Growth Performance of Rabbits Reared under Tropical Condition

3.5.1 Experimental Animals and Diets

A total of 30 weaned rabbits were used in this study. The rabbits were divided into five groups, with six rabbits per treatment in a completely randomized design. The rabbits were each randomly allotted to one of the five treatments based on BFPM supplementation. Rabbits in the Group 1 were not supplemented and served as the control T1; while those in groups 2 – 5 were fed diets, containing graded levels of BFPM, T2, T3, T4 and T5, respectively. The diets were formulated to meet NRC (1995) recommendation for weaned rabbits. All animals were given access to feed and water *ad libitum*. All recommended managerial practices were dully observed.

3.5.2 Parameters to be monitored

Parameters monitored as growth performance, thermoregulatory response, serum metabolites and thyroxine hormone levels. The methods used were the same as in Experiment 1.

3.6 Experiment 4: Effect of Supplementing Graded levels of Baobab Fruit Pulp Meal on Reproductive Performance of Rabbits Reared under Tropical Condition

3.6.1 Experimental Animals and Diets

A total of 50 adult rabbits, consisting of 25 bucks and 25 does were used in this study. The rabbits were randomly allotted into the experimental treatments of five treatment groups, with ten (10) rabbits per treatment in a completely randomized design. Rabbits were arranged in treatment groups as stated in experiment 3 and were given access to feed and water *ad libitum*. All recommended managerial practices were dully observed.

3.6. 2 Parameters to be monitored

Parameters evaluated were reproductive performance, thermoregulatory response, serum metabolites, hormonal assay, semen quality, epididimal and gonadal sperm characteristics. The procedures were the same as in Experiment 2.

3.7. Statistical Analysis

Data obtained from all the experiments were subjected to analysis of variance, using the General Liner Model Procedure of SAS (2002). Significant differences among treatment means were separated using the pair wise difference (Pdiff) in the SAS package. Values of $P < 0.05$ were considered significant.

4.0 RESULTS

Experiment 1:

4.1 Effect of Buffer, Vitamin C and BFPM on Performance of Growing Rabbits

4.1.01 Monthly Temperature Humidity Index for Year 1

The THI inside the rabbitry during the experimental period is shown in Figure 4.01. THI in the mornings averaged 26.44^{o}C while the afternoon it averaged 28.74^{o}C. THI values progressively increased from the month of February and peaked in May, but declined in the the month of June.

4.1.02 Thermoregulatory Response of Growing Rabbit

The result of the thermoregulatory response (Table 4.01) shows that respiratory rate, rectal and ear temperatures significantly ($P<0.05$) reduced by the administration of potassium bicarbonate buffer ($KHCO_3$), sodium bicarbonate buffer (Na_2CO_3), vitamin C and baobab fruit pulp meal (BFPM). Vitamin C and BFPM were more effective in reducing these parameters than $KHCO_3$ and Na_2CO_3 buffers. The buffers increased heart rate (143.36 and 143.36 bpm) more than the control (141.03 bpm) and treatments with vitamin C (141.35 bpm) and BFPM (141.29 bpm) while Na_2CO_3 and the control recorded a high ear temperature compared to the rest of the treatments.

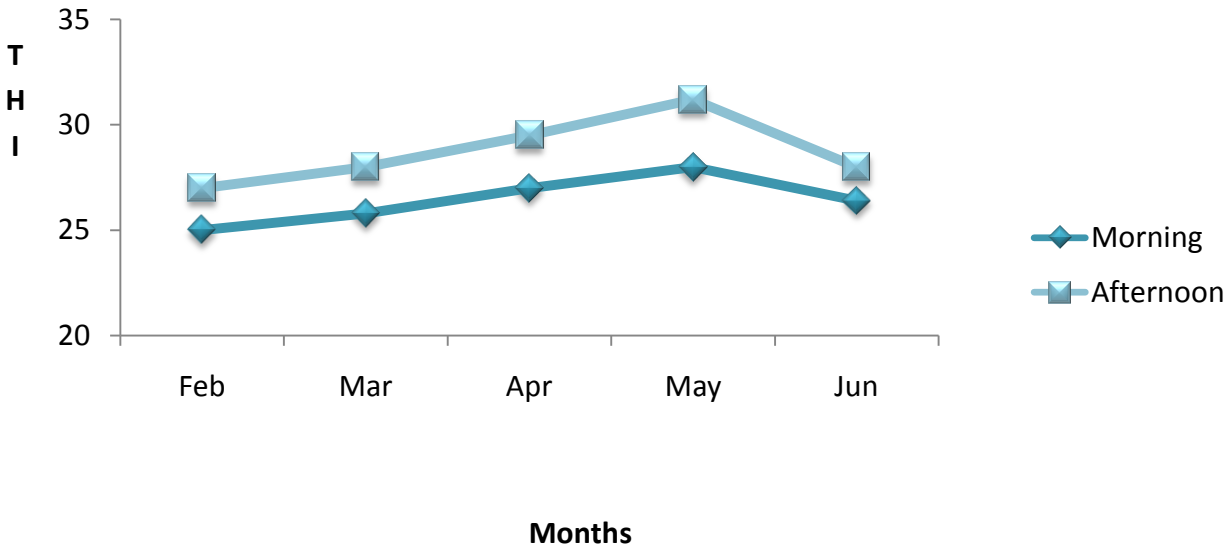


Figure 4.01: Monthly Temperature Humidity Index for Year 1

Table 4.01 Effects of Bicarbonate Buffers, Vit C and BFPM on Thermoregulatory Response of Growing Rabbits

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Respiratory Rate (Cnt/min)	97.94^{a}	79.56^{b}	83.45^{b}	78.11^{bc}	76.59^{c}	2.60
Heart Rate (beats/min)	141.03^{b}	143.36^{a}	143.19^{a}	141.35^{b}	141.29^{b}	0.53
Rectal Temperature (^{o}C)	37.68^{b}	36.56^{bc}	37.27^{a}	35.87^{c}	35.73^{c}	0.31
Ear Temperature (^{o}C)	35.68^{a}	34.13^{b}	35.48^{a}	33.96^{b}	33.23^{c}	0.56

Means within rows with different superscripts are significantly different: P<=0.05

4.1.03 Growth Performance of Growing Rabbits

Table 4.02 shows the effect of bicarbonate buffers, Vitamin C and BFPM on growth performance of growing rabbits. The treatments (buffers, Vitamin C and BFPM) significantly ($P < 0.05$) increased feed intake during this period. Rabbits on sodium bicarbonate buffer (Na_2CO_3), vitamin C and baobab fruit pulp meal (BFPM) had improved performance, compared to those on potassium bi-carbonate buffer ($KHCO_3$) treatment and the control. Final weight and daily weight gain did not show any significant difference.

Table 4.02: Effects of Bicarbonate Buffers, Vit C and BFPM on Growth Performance of Growing Rabbits

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Initial weight (g)	866.70	908.30	833.30	800.00	800.00	121.88
Final weight (g)	1533.30	1633.90	1400.00	1345.50	1365.00	117.58
Weight gain (g/day)	13.96	16.99	14.58	13.37	15.28	1.82
Feed intake (g/day)	30.76^{b}	30.93^{b}	42.39^{a}	37.59^{a}	36.77^{a}	1.88

Means within rows with different superscripts are significantly different: $P < 0.05$

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.1.04 Serum Metabolite of Growing Rabbits

Table 4.03 shows the effect of buffer, vitamin C and BFPM on concentration of serum metabolites of growing rabbits. The initial values of serum metabolites of the rabbits were lower than the final values. In the initial value, total protein showed significant ($P < 0.05$) difference between treatments with rabbits on BFPM recording the highest values. Triglyceride also showed a significant difference in the initial values, with the highest value obtained in animals on BFPM. The treatment with BFPM and vitamin C significantly increased triglyceride and serum calcium of the rabbits. Other parameters such as glucose, total protein, albumin, cholesterol and phosphorous did not show any significant difference.

4.1.05 Thyroxine Levels in Growing Rabbits

The effect of buffer, vitamin C and BFPM on thyroxine levels of growing rabbits in Fig. 4.02 revealed that the initial thyrosine levels of growing rabbits did not show any significant difference among the treatments group (fig. 4.2). However, the treatment with Na_2CO_3 recorded higher thyroxine level, compared to the rest of the treatments. On termination of the experiment, the serum values of thyroxine obtained from the serum shows that vitamin C significantly ($P < 0.05$) reduced thyroxine levels, compared to other treatments with the buffer treatments had the highest values.

Table 4.03 Effects of Bicarbonate Buffers, Vit C and BFPM on Serum Metabolite of Growing Rabbits

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Initial						
Glucose (mg/dl)	3.43	3.33	3.23	3.37	3.20	0.14
Total Protein (mg/dl)	60.67^{b}	60.00^{b}	63.35^{b}	63.33^{ab}	64.33^{a}	0.73
Albumin (mg/dl)	32.00	33.33	31.67	32.33	32.00	0.75
Cholesterol (mg/dl)	1.33	1.27	1.27	1.37	1.33	0.05
Triglyceride (mg/dl)	0.80^{b}	0.97^{ab}	0.90^{ab}	0.91^{ab}	1.00^{a}	0.04
Calcium (mg/dl)	2.25	2.39	2.28	2.42	2.25	0.60
Phosphorous (mg/dl)	1.10	1.07	1.08	1.08	1.06	0.03
After						
Glucose (mg/dl)	3.50	3.30	3.60	4.80	5.00	0.12
Total Protein (mg/dl)	66.67	65.67	72.00	69.67	72.67	0.38
Albumin (mg/dl)	34.67	36.00	37.67	37.33	38.67	1.38
Cholesterol (mg/dl)	1.33	1.30	1.33	1.43	1.43	0.04
Triglyceride (mg/dl)	0.97^{b}	1.00^{ab}	1.00^{ab}	1.30^{ab}	1.40^{a}	0.08
Calcium (mg/dl)	2.39^{ab}	2.31^{ab}	2.30^{ab}	2.33^{a}	2.26^{b}	0.01
Phosphorous (mg/dl)	1.08	1.13	1.31	1.16	1.09	0.01

Means within rows with different superscripts are significantly different: $P < 0.05$

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

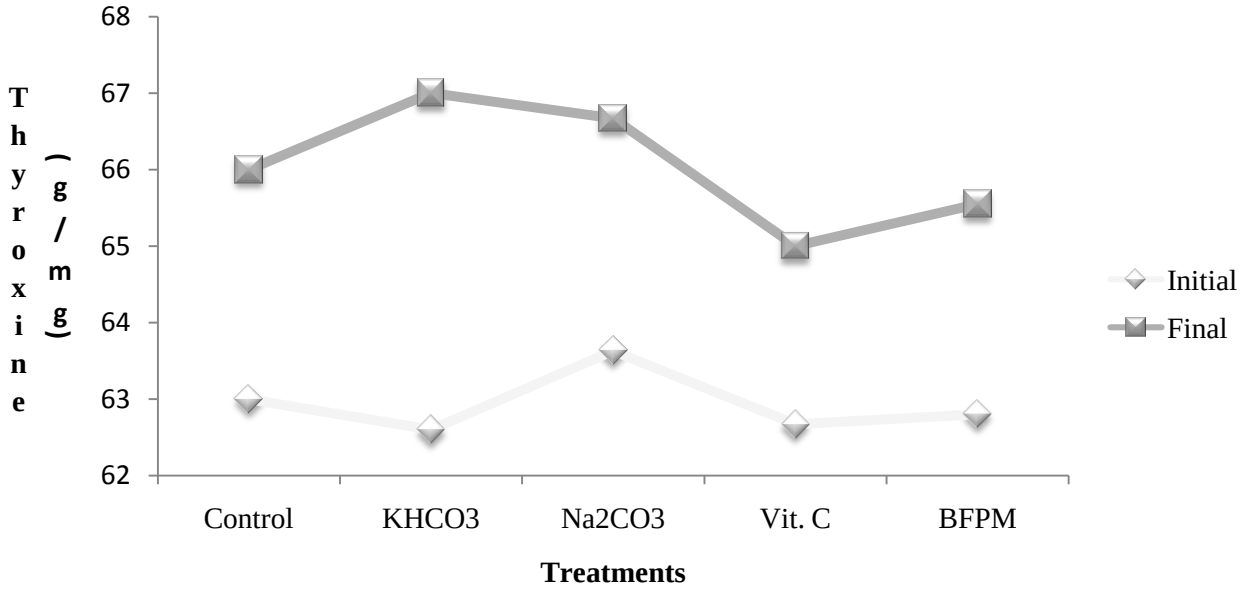


Fig. 4.02 Effects of Bicarbonate Buffers, Vit C and BFPM on Thyroxine Levels in Growing Rabbits

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Experiment Two:

4.2 Effect of Buffer, Vitamin C and BFPM on Reproductive Performance of Adult Rabbit Bucks

4.2.01 Thermoregulatory Response of Adult Rabbit Bucks

The result of the thermoregulatory response (Table 4.04) shows that Vitamin C and BFPM significantly ($P < 0.05$) reduced heart rate, rectal and ear temperature in adult bucks. The respiratory rate did not show any significant difference. $KHCO_3$ increased heart rate and Na_2CO_3 increased rectal temperature of bucks. The buffers $KHCO_3$ and Na_2CO_3 increased heart rate to 143.23 bpm and 143.53 bpm, respectively, and the values were higher than those of the control (141.03 bpm) and treatments with vitamin C (140.99 bpm) and BFPM (141.03 bpm).

4.2.02 Serum Metabolites of Adult Rabbit Bucks

Serum metabolites of rabbits treated with buffer, vitamin C and BFPM (Table 4.05) showed that triglyceride and phosphorous were significantly ($P<0.05$) different among the treatment groups. The final serum values in BFPM-treated rabbits significantly ($P < 0.05$) improved serum albumin; the control recorded significantly higher serum glucose while Na_2CO_3 significantly increased serum calcium level, but other metabolites did not show any significant difference. For the values that showed significant difference, it was observed that apart from the control group and the treatment with Na_2CO_3, initial glucose was higher than the final glucose. The control group and the treatments with the buffers recorded reduced serum albumin in the final values, compared to the initial values. Vitamin C and BFPM had higher final albumin compared to initial serum albumin. For serum calcium, a reduction in the final values was recorded in the control group and the treatments with vitamin C.

Table 4.04 Effects of Bicarbonate Buffers, Vit C and BFPM on Thermoregulatory Response of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Respiratory Rate (counts/min)	158.34	154.02	148.91	150.58	141.03	3.78
Heart Rate (beat/min)	141.03^{b}	143.23^{ab}	143.53^{a}	140.99^{b}	141.03^{ab}	0.67
Rectal Temperature (^{o}C)	38.73^{a}	38.04^{a}	38.29^{a}	37.97^{ab}	37.04^{b}	0.28
Ear Temperature (^{o}C)	36.79^{a}	35.59^{ab}	36.13^{ab}	35.23^{ab}	34.99^{b}	0.47

Means within rows with different superscripts are significantly different: P<=0.05

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 4.05 Effects of Bicarbonate Buffers, Vit C and BFPM on Serum Metabolite of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Initial						
Glucose (mg/dl)	4.17	5.03	4.12	4.60	4.70	0.33
Total Protein (mg/dl)	65.00	67.00	63.37	66.80	67.23	1.67
Albumin (mg/dl)	35.00	36.67	39.00	37.33	40.00	1.53
Cholesterol (mg/dl)	1.37	1.10	1.23	1.20	1.30	0.56
Triglyceride (mg/dl)	0.70^{b}	1.07^{ab}	0.98^{ab}	0.87^{ab}	1.17^{a}	0.09
Calcium (mg/dl)	2.38	2.32	2.37	2.38	2.32	0.06
Phosphorous (mg/dl)	0.87^{b}	1.15^{a}	1.07^{ab}	1.00^{ab}	1.00^{ab}	0.04
After						
Glucose (mg/dl)	4.70^{a}	4.13^{b}	4.20^{b}	4.47^{b}	4.50^{b}	3.57
Total Protein (mg/dl)	64.00	64.00	67.33	69.33	70.67	1.58
Albumin (mg/dl)	33.67^{b}	32.67^{c}	33.33^{b}	38.66^{ab}	40.00^{a}	1.33
Cholesterol (mg/dl)	1.50	1.37	1.38	1.55	1.53	0.07
Triglyceride (mg/dl)	2.90	1.33	1.27	1.27	1.37	0.71
Calcium (mg/dl)	2.30^{b}	2.29^{bc}	2.44^{a}	2.25^{c}	2.42^{ab}	0.03
Phosphorous (mg/dl)	1.00	1.07	0.90	1.00	1.06	0.04

Means within rows with different superscripts are significantly different: P<=0.05

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.2.03 Thyroxine Levels in Adult Rabbit Bucks

The effect of buffer, vitamin C and BFPM on thyroxine levels of adult male rabbits (Fig. 4.03) revealed that initial thyrosine levels of the rabbits did not differ significantly when compared to those of the treatment groups. The control group and Na_2CO_3 group recorded the lowest thyroxine levels compared to the rest of the treatments. On termination of the experiment, the values of thyrosine obtained from the serum showed that vitamin C and BFPM increased significantly ($P<0.05$) thyroxine levels compared to other treatments, and with the control group recorded the least value.

4.2.04 Testosterone Levels in Adult Rabbit Bucks

The initial testosterone concentration of rabbits treated with buffer, vitamin C and BFPM in Fig. 4.04 did not show any significant difference among the treatments. It was observed that vitamin C and BFPM increased significantly ($P < 0.05$) testosterone concentration compared to the rest of the treatments whose values were lower than that recorded initially. The treatment with $KCHO_3$ buffer recorded significantly ($P < 0.05$) lower testosterone levels than the rest of the treatments.

4.2.05 Semen Quality Characteristics of Adult Rabbit Bucks

The buffer, vitamin C and BFPM on semen characteristics of adult buck (Table 4.06) shows that BFPM significantly ($P<0.05$) improved semen volume, colour, mortility pH and concentration. The values of 6.94 and 32.56 x 10^6 for semen concentration obtained in the control and Na_2CO_3 treatment groups, respectively were lower compared to 74.23, 85.00 and 118.78 x 10^6 recorded in rabbits treated with $KHCO_3$, vitamin C and BFPM with respectively.

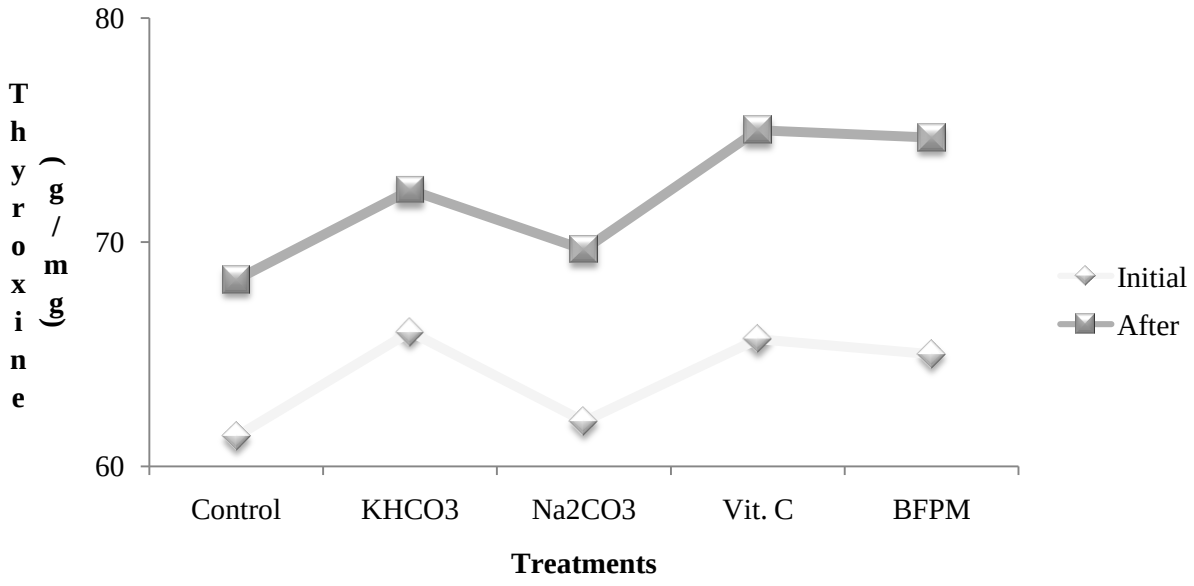
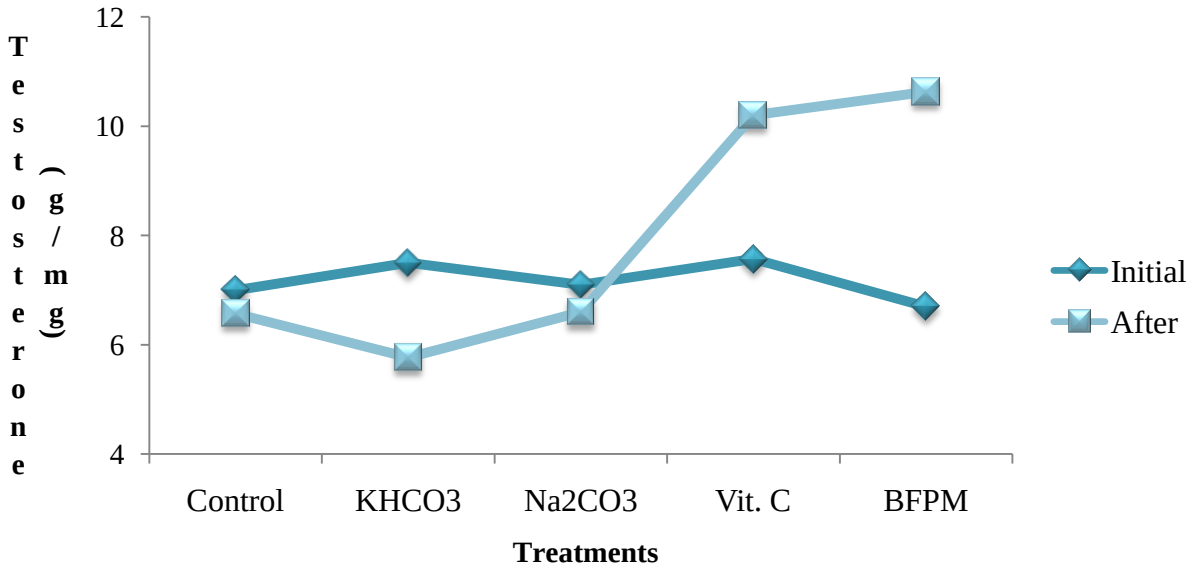


Fig. 4.03 Effects of Bicarbonate Buffers, Vit C and BFPM on Thyroxine Levels in Adult Rabbit Bucks

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal



Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Fig. 4.04 Effects of Bicarbonate Buffers, Vit C and BFPM on Testosterone Levels in Adult Rabbit Bucks

Table 4.06 Effects of Bicarbonate Buffers, Vit C and BFPM on Semen Characteristics of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Volume (ml)	0.50^{bc}	0.39^{c}	0.79^{abc}	1.07^{ab}	1.19^{a}	0.15
Colour	1.27^{b}	2.17^{a}	1.93^{a}	1.0^{b}	1.07^{b}	0.09
Mortility (%)	26.67^{b}	49.00^{ab}	52.00^{ab}	52.00^{ab}	58.33^{a}	6.13
pH	7.18	7.15	7.08	7.22	7.03	0.80
Concentration ($x10^6$)	6.94^{d}	74.23^{b}	32.56^{c}	85.00^{ab}	118.78^{a}	15.10

Means within rows with different superscripts are significantly different: P<=0.05

Semen colour= Milky: 1.00 – 1.5, Creamy: 1.51- 2.00 and Watery 2.10 and above.

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.2.06 Sperm Morphology of Adult Rabbits Bucks

The effects of buffer, vitamin C and BFPM on sperm morphology of adult rabbits are shown in Table 4.07. $KHCO_{3,}$ vitamin C and BFPM significantly ($P < 0.05$) improved most of the sperm morphological characteristics of the rabbits, compared to Na_2CO_3 and the control rabbits. Values recorded for live cells in rabbits treated with $KHCO_{3,}$ vitamin C and BFPM were 66.67, 76.67 and 72.33, respectively, and the counts for dead cells were 37.33, 23.33 and 27.67 in that order. Rabbits treated with Vitamin C and $KHCO_3$ recorded significantly ($P < 0.05$) higher percentages of 77.33% and 73.00% for normal cells, compared to the rest of the treatments. The treatments with vitamin C and BFPM also significantly ($P<0.05$) reduced the number of spermatozoa with deformed cells such as detached head, free tail and coil tail, which were significantly ($P < 0.05$) higher in rabbits treated with $KHCO_3$. Sperm with bent tail did not show any significant difference among the treatments.

4.2.07 Gonadal Morphometry of Adult Rabbit Bucks

In Table 4.08, treatments with buffer, vitamin C and BFPM in adult rabbit bucks significantly ($P<0.05$) increased the length of the left testis, weight of the left testis and filtrate volume of the right testis. This result was similar to the control for testis weight and filtrate volume of the right testis respectively. BFPM increased the filtrate volume of the left testis and testicular length. The length and weight of the left testis were larger than those of the right testis across the treatments.

Table 4.07: Effects of Bicarbonate Buffers, Vit C and BFPM on Sperm Morphology of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Live cells (%)	62.67^{bc}	57.67^{c}	66.67^{abc}	76.67^{a}	72.33^{ab}	3.21
Dead cells (%)	37.33^{b}	43.67^{a}	37.33^{b}	23.33^{d}	27.67^{c}	3.25
Normal Cells (%)	69.67^{ab}	73.00^{ab}	57.00^{b}	77.33^{a}	69.66^{ab}	2.79
Detached Head (%)	12.00^{a}	8.67^{b}	4.33^{c}	6.66^{bc}	8.33^{b}	1.74
Free tail (%)	6.33^{ab}	9.00^{a}	6.67^{ab}	6.33^{ab}	4.68^{b}	0.94
Coil Tail (%)	3.00^{b}	5.68^{a}	5.67^{a}	2.67^{b}	3.33^{b}	0.54
Bent Tail (%)	4.00	5.68	5.67	3.33	5.33	0.88

Means within rows with different superscripts are significantly different: $P < 0.05$

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 4.08: Effects of Bicarbonate Buffers, Vit C and BFPM on Gonadal Morphometry of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Testis Length (cm)						
R	3.17^{b}	4.33^{a}	4.47^{a}	2.53^{c}	2.80^{c}	0.08
L	0.37	0.17	0.43	0.21	0.23	0.14
Testis weight (g)						
R	2.03^{a}	1.84^{b}	0.74^{d}	0.66^{e}	0.98^{c}	0.02
L	2.63^{b}	2.30^{bc}	3.13^{a}	2.10^{c}	2.42^{bc}	0.08
Filtrate Volume (ml)						
R	9.47^{a}	9.00^{b}	9.50^{a}	9.00^{b}	9.00^{b}	0.28
L	9.17^{b}	9.00^{b}	9.00^{b}	9.17^{b}	9.90^{a}	0.01

Means within rows with different superscripts are significantly different: $P < 0.05$

R = Right testis; L = Left testis

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.2.08 Epididymis Morphometry of Adult Rabbit Bucks

The result of the effect of buffer, vitamin C and BFPM on the epididymis morphometry of adult rabbit bucks (Table 4.08) shows that the epididymal morphometry values (epididymis length, epididymis weight (left) and epididymis filtrate volume) were significantly ($P<0.05$) higher in the treatments with BFPM. The values were similar to those recorded in the control and $KHCO_3$. The size and weight of the left epididymis across the treatments was larger than those of the right epididymis. For the length and weight of the epididymis, it was observed that the length of the caput and corpus epididymis was longer than those of the cauda epididymis. On the other way round, the weight of the cauda epididymis was higher than those of the caput or corpus epididymis.

4.2.09 Gonadal and Epididymis Sperm Reserves of Adult Rabbit Bucks

The effect of buffer, vitamin C and BFPM on gonadal and epididymis sperm reserve of adult rabbits (bucks) are shown in Table 4.09 BFPM and vitamin C significantly improve testicular volume, and sperm concentration at the caput region of the epididymis. The treatments with $KHCO_3$ however significantly improved sperm concentration in the testis. Sperm concentration which happened to be high in the cauda epididymis significantly improved by the buffers ($KHCO_3$ and Na_2CO_3), compared to other treatments.

Table 4.09: Effects of Bicarbonate Buffers, Vit C and BFPM on Epididymis Morphometric of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Epididymis Length (cm)						
R	2.00^{a}	2.00^{a}	1.53^{b}	1.47^{b}	2.10^{a}	0.12
L	6.2^{a}	5.67^{b}	5.67^{b}	5.30^{c}	5.97^{ab}	0.07
Epididymis Weight (g)						
R	0.82	0.81	0.69	0.45	0.77	0.72
L	1.83^{a}	1.80^{ab}	1.70^{c}	1.47^{d}	1.75^{b}	0.01
Caput Length (cm)						
R	1.03^{c}	2.03^{ab}	1.85^{b}	2.07^{ab}	2.17^{a}	0.07
L	2.7^{c}	4.23^{a}	4.33^{a}	3.30^{b}	2.97^{bc}	0.08
Corpus Length (cm)						
R	1.40^{b}	4.03^{a}	4.23^{a}	4.17^{a}	3.53^{a}	0.17
L	2.4	2.97	2.47	2.93	2.93	0.20
Cauda Length (cm)						
R	0.27^{bc}	0.42^{b}	0.63^{a}	0.17^{c}	0.22^{c}	0.40
L	2.00^{a}	1.67^{b}	1.53^{b}	1.63^{d}	1.53^{b}	0.07
Caput Weight (g)						
R	0.21^{b}	0.33^{a}	0.32^{b}	0.16^{c}	0.25^{b}	0.01
L	0.83^{a}	0.71^{c}	0.63^{d}	0.43^{e}	0.83^{a}	0.01
Corpus Weight (g)						
R	0.01^{b}	0.02^{ab}	0.02^{ab}	0.04^{ab}	0.05^{a}	0.01
L	0.01	0.06	0.01	0.05	0.06	0.09
Cauda Weight (g)						
R	0.52^{b}	0.60^{a}	0.50^{b}	0.59^{a}	0.53^{ab}	0.13
L	0.60	0.64	0.54	0.59	0.70	0.38

Means within rows with different superscripts are significantly different: $P < 0.05$

R = Right testis; L = Left testis

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 4.09: Effects of Bicarbonate Buffers, Vit C and BFPM on Epididymis Morphometric of Adult Rabbit Bucks Cont…

Parameters	Control	$KHCO_3$	Na_2CO_3	Vit. C	BFPM	SEM
Epididymis Filtrate Volume (ml)						
Caput						
R	4.80^{a}	4.53^{b}	4.53^{b}	3.47^{c}	4.43^{b}	0.03
L	4.57^{a}	4.63^{a}	3.50^{b}	3.57^{b}	4.53^{a}	0.03
Corpus						
R	4.57^{a}	4.53^{a}	4.53^{a}	3.47^{b}	4.53^{a}	0.27
L	4.43^{b}	4.60^{a}	3.50^{c}	3.57^{c}	4.53^{ab}	0.03
Cauda						
R	4.47^{b}	4.80^{a}	4.80^{a}	3.63^{c}	4.53^{ab}	0.02
L	4.60^{b}	4.83^{a}	3.57^{c}	3.66^{c}	4.53^{b}	0.03

Means within rows with different superscripts are significantly different: $P < 0.05$ R = Right testis; L = Left testis

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 4.10 Effects of Bicarbonate Buffers, Vit C and BFPM on Gonadal and Epididymis Sperm Reserves of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Testis sperm volume (ml)						
R	1.00^{b}	3.33^{ab}	2.33^{b}	3.67^{a}	4.00^{a}	0.02
L	0.54^{b}	0.62^{a}	0.61^{a}	0.56^{b}	0.63^{a}	0.08
Testis sperm Reserve. (x 10^6)						
R	25.67^{c}	38.67^{a}	21.00^{e}	32.00^{b}	14.33^{d}	1.35
L	22.33^{d}	25.00^{c}	31.00^{b}	34.67^{c}	17.67^{e}	0.49
Epididymis Reserves						
Caput (x 10^6)						
R	9.67^{c}	14.67^{b}	14.33^{b}	13.00^{b}	29.33^{a}	0.46
L	9.33^{d}	15.67^{b}	12.33^{c}	20.33^{a}	11.33^{c}	0.26
Corpus (x 10^6)						
R	3.67	3.67	3.00	2.67	4.67	0.46
L	0.67	0.67	1.00	0.67	1.67	0.03
Cauda (x 10^6)						
R	150.33^{c}	196^{a}	73.67^{e}	185.67^{b}	123.67^{d}	2.12
L	105.66^{d}	105.00^{d}	184.67^{a}	152.67^{c}	156.00^{b}	0.80

Means within rows with different superscripts are significantly different: P = 0.05 Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.2.10 Serum Metabolite of Adult Rabbit Does

Table 4.11 shows the effect of buffer, vitamin C and BFPM on serum metabolites of adult female rabbits before, during and after pregnancy. Apart from calcium, serum metabolites before pregnancy like glucose, total protein, albumin, cholesterol, triglyceride and phosphorous did not show any significant difference across the treatments. During pregnancy, Na_2CO_3 and BFPM significantly ($P < 0.05$) increased albumin and triglyceride; other metabolites did not show any significant difference. Apart from the treatment with Na_2CO_3 and BFPM, serum glucose reduced at the final stage compared to the initial state and during pregnancy. Vitamin C and BFPM significantly ($P<0.05$) increased serum glucose, total protein, albumin and phosphorous; while the treatments with the buffers significantly increased serum albumin, triglyceride and calcium in the final values evaluated. Cholesterol did not show any significant difference in all the stages of pregnancy across the treatments. Generally, most of the serum metabolites reduced during pregnancy, compared to values recorded before and after pregnancy.

Table 4.11 Effects of Bicarbonate Buffers, Vit C and BFPM on Serum Metabolite of Adult Rabbit Does

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
a. Glucose (mg/dl)	4.77	4.90	4.63	4.47	5.0	0.30
b. Glucose (mg/dl)	4.30	5.13	4.47	5.00	5.22	0.21
c. Glucose (mg/dl)	4.13^{c}	4.10^{c}	4.57^{b}	4.73^{ab}	5.27^{a}	0.14
a. Total Protein(mg/dl)	63.63	67.33	66.00	66.67	67.00	1.54
b. Total Protein (mg/dl)	61.67	62.00	64.34	64.00	63.33	1.48
c. Total Protein (mg/dl)	67.33^{abc}	65.00^{b}	68.00^{ab}	69.33^{a}	64.33^{c}	0.72
a. Albumin (mg/dl)	35.67	34.67	37.00	37.33	36.00	0.77
b. Albumin (mg/dl)	37.33^{ab}	34.00^{b}	39.00^{a}	38.08^{ab}	41.17^{a}	1.13
c. Albumin (mg/dl)	37.17^{ab}	34.33^{b}	40.35^{a}	41.33^{a}	38.00^{ab}	1.21
a. Cholesterol (mg/dl)	1.60	1.63	1.60	1.43	1.30	0.09
b. Cholesterol (mg/dl)	1.70	1.73	1.63	1.57	1.57	0.08
c. Cholesterol (mg/dl)	1.46	1.42	1.47	1.47	1.37	0.05
a. Triglyceride (mg/dl)	0.93	0.92	0.86	0.83	0.87	0.09
b. Triglyceride (mg/dl)	1.03^{ab}	0.97^{b}	1.43^{a}	1.20^{ab}	1.30^{ab}	0.10
c. Triglyceride (mg/dl)	0.87^{ab}	1.17^{a}	$0.8b^{ab}$	0.77^{b}	0.92^{ab}	0.06
a. Calcium (mg/dl)	2.34^{ab}	2.57^{a}	2.21^{b}	2.39^{ab}	2.34^{ab}	0.06
b. Calcium (mg/dl)	2.28	2.31	2.36	2.41	2.35	0.04
c. Calcium (mg/dl)	2.43^{a}	2.34^{ab}	2.21^{b}	2.34^{ab}	2.19^{b}	0.04
a. Phosphorous (mg/dl)	1.05	1.04	1.00	1.10	1.11	0.06
b. Phosphorous (mg/dl)	0.95^{ab}	0.94^{ab}	0.52^{b}	1.14^{a}	1.17^{a}	0.08
c. Phosphorous (mg/dl)	1.01	1.00	1.08	1.07	1.09	0.02

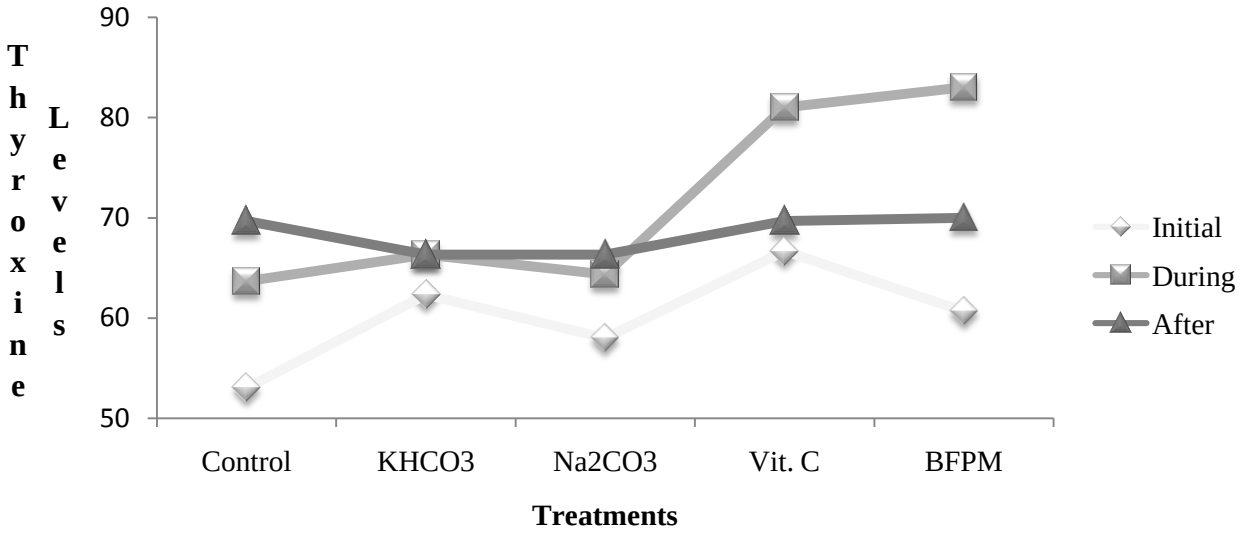
Means within rows with different superscripts are significantly different: $P < 0.05$ a: before pregnancy, b: during pregnancy, c: after delivery. Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

4.2.11 Thyroxine Levels in Adult Rabbit Does

The effect of buffer, vitamin C and BFPM on thyroxine levels of adult rabbit does (Fig. 4.4) revealed that initial thyroxine levels of the rabbits were low, compared to the values obtained during and after pregnancy. Initial thyrosine levels did not show any significant difference among the treatment groups. During pregnancy, thyroxine levels increased a little above the initial thyroxine value. After kindling, vitamin C and BFPM significantly increased thyroxine levels, compared to other treatments while the treatment, with Na_2CO3 recorded the least.

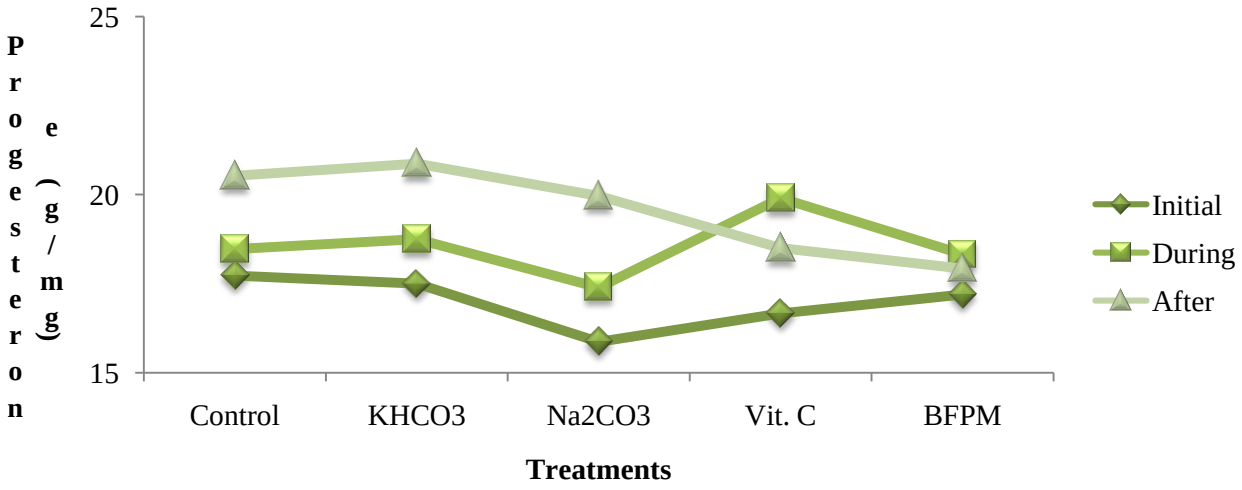
4.2.12 Progesterone Levels in Adult Rabbit Does

The effects of buffer, vitamin C and BFPM on progesterone levels of adult rabbit does are shown in Fig. 4.05 Initial progesterone levels of the rabbits were low across the treatment group compared to the values obtained during and after pregnancy. The values did not show any significant difference among the treatment groups. During pregnancy, progesterone significantly ($P<0.05$) increased in vitamin C and BFPM treatment groups. After kindling, vitamin C and BFPM significantly ($P < 0.05$) reduced the values of progesterone while the values in the control, $KHCO_3$ and Na_2CO3 groups increased above the initial progesterone values and those recorded during pregnancy.



Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Fig. 4.05 Effects of Bicarbonate Buffers, Vit C and BFPM on Thyroxine Levels in Adult Rabbit Does



Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Fig. 4.06 Effects of Bicarbonate Buffers, Vit C and BFPM on Progesterone Levels in Adult Rabbit Does

4.2.13 Reproductive Performance of Adult Rabbit Does

The result of the effect of buffer, vitamin C and BFPM on the reproductive performance of rabbit does are shown in Table 4.10. The buffers significantly ($P<0.05$) reduced reproductive performance of the rabbits does compared to the treatments with vitamin C, BFPM and the control. Rabbits treated with $KHCO_3$ did not conceive at all even after the third mating trial. Littre size at birth and kit weight at weaning obtained from rabbits treated with vitamin C and BFPM did not show any significant difference compared with the control. Litter size at weaning was significantly ($P < 0.05$) higher in the treatment with BFPM and the control, compared to the rest of the treatments.

Table 4.12 Effects of Bicarbonate Buffers, Vit C and BFPM on Reproductive Performance of Adult Rabbit Does

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Gestation period	30.00[a]	00.00[b]	30.00[a]	30.00[a]	30.00[a]	0.10
Litter size at birth	5.67[a]	00.00[c]	4.00[b]	5.00[a]	5.60[a]	0.23
Weight of litter (g)	236.67[a]	00.00[c]	180.00[b]	200.00[b]	200.00[b]	6.68
Weight of kit (g)	41.78[b]	00.00[c]	45.00[a]	40.00[b]	40.00[b]	0.06
Litter size at weaning	4.00[a]	00.00[c]	0.67[b]	2.67[b]	4.67[a]	0.29
Kit weight at weaning (g)	519.23[a]	00.00[b]	458.33[a]	426.67[a]	504.00[a]	70.23

Means within rows with different superscripts are significantly different: P<=0.05

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Experiment 3:

4.3 Effect of Graded Level of BFPM on Performance of Growing Rabbits

4.3.01 Temperature Humidity Index for Year 2

The monthly THI inside the rabbitry during the experimental period is shown in Figure 1. THI in the mornings averaged 26.44^{o}C while the Afternoon THI averaged 28.74^{o}C. The THI values kept increasing from the month of February with a peak in May. There was a decline in THI in June.

4.3.02 Thermoregulatory Response of Growing Rabbits

The effect of graded levels of BFPM on thermoregulatory response of growing rabbits is shown in Table 4.13. The BFPM significantly ($P<0.05$) reduced rectal and ear temperatures of the rabbits at 4.5 and 5.5% inclusion levels. Respiratory rate and heart rate did not show any difference. As the levels of BFPM increased in the diets, rectal and ear temperature rose. However, respiratory rate and heart rate reduced up to 3.5% inclusion level and increased again at 4.5 and 5.5% inclusion levels, although the values were not significant.

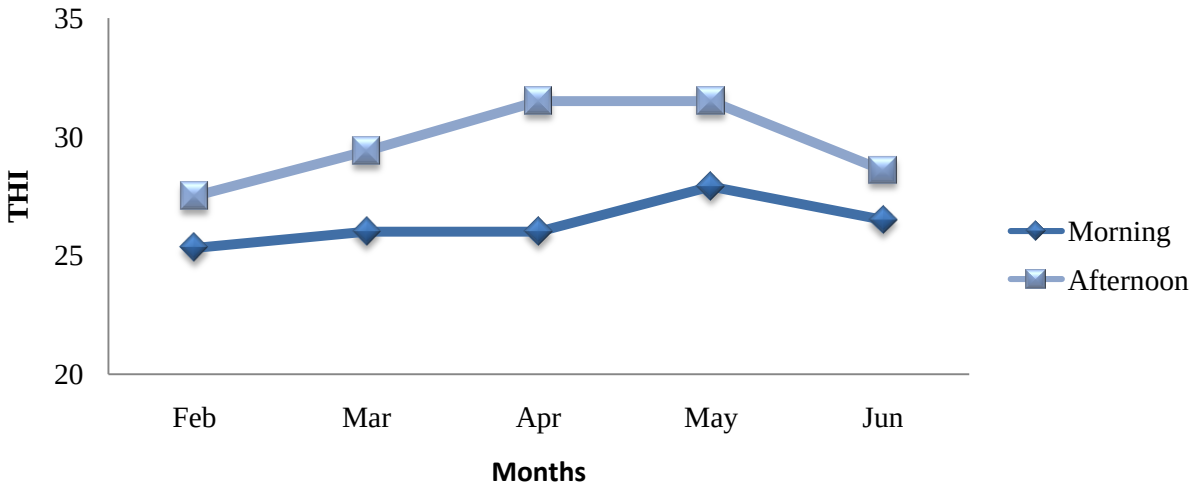


Figure 4.07: Monthly Temperature Humidity Index inside the Rabbit house during the Experimental Period for Year 2

Table 4.13 Effect of Graded Levels of BFPM on Thermoregulatory Response of Growing Rabbits

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Respiratory Rate (counts/min)	106.81^{a}	94.08^{b}	91.33^{b}	96.92^{b}	107.50^{a}	5.60
Heart Rate (beats/min)	137.44	139.16	136.94	135.75	141.10	2.25
Rectal Temperature (^{o}C)	38.51^{a}	37.80^{b}	37.62^{bc}	37.20^{c}	36.90^{d}	0.14
Ear Temperature (^{o}C)	35.83^{a}	35.20^{ab}	34.88^{bc}	34.30^{c}	33.97^{d}	0.19

Means within rows with different superscripts are significantly different: $P < 0.05$

4.3.03 Growth Performance of Growing Rabbits

The graded levels of BFPM significantly ($P < 0.05$) improved weight gain and final weight at 2.5 and 3.5% inclusion levels, compared to 4.5 and 5.5%. BFPM treated rabbits also recorded a significantly ($P < 0.05$) high feed intake at 5.5% inclusion levels. Feed intake rose with increase in the inclusion levels of BFPM and a reverse trend was observed with weight gain. Weight gain decreased as the levels of BFPM in the diet increased. Weight gain increased up to 3.5% inclusion level before declining. At 3.5% inclusion level, BFPM recorded a value of 1310.00, which declined to 1050.83 (4.5%) and 970.00 (5.5%), respectively.

4.3.04 Serum Metabolite of Growing Rabbits

Serum metabolites of growing rabbits fed graded levels of BFPM show that the initial values of serum metabolites did not show any significant difference among the treatment groups (Table 4.15). In the final value, 3.5 and 5.5% BFPM significantly increased serum glucose, total protein and albumin concentration. The treatments with 2.5% BFPM significantly increased serum phosphorous, while the control significantly increased serum calcium. Serum metabolites improved in the final evaluation compared to the initial values.

Table 4.14 Effect of Graded Levels of BFPM on Growth Performance of Growing Rabbits

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Initial weight (g)	658.33	658.33	658.33	616.66	641.66	-
Final weight (g)	1120.00^{ab}	1279.30^{a}	1310.00^{a}	1050.83^{b}	970.00^{c}	77.07
Weight gain (g/day)	9.04	12.24	12.68	8.66	6.16	0.95
Feed intake (g/day)	50.13^{e}	55.25^{d}	63.25^{c}	70.25^{b}	82.92^{a}	0.10

Means within rows with different superscripts are significantly different: $P < 0.05$

Table 4.15 Effect of Graded Levels of BFPM on Serum Metabolite of Growing Rabbits

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Initial						
Glucose (mg/dl)	3.63	3.53	3.23	3.07	3.50	0.53
Total Protein (mg/dl)	64.33	60.00	63.00	63.00	63.00	0.04
Albumin (mg/dl)	33.00	32.67	32.33	33.67	33.33	0.04
Cholesterol (mg/dl)	1.26	1.33	1.27	1.33	1.33	0.78
Triglyceride (mg/dl)	0.90	1.00	0.93	0.90	1.00	0.06
Calcium (mg/dl)	2.42	2.38	2.25	2.29	2.25	0.03
Phosphorous (mg/dl)	1.08	1.07	1.07	1.07	1.08	0.13
After						
Glucose (mg/dl)	3.53^{c}	3.23^{c}	4.70^{ab}	4.43^{b}	5.07^{a}	0.16
Total Protein (mg/dl)	68.00^{b}	65.67^{c}	72.00^{a}	64.33^{c}	60.00^{d}	3.06
Albumin (mg/dl)	35.67^{ab}	37.66^{ab}	38.67^{a}	31.66^{b}	32.33^{b}	1.12
Cholesterol (mg/dl)	1.53^{a}	1.37^{ab}	1.33^{b}	1.27^{b}	1.33^{b}	0.06
Triglyceride (mg/dl)	0.83	0.90	1.00	0.90	0.70	0.10
Calcium (mg/dl)	2.32^{a}	2.25^{b}	2.31^{ab}	2.29^{ab}	2.29^{ab}	0.01
Phosphorous (mg/dl)	1.13^{c}	1.18^{a}	1.16^{b}	1.04^{c}	1.09^{d}	0.07

Means within rows with different superscripts are significantly different: $P < 0.05$

4.23 Thyroxine Levels in Growing Rabbits

The effect of graded levels of BFPM on growing rabbits (Fig. 4.08) revealed that initial thyrosine levels of the rabbits showed significant ($P<0.05$) difference across the treatments, with the control, 3.5 and 5.5% BFPM showing the highest values. However, initial thyroxine levels were low compared to those obtained at the end of the experiment. At the end of the experiment, thyroxine levels increased across the treatments, and 2.5 and 3.5% BFPM significantly recorded the highest values. Thyroxine levels reduced at 4.5 to 5.5% BFPM levels.

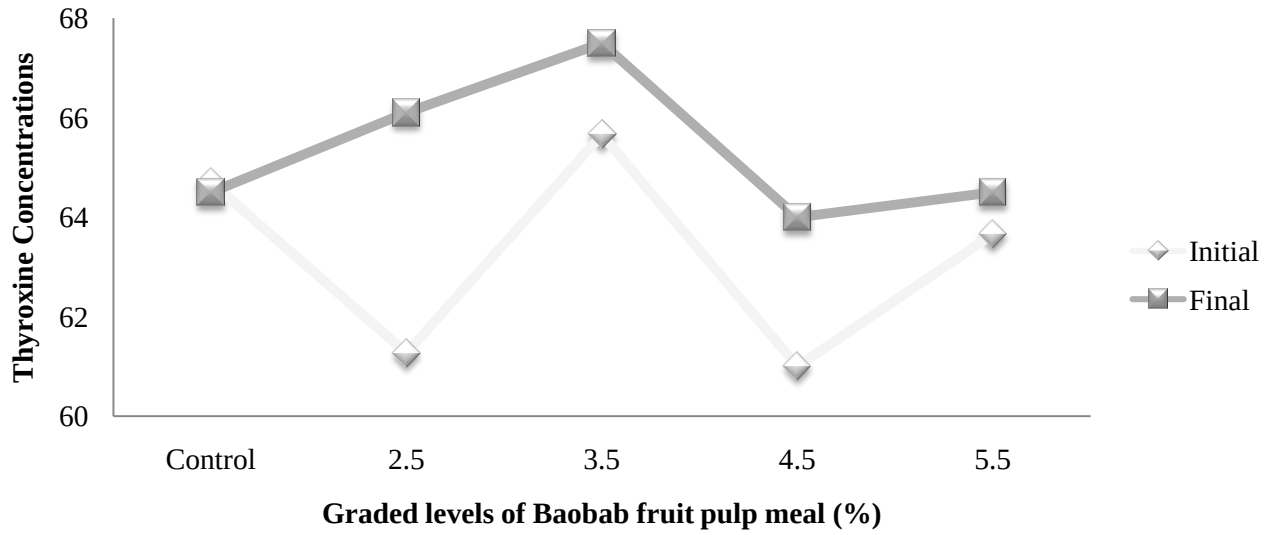


Fig. 4.08 Effect of Graded Levels of BFPM on Thyroxine Levels in Growing Rabbits

Experiment 4

4.4 Effect of Graded Levels of BFPM on Reproductive Performance of Adult Rabbits

4.4.01 Thermoregulatory Response of Adult Rabbits Bucks

Table 4.16 shows the effect of graded levels of BFPM on thermoregulatory response of adult rabbit bucks. Increasing levels of BFPM ($P < 0.05$) significantly reduced all the thermoregulatory parameters; respiratory rate, heart rate, rectal temperature and ear temperature. The treatments with 4.5 or 5.5% inclusion levels of BFPM had better improvements in all the parameters compared to other treatments. Respiratory rate was reduced from 159.63 (cpm) recorded in the control to 137.63 (cpm) and 137.78 (cpm) in 4.5% and 5.5% levels of BFPM in the diet. Heart rate was also reduced from 154.60 (bpm) in the control to 137.63 (bpm) on 5.5% level of BFPM in the diet.

Table 4.16 Effect of Graded Levels of BFPM on Thermoregulatory Response of Adult male Rabbits

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
RespiratoryRate (counts/min)	159.63^{ab}	165.22^{a}	149.44^{b}	137.63^{c}	137.78^{c}	2.44
Heart Rate (beat/min)	154.60^{a}	143.69^{b}	144.70^{b}	141.30^{b}	137.63^{c}	2.74
Rectal Temperature (^{o}C)	39.27^{a}	38.62^{a}	38.32^{b}	37.90^{c}	37.76^{c}	0.12
Ear Temperature (^{o}C)	35.83^{a}	35.20^{ab}	34.88^{bc}	34.30^{c}	33.97^{d}	0.16

Means within rows with different superscripts are significantly different: P<=0.05

4.4.02 Serum Metabolite of Adult Rabbit Bucks

The values of serum metabolites (glucose, total protein, albumin, cholesterol, triglyceride, calcium and phosphorous) obtained before the experiment did not show any significant difference among the treatment groups (Table 4.17). It was however revealed that at the end of the experiment BFPM significantly decreased cholesterol, triglycerides and calcium levels. The treatment with 2.5% BFPM recorded significantly higher (1.67) cholesterol level, while the least (1.37) was recorded in rabbits treated with 5.5% BFPM. BFPM at 4.5% improved triglyceride (1.03) compared to the rest of the treatments, while 2.5 and 3.5% treatments recorded the highest calcium content, compared to the rest of the treatments. Values of serum metabolites recorded in this result did not follow a particular pattern. However, most of the metabolites whose values were low before the commencement of the experiment were increased by treatment with 5.5% BFPM at the end of the experiment. Serum metabolites did not follow a particular pattern. However, most of the metabolites whose values were low before the commencement of the experiment were increased by the treatment with 5.5% BFPM at the end of the experiment.

4.4.03 Thyroxine Levels in Adult Rabbit Bucks

The result of graded levels of BFPM on thyroxine levels in adult rabbit bucks (Figure 4.08) shows that thyroxine level was low at the commencement of the experiment and increased at the end of the experiment. In both instances, the values did not show any significant difference among the treatments. The trend after the experiment was similar to that recorded at the end of the experiment. Rabbits on 2.5% BFPM had higher thyroxine level both at the beginning and end of the experiment.

Table 4.17 Effect of Graded Levels of BFPM on Serum Metabolite of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Initial						
Glucose (mg/dl)	4.77	4.27	4.17	4.03	4.13	0.33
Total Protein (mg/dl)	68.33	66.00	65.00	68.33	67.00	1.76
Albumin (mg/dl)	37.33	35.00	35.00	37.33	36.00	1.55
Cholesterol (mg/dl)	1.33	1.47	1.28	1.33	1.57	0.10
Triglyceride (mg/dl)	0.87	0.77	1.12	0.77	0.93	0.08
Calcium (mg/dl)	2.40	2.17	2.38	2.37	2.45	0.03
Phosphorous (mg/dl)	1.00	1.06	1.05	1.03	0.99	0.04
After						
Glucose (mg/dl)	4.30	5.13	5.20	5.40	5.33	0.13
Total Protein (mg/dl)	66.67	64.67	68.33	62.33	70.00	1.75
Albumin (mg/dl)	35.67	35.67	38.57	33.33	38.00	1.75
Cholesterol (mg/dl)	1.47^{ab}	1.67^{a}	1.40^{ab}	1.47^{ab}	1.37^{b}	0.06
Triglyceride (mg/dl)	0.70^{bc}	0.73^{b}	0.90^{ab}	1.03^{a}	0.97^{a}	0.06
Calcium (mg/dl)	2.26^{b}	2.42^{a}	2.45^{a}	2.27^{b}	2.32^{ab}	0.03
Phosphorous (mg/dl)	1.01	1.06	0.93	1.08	1.05	0.04

Means within rows with different superscript lettes are significantly $P < 0.05$ different:

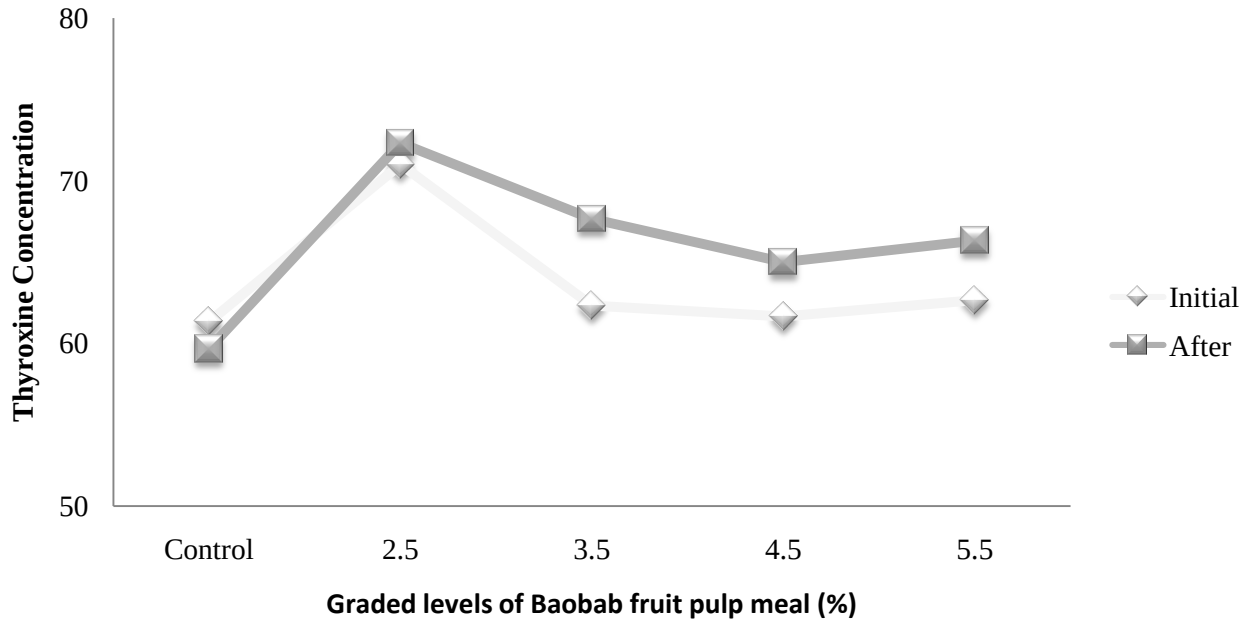


Fig. 4.09 Effect of Graded Levels of BFPM on Thyroxine Levels in Adult Rabbit Bucks

4.4.04 Testosterone Levels in Adult Rabbit Bucks

Testosterone level at the commencement of the experiment was lower than that recorded at the end of the experiment (Fig. 10). There was no significant difference in initial testosterone level among the treatments but treatment with 5.5% BFPM significantly ($P<0.05$) increased testosterone level from 7.4 ml/ng to 13.7ml/ng. Treatment of rabbits with 2.5% BFPM increased testosterone level from 7.4 ml/ng to 12.24ml/ng.

4.4.05 Semen Quality Characteristics of Adult Rabbit Bucks

The effect of graded levels of baobab fruit meal on semen characteristics of adult rabbits is shown in Table 4.18. BFPM significantly ($P<0.05$) improved semen characteristics of rabbit bucks, especially at 4.5% inclusion. At 4.5% inclusion BFPM recorded higher semen concentration of about 102.50×10^6, better pH, motility, colour and volume. The quality of the semen increased as the level of BFPM in the diet rose with a peak at 4.5% inclusion level and thereafter declined at 5.5%. Inclusion above 5.5% level of BFPM did not support semen characteristics.

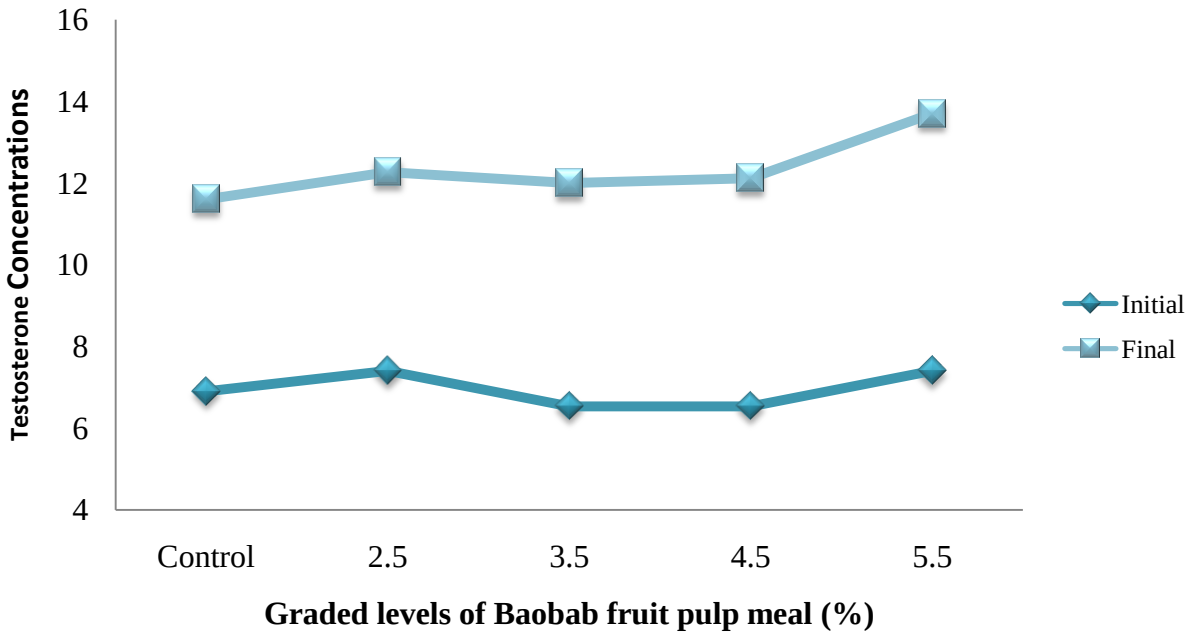


Fig. 4.10 Effect of Graded Levels of BFPM on Testosterone Levels in Adult Rabbit Bucks

Table 4.18 Effect of Graded Levels of BFPM on Semen Characteristics of Adult Buck

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Volume (ml)	0.55^{b}	0.94^{a}	1.00^{a}	0.96^{a}	1.03^{a}	0.22
Colour	1.50^{ab}	1.93^{a}	1.17^{a}	1.2^{ab}	1.0^{b}	0.18
Mortility (%)	28.33^{c}	65.67^{ab}	66.25^{ab}	77.09^{a}	51.88^{b}	4.13
pH	7.19^{ab}	7.30^{a}	6.75^{ab}	6.75^{ab}	6.71^{b}	0.12
Concentration ($x10^{6}$)	6.61^{c}	82.71^{b}	86.88^{b}	102.50^{a}	71.92^{b}	9.58

Means within rows with different superscript letters are significantly $P < 0.05$ different

Semen colour = Milky: 1.00 – 1.5, Creamy: 1.51- 2.00, and Watery, 2.10 and above.

4.4.06 Sperm Morphology of Adult Rabbit Bucks

The effect of graded leves of BFPM on sperm morphology of adult rabbits is shown in Table 4.19. Sperm morphological characteristics improved significantly ($P<0.05$) with increasing levels of BFPM up to 4.5%. At this 4.5% BFPM, there was an increase in number of live cells (74.38%) but dead cells reduced (25.62%), and increased number of normal cells rose (77.71%), compared to 65.67%, 34.33% and 60.67% recorded by the control for live cells, dead cells and normal cells, respectively. This poor performance recorded in the control was also similar to those recorded by rabbits treated with 5.5% BFPM. The treatment with 4.5% BFPM also significantly ($P < 0.05$) reduced the number of deformed cells, such as detached head, free tail and coil tail. The treatment with 5.5% BFPM recorded high numbers of coil tail and free tail respectively. Bent tail did not show any significant difference among the treatments.

4.4.07 Gonadal Morphometry of Adult Rabbit Bucks

Table 4.20 shows the effect of graded levels of BFPM on gonadal morphometry of adult rabbit bucks. Treatments with 3.5% BFPM significantly ($P<0.05$) increased testicular length, testicular weight and filtrate volume. The values were similar with those recorded in rabbits treated with 4.5 and 5.5% BFPM, compared to the rest of the treatments. Across the treatments the values of the right testis were generally higher than those of the left testis, but the weight of the left testis did not show any significant difference.

Table 4.19 Effect of Graded Levels of BFPM on Sperm Morphology of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Live cells (%)	65.67^{b}	68.96^{ab}	67.92^{ab}	74.38^{a}	65.21^{b}	2.30
Dead cells (%)	34.33^{b}	31.71^{b}	32.77^{a}	25.62^{a}	33.96^{b}	3.95
Normal Cells (%)	60.67^{c}	70.33^{b}	71.29^{ab}	77.71^{a}	68.34^{b}	1.73
Detached Head (%)	17.00^{a}	13.33^{abc}	12.54^{bc}	10.25^{c}	14.38^{ab}	0.54
Free tail (%)	6.98^{b}	6.82^{b}	7.11^{ab}	5.70^{c}	8.43^{a}	0.77
Coil Tail (%)	5.50^{a}	4.34^{a}	5.63^{a}	3.34^{b}	3.83^{b}	0.73
Bent Tail (%)	4.77	3.88	5.61	3.56	4.78	0.61

Means within rows with different superscripts are significantly different: $P < 0.05$

Table 4.20: Effect of Graded Levels of BFPM on Gonadal Morphometric of Adult Rabbits Bucks

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Testis Length (cm)						
Right	2.40^{b}	2.47^{b}	3.16^{a}	2.73^{ab}	2.63^{b}	0.11
Left	2.30^{b}	2.70^{ab}	3.33^{a}	3.07^{a}	2.67^{ab}	0.17
Testis weight (g)						
Right	0.53^{c}	1.48^{abc}	2.40^{a}	2.22^{ab}	1.11^{b}	0.26
Left	0.45	1.28	1.64	1.89	1.12	0.34
Filtrate Volume (ml)						
Right	8.67^{b}	9.00^{ab}	9.16^{ab}	9.17^{ab}	9.47^{a}	0.99
Left	8.33^{bc}	8.00^{c}	9.63^{a}	9.00^{ab}	9.60^{a}	0.83

Means within rows with different superscripts are significantly different: $P < 0.05$

4.4.08 Epididymis Morphometry of Adult Rabbit Bucks

The result of the effect of graded levels of BFPM on the epididymis morphometry of adult rabbit bucks is shown in Table 4.21. The treatment with 3.5% BFPM significantly (P<0.05) increased epididymis length and epididymis weight (right) while the control, 2.5 and 5.5% significantly increased epididymis filtrate volume. The weight of the left epididymis, length of caput and corpus epididymis (right) and weight of the caput and corpus epididymis, did not show any significant difference. The values of the right epididymis across the treatments were larger than those of the left epididymis.

4.4.09 Gonadal and Epididymal Sperm Reserve of Adult Rabbit Bucks

The effect of graded levels of BFPM on gonadal and epididymal sperm characteristics of adult rabbit bucks is shown in Table 4.22 Inclusion of BFPM in the diet significantly (P < 0.05) increased testicular volume and sperm concentration, compared to the rest of the treatments while 5.5% BFPM significantly (P < 0.05) improved testicular sperm concentration of the right testis. Epididymal sperm concentration did not follow any pattern. For instance, 3.5% BFPM significantly (P<0.05) increased the right caput and cauda epididymis sperm concentration, compared to the rest of the treatments while 5.5% BFPM significantly (P<0.05) increase the left caput epididymis. Corpus and cauda epididymis sperm concentration significantly increase with bucks treated with 2.5% BFPM and in the control. The control rabbits recorded poor sperm characteristics, compared to the treatments with BFPM.

Table 4.21: Effect of Graded Levels of BFPM on Epididymis Morphometry of Adult Bucks

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Epididymis Length (cm)						
Right	3.42^{b}	5.10^{ab}	6.50^{a}	5.77^{ab}	3.92^{a}	0.69
Left	2.88^{b}	4.67^{ab}	7.17^{a}	6.50^{ab}	4.21^{ab}	0.91
Epididymis Weight (g)						
Right	0.38^{c}	0.79^{bc}	1.18^{a}	1.10^{ab}	0.55^{b}	0.13
Left	0.55	0.71	0.92	0.95	0.85	0.16
Caput Length (cm)						
Right	1.27	1.37	1.67	1.67	1.64	0.17
Left	1.00^{c}	1.10^{b}	1.37^{a}	1.27^{ab}	1.13^{b}	0.05
Corpus Length (cm)						
Right	2.77	2.17	2.47	2.07	2.60	0.71
Left	1.70^{ab}	1.47^{b}	2.27^{a}	1.73^{ab}	1.70^{ab}	0.20
Cauda Length (cm)						
Right	1.23^{b}	1.28^{b}	1.70^{a}	1.50^{ab}	1.40^{ab}	0.08
Left	1.57	1.57	1.67	1.53	1.68	0.09
Caput Weight (g)						
Right	1.13	0.22	0.30	0.30	1.17	0.50
Left	0.10	0.80	0.46	0.40	0.22	0.05
Corpus Weight (g)						
Right	0.04	0.02	0.04	0.05	0.03	0.01
Left	0.01	0.01	0.01	0.02	0.01	0.01
Cauda Weight						
Right	0.36^{b}	0.47^{b}	0.65^{a}	0.66^{a}	0.46^{ab}	0.06
Left	0.28^{b}	0.43^{ab}	0.65^{a}	0.61^{a}	0.42^{ab}	0.07
Epididymis Filtrate Volume (cm)						
Caput						
Right	4.27^{ab}	4.17^{ab}	4.37^{ab}	4.00^{b}	4.57^{a}	0.13
Left	4.73^{a}	4.77^{a}	4.50^{ab}	3.79^{b}	4.23^{ab}	0.17
Corpus						
Right	4.33	5.00	4.56	3.90	4.67	0.28
Left	4.76^{a}	4.73^{a}	4.60^{ab}	3.90^{b}	4.37^{ab}	0.19
Cauda						
Right	4.53^{ab}	4.00^{b}	4.37^{abc}	3.87^{c}	4.63^{a}	0.13
Left	4.66^{a}	4.70^{a}	4.40^{ab}	3.90^{b}	4.20^{b}	0.15

Table 4.22: Effect of Graded Levels of BFPM on Gonadal and Epididymal Sperm Reserve of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Testis sperm volume (ml)						
Right	0.90^{b}	4.50^{ab}	5.00^{ab}	7.67^{a}	2.70^{b}	0.17
Left	0.80^{b}	3.17^{ab}	4.33^{ab}	5.67^{a}	1.97^{b}	0.17
Testis sperm conc. (x 10^6)						
Right	20.33^{bc}	27.33^{ab}	17.67^{c}	33.67^{a}	32.67^{a}	2.13
Left	19.33^{c}	25.00^{b}	16.67^{c}	33.33^{a}	25.33^{b}	1.36
Epididymis Caput (x 10^6)						
Right	5.33^{b}	6.00^{b}	20.33^{a}	13.00^{ab}	12.00^{ab}	3.25
Left	7.33^{c}	8.00^{b}	9.67^{abc}	16.00^{ab}	17.33^{a}	1.92
Corpus (x 10^6)						
Right	2.56^{b}	6.00^{a}	4.00^{ab}	6.00^{a}	2.67^{b}	0.55
Left	5.33^{ab}	7.00^{a}	2.00^{b}	5.33^{ab}	3.33^{ab}	1.00
Cauda (x 10^6)						
Right	124.00^{c}	137.33^{ab}	154.33^{a}	143.00^{a}	132.33^{b}	21.26
Left	110.00^{c}	107.67^{c}	173.67^{a}	17.67^{a}	165.33^{b}	22.74

Means within rows with different superscript letters are significantly $P < 0.05$ different

4.4.10 Serum Metabolites of Adult Rabbit Does

The effect of graded levels of BFPM on serum metabolites concentration of adult female rabbits is shown in Table 4.23. Graded levels of BFPM significantly ($P<0.05$) increased some serum metabolites such as glucose, total protein compared to the control, 3.5% and 5.5% BFPM, and triglyceride. Total protein, phosphorous and triglyceride significantly improved with graded levels of BFPM after pregnancy. It was revealed that values of metabolites recorded before pregnancy were generally low and similar to those recorded in the control throughout the experiment.

4.4.11 Thyroxine Levels in Adult Rabbit Does Before, During and After Pregnancy

The effect of graded levels of BFPM on thyroxine levels in adult female rabbits before, during and after pregnancy is shown in Fig. 4.11. Thyroxine levels were low in all the treatment groups, compared to thyroxine levels recorded during and after pregnancy. During pregnancy, thyroxine levels significantly ($P<0.05$) rose in in rabbits 4.5 and 5.5% BFPM levels. Thyrosine levels in (4.5 and 5.5% BFPM) in treatments were higher than thyroxine levels after pregnancy, after pregnancy, thyroxine levels did not show any significant difference among the treatment groups. Thyroxine levels after pregnancy were higher than obtained before and during pregnancy in the control group, Treatments with 2.5% and 3.5% BFPM. Thereafter the levels declined in treatment 4.5 and 5.5% inclusion levels but not above the values recorded after pregnancy.

Table 4.23 Effect of Graded Levels of BFPM on Serum Metabolites of Adult Rabbit Does

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
a. Glucose (mg/dl)	5.20	4.93	4.77	4.47	5.0	0.35
b. Glucose (mg/dl)	4.20^{b}	5.53^{a}	5.25^{a}	5.83^{a}	5.30^{a}	0.06
c. Glucose (mg/dl)	4.57	5.33	4.50	4.00	4.40	0.14
a. Total Protein (mg/dl)	63.00	66.67	66.00	67.67	67.33	1.43
b. Total Protein (mg/dl)	68.33^{a}	62.00^{b}	67.00^{a}	65.00^{ab}	68.67^{a}	1.03
c. Total Protein (mg/dl)	68.67^{a}	64.33^{c}	68.00^{ab}	68.00^{ab}	65.00^{bc}	0.79
a. Albumin (mg/dl)	36.07	35.67	39.67	37.67	37.33	1.12
b. Albumin (mg/dl)	37.00	32.00	38.33	36.00	38.00	1.45
c. Albumin(mg/dl)	42.00^{a}	35.33^{b}	40.33^{a}	39.17^{a}	34.33^{b}	0.84
a. Cholesterol (mg/dl)	1.57	1.53	1.47	1.47	1.53	0.11
b. Cholesterol (mg/dl)	1.5	1.43	1.35	1.43	1.40	0.05
c. Cholesterol (mg/dl)	1.43	1.50	1.40	1.35	1.50	0.04
a. Triglyceride (mg/dl)	0.93	1.00	0.90	0.87	0.67	0.10
b. Triglyceride (mg/dl)	1.0^{a}	0.73^{b}	0.90^{ab}	1.03^{a}	0.97^{a}	0.05
c. Triglyceride (mg/dl)	0.85^{bc}	1.10^{a}	0.85^{b}	0.70^{c}	0.94^{ab}	0.04
a. Calcium (mg/dl)	2.33	2.40	2.27	2.56	2.29	0.07
b. Calcium (mg/dl)	2.31^{ab}	2.37^{ab}	2.40^{a}	2.40^{a}	2.24^{b}	0.03
c. Calcium (mg/dl)	2.17	2.31	2.43	2.41	2.18	0.02
a. Phosphorous (mg/dl)	1.05	1.02	1.16	1.13	1.01	0.06
b. Phosphorous (mg/dl)	0.97^{ab}	0.93^{ab}	0.62^{b}	1.11^{a}	1.16^{a}	0.08
c. Phosphorous (mg/dl)	1.11^{a}	1.0^{b}	1.06^{a}	1.0^{b}	1.0^{b}	0.01

Means within rows with different superscripts are significantly different: $P < 0.05$

a: before pregnancy, b: during pregnancy, c: after delivery

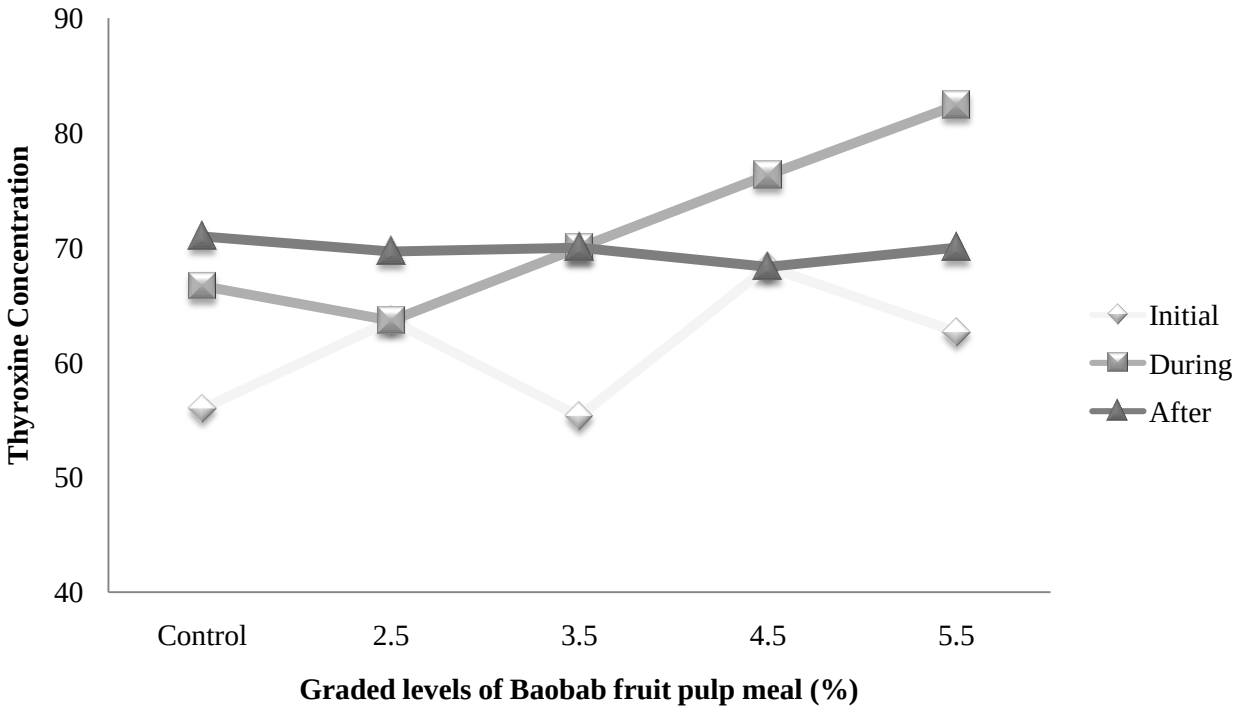


Fig. 4.11 Effect of Graded Levels of BFPM on Thyroxine Levels in Adult Rabbit Does

4.4.12 Progesterone Levels in Adult Rabbit Does Before, During and After Pregnancy

Fig 4.12 shows the effect of graded levels of BFPM on progesterone levels in adult rabbit does before, during and after pregnancy. Progesterone levels were generally low before pregnancy and were not significantly different among the treatment groups. However, BFPM significantly ($P<0.05$) increase progesterone levels in the female rabbits during and after pregnancy. During pregnancy, 2.5% BFPM increased progesterone level to 20.87 and was reduced at 5.5% BFPM. During and after pregnancy, progesterone levels decreased in the rabbits with increasing levels of BFPM in the diets.

4.4.13 Reproductive Performance of Adult Rabbit Does

The effect of graded levels of BFPM on reproductive performance of rabbits does is shown in Table 4.24. There was a significant ($P<0.05$) improvement in litter size and weight of litter at 3.5 and 4.5% BFPM levels respectively. Weight of kit increased in 2.5 and 4.5% BFPM, while litter size at weaning was larger in 4.5 and 5.5% BFPM. Gestation period and kit weight at weaning did not show any significant difference.

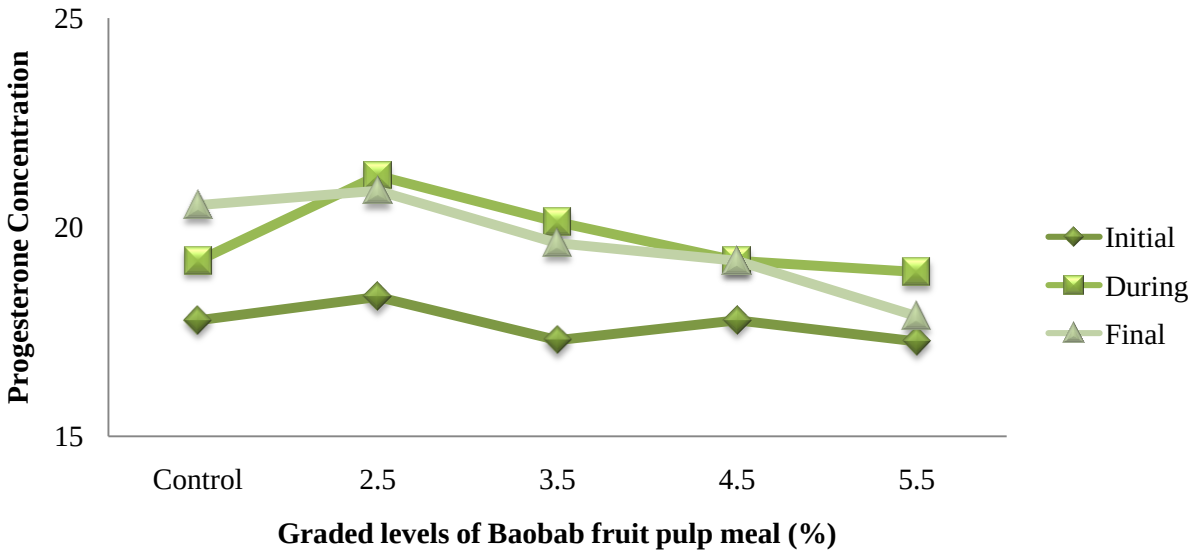


Fig. 4.12 Effect of Graded Levels of BFPM on Progesterone Levels in Adult Rabbits Does Before, During and After Pregnancy

Table 4.24 Effect of Graded Levels of BFPM on Reproductive Performance of Adult Rabbit Does

	Treatments					
Parameters	**Control**	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Gestation period	30.00	30.00	30.00	30.67	30.33	0.17
Litter size at birth	4.00^{bc}	3.67^{c}	5.66^{a}	5.33^{ab}	5.33^{ab}	0.37
Weight of litter (g)	186.67^{b}	176.67^{b}	233.33^{a}	218.33^{ab}	250.00^{a}	10.95
Weight of kit (g)	47.33^{ab}	48.89^{a}	40.00^{b}	40.00^{b}	48.33^{a}	2.60
Litter size at weaning	3.67^{ab}	3.33^{b}	4.68^{ab}	5.00^{a}	5.00^{a}	0.34
Kit weight at weaning (g)	502..57	502.00	426.67	504.00	436.67	20.59

Means within rows with different superscripts are significantly $P < 0.05$ different

CHAPTER FIVE

5.0 Discussion

Experiment 1

5.1 Ameliorative effect of bicarbonate buffer, vitamin C and baobab on growth performance of rabbits under tropical environment

5.1.01 Temperature Humidity Index for Year A

The THI value of 27^{o}C (Feb) indicated that the month of February had absence of heat stress in the rabbit house, while the THI values of 28^{o}C (March and June), 29.5^{o}C (April) and 31.2^{o}C (May) and 28^{o}C (June) are indications that the rabbit house was moderately thermally-stressful, severely thermally stressful and very severely-thermally stressful (Marai, 2001) respectively in these months. The average THI of 28.74^{o}C during the experimental period indicated that the rabbit house was thermally stressful and may have adverse effects on the rabbits (Marai, 2001). Overall data obtained indicated that THI in the afternoon was higher by 1.24 % than THI in the morning.

5.1.02 Thermoregulatory Response of Growing Rabbit

The result obtained this study showed that supplementing vitamin C and BFPM in rabbit diets effectively reduced respiratory rate, heart rate, rectal and ear temperature compared to using buffers ($KHCO_3$ and Na_2CO_3). It was also observed that heart rate and respiratory rate increased in treatments with $KHCO_3$ and Na_2CO_3. This result is in concordance with the reports of Teeter *et al.* (1985), who reported that $NaHCO_3$ could increase respiratory alkaloisis severity, which might have led to the increased heart rate and respiratory rate recorded in this study. Contrary to the findings of this study, Marai *et al.* (1994) found that 1.25 or 2.5% $NaHCO_3$ improved rectal temperature, respiration rate and blood components and thus corrected acid–base balance disturbances, occurring under stress conditions. Haydon and West (1990) suggested that the improvement resulted from adding $NaHCO_3$ to heat stressed rabbits may be due to increase in

blood buffering capacity. Supplemental vitamin C has been reported to be effective in ameliorating heat stress as was recorded in this study. Verde and Piquer (1986) showed that the plasma vitamin C concentration significantly reduced in animals exposed to heat stress. Supplementary vitamin C has been shown to be beneficial in reducing the effects of stress. Vitamin C as a metabolic factor is essential for growth and counteracting infections, caused by pathogenic bacteria and viruses. Therefore means the metabolic requirement for vitamin C increases under certain conditions. BFPM has been reported to lower rectal temperature of birds than the value obtained with the control diet (Adeosun, 2012). This could be due to the high content vitamin C in BFPM (Agbessi Dos-Santos, 1987) which may also have antioxidant properties, and scavenges for free radicals as vitamin C does.

5.1.03 Growth Performance of Growing Rabbits

Stress in rabbits results in a decline in body weight, feed consumption and overall feed efficiency. However, supplementation of antioxidants along with the basal diet has been demonstrated to improve growth and performance in rabbits (Yassein *et al.,* 2011) and birds (Sahin and Kucuk, 2001). It was observed in this study that the buffers (Na_2CO_3 and $KHCO_3$), vitamin C and BFPM significantly improved feed intake and weight gain. This result agrees with the findings of Yassein *et al.* (2011), who reported that Na_2CO_3 and $KHCO_3$ serve as appetizer supplements to rabbit's diets and may stimulate the appetite, increase fiber digestibility and improve feed efficiency. Similar results were obtained by Marai *et al.* (1994) who found that 1.25% or 2.5% NaHCO3 improved growth performance, rectal temperature, respiration rate and blood components by correcting acid –base balance disturbances, such as occur under stress conditions. Vitamin C has been reported to be effective in growth performance of rabbits,

especially during heat stress (Rao and Sharma 2001). Puron *et al.* (1994) showed that addition of 200-600 mg of vitamin C in diets, improves growth, feed efficiency, and livability in in broilers exposed to heat stress. Bain (1996) revealed that vitamin C aids conversion of protein, and fat into energy for production and survivability through increased corticosteroid secretion. Vitamin C supplementation also increases performance, yield better carcass trait in broilers, reared under heat stress conditions (32^{o}C) (Sahin, and Kucuk, 2001). It has been reported that broiler chicken fed BFPM diets during hot season performed better than those fed the vitamin-C supplemented diets as well as the control diet (Adeosun, 2012). A similar trend was observed in this study. Supplementation wirh BFPM in diets up to 3.5 % gave better performance and egg quality characteristics of layers than the control diet during the hot-dry season (Adeosun, 2012).

5.1.04 Serum Metabolite of Growing Rabbits

The values of final serum metabolites in this experiment followed the trend of their growth performance records (feed intake, weight gain and final body weight). For instance, BFPM recorded significantly high serum triglycerides which was similar to the treatments with vitamin C and bicarbonate buffer, compared to the control. These treatments were the ones that showed a better feed intake and weight gain, compared to the control. Triglycerides are important component of the body's adipose tissue. Lebas *et al.* (1986), Chiericato *et al.* (1996) and Marai *et al.* (2001) reported a reduction in live body weight and daily body weight gain due to heat-stressed conditions attributed to the negative effects of heat-stress on appetite and consequently a decrease in feed consumption. Low feed intake must have affected the low serum metabolites recorded in the control group. Bicarbonate buffers might have triggered the endocrine gland to increased thyroxine secretions, and thyrosine is known to increase the metabolic activities of

almost all the tissues of the body. The growth rate of young people is greatly accelerated. The mental processes are excited, and the activities of most of the other endocrine glands are increased (Burger, 2004). Vitamins are known to be essential parts of some of the enzymes or coenzymes (Burger, 2004) and enzymes are known to be important in food digestion making micronutrients to be available in the body of animls.

5.1.05 Thyroxine Levels in Growing Rabbits

The final thyroxine level in this study was higher than the value recorded at the beginning of the experiment. The difference in the value may be attributed to changes in age and body metabolism of the rabbits. Body metabolism increases with age (Burger, 2004); and the increasing body functioning together and adipose tissue deposition can increase heat load leading to the demand for more thyroxine (T_4) level in the bloodstream. It has been reported that T_4 increases with age in chicks (Leenstra *et al*., 1991). The thyroid hormones increase the metabolic activities of almost all the tissues of the body. The basal metabolic rate can increase by 60 to 100 percent above normal when large quantities of the hormones are secreted. The rate of utilization of foods for energy is greatly accelerated. Although the rate of protein synthesis is increased, at the same time the rate of protein catabolism is also increased. The growth rate of the young is greatly accelerated (Burger, 2004). The reduction in T_4 at the final stage by the treatments with vitamin C and BFPM meal diets could be because the supplemented vitamin in the vitamin pool was maintained and body metabolism was retarded. According to (Burger, 2004), thyroid hormone increases the activities of many bodily enzymes and because vitamins are essential parts of some of the enzymes or co-enzymes, thyroid hormone causes increased need for vitamins. Therefore, a relative vitamin deficiency can occur when excess thyroid hormone is

secreted, unless at the same time increased quantities of vitamins are made available. The treatments with the buffer may have triggered the activity of the thyroid gland to increase thyroxine secretion, and this reason may be responsible for the increase in thyroxine secretion recorded in these treatments.

5.2 Experiment 2: Reproductive Performance Trial

5.2.01 Thermoregulatory Response of Adult Rabbit Bucks

Results in this study showed that adult rabbits recorded an averagely high respiratory rate, rectal temperature and ear temperature, compared to the corresponding values recorded in growing rabbits administered the same treatments. For instance a range of 141.03 – 158.34 cpm was recorded for respiratory rate in adult male rabbits, compared to 76.59 – 97.94 (cpm) recorded in growing rabbits administered the same treatments. This result agrees with the reports of Anoh *et al.* (2014), who reported that adult rabbits are more prone to heat stress, compared to growing rabbits. Vitamin C and BFPM significantly reduced thermoregulatory parameters compared to $KHCO_3$ and Na_2CO_3 or the control. The result shows that $NaHCO_3$ could increase respiratory alkaloisis severity (Teeter *et al.,* 1985), which may be responsible for the high values of heart rate, rectal and ear temperature recorded in rabbits treated with the buffers. The effectiveness of vitamin C and BFPM observed in this study agrees with the finding of Ayo *et al.* (2011) who reported reduced rectal temperature values in transported rabbits due to vitamin C induced amelioration. Vitamin C protects the cells from the damage caused by reactive oxygen species and mutagens (Yarube *et al*., 2009). One of the most widely accepted functions of vitamin C is that it acts as an antioxidant by interrupting free-radical chain reactions in the (Yarube *et al*., 2009) and, thus, significantly decreases the levels of reactive oxygen species in the body (Dröge, 2002; Whitehead and Keller, 2003).

5.2.02 Serum Metabolites of Adult Rabbit Bucks

The significant increase in final serum albumin in male rabbits may be due to the fact that vitamins are essential parts of some of the enzymes or co-enzymes (Burger, 2004) and enzymes are important in food digestion, making micro nutrients to be available for growth and other body functions. The significant increase in final serum calcium in the treatment with $NaHCO_3$ may be due to the increase in body metabolism and excitation caused by the buffers, thereby increasing blood calcium (Burger, 2004). It should be recalled that bicarbonate buffers increased thyroxine levels in growing rabbits in this experiment, and thyroxine is known to increase metabolic activities of almost all the tissues of the body. The trend in serum glucose recorded in this experiment agrees with the reports of Ondruska *et al.* (2011). It was attributed to be due to increase in glucose utilization during muscular movements required for high respiratory activity (Habeeb *et al*., 1992; Okab et al., 2008), or due to increases in corticosteroid concentrations (Habeeb *et al*., 1997).

5.2.03 Thyroxine Levels in Adult Rabbit Bucks

The lower thyrosine levels recorded in the control reveals that the control group was heat-stressed and metabolic activities may have been lowered. This result agrees with the reports of Yahav *et al*. (1996) who reported reduced concentrations of thyroid hormones (triiodothyronine and thyroxine) in the blood plasma and a decreased metabolic rate of pullets and cocks reared under a high ambient temperature. The reduction in thyroxine levels after the experiment compared to the initial values can be attributed to two factors; acclimatization of the animals for the control and amelioration of heat stress by the buffers and the vitamin. The buffers especially $KCHO_3$, may have triggered the stimulation of the thyroid glands leading to the secretion of more

thyrosine compared to the control while the exogenous antioxidant vitamin sources (vitamin C and BFPM) may have reduced oxidative stress and improved body metabolism, leading to the increase in serum thyrosine. Vitamin C has been reported to be effective in the increase the reduction in thyroid functions (Coates, 1984). Vitamin C synthesized in rabbit liver has been demonstrated to protect the animal from heat stress and improve disease resistance in rabbits by optimizing the function of the immune system (Bain, 1996), but during stress vitamin C produced is rapidly consumed and amount synthesized fall below animal requirements. Supplemental vitamin C may, therefore reinstate normal metabolic functions during heat stress.

5.2.04 Testosterone Levels in Adult Rabbit Bucks

The final testosterone values of the control thatwere lower than the initial values may be attributed to heat stress, which may affect the Leydig cells. Jelodar and Zare (2008) reported a decrease in serum testosterone concentration of rats exposed to radiation from phones; and attributed the decrease to the effect of radiation on Leydig cells, pituitary or hypothalamus and alteration of gonadotropin secretion. It is also possible that the buffers, especially $KHO_{3,}$ also had a negative effect on the Leydig cells, responsible for the production of testosterone. Stress can also cause structural and pathological changes in the Leydig cells. Apoptosis associated with nuclear damage of the cells can lead to a decrease in testosterone and estrogen production (Hamada *et al*., 2011). It is also possible that the buffers interfered with the transfer of free cholesterol to mitochondria of Leydig cells, which is an important step in steroidogenesis, and also disrupted the conversion of cholesterol to testosterone by imparing the activity of key regulatory enzymes in steroidogenesis (Ebomoyi and Ahumibe, 2010). The significant improvement recorded in rabbits treated with vitamin C and BFPM could be the vitamins

counteracted free radicals that may limit normal functioning of the Leydig cells from functioning properly. Vitamin C has been reported to be an effective antioxidant.

5.2.05 Semen Characteristics of Adult Rabbit Bucks

The result of semen characteristics showed that vitamin C and BFPM improved semen volume, colour, motility and concentration compared to $KHCO_3$, Na_2CO_3 and the control. The low semen volume, motility and concentration observed in the control may be due to heat stress. This result of the present study is similar to the reports of Theau-Clement *et al.* (1995); Marai *et al.* (2002); Roca *et al.* (2005). Marai *et al.* (2002) attributed the increase in abnormal spermatozoa rate in the summer ambient conditions to defects of the spermatogenesis, particularly in the last stage of differentiation of spermatids. The changes in ejaculate volume may be due to low sperm concentration and a decrease in the volume of seminal plasma as a result of hypoactivity of the accessory glands and the testes, due to the adverse effect of high ambient temperature (Theau-Clement *et al.,* 1995). Motility and concentration are one of the most sensitive indicators of heat stress. The negative effects of heat stress on semen quality have also been reported in mice (Ren *et al*., 2006; Perez-Crespo *et al*., 2008) and boars (McNitt and First, 1970; Stone 1981; Malmgren and Larsson 1984). The reduction in progressive in rabbits, spermatozoa motility under high ambient temperature (Jannes *et al.,* 1998; Armstrong *et al.,* 1999; Roca *et al*., 2005) is related to the production of reactive oxygen species (Aitken *et al*., 1989). Vitamin C and BFPM (3.5%) increased ejaculate volume, motility and sperm concentration. This result agrees with the findings of Najjar *et al,* (2013).

5.2.06 Sperm Morphology of Adult Rabbit Bucks

Vitamin C and BFPM at 3.5% significantly increased the number of live cells and reduced the number of dead cells. This result is contrary to the findings of El-Masry *et al.* (1994), who did not find a positive effect of vitamin E (40 mg/kg) and selenium (0.7 mg/kg) on sperm motility and reproductive performance of bucks. Najjar *et al.* (2009) found that vitamin C and E did not improve semen qualities, attributed to the short duration (six weeks) of their study, whereas this study lasted for nine weeks. The present study is in agreement with the finding of Youssef *et al.* (2003) that vitamin C and E improved rabbit male fertility by increasing sperm concentration and total motile sperm but decrease abnormal and dead sperm after 12 weeks of its administration. Vitamin C has been reported to protect cells from oxidation of substrates such as proteins, fatty acids, and DNA (Pincemail *et al*., 1998). In fact, antioxidant supplementation in drinking water has been observed as an interesting application to improve sperm quality in rabbits (Mangiagalli *et al*., 2012). Both sodium bicarbonate buffer and potassium bicarbonate buffer increased the numbers of deformed sperm in this study, especially sodium bicarbonate buffer (Na_2CO_3) compared to the control. This finding agrees with the established fact that almost all biological processes are pH-dependent. Even a slight change in pH can result in metabolic acidosis or alkalosis, resulting in severe metabolic complications (Chandra, 2006) which may be responsible for the high numbers of deformed sperm cells recorded in this study.

5.2.07 Gonadal Morphometry of Adult Rabbit Bucks

The observed differences in testis length and weight recorded in this study may not have been affected by heat stress as the control treatment also recorded a significantly high weight of the right testis. Rather, the observed differences may be attributed to differences in breed and body

size of the animals. The animals were not of the same breed and weight, hence the observed differences in their gonadal morphometry. The influence of age, body weight and breed has been reported on testicular integrity (Obidi *et al,.* 2008). Secondly, the observed variation may also be influenced by genetic factors. The influences of these factors on testicular development in domestic animals have been well documented (Rekwot *et al*., 1987; Osinowo *et al*., 1981; Lustra *et al*., 1979). On the observed differences in length and weight of the right and left testis, the result was similar to the findings of Etches (1996) in Plymouth Rock breeder cocks, and those of Obidi *et al.* (2008) in Shikabrown breeder cocks. The report of Thurston and Korn (2000) in the turkey tom also supports the finding of the present study.

5.2.08 Epididymis Morphometry of Adult Rabbit Bucks

The values of weight and length of the epididymis observed in this study cannot be said to have been affected by heat stress as the control also recorded a higher weight and length of the epididymis. The trend in the values of epididymis is a reflection of the values that were recorded for the testis. Variation in the testis weight and length has been attributed be influenced by age and genetic factors (Osinowo *et al*., 1981). The influences of these factors on testicular development in domestic animals have been reported (Rekwot *et al*., 1987; Osinowo *et al*., 1981; Lustra *et al*., 1979). On the differences in weight and length of the caput, corpus and cauda epididymis, it is expected for the cauda epididymis to weigh more than the caput and corpus because the cauda epididymis is the reservoir for produced sperm. The longer length of the corpus epididymis compared to the cauda epididymis is because the corpus epididymis is the body and neck of the epididymis.

5.2.09 Gonadal and Epididymis Sperm Reserves of Adult Rabbit Bucks

The improvement noticed in the gonadal and epididymal sperm reserve in the treatments with BFPM and vitamin C compared to the control is an indication that the sperm concentration of the control was affected by heat stress. This could be explained by the degeneration of the germinal epithelium and to the partial atrophy of seminiferous tubules (Marai *et al.,* 2002). Increasing the temperature of the testes can prevent spermatogenesis by causing degeneration of most cells of the seminiferous tubules besides the spermatogonia (de Kretser, 2004). Marai *et al.* (2002) attributed the increase of abnormal sperm rate in the summer ambient conditions to defects of spermatogenesis, particularly in the last stage of differentiation of spermatids. The cauda epididymis sperm concentration obtained in this study in the treatment with vitamin C and BFPM range from 123.67 to 185.67x10^6/ml and were comparable to the range of 126.00 to 154.00 x10^6/ml reported by Ajayi *et al*. (2009). Oyeyemi and Okendran (2007) showed that high concentration of spermatozoa is an indication of possible high fertility rate by reason of the number of spermatozoa, available at the time of copulation or insemination. The improvement observed in the treatments with vitamin C and BFPM agrees with the report of Mangiagalli *et al*. (2012). Vitamin C and BFPM may reduce the negative effect of heat stress on these variables by reducing the damage caused by ROS or by sparing the effect of enzymes, involved in the antioxidant systems of the testicle. The importance of vitamins in protecting the sperm membrane against lipid peroxidation may be more important in late spermiogenesis, when sperm drop most of their cytoplasm, leading to lowered concentrations of cytoplasmic defensive enzymes (Aitken *et al*., 1989).

5.2.10 Serum Metabolite of Adult Rabbit Does

The general reduction in most of the serum metabolites during pregnancy compared to what was recorded before and after pregnancy agrees with the reports of Marai *et al*. (2007). During pregnancy, reductions in blood serum total proteins, globulin, glucose, cholesterol, alkaline phosphatase, acid phosphates and T3 hormone may be due to the decrease in food intake of dams (Marai *et al.,* 1994) and increase in water retention (Marai *et al.,* 1994), and the high demand of the foetus at late stages of pregnancy (Marai *et al.,* 1994). Particularly, the decrease in glucose in blood may be due to the increase in each glomerular filtration rate (GFR) (Marai *et al.,* 1994) and foetal consumption and conversion of glucose to lactose of milk (Marai *et al.,* 1994), and the decrease in each of renal threshold of glucose and capacity of renal tubules to absorb the glucose.

5.2.11 Thyroxine Levels in Adult Rabbit Does

The increase in thyroxine hormone level during pregnancy than before and after pregnancy may be due to the important role the hormone play in body metabolism, leading to increase in serum protein, fat and carbohydrate to fulfill the high demand of the foetus from glucose and other components. El-Masry and Habeeb (1989) reported that thyroxine is considered necessary for cellular metabolism of the mammary gland and energy utilization which could be considered as important factors in milk biosynthesis. The increase in thyroxine levels in the treatments with vitamin C and BFPM compared to other treatments could be that the vitamin sources (vitamin C and BFPM) may have reduced oxidative stress and improved body metabolism, leading to the increase in serum thyroxine. Vitamin C has been reported to be effective in the alleviation of retardation in thyroid functions (Coates, 1984).

5.2.12 Progesterone Levels in Adult Rabbit Does

The trend in progesterone levels obseved in this study during and after pregnancy cannot be attributed to heat stress but to the physiological status of the rabbits. Maria (2002) reported that the effect of heat stress on plasma progesterone and LH concentration is controversial. Some studies found that heat stress has no effect on progesterone levels (Wilson *et al*., 1998), although other studies have reported reduced (Wolfenson *et al*., 1995; Johnson *et al*., 1987) increased (Trout *et al*., 1998) or unchanged (Roth *et al*., 2001) concentration during summer in dairy cows. Increased progesterone levels during pregnancy by the treatments with vitamin C and BFPM may be attributed to the fact that vitamin C and BFPM stimulated the follicles to shed more ova, creating more CL sites, which were responsible for more progesterone secretion. The CL is a transient endocrine gland that secretes progesterone to support pregnancy in rabbits, the CL, is maintained throughout the gestation, a characteristic that differentiate the rabbits from other species (Theau-clement *et al*., 2000). The CL are formed from ovulated follicles in a process that involves angiogenesis and tissue remolding under the influence of several endothelial-derived factors, including vascular endothelial growth fact, transforming growth factor and fibroblast growth factor acting locally in a paracrine/autocrine manner (Theau-clement *et al*., 2000).

Two factors may have been responsible for the increase in progesterone after pregnancy in the control and the treatments with bi-carbonate buffers. For the control and $NaHCO_3$, at the time of collection of blood samples, only about 40% of the rabbits had kindled while the remaining 60% were yet to kindle and were in their last trimester of pregnancy this is in contrsast to the treatments with vitamin C and BFPM with about 90% of rabbits that had already kindled. For the treatment with KHO_3 buffer, the rabbits could have been pseudopregnant, because no rabbit in

this group kindled even after three consecutive mating trials. Progesterone is believed to be high during pseudo pregnancy and reduces at the end of pseudopregnancy (Theau-clement *et al*., 2000). The high progesterone level during pseudo pregnancy may arise as a result of the alteration in dominant follicles, failure of implantation and early embryonic death. It is expected that when this happens, there will be active sites of CL hence more progesterone secreted before luteolysis.

5.2.13 Reproductive Performance of Adult Rabbit Does

It was found in this study that vitamin C and BFPM improved litter size at birth, weight of litter and litter weight at weaning compared to the treatments with buffers ($KHCO_3$ and Na_2CO_3). The values of litter size at birth, weight of litter and litter weight at weaning are similar to the values recorded by Azza, (2015) when vitamin C was used to improve reproductive performance of female rabbits raised during hot environmental conditions. The effect of vitamin C may be due to reducing effects of stress, increasing the immunological traits (Coates, 1984). Improvement in the reproductive performance of doe rabbits, supplemented with ascorbic acid may be due to the prevention of oxidation break-down of cell membranes associated with the hydroperoides of polyunsaturated fatty acid (Hughes, 1999). Ismail *et al.* (1992) and Gad-Alla *et al.* (2002) obtained the same results on litter size and Afifi and Makled (1995) on gestation period, litter size, litter weight and mortality rate. The zero conception recoreded in the treatment with $KHCO_3$ may be due to the fact that the buffer may have made the medium in the oviduct to be acidic, making the semen not to be motile. The vaginal secretions of the female are acidic (pH of 3.5 to 4.0). Sperm do not become optimally motile until the pH of the surrounding fluids rises to about 6.0 to 6.5 (Smith *et al.,* 2003). Consequently, it is probable that the slightly alkaline

prostatic fluid helps to neutralize the acidity of the other seminal fluids during ejaculation, and thus enhances the motility and fertility of the sperm (Guyton and Hall, 2006) or early embryonic abortion may have occurred. During heat period, semen tends to be slightly acidic (6.0 – 6.7). The buffer may have made vaginal secretions and medium of the female to be more acidic (<3.5) due to acidosis, hence, leading to infertility. Almost all biological processes are pH-dependent. Even a slight change in pH can induce metabolic acidosis or alkalosis, resulting in severe physiological complications (Chandra, 2006).

Experimental Period for Year 2

5.3 Effect of graded levels of baobab fruit pulp meal on growth performance of rabbits under tropical environment

5.3.01 Temperature Humidity Index inside the Rabbit house

The THI for year 2 was similar to the THI for year 1. THI of 27 in the year was similar to 27.5 recorded for the month of February in year 1. The values indicated that the month of February had absence of heat stress in the rabbit house. On the average, year 2 was hotter than year 1. The THI values of 29.4 (March), 30 (April), 31.5 (May) and 28.49 (June) indicated that the rabbit house was thermally severely stressful and very severely stressful (Marai *et al.*, 2001) in these months. The averaged THI of 29.85 during the experimental period showed that the rabbit house was thermally stressful and may exert adverse effects on the rabbits (Marai, 2001). Overall, data obtained indicated that THI in the afternoon was higher by 1.45 % than THI in the morning.

5.3.02 Thermoregulatory Response of Growing Rabbits

It was found that BFPM was effective in reducing rectal and ear temperatures, and more effective as the quantity increased in the diet. This could be because BFPM contained high amount of vitamin C. BFPM has been reported to lower rectal temperature of birds than the

value obtained with the control diet (Adeosun, 2012). This could be due to the high content of vitamin C in baobab is reported to contain (Agbessi Dos-Santos, 1987), which may enhance its antioxidant properties that scavenges for free radicals, as vitamin C does. This result is similar to the findings of Ayo *et al.* (2011), who reported a reduced rectal temperature for transported rabbits ameliorated with vitamin C. Vitamin C protects the cells from the damage caused by reactive oxygen species and mutagens (Yarube *et al*., 2009). One of the most widely accepted functions of vitamin C is that it acts as an antioxidant by interrupting free-radical chain reactions in the body (Powers and Jackson, 2008) and, thus, significantly decreases the levels of reactive oxygen species in the body (Dröge, 2002; Whitehead and Keller, 2003).

5.3.03 Growth Performance of Growing Rabbits

It was observed that feed intake increased as the levels of BFPM in the diet increased. It is possible that BFPM improved the palatability of the diets thereby stimulating more feed intake. Weight gain increased up to 3.5% and declined thereafter. At this point exogenous substance may have affected digestion. It is also possible that at 4.5% and 5.5%, BFPM may dilute other nutrients that would have been responsible for weight gain. This result did not support the reports of Oladunjoye *et al.* (2014), who did not record any significant effect in the final weight, total weight gain, average daily gain, feed intake, feed conversion ratio and mortality of the rabbits fed graded levels of baobab fruit pulp and seed meal. This result agrees with the findings of Adeosun (2012) who reported that increasing the levels of BFPM beyond 3.5% resulted in depressed growth of birds during the cool-wet season. The higher levels of BFPM supplementation (7.0 and 10.5%) resulted in poorer growth of broilers in the finisher phase during the cool-wet season in her experiment.

5.3.04 Serum Metabolite of Growing Rabbits

The increase in final serum metabolites in the treatments with 3.5 - 5.5% BFPM may be due to the fact that vitamin C in BFPM may have suppressed the effect of heat stress on the appetite centre in the hypothalamus leading to increased feed intake. This phenomenon can lead to increased serum glucose, albumin and triglyceride as was observed in this study. The pattern in feed intake might have reflected in the values of serum metabolites recorded in this study. Vitamins are known to be essential parts of some of the enzymes or coenzymes (Guyton and Hall, 2006) and enzymes are known to be important in food digestion making micro-nutrients to be available for body functionings.

5.3.05 Thyroxine Levels in Growing Rabbits

The trend in thyroxine secretion in this study was a reflection of serum glucose secretion. The treatment with 2.5% BFPM recorded the highest differences in initial and final thyrosine (61.27g/mg) for initial and 66.10g/mg for final), recorded a low serum glucose, which may be attributed to high body metabolism and high demand for glucose. High body metabolism is a function of high thyroxine secretion. This result agrees with the fact that thyroid hormones increase the metabolic activities of almost all the tissues of the body. The basal metabolic rate can increase from 60 to 100 percent above normal when large quantities of the hormones are secreted. The rate of utilization of energy is greatly accelerated (El-Masry and Habeeb1989). This could also so be responsible for the drop in thyroxine in the treatment with 5.5% BFPM. The glucose in this treatment was high because metabolic process was slowed down, less glucose was utilized because thyrosine level was reduced. This could be the reason for the lower growth performance in this treatment.

Heat stress may have affected the thyroxine level in the control. This result agrees with the reports of Yahav *et al*. (1996), who found that both pullets and cocks reared under high ambient temperature had reduced concentrations of thyroid hormones (triiodothyronine and thyroxine) in the blood plasma and a decreased metabolic rate.

5.4 Reproductive Performance Trials

5.4.01 Thermoregulatory Response of Adult Rabbit Bucks

The treatments with 4.5% and 5.5% inclusion levels of BFPM had better improvements in all the parameters compared to other treatments. Respiratory rate was reduced from 159.63 (cpm) recorded in the control to 137.63 and 137.78 in 4.5% and 5.5% BFPM in the diet. Heart rate was also reduced from 154.60 (cpm) in the control to 137.63 (cpm) in 5.5% BFPM in the diet, respectively. It could be because BFPM beyond 3.5% reduced the adipose tissue and body weight as was obsreved in the growth performance of growing rabbits, thereby reducing heat load. At these levels of BFPM (4.5 and 5.5%), it is possible that the adequate quantity of vitamin C was attained that was able to counteract the reactive oxygen that may have been present in the system of the rabbits (Yarube *et al*., 2009).

5.4.02 Serum Metabolite of Adult Rabbit Bucks

The reduction in serum glucose, total protein, albumin, triglyceride and calcium in the control group at the end of the experiment compared to the initial values may be attributed to heat stress. This result disagrees with the findings of Okab *et al.* (2008), who found that the metabolites

increased during summer, compared to the values recorded during spring period. The report was similar to the findings of Ondruska *et al.* (2011) who reported a decline in plasma TP with rising temperature which was attributed to dilution of plasma TP caused by the increased water consumption (Okab *et al*., 2008), and/or increases in protein utilization and amino acid transamination in the heat-stressed rabbits (Habeeb *et al*., 1993). Decreased glucose levels in the heat-stressed adult rabbits have been reported (Ondruska *et al.* 2011), attributed to increase in glucose utilization during muscular movements required for high respiratory activity (Habeeb *et al*., 1992; Okab *et al*., 2008), or due to increases in corticosteroid concentrations (Habeeb *et al*., 1997). Plasma cholesterol concentrations have been reported to decrease due to increases in total body water, resulting from exposure to elevated temperature (Marai *et al*., 1995; Habeeb *et al*., 1996). The increase in glucose, total protein, albumin, cholesterol, triglyceride, calcium and phosphorous that was later observed in the treatments with 4.5% and 5.5% BFPM could be because baobab pulp ameliorated heat stress to reduce both heart and respiratory rates which are energy-demanding thereby reserving some of the metabolites.

5.4.03 Thyroxine Levels in Adult Rabbit Bucks

It was discovered that thyroxine level was low at the commencement of the experiment, but increased at the end of the experiment with 2.5% BFPM recording the highest value. The result agrees with the findings that heat stress reduces thyroxine levels in rabbits (Marai *et al.*, 2007). The reduction in thyroxine before the commencement of the experiment and in the control at the

end of the experiment agrees with the findings obtained on serum metabolites in this study; where most of the metabolites decreased before the commencement of the experiment and in the control at the end of the experiment. Decrease in thyroxine secretion has been reported to reduce energy metabolism (gluconeogenesis and glycogenolysis) during heat exposure (Herbein *et al*., 1985; Habeeb *et al*., 1996).

5.4.04 Testosterone Levels in Adult Rabbit Bucks

The significant increase recorded in the treatments with BFPM could be that the vitamin in BFPM counteracted free radicals that may impared the Leydig cell functioning and also suppress heat stress. Vitamin C has been reported to be an effective antioxidant. Heat stress causes the degeneration of the germinal epithelium and partial atrophy of seminiferous tubules (Marai *et al.,* 2002). Increasing the temperature of the testes can prevent spermatogenesis; reduce testosterone secretion by causing degeneration of most cells like the leydig cells and cells of the seminiferous tubules besides the spermatogonia (de Kretser 2004). BFPM may have reduced the negative effect of heat stress on these variables because of it high vitamin C content by reducing the damage caused by reactive oxygen species or by sparing the effect of enzymes involved in the antioxidant systems of the testes. It was more effective with increasing levels of BFPM in the diet.

5.4.05 Semen Quality Characteristics of Adult Rabbit Bucks

Semen quality in the control group was poor; this could be attributed to heat stress and could be explained by the degeneration of the germinal epithelium, and to the partial atrophy of seminiferous tubules (Marai *et al.,* 2002). Theau-Clement *et al.* (1995); Marai *et al.* (2002) and

Roca *et al.* (2005) have attributed the increased abnormal sperms rate in the summer ambient conditions to defects of spermatogenesis, particularly in the last stage of differentiation of spermatids. The changes in ejaculate volume may be due to low sperm concentration and a decrease in the volume of seminal plasma as a result of hypoactivity of the accessory glands and the testes due to the adverse effect of high ambient temperature (Roca *et al.,* 2005). Motility and concentration are some of the most sensitive indicators of heat stress. This negative effects on semen quality have also been reported for mice (Ren *et al.,* 2006); Perez-Crespo *et al*., 2008) and boars (McNih and First, 1970; Stone 1981; Malmgren and Larsson 1984). BFPM was proven to be effective in improving semen characteristics up to 4.5% inclusion level in this study; this could be due to the antioxidant properties of BFPM. Castellini *et al.* (1999; 2003); Castellini (2008), reported that the administration of antioxidants such as vitamin E, selenium, vitamin C, and carotenoids may reduce oxidative stress and improve sperm motility. The role of antioxidants is to counteract the spermatic cell membrane lipid peroxidation and sperm DNA fragmentation, caused by reactive oxygen species and responsible for male infertility in animals and man (Aitken *et al.,* 1989; Baker *et al.,* 1996; Greco *et al.,* 2005).

5.4.06 Sperm Morphology of Adult Rabbit Bucks

The treatment with 4.5% BFPM increased the numbers of live cells (74.38%) reduced dead cells (25.62%), and increased number of normal cells (77.71%), compared to 65.67%, 34.33%, and

60.67% recorded by the control for live cells, dead cells and normal cells, respectively. This result is similar to the findings of Youssef *et al.* (2003) that vitamin C and E improve rabbit male fertility by increasing sperm concentration and total motile sperm, but decreasing abnormal and dead sperm after 12 weeks. Vitamin C has been reported to protect cells from oxidation of substrates such as proteins, fatty acids and DNA (Pincemail *et al*., 1998). Antioxidant supplementation in drinking water has been observed to improve sperm quality in rabbits (Mangiagalli *et al*., 2012).

5.4.07 Gonadal Morphometry of Adult Rabbit Bucks

It was also observed that the right testis was generally bigger than that of the left testis across the treatments. This result disagrees with the finding of Etches (1996) in Plymouth Rock breeder cocks, and Obidi *et al.* (2008) in Shikabrown breeder cocks, and the reports of Thurston and Korn (2000) in the turkey tom. The reduction in testis weight in the control group may be attributed to heat stress. The effect of heat stress on scrotal temperature has been reported to be a decrease in testis weight (Finzi *et al.,* 2000), and the testis can remain reduced if there is severe heat stress (Habeeb *et al.,* 1993). The effect has been reported for mice (Habeeb *et al.,* 1993), Jannes *et al.,* 1998), rats and rabbits (Hamada *et al.,* 2011). This reduction suggests degeneration in the germinal epithelium and partial atrophy in the seminiferous tubules (El-Shery *et al*., 1980)

The improvement in gondal morphomery in the treatments with BFPM suggests that the content of vitamin C content in BFPM may be involved in the reduction of the negative effects of heat stress on the variables. This reduction may decrease the damage caused by reactive species of oxygen, or by sparing the effects of enzymes, involved in the antioxidant systems of the testicle.

5.4.08 Epididymis Morphometry of Adult Rabbit Bucks

The trend in epididymis morphometry is a reflection of the values for gonadal morphometry of rabbits fed graded levels of BFPM in this study. It was observed that the values of gonadal weight and length of the right testis were higher than those of the left. This findings was the same trend for epididymal morphometry. This finding disagrees with the reports of Etches (1996), Thurston and Korn (2000) and Obidi *et al,* (2008). Heat stress may be responsible for the reduction in weight and length of the epidimis. El-Shery *et al.* (1980) attribute the reduction in goadal and epidiymal weight to heat stress caused by the degeneration in the germinal epithelium and partial atrophy in the seminiferous tubules. The improvements in epididymal morphomery in the treatments with BFPM suggest that the vitamin C content in BFPM may have reduced the negative effect of heat stress on these variables. This reduction apparently results in decrease in the damage caused by reactive species of oxygen or by sparing the effect of enzymes, involved in the antioxidant systems of the testicle.

5.4.09 Gonadal and Epididymis Sperm Reserve of Adult Rabbit Bucks

The epididymis sperm concentration did not follow any pattern for the treatments with BFPM; rarher their values were better than the control. The reduction in sperm reserve of the control may be attributed to heat stress. Increasing the temperature of the testes can prevent spermatogenesis by causing degeneration of most cells of the seminiferous tubules besides the spermatogonia (de Kretser 2004). Marai *et al.* (1991) attributed the increase in the abnormal sperms rate in the summer ambient conditions to defects in the spermatogenesis, particularly in the last stage of differentiation of spermatids. BFPM might have reduced heat stress and improved sperm reserve because of its vitamin C content, which protect cells from oxidation of

substrates such as proteins, fatty acids, and DNA (Pincemail *et al*., 1998). Supplementation of vitamin C and other antioxidant in drinking water improved sperm quality in rabbits (Mangiagalli *et al*., 2012).

5.4.10 Serum Metabolites of Adult Rabbit Does

It was revealed that values of metabolites recorded before pregnancy were generally low and were similar to those recorded in the control rabbits throughout the experiment. The low serum metabolites before pregnancy were similar to the findings of Marai *et al.* (2007). The decrease in glucose in blood is particularly due to the increase in Glomerular filtration rate (GFR) (Guyton, 1992), foetal consumption and conversion of glucose to lactose of milk (Marai *et al.*, 1994) The decrease in each of the renal thresholds of glucose, and capacity of renal tubules to absorb the glucose. The decrease in cholesterol level may be due to the decrease in protein synthesis (lipid is transported as lipoprotein).

5.4.11Thyroxine Levels in Adult Rabbit Does Before, During and After Pregnancy

It was observed that thyroxine level increased as the levels of the BFPM in the diets increased. The trend in thyroxine was a reflection of the serum metabolite of this research. This finding may be due to the important role the hormone plays in body metabolism leading to increase in serum protein, fat and carbohydrate to fulfill the high demand of the foetus in glucose and other components. El-Masry and Habeeb (1989) reported that thyroxine is necessary for cellular metabolism of the mammary gland and energy utilization, which could be considered as important factors in milk biosynthesis. Higher levels of BFPM (4.5 and 5.5%) increased thyroxine levels in this study, and this agrees with the reports of Coates (1984), who reported that vitamin C and other antioxidant have the ability to reduce oxidative stress and improve body

metabolism. They also alleviate retardation in thyroid functions, leading to the increase in serum thyroxine.

5.4.12 Progesterone Levels in Adult Rabbit Does Before, During and After Pregnancy

The trend in progesterone secretion in this study, which shows low progesterone in the control, compared to the treatments with BFPM, could be as an indirect effect of heat stress. Heat stress may reduce the number of mature follicles. Maria (2002) reported that the effect of heat stress on plasma progesterone and LH concentration is controversial. BFPM diets may stimulate the follicles to shed more ova, creating more corpus lutuem sites which were responsible for more progesterone secreted. The CL is a transient endocrine gland that secretes progesterone to support pregnancy in rabbits. The CL is maintained throughout the gestation, a characteristic that differentiate the rabbits from other species (Theau-clement *et al*., 2000).

5.4.13 Reproductive Performance of Adult Rabbit Does

The BFPM significantly improved litter size and weight of litter at 3.5 and 4.5% inclusion levels respectively. Weight of kit was shown to increase in 2.5 and 4.5% BFPM. The reason for the increase in weight, recorded in treatments with 2.5 and 4.5% BFPM diets could be because of their small litter size. The poor performance observed in the control and 2.5% BFPM agrees with the findings of Marai *et al.* (2001) who reported low conception rate during the hot conditions. Lebas *et al.* (1986) clarified that the lower prolificacy of does, reared in hot climates (30-31^{o}C) would appear to be due to a reduction in body weight, and not so much to the temperature itself. Particularly, such phenomena may be due to the marked decline in post-coital ovulation (Marai *et al.* 2001) ovulation rate (Lebas *et al.* 1986), number of implantation sites per doe, and number of viable embryos per doe (Abdel – Monem, 2001). The low conception rate may also be a result

of either fertilization failure or early embryonic mortality (Abdel – Monem, 2001). The decrease in receptivity and percentage of voluntary mating may be another reason for decline in fertility during hot conditions. Thus 3.5 to 5.5% BFPM improved rabbit's doe performance. Similar results were obtained by Abdel – Monem (2001) who found that feeding doe rabbits on diets containing vitamin C improves feed intake, litter size at birth, 21 days and weaning, litter weight at birth, 21 days and weaning and milk yield. The effect of ascorbic acid may be due to reducing effects on stress, increasing the immunological traits (Coates, 1984).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Four experiments were conducted with growing and adult rabbits. The first experiment was conducted to evaluate the ameliorative effect of bi-carbonate buffer, vitamin C and BFPM on growth performance of rabbits under tropical environment. It was observed that vitamin C and BFPM had superior results on thermoregulatory parameters, growth performance serum metabolites and thyroxine hormone levels. Experiment two was conducted to evaluate the ameliorative effect of bi-carbonate buffer, vitamin C and BFPM on reproductive performance of rabbits under tropical environment. It was observed that vitamin C and BFPM performed better in thermoregulatory parameters, serum metabolites, hormones evaluated (thyroxine, testosterone and progesterone), semen characteristics, and reproductive performance of the does. BFPM was superior to vitamin C in most of the parameters. Experiment three was conducted to evaluate the ameliorative effect of graded levels of BFPM on growth performance of rabbits under tropical environment and it was revealed that 3.5 - 5.5% had superior performance on thermoregulatory parameters, 4.5% for growth performance and 3.5 – 5.5% for serum metabolites and thyroxine hormone levels. Experiment four was conducted to evaluate the ameliorative effect of graded levels of baobab fruit pulp meal (BFPM) on reproductive performance of rabbits under tropical environment. It was also observed that 3.5% - 5.5% BFPM performed better in thermoregulatory parameters, serum metabolites, hormones evaluated (thyrosine, testosterone and progesterone), semen characteristics, and reproductive performance of the does.

6.2 Conclusion

From the results obtained from this study, the following conclusions on the use of bicarbonate buffer, vitamin C and BFPM in ameliorating heat stress in rabbits under tropical environment were made.

i. Vitamin C and BFPM reduced thermoregulatory parameters, the buffers increased heart rate of the rabbits

ii. Sodium bi-carbonate buffer, vitamin C and BFPM were effective on the growth performance of growing rabbits.

iii. Vitamin C and BFPM improved most of the serum metabolites especially glucose, total protein, albumin and phosphorous; increased testosterone and progesterone levels, while the buffers increased serum thyrosine for growing rabbits.

iv. BFPM performed better than the rest of the treatments in improvement of semen and sperm quality characteristic, compared to the rest of the treatments. Vitamin C was similar to BFPM in performance.

v. On reproductive performance of the female rabbits, BFPM and vitamin C also improved reproductive performance by increasing birth weight and weaning weight. The buffers were not effective in reproduction as rabbits on potassium bi-carbonate buffer did not conceive at all.

vi. The treatments with 3.5 and 4.5% BFPM improved feed intake and weight gain of growing rabbits, 5.5% BFPM only improved and recorded the highest feed intake but poor weight gain compared to 3.5 and 4.5% BFPM. The treatments with 2.5% performed poorly as the control.

vii. 3.5 - 5.5% BFPM improved thermoregulations, serum metabolites, hormones, semen and sperm quality, and reproductive performance of the does.

6.3 Recommendations

The following recommendations were made for further study to enhance the utilisation of bi-carbonate buffer, vitamin C and baobab fruit pulp meal in ameliorating heat stress in rabbit production under tropical environment.

i. Buffers are effective for rabbit growth, but should not be used for rabbit reproduction as buffers reduced fertility in rabbits in this study.

ii. A trial involving buffer and vitamin E on reproductive performance is recommended.

iii. BFPM should be included in all categories of rabbit meal especially during hot periods of the year for optimum production.

iv. The use of BFPM should be encouraged among farmers rather than vitamin C because it is cost effective, readily available in the study area, biologically safe as an organic source of vitamin C and can easily be identified by the farmers.

v. The use of BFPM should not exceed 4.5% for growth and 5.5% for reproduction because results obtained revealed that beyond these levels, growth and reproductive performance may be affected negatively.

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