ISSN 0189-0514
J. Anim. Prod. Res. (2016) 28(2):61-65

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EFFECT OF DIETARY PROTEIN, SELENIUM AND TEMPERATURE HUMIDITY INDEX ON REPRODUCTIVE TRAITS OF MALE RABBITS IN A TROPICAL ENVIRONMENT

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ABSTRACT

The study aimed to evaluate the effects of dietary protein and selenium supplementation and temperature humidity index (THI) on male rabbit reproductive traits. Fourty eight male New Zealand White rabbits (23 ± 1.4 weeks of age) weighing 2.8 ± 1.13kg, were randomly allocated to one of six isocaloric diets (n = 8 animals/ treatment) that differed in either protein content (14g/100g, 18g/100g and 22g/100g) or selenium content (0.4 and 0.7 mg Se/ kg diet). The experimental design was 3 x 2 factorial. The study ran from April 2012 to September 2012. Significant (P= 0.001) differences observed in semen pH levels (R^2 = 0.80, P= 0.010), reaction time [(libido)(R^2= 0.85, P= 0.006)] and the proportion of abnormal sperm (R^2= 0.44, P= 0.089) as time advanced suggesting positive relation with the changes in environmental THI. There were significant (P= 0.001) interactions between dietary protein level and Se on testis characteristics which seem to improve in Se supplemented group as dietary protein increased. The levels of THI experienced in this study were not sufficiently high enough to result in more pronounced responses on reproductive performance. Therefore there were no adverse effects on the rabbits reproductive traits fed dietary supplements in the tropics.

***Keywords*:** Dietary protein; dietary selenium; environmental temperature; male rabbits; reproductive traits.

INTRODUCTION

Rabbit production has enormous potential in alleviating the problem of animal protein supply in developing countries (Oseni, 2012) and some peri-urban parts of developed nations (Attia *et al*., 2011). Currently, rabbits are considered as a novel production animal, hence its potential and important contribution to future animal protein supply to human should not be underestimated (Attia and Kamal, 2012; Boland et al., 2013). In addition, low levels of technical and management skills in rabbit production, climatic factors, availability and quality of forage are constraints in developing countries that are mostly located in hot climatic regions (NRC, 1991, Lukefahr, 2007, Oseni, 2012).

The increase in environmental temperatures coupled with the non specified nutrient supply or rather requirement to male breeder rabbits has the potential to challenge the productive potential of rabbit farming, not only in tropical regions but also temperate areas. In tropical and subtropical areas, heat stress appears to be the major constraint to livestock production (Marai *et al*., 2007; Ganaie *et al*., 2013), heat stress has the capability to adversely affect reproductive performance of rabbits and in extreme instances can result in animal death. A number of researchers have reported deterioration in semen quality as a result of prolonged or

intense heat stress (Roca *et al*., 2005, Marai *et al*., 2008). Hafez and Hafez (2000) reported that semen quality was reduced in bulls, rams and boars exposed to high environmental temperatures (at or above 35^{o}C). In fact, detrimental effects on reproductive traits have been reported at ambient temperature above 25^{o}C (El- Raffa, 2004; Safaa *et al*., 2008). Marai *et al*. (2008) stated the upper tolerable temperature for spermatogenesis is 30^{o}C, above which damage to the germinal epithelium and sperm cells in the epididymis could occur.

Changes in seminal characteristics of bull was observed within two weeks of exposure to heat stress (41^{o}C) (Hansen, 2009), although Talebi *et al*. (2009) did not observe any adverse effects of the high ambient temperature (33 ^{o}C) on semen characteristics of goats, however noted increases in semen volume, whereas others reported reduced semen volume during the same period (Safaa *et al*., 2008; Ain-Baziz *et al*., 2012). However, Okab (2007) did not observe any difference in semen volume between spring (27 ^{o}C) and summer (32 ^{o}C) but noted a reduction in sperm concentration. Summer heat stress (36 ^{o}C) partially affected semen quality of rabbit bucks but within fertile limit (El- Tohamy et al., 2013).

Nutrition modulates growth performance and reproductive functions in animals. It influences testicular growth, sperm production capacity, fertility and libido, as well as testosterone production (Elmaz *et al*., 2007). Incorporation of macro and micronutrients into feeds has positive effects on the performance of livestock. Under nutritional stress or rather inadequate nutrient supply, protein supplementation decreases the age at puberty of bulls and improves subsequent semen quality (Tegegne *et al*., 1992, Rekwot et al., 1987). It was indicated that group of rabbits fed 14% dietary protein levels had reduced testicular size with decline in sperm production (Ladokun et al., 2006). However, it has been shown that feeding diets with an excessive amount of protein can have deleterious effects on semen characteristics (Elmaz *et al*., 2007), and testicular tissues. Thus there seem to be inconsistency in crude protein requirement in rabbit diets.

Selenium (Se) enhances the productivity of animals and antioxidant status, particularly in hot summer environments (Marai *et al*., 2009; Mahima *et al*., 2013). In mammals, Se is essential for spermatogenesis and its deficiency results in impaired sperm motility and morphological abnormalities in rodents (Kehr *et al*., 2009). Therefore, the addition of supplementary protein (dietary protein concentrations) and/or Se supplementation at appropriate levels also provides thermo-protection to animals under high temperature conditions, which may subsequently enhance reproductive performance. Protein supplements compensate the low feed intake under high ambient temperatures, but the optimum level for male rabbits in the tropics has not been established. The objective of this study was to determine the effects of dietary protein concentrations and/ or Se supplementation on reproductive traits of male rabbits kept under tropical climates.

MATERIALS AND METHODS

The study was carried out at the rabbit research farm of the National Animal Production Research Institute (NAPRI) Shika, Zaria, Nigeria. The area is located in the northern Guinea Savannah ecological zone (latitude 10^{0} 11'N, longitude 7^{0}8'E; 650m above sea level) which has an annual rainfall of 1100 mm, which falls from April to October. The duration of the experiment was six months (April to September). Meteorological data (ambient temperature and relative humidity) was recorded throughout the experimental period using a digital thermo-hygrometer (Mextech TM-1, China) (Table 1).Temperature-humidity index (THI) was considered as an explanatory factor which changes over time and was computed using

the standard formula (Marai *et al*., 2002): THI = t - {(0.31 - 0.31RH)(t - 14.4)}where t is the temperature (^{o}C) in Celsius and RH is the relative humidity (%) .

Table 1: Average ± SD air temperatures and relative humidities of the study area

Months (d)	Temperature (^{o}C)	Relative humidity (%)	Temperature humidity index (THI)
April	32.6 ± 2.05	47.1 ± 9.24	30.0 ± 5.64
May	27.8 ± 2.09	69.3 ± 9.03	26.5 ± 5.56
June	26.0 ± 1.36	80.3 ± 2.47	25.3 ± 1.91
July	25.7 ± 1.17	86.0 ± 2.30	25.2 ± 1.73
August	25.3 ± 1.25	86.0 ± 3.43	25.0 ± 2.34
September	27.4 ± 1.80	81.8 ± 5.45	27.0 ± 3.63

Experimental design and diets

Experimental design was completely randomized and factorial arrangement 3 x 2, with three dietary protein levels (140, 180, and 220 mg/kg) and 2 Se levels (0.4 and 0.7 mg/kg). Treatments consisted of six isocaloric diets formulated according to NRC (1977) (Table 2) and these diets constitute three with background Se (0.4mg/kgDM) and the other three with additional Se (0.3mg/kgDM). The proximate analyses (Table 3) of the diets were done according to AOAC (1990) methods. The supplementary Se was in the organic form (selenomethionine).

Table 2: Ingredient composition of the experimental diet

	Protein levels (g/kg)		
Ingredients(g/kg)	**140**	**180**	**220**
Maize	600.00	490.00	370.00
Soybean meal	100.00	170.00	250.00
Wheat offal	205.00	210.00	225.00
Groundnut cake	45.00	80.00	105.00
Limestone	15.00	15.00	15.00
Bone meal	20.00	20.00	20.00
Salt	5.00	5.00	5.00
Vit-min premix*	5.00	5.00	5.00
DL-Methionine	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00

*Each kilogram of vitamins mineral premix contains: Vit. A 4000000 iu, Vit. D3 800,000 iu, Vit. E 9200 mg, Vit. K3 800 mg,Vit. B1 720 mg, Vit. B2 2000 mg, Niacin 11 mg, Pantothenic acid 3000 mg, Vit. B6 1200 mg,Vit. B12 6 mg, Folic Acid 300 mg, Biotin H2 24 mg, Choline chloride 120,000 mg, Cobalt 80 mg, Copper 1200 mg, Iodine 400 mg, Iron 800 mg, Manganese 16000 mg, Selenium 80 mg, Zinc 1200 mg, Antioxidant 500 mg.

Additional Se inclusion (0.3 mg/kg DM) was determined by experimental treatment, in which three were supplemented and the other three unsupplemented, but contain only background/basic Se(0.4mg/kg DM).

Table 3: Chemical Analysis of the Experimental Diets (means ± SD)

	Dietary treatment		
Parameters (g/kg)	140g/kg	180g/kg	220g/kg
Dry matter	948.7 ± 0.10	926.6 ± 1.19	933.6 ± 1.28
Crude protein	151.2 ± 0.55	191.1 ± 0.31	230.6 ± 0.15
Crude fibre	121.3 ± 1.63	152.0 ± 0.88	123.9 ± 0.84
Ether extract	21.1 ± 0.25	23.9 ± 0.48	25.9 ± 0.18
Ash	65.6 ± 0.25	61.9 ± 0.56	65.1 ± 0.71
**NFE	640.8 ± 1.47	571.1 ± 1.21	554.5 ± 1.23
*Energy ME(Mj/kg)	12.6	12.3	12.7

*Calculated using Pauzenga method (1985)
**Nitrogen Free Extract

Experimental Animals and Management

Forty eight mature New Zealand White rabbit bucks, mean age 23±1.4 weeks and mean weight 2.8±1.13 kg were used. Before the onset of the experimental period, animals were quarantined, physically examined (eye and coat condition, and animal conformation), and all necessary medications and vaccinations were administered. Animals were housed individually in conventional rabbit cages with floor dimension of 1.2m x 0.8m, each equipped with feeder and drinker in a naturally well ventilated building.

Rabbits were blocked according to their initial live-weight and randomly allocated to one of the six treatments. Pelletized feeds and forage (*Brachiaria brisantha*) were offered *ad libitum* throughout the experimental period. Daily feed intake of individual rabbits was recorded throughout the study.

Semen collection (ejaculate sampling)

Semen samples were collected from individual bucks using a plastic cone artificial vagina (AV) designed and constructed for rabbit bucks. Semen was collected on a monthly basis throughout the experimental period. To simulate a natural vagina the assembled AV was dipped into a beaker of warm water (40-42^{o}C) for 10-15 minutes after which it was cleaned and dried. The inner sleeve was lubricated with glycerol. A teaser doe was introduced to the buck's pen at the time of collection, as the buck mounted the teaser, the AV was introduced and the ejaculate collected. Semen was collected into a calibrated tube attached to the AV. Rabbit libido was evaluated by determining the buck's reaction time. Reaction time was defined as the duration between introduction of the teaser doe and ejaculation time following copulation.

Semen evaluation

Semen was evaluated as described by Hafez and Hafez (2000). Semen colour was evaluated visually by observing the appearance of the ejaculate contained within the graduated tubes.

Reddish (presence of blood) or yellowish/brownish (presence of urine) semen sample was considered to be contaminated and discarded. The ejaculate volume was determined by reading the volume directly from the calibrated collecting tube and the gel free ejaculate volume recorded.

Progressive sperm motility w*a*s determined immediately following semen collection by the method of Hafez (1985). Semen sample was diluted at 1/20 with normal saline/sodium citrate solution; a drop of semen was placed on a glass slide and the diluent added drop-wise as required. A cover slip was put on the smear and observed under x100 objective lens using an Olympus CX21 microscope (model: CX21FS1, Olympus Corporation, Tokyo, Japan). The number of motile sperm cells with forward progression versus immotile were noted. Ejaculate pH was determined immediately following collection using pH paper (Spezial-Indikatorpapier pH 5.5 - 9.0, Macherey-Nagel, Germany).

Sperm cell concentration, defined as the number of spermatozoa in one ml of ejaculate, was determined in freshly collected semen. A 10- fold dilution was made by mixing one drop of semen to 9 drops of spermicide (formal saline). The concentration of spermatozoa in semen was determined by haemocytometric counts using a Neubauer haemocytometer (Neubauer Improved – Marinfeld, Germany). Sperm concentration was estimated using the standard formula of Hafez (1985) C = N x D x 50,000, where C = concentration of spermatozoa per ml ($x10^6$/ml). N - number of spermatozoa counted and D - dilution factor = 20.

Percentage live/dead spermatozoa were determined using eosin nigrosin stained smears. Semen smears were prepared using one drop of eosin stain on a clean glass slide which was then dried at room temperature. The slide was examined at x400 magnification, using an Olympus CX21 microscope, (Olympus Corporation, Tokyo, Japan). At least 100 cells were counted and the percentage calculated.

Sperm morphological abnormalities were evaluated using the stained semen smears. At least 200 spermatozoa were manually counted on each slide and examined at x400 magnification, using an Olympus CX21 microscope, (Olympus Corporation, Tokyo, Japan). The percentage of normal and abnormal sperm cells was counted. Sperm abnormalities were observed based on average classes, these included; acrosomal damage (an abnormal apical ridge), abnormal heads, proximal and distal cytoplasmic droplets, abnormal tail formations, and abnormal mid-piece. Abnormality of sperm was graded based on percentage scale where 0% and 100% of abnormalities was considered excellent and very poor, respectively.

Testicular Measurements

Testicular measurements were recorded monthly from each individual buck. Testis length (TL) and width (TW) were measured using a flexible measuring tape calibrated in centimetres and millimeters. Testis weight (TM) and testis volume (TV) were estimated using the mathematical model of Bailey *et al*. (1996), where TM - $0.5533*(TL)*(TW)^2$ and TV - $0.5236*(TL)*(TW)^2$.

Statistical analysis

Data were analysed by ANOVA using the Mixed Model procedure in SAS (version 9.2). The model included main effects such as dietary crude protein (2 d.f) and Se (1d.f) levels, the period/THI (5d.f) levels as an explanatory factor, and animal as experimental unit. There were eight experimental units per treatment. First and second order interactions were also included in the model, that is effects of dietary treatments and their interactions as well as changes of these diets in relation to temperature variations. The treatment means were compared using Tukey test at 5% probability. To evaluate the period/THI effects on semen quality polynomial regression was used when F was significant. .

RESULTS

Changes in semen characteristics over time are presented in Table 4. Although there were significant (P=0.046) differences in motility between different months these differences were not related to changes in THI and/ or period, as regression analysis did not show any relationship between THI and /or period and motility. Similarly semen sperm concentration also differed significantly between different months (P=0.023) but subsequent regression analysis indicated that these differences too were not related to THI and /or period.

However, there were significant (P=0.001) differences in semen pH values between different months that regression analysis indicated were related to changes in THI and /or period; pH values increased with increasing THI and/ or period (R^2 = 0.80; P = 0.010).

In addition there were significant (P=0.001) differences between different months in reaction time which was related to changes in THI and/ or period; reaction time was found to increase with rise in THI (R^2 = 0.85; P = 0.006) indicating a reduction in libido with rising THI and/ or period.

Incidence of abnormal sperm differed between different months (P=0.001). Regression analysis indicated that there was a tentative relationship with THI and /or period (R^2 = 0.44; P= 0.089) but there were no patterns in the data that would be indicative of time dependent changes.

There was a significant interactive effect (P = 0.001) of dietary protein and Se on testis characteristics (Table 6), and this was observed on testis length, width, weight and volume with higher mean values observed under the influence of Se supplemented group and 220g/kg protein level group. Suggesting effect of Se supplemented treatment with increasing order on testis length and testis width as dietary protein concentration increased. Although the Se supplemented group have high testis length at lower protein concentration.

Effects of protein and Se interaction on reproductive traits are presented in Table 6. There was a significant (P = 0.005) interaction between protein level and Se supplementation on semen volume, TL, TW, TM and TV.

Despite there being differences between different months in testis length, width, weight and volume, regression analysis (R^2 = 0.22, P = 0.001) indicated that these were unrelated to changes in THI and/ or period.

Effects of protein

There were no appreciable differences between dietary protein levels in semen characteristics (Table 5). There was an effect of dietary protein level on testis length (P=0.001) whereby testis length was greater in the 220g/kg protein diet when compared to the other two treatments. However this effect was not apparent on any other testis parameter.

There were no effects of dietary protein concentration on semen pH as these failed to achieve statistical significance (P=0.062). There were no effects of dietary protein level on any other parameters.

Effects of Selenium

There were no significant effects of selenium supplementation on the percentage of live sperm, although there were marginal differences (P=0.074) such that higher percentage live sperm was observed on Se supplemented group as compared to unsupplemented.

There were no effects of Se on all other parameters.

Table 4: Effect of THI/ period on reproductive traits in male rabbits fed dietary protein and selenium supplements

Parameters	Temperature humidity index/ period							
	April	May	June	July	Aug	Sept		
	30.0	26.5	25.3	25.2	25.0	27.0	SEM	P value
Volume (ml)	1.11	0.92	0.97	1.31	0.89	0.79	0.129	0.072
Motility (%)	74.41^{b}	65.52^{d}	73.87^{b}	76.56^{a}	77.03^{a}	71.06^{c}	2.849	0.046
Concentration($x10^6$/ml)	85.59^{b}	114.27^{ab}	125.78^{a}	82.85^{b}	103.52^{ab}	102.84^{ab}	10.029	0.023
pH	7.30^{a}	7.07^{ab}	6.92^{bc}	6.77^{c}	6.84^{bc}	6.92^{bc}	0.075	0.001
Reaction time (sec)	9.32^{a}	4.83^{c}	5.36^{bc}	3.82^{c}	3.96^{c}	7.05^{b}	0.463	0.001
Live Sperm (%)	79.29	79.06	80.16	80.53	76.88	81.83	1.717	0.157
Abnormal Sperm (%)	14.74^{a}	11.14^{b}	12.46^{ab}	11.07^{b}	7.68^{c}	10.95^{b}	0.634	0.001
Testis Length (cm)	7.12^{bc}	6.48^{c}	7.34^{ab}	6.99^{bc}	7.97^{a}	7.28^{ab}	0.161	0.001
Testis Width (cm)	3.30^{ab}	2.92^{c}	3.04^{bc}	3.59^{a}	3.60^{a}	3.22^{bc}	0.084	0.001
Testis Weight (g)	45.64^{bc}	31.63^{d}	39.01^{cd}	52.91^{ab}	59.37^{a}	43.57^{bcd}	2.986	0.001
Testis Volume (cc)	43.19^{bc}	29.93^{d}	36.92^{cd}	50.07^{ab}	56.18^{a}	41.23^{bcd}	2.826	0.001

Means with different superscripts in the same row are significantly different (P<0.05; P<0.001)

Table 5: The effect of either dietary protein or supplementary selenium on reproductive traits of male rabbits

Parameters	Protein Concentration (g/kg)					Selenium (mg/kg)			
	140	180	220	SEM	P value	0.4	0.7	SEM	P value
Mot (%)	72.38	72.93	73.90	2.015	0.862	74.82	71.33	1.646	0.134
Con($x10^6$/ml)	99.98	95.66	111.78	7.087	0.249	107.96	96.99	5.792	0.182
pH	6.94	6.91	7.07	0.053	0.062	7.01	6.94	0.043	0.285
RT (sec)	5.45	5.88	5.84	0.327	0.593	5.91	5.53	0.267	0.321
LS (%)	80.19	77.59	79.60	1.213	0.279	77.87	80.38	0.991	0.074
AS (%)	11.10	11.23	11.57	0.448	0.750	11.49	11.11	0.366	0.464
TL (cm)	7.04^{b}	6.93^{b}	7.53^{a}	0.114	0.001	7.17	7.16	0.093	0.951

Means with different superscript in the same row are significantly different (P < 0.001)
Mot = sperm motility; Con = sperm concentration; RT = reaction time; LS = live sperm; AS = abnormal sperm; TL = Testis length; SEM = standard error of the mean

Table 6: Effect of protein and selenium interaction on reproductive performance of male rabbits

	Selenium level (mg/kg)							
	0.4(basic)			**0.7(supplementary)**				
	Crude protein (g/kg)							
Parameters	140	180	220	140	180	220	SEM	P Value
Vol. (ml)	1.1^{ab}	0.8^{b}	1.0^{ab}	1.0^{ab}	1.4^{a}	0.8^{b}	0.13	0.005
TL (cm)	7.4^{ab}	6.9^{b}	7.1^{ab}	6.8^{b}	7.0^{ab}	7.6^{a}	0.17	0.005
TW (cm)	3.3^{ab}	3.4^{ab}	3.1^{b}	3.2^{ab}	3.2^{ab}	3.5^{a}	0.08	0.001
TM (cm)	50.4^{ab}	45.6^{abc}	41.5^{bc}	37.8^{c}	42.2^{bc}	54.5^{a}	2.99	0.001
TV (cc)	47.7^{ab}	43.2^{abc}	39.3^{bc}	35.8^{c}	40.0^{bc}	51.6^{a}	2.83	0.001

Means with different superscript in the same row are significantly different ($P < 0.001$)
Vol = Semen volume; TL = Testis Length; TW = Testis Width; TM = Testis Weight; TV= Testis Volume
SEM= Standard error of the mean

DISCUSSION

Effects of period/ THI:

Semen characteristics

The significantly higher percentage value of motility observed during the fifth month does not seem to have been affected by the THI, because the best values were obtained at lower THI values. Other studies reported that sperm motility was not affected by seasonal changes or variations in environmental temperatures (Roca *et al*, 2005; Okab, 2007; Elnagar, 2010). Hence the changes in motility as time advanced could be due to other physiological alterations such as change in viscosity. Considering the fact that Hafez and Hafez (2000), indicated that biophysical and physiological factors such as pH and viscosity of the semen could affects the motility of the sperm cells as a consequence of agglutination. Furthermore these slight alterations in sperm motility observed may likely depend on the nature and type of feed consumed, making of smear and possibly advancing age of animal (Pascual *et al.*, 2004). `

The changes in sperm concentration observed across different periods may not be attributed to effect of THI. Owing to the fact that regression analysis output presented no relationship with period/THI. Similarly, other scientific reports revealed that, sperm concentration was unaffected by changes in ambient temperature (Aguirre et al., 2007; Roca et al., 2005).

The pH was slightly affected in direct proportion to changes in THI value with advancing age. However the values fall within normal range, even though period with relatively high THI recorded corresponding high pH values. This is in agreement with the findings of Nizza *et al*., (2003) and El-Raffa (2004) who also observed increase in semen pH under high environmental temperatures. Ayo *et al*. (2011), have shown that ionic composition of the seminal plasma could be altered under high ambient temperature. Hence increase in semen pH under hot conditions may have the potential to causes changes in ionic balance of seminal plasma, which could subsequently affect the cellular fluid, membrane integrity and nutrient reserves. Nevertheless the pH values in this study fall within the normal published range of 6.5-7.5 (Osinowo, 1979). Therefore, the level of THI was not high enough to elicit drastic

change in the semen pH. However, the pH changes seem to be related to the humidity, as there could be an increase in citric acid content and other macro-minerals (Ca, P and Mg) in semen during rainy season. Nevertheless, other related studies observed no effects of THI or season on semen pH (Roca *et al.*, 2005; Marai *et al*., 2009).

Reaction time or libido was higher during periods when the THI value was also high. Hence there were direct or rather positive relation of reaction time (libido) and environmental THI as age of animal advanced. Similar observations have also been reported by Nizza et al.(2003), El-Raffa (2004), Safaa *et al*. (2008) and Ain-Baziz *et al*. (2012), stressing that sexual activity/ drive was reduced in rabbits exposed to summer periods when compared to those during winter periods. Thus slight reduction in libido or rather slight increased reaction time observed in this study, could be attributable to high ambient temperatures, although the changes were mild, owing to the fact that when the ambient temperature was high, the relative humidity was low, hence the environmental THI was not extreme. The values of the reaction time observed were within normal range (Marai *et al*., 2009). Other scientific reports, have shown that sex drive or libido may also be influenced by several factors, such as bio-stimulation, Rodriguez-De lara (2008), condition of the AV, Boiti *et al*. (2005), nutrient intake Elmaz *et al*. (2007); Attia et al. (2011) as well as ambient temperature Safaa *et al*. (2008), Marai *et al*. (2009), or the interaction between these factors (Attia and Kamel, 2012). Although Talebi *et al*. (2009), pointed out that high ambient temperature during the summer had no effect on semen quality in male goat, rather the breeding season. However, high ambient temperature seems to be the most important, since it could adversely affect the biological function of the body, though depending on intensity and duration of exposure (Marai and Habeeb, 2010).

Significantly high percentage of sperm abnormality was observed when the THI value was high as compared to other periods. However the values were within normal range. This would suggest that the incidence of abnormal sperm, although influenced to some degree by THI may be attributable to a number of other factors such as slight mouldiness of feed caused by aflatoxins due to high relative humidity (Marai and Asker, 2008). Therefore, sperm morphology was not adversely affected by high ambient temperature in this study. Similarly Talebi *et al*. (2009) and Okab (2007), did not observe any detrimental effect of high ambient temperature (30-35^{o}C) on semen characteristics in goats and sperm abnormality in rabbits respectively. On the contrary detrimental effects of heat or high ambient temperature (30-32^{o}C) had been shown to increase sperm abnormality in rams (Marai *et al*., 2009) and in rabbits (Marai *et al*.,2002; El-Raffa, 2004, Bodnar, 2000), thereby decreasing semen quality. Apparently, the degree of adverse effect of heat may depend on duration of exposure and physiological state of the animal.

The semen volume values were not statistically affected by period or rather THI.

Testis characteristics

The testicular length and width values observed although affected by period, but these changes were not due to THI effects as indicated by regression analysis, but most probably unidentified environmental or physiological and genetic factors that could not have been controlled. Similar increased in testis length and width with advancing age of the animal was observed in bulls (Towhidi and Gholami, 2006), and testis length was correlated to testis width and both correlated to body weight. Hence changes in body weight could have the potential to increase the testis size as age advanced. More over increased testis size has been shown to positively correlated to increase in seminiferous tubule diameter, sertoli cell number and testosterone production with subsequent improvement on semen production (Thompson and Berndtson, 1993; Bailey *et al*., 1996). The increased in testicular parameters with

advancing age may be attributed to improvement in the body weight and subsequent sperm production of the rabbits as time advanced.

Effects of protein and Se interaction:

Semen volume

There were significant effects of interaction of Se supplemented treatment with increasing order on semen volume as the dietary protein concentration increases upto 180g/100g. Similarly, Se was shown to improve the biopotency of some enzymes such as glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) , however their activity has not been improved when the dietary Se was higher than 0.59mg/kg(Zhang et al., 2011). Since selenoproteins are enzymes, there may be possible saturation effect, therefore may also be required at specific amount for optimum productive and reproductive functions. Se has been shown to enhance the uptake of proteins and subsequent formations of amino acids /selenoproteins in the body via Se-Sulphur intermediates (Ganther, 1999). Therefore both Se and protein supplementation at required level could improve the reproductive process as well as protection against oxidative stress, by enhancing and regulating the quality and quantity of secretions of seminal fluid (Marai et al., 2009; WHO, 2007; Sahin et al., 2013).

Testes characteristics

The testes length, width, weight and volume seem to change with increased in Se supplemented group as dietary protein increased. Similarly increased testis length and sperm production had been reported on rabbits fed dietary protein with 24 g/100gCP (Ladokun et al., 2006) and rams fed dietary Se with 0.1mgSe/kgDM (Marai et al., 2009). In addition increased in testis size has been shown to positively correlated to increase in sertoli cell number with subsequent improvement on semen production (Johnson et al., 2008). Several studies have shown proper and effective action of nutrition on reproductive functions, particularly protein and selenium. Since it has been reported that Se is an essential nutrient and a component of the amino acid, selenomethionine that has potential influence on growth, health and well being (Mclntosh, 2008). Protein utilisation has also been reported to enhance the synthesis of thioredoxin reductase-3, a selenoprotein which is specifically localized in the testis and regulates the intracellular redox reaction and enhances antioxidant function (Fairweather-Tait et al., 2010). These authors also indicated that, Se being absorbed in the body could regulate the metabolism of thyroid hormones through the action of selenoprotein (Iodothyronine 5' deiodinase-1), hence may improve protein metabolism (Fairweather-Trait *et al*., 2010), particularly in extreme Se deficiency disorder. Therefore the interactive functions of protein and Se towards optimum productivity is indispensable.

Effects of protein:

The significant effect of dietary protein treatment on testis length, with relatively high value observed at 22 g/100g protein concentration may be attributed to the changes in the entire testis size. As these findings was in line with the report by Elmaz et al., (2007), stated that dietary protein could play a vital role in modulating the effects of IGF-I on testicular tissue growth and development in ram. Similarly Ladokun *et al*. (2006) also indicated that male rabbits require high dietary protein level above 20g/100g CP for optimum reproductive functions in humid tropics, stressing that when rabbits were fed diets containing 14g/100g CP for certain period of time, the testis were shrunken with subsequent narrower seminiferous tubules. However, recommendation in NRC 1977 about 16g/100g of protein is required for adult rabbit, hence regarding to the present study a slight increase on the required level at 18g/100g seems to be optimal for rabbits in tropical conditions. Since higher protein level could lead to increase in cost of diet and also nitrogen emission into the environment, with

subsequent rise in pollution. In addition it has been shown that dietary inclusion of more than 15g/100g level of crude protein was suggested to ensure optimum sperm production in rabbits (Nizza *et al.,* 2000). These findings were in line with the report by Elmaz *et al*. (2007), stated that dietary protein could play a vital role in modulating the effects of IGF-I on testicular tissue growth and development in ram. The improvement on testis length could probably be as a response of amino acid uptake for subsequent tissue development, hence the gonadal sperm may be improved, since relatively high level of dietary protein diet appears to be effective for proper function of the male reproductive system. Since changes in testes size has been known to be positively influenced by changes in body weight and closely related to body growth (Elmaz *et al.*, 2007, Ladokun *et al*., 2006; Oyeyemi and Okediran, 2007). Dietary supplementation has been implicated in enhancing hormonal secretions, enzymatic actions and antioxidant status, with subsequent improvement on reproductive tracts of an animal (WHO, 2007; Hafez and Hafez, 2000; Attia *et al.*, 2011; Attia and Kamel, 2012; Castellini, 2008; Sahin *et al*., 2013).

A relatively high pH value on 22g/100g protein concentration as compared to other levels of protein was noted, although these differences failed to reach statistical significance. However, these variations were marginal and the values are within normal range.

Effects of Selenium:

The percentage live sperm values although did not differ statistically, but higher value was observed on Se supplemented group. However, Zhang *et al*., (2011), have reported that increased Se intake resulted in better spermatid development that were attributed to improvements in selenoprotein expression. Selenium was reported to be essential to male fertility, as it had been considered an integral component of sperm structure, in form of structural selenoprotein, phospholipid GSH-Px 4 (Fairweather, *et al*., 2010). However, the percentage live sperm were not affected by dietary Se treatment, hence the values were within normal range. Additionally, several reports, had implicated positive impact of dietary Se on semen quality and quantity in different species, such Se in bulls (El-tohamy et al., 2013), Se in rams (Marai *et al*.,2009), Se in humans (BNF,2001), Se in rats (Yeh *et al*., 1997). The lack of significant effect of Se supplementation in the present work, may be due to the basal content/ background adequate Se contents that fulfilled the animal requirements for reproductive functions.

CONCLUSION

There was an improvement on the testicular measurements as age advanced with decreasing THI, which may be attributed to influence of protein and possibly Se in enhancement on testicular tissue and hormonal profile. The positive effect of Se and dietary protein was manifested on testicular parameters. Therefore 18g/100g dietary protein and 0.4mgSe/kgDM seems to be optimum for male rabbit reproductive performance. Hence higher levels of supplementation of these diets may not be economical.

Additionally, supplementation with 22g/100g dietary protein and 0.7mgSe/kg DM did not have any adverse effect on the reproductive functions. Although the rabbits did not show any response to dietary Se. Considering the fact that, most of the parameters were maintained within normal range. Although, the few affected parameters fluctuated as feeding period progresses with advancing age of the animals, most probably in response to certain physiological alterations due to other unidentified factors that might not have been controlled, such as semen handling and processing, slight mould infections on feedstuffs due to high humidity and or changes in seminal plasma enzymes and proteins.

ACKNOWLEDGEMENTS

The authors appreciated the National Animal Production Research Institute (NAPRI)/ ABU, Shika, Zaria, Nigeria, for providing appropriate study environment and materials for the study. This study was supported by Education Trust Fund (ETF), Nigeria and Ahmadu Bello University (ABU), Zaria, Nigeria.

REFERENCES

Aguirre V., Orihuela, A. and Vazquez, R. (2007). Seasonal variations in sexual behaviour, testosterone, testicular size and semen characteristics, as affected by social dominance, of tropical hair rams (Ovis aries). *Animal Science Journal,* 78, 417 – 423.

Ain-Baziz, H., Boulbina, I., Iles, I., Belabbas, R., Zenia, S. and Temim, S. (2012). Influence of environmental temperature and relative humidity on semen characteristics in male rabbit (Oryctolagus Cuniculus) of local Algerian population. *Proceedings 10th World Rabbit Congress,* September 3 – 6, Sharm El – Sheik, Egypt, pp. 347 – 350.

AOAC (1990). Association of Official Analytical Chemist. Official Methods of Analysis.15th Edn. AOAC. Inc. Arlington, Virginia 22201.

Attia, Y.A., Al-Hanoun, A. and Bovera, F. (2011). Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. *Journal of Animal Physiology and Animal Nutrition.* 95 (1): 17–26.

Attia, Y.A. and Kamel, K.I. (2012). Semen quality, testosterone, seminal plasma biochemical and antioxidant profiles of rabbit bucks fed diets supplemented with different concentrations of soybean lecithin. *Animal,* 6 (5): 824 - 833.

Ayo, J.O., Obidi, J.A. and Rekwot, P.I. (2011). Effects of Heat Stress on the Well-Being, Fertility, and Hatchability of Chickens in the Northern Guinea Savannah Zone of Nigeria: A Review. *International Scholarly Research Network, ISRN Veterinary Science*. Volume 2011, Article ID 838606, 10 pages.

Bailey, T.L., Monke, D., Hudson, R.S., Wolfe, D.F., Carson, R.L. and Riddell, M.G. (1996). Testicular shape and its relationship to sperm production in mature Holstein bulls. *Theriogenology,* 46: (3), 881 – 887.

Bodnar, K., Szendro, Z., Nemeth, E.B., Eiben, C. and Radnai, I. (2000). Comparative evaluation of abnormal spermatozoa in Pannon White, New Zealand White and Angora rabbit semen (short communication). *Arch. Tierz., Dummerstorf.* 43: 5, 507- 512.

Boiti, C., Castellini, C., Theau- Clement, M. , Besenfelder, U., Liguori, L., Renieri, T. and Pizzi, F. (2005). International Rabbit Reproduction Group. Guidelines for the handling of rabbit bucks. *World Rabbit Science,* 13, 71-91.

Boland, M.J., Rae, A.N., Vereijken, J.M., Meuwissen, M.P.M., Fischer, A.R.H., Boekel, M.A.J.S., Rutherfurd, S.M., Gruppen, H., Moughan, P.J. and Hendriks, W.H. (2013). The future supply of animal - derived protein for human consumption. *Trends in Food Science and Technology,* 29, 62 – 73.

Castellini, C. (2008). Semen Production and Management of Rabbit Bucks. *Proceedings of the 9th World Rabbit Congress,* Verona, Italy, pp. 265 - 273.

Elmaz, O., Cirit, U., Keser, O., Gurbulak, K., Guvenc, K. and Kutay, C. (2007). Effect of two dietary protein levels on testosterone, testicular parameters and semen quality in ram lambs during pubertal development. *Medycyna Wet*. 63: (10), 1177-1180.

Elnagar, S.A. (2010). Royal jelly counteracts bucks summer infertility. *Animal Reproduction Science*, 121, 174 – 180.

El-Raffa, A.M. (2004). Rabbit Production in the Tropics. *Proceedings of the 8th World Rabbit Congress*, 7-10, September, Puebla, Mexico. 1172 - 1180.

El-Tohamy, M.M., Kotp, M.S.Z., EL- Nattat, W.S., Amira, H.M. and Soliman, S.I. (2013). An attempt at alleviating heat stress infertility in male rabbits with some antioxidants. *Factory Pro trial version. www.pdffactory. com* (accessed 12/11/2013).

Fairweather –Tait, S.J., Collings, R. and Hurst, R. (2010). Selenium bioavailability: current knowledge and future research requirements. *American Journal of Clinical Nutrition*, 91(suppl): pp. 1484S- 91S.

Finzi, A., Morera, P. and Kuzminsky, G. (1995). Sperm Abnormalities as Possible Indicators of Rabbit Chronic Heat Stress. *World Rabbit Science*, 3(4), 157-161.

Ganaie, A.H., Shanker, G., Bumla, A.N., Ghasura, R.S., Mir, N.A., Wani, S.A. and Dudhatra, G.B. (2013). Biochemical and Physiological Changes during Thermal Stress in Bovines. *Journal of Veterinary Science and Technology*, 4: (1), 1-6.

Ganther, H.E. (1999). Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis*, 20(9): 1657- 66.

Hafez, B. and Hafez, E.S.E. (2000). Semen Evaluation. In: Reproduction in farm animals. 7thedn. Lippincott Williams & Wilkins. Philadelphia, Pennsylvania. 19106-3621 USA, pp. 365-375.

Hafez, E.S.E. (1985).'Semen Characteristics.'In Reproduction in Farm Animals. 5th edn. Lea and Fabiger. Philadelpia. pp. 189-207, 388-397, 455-480.

Hansen, P.J. (2009). Effects of Heat Stress on mammalian reproduction. Philosophical Transactions of the Royal Society B. pp 364, 3341-3350.

Johnson, L., Thompson Jr, L., Varner, D.D. (2008). Role of Sertoli cell number and function on regulation of spermatogenesis. *Animal Reproduction Science*, 105, 23 – 51.

Kehr, S., Malinouski, M., Finney, L., Vogt, S., Labunskyy, V.M., Kasaikina, M.V., Carison, B.A., Zhou, Y., Hatfield, D.L. and Gladyshev, V.N. (2009). X-Ray Fluorescence Microscopy Reveals the Role of Selenium in Spermatogenesis. *Journal of Molecular Biology*, 389, 808-818.

Ladokun, A.O., Egbunike, G.N., Adejumo, D.O. and Sokunbi, O.A. (2006). The Effect of Three Dietary Crude Protein Levels on Digestibility and Tests Function in Male Pubertal Rabbits. *Tropicultura*, 24: (1), 3-6.

Lukefahr, S.D. (2007). The small-scale rabbit production model: Intermediate factors. *Livestock Research for Rural Development*, 19 (5) . http://lrrd.cipav.org.co/lrrd19/5/luke19069.htm.(Accessed 17/03/2011).

Mahima, A. K., Amit, K., Anu, R., Vinod, K. And Debashis, R.(2012). Inorganic Versus Organic Selenium Supplementation: A Review. *Pakistan Journal of Biological Sciences*, 15 (9): 418 – 425.

Marai, I.F.M. and Asker, A.A. (2008). Aflatoxins in rabbit production: Hazards and control. A review. *Tropical and Subtropical Agroecosystem,* 8: 1- 28.

Marai, I.F.M., El- Darawany, A.A., Fadiel, A., Abdel- Hafez, M.A.M. (2007). Physiological traits as affected by heat stress in sheep – A review. *Small Ruminant Research*, 71, 1 – 12.

Marai, I.F.M., El- Darawany, A.A., Fadiel, A. and Abdel- Hafez, M.A.M.(2008). Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Tropical and Subtropical Agroecosystems,* 8: (3), 209-234.

Marai, I.F.M., El – Darawany, A.A., Abou-Fandoud, E.I. and Abdel-Hafez M.A.M. (2009). Reproductive and physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation during the subtropical environment of Egypt. *Livestock Research for Rural Development,* 21 (10). www.lrrd.org/lrrd21/10/mara21157. htm .(Accessed on 17/11/2010).

Marai, I.F.M. and Habeeb, A.A.M. (2010). Buffalo's biological functions as affected by heat stress- A review. *Livestock Science,*127: 89-109.

Marai, I.F.M., Habeeb, A.A.M. and Gad, A.E. (2002). Reproductive traits of male rabbits as affected by climatic conditions, in the subtropical environment of Egypt. *Animal Science*, 75, 451-458.

Nizza, A., DiMeo, C. and Taranto, S. (2003). Effect of Collection Rhythms and Season on Rabbit Semen Production. *Reproduction Dom Animal,* 38, 436-439.

National Research Council (NRC). (1977). Nutrient Requirements of Rabbits. Second Revised Edition. National Academy of Sciences, 2101 Constitution Avenue, N.W. Washington, D.C. 20418. pp. 30.

National Research Council (NRC). (1991). Microlivestock: Little-known Small Animals with a promising Economic Future. National Academy Press, Washington, D.C.

Okab, A.B. (2007). Semen Characteristics and Plasma Testosterone of New Zealand Male Rabbits as Affected by Environmental Temperatures. *Slovakia Journal of Animal Science,* 40: (4), 161-167.

Oseni, S.O. (2012). Rabbit Production in Low-input Systems in Africa – Prospects, Challenges and Opportunities. *Proceedings of the 10th World Rabbit Congress,* 3-6 September, Sharm EL-Sheikh, Egypt, pp. 719 – 731.

Osinowo, O.A. (1979). Testicular Growth and Semen Qualities of West African Bulls. *PhD Thesis*. Faculty of Veterinary Medicine **,** Ahmadu Bello University, Zaria, Nigeria.

Oyeyemi, M.O. and Okediran, B.S. (2007). Testicular parameters and sperm morphology of chinchilla rabbit fed with different planes of soyameal. *International Journal of Morphology,* 25(1): 139 – 144.

Pascual, J.J., Garcia, C., Martinez, E., Moce, E. and Vicente, J.S. (2004). Rearing management of rabbit males selected by high growth rate: The effect of diet and season on semen characteristics. *Reproduction Nutrition Development,* 44: 49 – 63.

Pauzenga, U. (1985). Feeding Parent stock. *Zootecnica International*. pp. 22-24.

Rekwot, P.I., Oyedipe, E.O., Akerejola, O.O., Kurni-Diaka, J. and Umoh, J.E. (1987). The effect of protein intake on the onset of puberty in Bunaji and Friesian X Bunaji bulls in Nigeria. *Theriogenology,* 28 (4): 427-443.

Roca, J., Martinez, S., Orengo, J., Parilla, I., Vazquez, M.J. and Martinez, A.E. (2005). Influence of constant long days on ejaculate parameters of rabbits reared under natural environment conditions of Mediterranean area. *Livestock Production Science*, 94, 169-177.

Rodriguez-De Lara, R., Fallas – Lopez, M., Rangel-Santos, R., Mariscal- Aguayo, V. Martinez- Hernandez, P.A. and Garcia Muniz, J.G. (2008). Influence of doe exposure and season on reaction time and semen quality of male rabbits. *Proceedings of the 9th World Rabbit Congress*, held in Verona, Italy, pp. 443- 447.

Safaa, H.M., Emarah, M.E. and Saleh, N.F.A. (2008). Seasonal effects on semen quality in Black Baladi and White New Zealand rabbit bucks. *World Rabbit Science*, 16, 13 - 20.

Sahin, K., Orhan, C., Smith, M.O. and Sahin, N. (2013). Molecular targets of dietary phytochemicals for the alleviation of heat stress in poultry. *Poultry Science Journal,* 69 (1): 113-133.

Tajangookeh, H.D., Shahneh, A.Z., Shahrebabak, M.M. and Shakeri, M. (2007). Monthly Variation of Plasma Concentrations of Testosterone and Thyroid Hormones and Reproductive Characteristics in Three Breeds of Iranian Fat-Tailed Rams Throughout One Year. *Pakistan Journal of Biological Sciences,* 10 (19): 3420-3424.

Talebi, J., Souri, M., Moghaddam, A., Karimi, I. And Mirmahmoodi, M. (2009). Characteristics and seasonal variation in the semen of Markhoz bucks in western Iran. *Small Ruminant Research*, 85, 18-22.

Tegegne, A., Enwistle, K.W. and MukasaMugerwa, E. (1992). Nutritional influence on growth and Onset of puberty in Boran and Boran x Friesian bulls in Ethiopia. *Theriogenology*, 37: 1004 -1016.

Thompson, T.L. and Berndtson, W.E. (1993). Testicular Weight, Sertoli Cell Number,Daily Sperm Production, and Sperm Output of Sexually Mature Rabbits after Neonatal or Prepubertal Hemicastration. *Biolology of Reproduction*, 48: 952-957.

Towhidi, A. and Gholami, H. (2006). Changes in testicular size, body weight and plasma testosterone concentration in Iranian Holstein bull during the growth period. *Proceedings of the British Society of Animal Science.* p.149.

WHO (2007). Protein and amino acid requirement in human nutrition: Report of a joint FAO/WHO/UNU expert. *Technical report series*, no 935. Geneva, Switzerland.

Yeh, Y., Vendeland, C., Gu, Q., Butler, J.A., Ou, B. and Whanger, P.D. (1997). Dietary Selenium Increases Selenoprotein W levels in Rat Tissues. *Journal of Nutrition*,127: 2165-2172.

Zhang, Y., Suzhen, Z., Wang, X., Chunyang, W. and Fuchang, L. (2011). The effect of dietary Selenium levels on growth performance, antioxidant capacity and glutathione peroxidase1(GSHPx1) mRNA expression in growing meat rabbits. *Animal Feed Science and Technology*, 169: 259-264.