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Physical Performance and Thermoregulatory Response of Male Breeder Rabbits Fed Dietary Protein and Selenium Supplementation under Tropical Condition.

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Abstract

The study was conducted to evaluate the effects of dietary protein and selenium supplementation of male rabbits on changes in physical performance and thermoregulatory response under tropical conditions. Fourty eight New Zealand rabbit bucks of 23 ± 1.4 weeks of age, weighing 2.8 ± 1.131kg, were allotted to six isocaloric dietary treatments (n = 8 animals/ treatment) that differed in either protein (14g/100g, 18g/100g and 22g/100g) or selenium (0.4 and 0.7 mg Se/ kg diet). Animals were distributed in a 2 x 3 factorial design, and housed individually in conventional rabbit cages. The temperature humidity index (THI) was determined on six different periods. Total body weight increases linearly ($P < 0.001$) with advancing time. Although values are within normal range, the rectal temperature and respiratory rates varies significantly ($P < 0.001$) as the THI changes. Daily feed intake, total body weight and feed intake scale significantly increases linearly ($P < 0.001$) as the dietary protein levels increased. There were significant interactive effects ($P < 0.001$) of protein and selenium dietary treatments on feed intake, with higher values on non selenium and higher protein supplemented groups. Most of the values were within normal range, hence the dietary treatments provided optimum performance.

Keywords: Physical performance, Protein, Selenium, Thermoregulation, Rabbits

Description of Problems

In hot climates rabbits require a higher protein diet in order to compensate for low feed intake. Therefore, modification of dietary protein levels has been shown to compensate for seasonal changes in feed intake (1). Different protein levels are already offered to rabbits experiencing differences in environmental temperatures, with a higher optimum level of 180 g/kg CP in the tropics as compared to 160 g/kg CP in more temperate climates (2).

Selenium could not only have the potential to prevent oxidative damage during heat stress, but could also influence maintenance of cell membrane integrity via phospholipid hydroperoxide glutathione peroxidase (3). Furthermore, it was reported to be implicated in body metabolism and growth via thyrotropic axis, since Se forms an integral part of the deiodinases (4), but, this particular changes only occurs, when the levels of dietary Se are extremely low or deficient within the body system, such that other selenoproteins might have been metabolically inadequate.

Both protein and selenium are important in supporting cellular processes of the body(5),through homeostasis. Body weight gain, feed conversion efficiency and antioxidant capacity of growing rabbits was improved when offered supplementary dietary Se at a rate of 0.24mgSe/kgDM (4). Conversely, (6) reported that Se supplementation in rabbits did not effectively influence the rate of growth, feed efficiency or carcass yield. Considering the discrepancies on reports, the bioavailability of dietary supplements may depend on age and availability of other nutrients in the diets, as well as environmental influence. It is thus considered expedient to exploit dietary Se supplementation as a form of nutritional strategy to counteract the adverse effects of heat stress in rabbit productive performance in order to enhance successful production all year round.

Rectal temperature and respiratory rates may reflect the physiological responsiveness of the animal to its internal and external environment, which include feed and feeding as well as ambient temperature and relative humidity. However, (7) reported that specific genetic effects such as growth traits could strongly influence a rabbit's environmental adaptability. These may include body weights, ear length and fur density (hair coat) (8). Therefore, investigating a range of biomarkers of hyperthermia such as rectal temperatures and respiratory rates, may be indicative of physiological stress and tissue damage that may provide a scientific means of determining indicators of metabolic adjustment and behavioural responses which might occur as a consequence of heat stress. Furthermore identifying variations that might have evolved between treatment groups and that of temporal effects, could be used to select and maintain adaptive traits.

The objective of this research study is to evaluate the effects of dietary protein and Se supplementation on male rabbit production and their ability to offset the deleterious effects of heat stress on productive and physiological traits in a tropical climate.

Materials and Methods

Experimental site

The study was conducted at the rabbit research farm of the National Animal Production Research Institute (NAPRI) Shika, Zaria, Nigeria. Proximate chemical analyses were carried out in the Central Laboratory (NAPRI – ABU). The mean temperature of the site during the coolest season is 15-18^{0}C and during the hot season is 27-32^{0}C. Relative humidity is between 55-75% during the dry season, and 80-85% during the wet season.

Experimental diets

Chemical Composition of forage administered, Ingredient composition and proximate analysis of the experimental diets are shown in Table 1, 2 and 4 respectively, and were formulated to meet the specific nutrient requirements of rabbits based on (9), (10), (11) and (12). All ingredients were procured/purchased in livestock feed industry/shops, then assembled, ground, mixed and formulated on dry weight basis in a standard feed mill and bagged. The manufactured feed remained in bags until distributing to the animals in their respective treatment groups.

The variation on the fibre content in the ingredient composition and proximate analysis of the test material, was due to the fact that one was determined based on calculated with values recommended by NRC and other relevant literatures, while the latter was determined from laboratory analysis of the test ingredients.

Table 1. The chemical composition of tropical forage administered to the experimental rabbits (dry matter basis)

Nutrients	Brachiaria brisantha
Gross energy (MJ/kg)	11.81
Crude protein(g/kg)	67.00
Ca(g/kg)	4.70
P(g/kg)	1.60
Ash(g/kg)	131.00
CF(g/kg)	368.10

Adapted from; FAO (13); Lukefahr an d Cheeke, (7); Aduku and Olukos (14). Ca = calcium; P= phosphorus; CF = crude fibre

Table 2: Ingredient composition of the experimental diet (g/kg unless otherwise stated)

	Dietary treatments		
Ingredients(g/kg)	140g/kg	180g/kg	220g/kg
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).

Meteorological data

Meteorological data (ambient temperature and relative humidity) of the study area was recorded throughout the experimental period using a digital thermo-hygrometer (Mextech TM-1, China). Temperature and relative humidity were measured at two different times daily (8.00hr and 14.00hr), within the building and outside, and monthly average were computed using basic statistics (Table 3).

The temperature-humidity index (THI) was computed (Table 3) using the standard formula by (15), as shown:

$$THI = t - \{(0.31 - 0.31RH)(t - 14.4)\}$$

Where t = temperature (°C) and RH = relative humidity (%)

Table 3: Monthly average ± SD of air temperature and relative humidity of the study area (2012)

Months (d)	Temperature (°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

THI calculated using standard formula (15).

Proximate analysis of the diets:

The proximate chemical analyses (Table 4) of the experimental diets were done according to (16) methods. This was conducted prior to the commencement of the feeding trial, in order to determine the specific nutritive value of the experimental diets.

Table 4: Proximate/Chemical Analysis of the Experimental Diets(dry matter basis)

Parameters (g/kg)	Dietary treatments		
	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 (17) : ME = 37 x CP + 81.8 x EE + 35.5 x NFE

**Nitrogen Free Extract

Values are means of three determinations ± SD

ME – Metabolisable energy CP – Crude protein

EE – Ether extract NFE – Nitrogen free extract

Experimental animals and Design

A total of 48 male New Zealand White [NZW] rabbits, with mean age of 23 ± 1.4 weeks and weighing 2.8 ±1.13 kg were used for the experiment. Animals were sourced from the National Animal Production Research Institute (NAPRI), Shika, Zaria-Nigeria. The male rabbits were used to single/individual cages before the experiment started. Prior to the commencement of the experiment animals were quarantined, physically examined (eye and coat condition, and animal conformation), and offered all necessary medication and vaccinations within two to three weeks of their arrival at the unit. The male rabbits (bucks) used were result of several generations and adapted to conditions in Nigeria. Upon arrival, animals were placed on the basal common diets for two weeks to allow for diet adaptation. All animals were individually weighed for initial body weight and at weekly intervals thereafter throughout the study. At the beginning of April, animals were blocked by weight and then randomly allocated to one of six dietary treatments (n = 8 animals/treatment) that differed in either protein content or selenium content in a 3 x 2 factorial design.

Housing management

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. The distribution of the experimental units was evenly spread across the various cages with fair representation of each treatment, in order to minimize or eliminate variation due to micro-climate.

Feeding management

Concentrates (experimental feeds) of known quantity were offered *adlibitum,* and much later of same day a dry forage (*Brachiaria brisantha*) was offered *adlibitum* as fed free choice to all rabbits. The forage was withheld so as the rabbits would consume much of the concentrate, thereafter consumed less of the forage. Hence, it was assumed that forage intake was low and the same between all rabbits. All rabbits had ready access to clean potable water at all times. Daily feed intake on concentrate feed of individual rabbits was recorded throughout the experimental period, by measuring the feed offered and the refusals. All animals were kept, maintained and treated in accordance with standard routine management practices.

Performance Records Excercise

Physical Performance Records

Feed intake (FI) and body weight (BW) were recorded daily and weekly in grams and kilogrammes, respectively. Feed offered and refused was weighed daily for each rabbit. BW was determined weekly by weighing individual rabbits using an analogue weighing scale (weight balance-hana : 20kg- The big boss, China). These data were used to determine the average weekly feed intake per rabbit and average weekly body weight per rabbit for each month according to their treatment group.

Physiological Records

Rectal Temperature was recorded weekly, using digital thermometer (^{o}C) (Kruuse Digi-Temp: DK-5290, Marslev, Denmark). The probe tip was inserted at about 4cm into the rectum for approximately one minute, when the measurement records displayed immediately after 4 long beeps. Respiratory rate was measured weekly by visually observing the number of flank movements per minute (n/min) at resting position using a stopwatch. These data were used to determine the four weekly average rectal temperature per rabbit and four weekly average respiratory rate per rabbit for each month according to their treatment group.

Statistical analyses

Data was analysed by Analysis of Variance (ANOVA), using Minitab vs 16 GLM procedure. Differences between means were established using Tukey pairwise comparison. Simple regression analysis was used to separate temporal effects from temperature effects.

Results

Effects of period on physical performance

Changes in physical performance over time are presented in Table 5.

There were significant differences in feed intake (P= 0.003) as time advanced. This changes may partially be attributed to THI and mostly by other factors, as confirmed by regression analysis (R^2= 0.59 ; P= 0.10). The total body weight increased linearly (P= 0.001) with advancing time. Suggesting possible increased in growth rate. However the regression analysis (R^2= 0.04) showed no effect of THI, rather dietary treatment effects may be responsible. The feed intake scale (P>0.05) was not affected by THI or rather period.

There were slight changes on rectal temperature (P= 0.001) as time advanced. However this changes may not be attributed to THI values, as confirmed by regression analysis (R^2= 0.03). There were significant effect of respiratory rate (P= 0.001) as time advanced in accordance with changes in THI, hence this parameter was partly affected by changes in environmental temperature and some other unknown factors. These changes were confirmed by regression analysis (R^2= 0.42, P = 0.001).

Table 5: Effects of period on physical performance in male rabbits fed dietary protein and selenium supplements

Parameters	Month 1	2	3	4	5	6	SEM	P value
FI/w/r (g)	784.4^{c}	787.1^{c}	839.0^{a}	824.7^{ab}	836.4^{b}	822.7^{ab}	12.65	0.003
TBW(g)	2802^{a}	3054^{ab}	3155^{ab}	3215^{bc}	3254^{bc}	3513^{b}	0.08	0.001
FIS(g/g^{mw})	2.1	2.0	2.0	2.0	2.0	2.0	0.07	0.808
RT(oC)	38.5^{a}	38.1^{bc}	38.3^{cd}	37.6^{d}	37.9^{d}	38.3^{cd}	0.10	0.001
RR(n/min)	130^{d}	112^{c}	97.9^{a}	97.2^{a}	99.2^{ab}	104^{b}	1.29	0.001

[abcd]Means with different superscripts in the same row are significantly different (P<0.05; P<0.001); FI – Feed intake; TBW – Total body weight; FIS – Feed intake scale; RT – Rectal temperature; RR – Respiratory rate

Effect of dietary protein on physical performance

Changes on physical performance on the respective dietary treatment groups are presented in Table 6. There were linear increased of feed intake (P= 0.001) with increased in protein concentration. This chages were confirmed by regression analysis (R^2 = 0.96, P= 0.12).Total body weight also increased linearly (P= 0.001) with increased in protein concentration. The regression analysis (R^2 = 0.98, P= 0.05) confirmed this trend. The rectal temperature also increased linearly (P= 0.001) with increased in protein concentration. This was confirmed by regression analysis (R^2= 0.99, P= 0.009). The respiratory rate (P> 0.05) was not affected by dietary treatments. There were no effect of dietary Se (P> 0.05) on all the physical performance parameters. There was an evidence of interaction between protein and time (P= 0.001) on feed intake with higher value during the third period on 22g/100g protein level(group). There was a weak interaction between time and Se (P= 0.06), with a slightly higher value average body weight during the sixth period on Se supplemented group. There was interaction between protein and time (P= 0.004), with higher value of rectal temperature during the sixth period on 22g/100g protein level (group).

Table 6: The effect of either dietary protein or supplementary selenium on physical performance of male rabbits

Parameters	Protein Concentration (g/100g)			SEM	P value	Selenium		SEM	P value
	14	18	22			NSe	Se		
FI/w/r (g)	755^{c}	801^{b}	891^{a}	7.60	0.001	813	819	7.12	0.535
T BW (g)	3022^{b}	3133^{b}	3343^{a}	0.04	0.001	3219	3113	0.03	0.515
F I S(g/g^{mw})	1.9^{b}	2.0^{b}	2.2^{a}	0.05	0.002	2.0	2.0	0.04	0.790
RT(oC)	37.9^{c}	38.1^{b}	38.3^{a}	0.05	0.001	38.1	38.1	0.05	0.181
RR(n/min)	107	106	106	0.69	0.214	106	107	0.60	0.607

^{abc}Means with different superscripts in the same row are significantly different (P<0.001), FI – Feed intake; TBW – Total body weight; FIS – Feed intake scale; RT – Rectal temperature; RR – Respiratory rate, NSe – No Selenium supplement; Se – Selenium supplement

Effect of protein x selenium interactions

The protein and Se interaction are presented on Table 7.

Significant interactive effect (P= 0.007) of dietary protein and Se was evident on feed intake with high value on NS22CP group as compared to other treatment groups. This suggests effects of varying unsupplemented Se treatment on feed intake with increasing order as the dietary protein concentration ascends. There was no interaction (P= 0.995) of dietary protein and Se on average body weight (BW) per rabbit. There was a mild effect (P = 0.063) of dietary protein and Se on feed intake scale with a linear increase as the dietary protein increased. There was no effect (P= 0.541) of dietary protein and selenium interaction on rectal temperature (RT), indicating lower value on Ns22cp as compared to other treatment groups.There was evidence of an interactive effect (P= 0.037) of dietary protein and selenium on respiratory rate (RR), with lower value on Ns18cp treatment group as compared to other groups

Table 7: Effect of protein and Se interaction on reproductive and physical performance of male rabbits

Variable	Protein(14g/100g)		Protein (18g/100g)		Protein (22g/100g)			Significance levels		
	Se1	Se2	Se1	Se2	Se1	Se2	SEM	Protein	Se	Interaction
FI /w/r (g)	791^{cd}	716^{d}	777^{cd}	816^{bc}	881^{bc}	907^{a}	19.36	0.001	0.535	0.007
BW (g)	2976	3068	3073	3193	3290	3396	148.62	0.001	0.515	0.995
FIS (g/g^{mw})	2.0^{ab}	1.8^{b}	1.9^{b}	2.0^{ab}	2.1^{ab}	2.2^{a}	0.07	0.002	0.790	0.063
RT (^{O}C)	37.8	38.0	38.1	38.1	38.3	38.3	0.09	0.001	0.181	0.541
RR (n/min)	106	107	107	104	105	106	0.82	0.214	0.607	0.037

^{abc}Means with different superscripts in the same row are significantly different (P< 0.001) ; SEM = Standard error of the mean, Se 1 = Selenium supplemented; Se 2 = No selenium supplement FI = Feed intake; BW = Body weight; FIS= Feed intake scale; RT= Rectal temperature; RR= Respiratory rate

Discussion

Effect of interaction

Body weight

There was no interactive effect (P= 0.995) of dietary protein and Se. However numerical variations exist with linear increased as the dietary protein increased. Thus, there may be effect of Se supplemented treatment with an increasing order on body weight as dietary protein concentration increases, suggesting proper protein utilization with subsequent amino acid uptake. Possibly as a consequence of improve protein absorption via selenomethionine uptake mechanism. However the value recorded fell within normal range. Zhang et al. (4), has shown that body weight gain in rabbit was improved in rabbits fed 0.24mgSe/kg. In addition, improved body weight was also observed in rabbits fed dietary protein with 24%CP (18) and this was correlated to testis weight and gonadal sperm reserve. Literally, it is important to supply adequate dietary protein in order to meet up the minimum protein requirement and also to maintain all those physiological and metabolic processes that requires amino acids for the optimum productive and reproductive performance.

Feed intake

Significant interactive effect (P= 0.001) of dietary protein and Se was evident with high value on NS22CP group as compared to other treatment groups. This suggests effects of varying unsupplemented Se treatment on feed intake with increasing order as the dietary protein concentration ascends. Even though the level of feed intake varies, it appears to be within normal range (120 - 150g/d/adult rabbit) considering the age and weight of the rabbits. Although supplemental Se treatment did not show any trend, it was known to improve protein utilization as well as feed intake. As it has been reported by (4), that average daily intake in rabbits had been improved, when fed dietary Se between 0.24 to 0.59mg/kg. In addition, adequate protein concentration in diets within required level seems to enhance feed intake, particularly under tropical conditions (2). Thus 22g/100g supplemental protein with low level of Se could enhance body biological functions due to subsequent improvement in feed intake.

Respiratory rate

A significant interactions (P= 0.037) of protein and Se was established on RR with lower mean value on Ns18cp as compared to other treatment combinations. Although there was no trend, both Se treatments seem to have affected the respiratory rate at 18g/100g. However, the mean values generally fell within normal range. As such there was no indication of hyperthermia on the rabbits. Therefore the dietary treatments had no any detrimental effects on respiratory rate.

Conclusion

However, the RR was partially affected by THI as the values fell above normal range (10 – 30 breath/minute), since the ambient temperature during the experimental period was slightly above the rabbits thermoneutrality (21°C), although not high enough to induce heat stress.

Additionally, supplementation with 22g/100g dietary protein and 0.7mgSe/kg DM does not pose any adverse effects on the physical performance, particularly under tropical conditions. The rabbits did not show any response to dietary Se.

In addition, most of the parameters were maintained within normal range. However, the affected parameters fluctuated as feeding period progresses with advancing time, most probably in response to certain physiological alterations due to other unidentified environmental factors that might not have been controlled, such as slight mould infections on feedstuffs and or changes in seminal plasma enzyme.

Application

Since the rabbits did not show any response to dietary Se, therefore lower level of Se supplementation may be investigated. Although the required concentration of Se in diet for most farm animals was however suggested to be within the range of 0.05 to 0.3 mg Se/kg of diet (19), in order to avoid toxicity, but the Se supplementation in rabbits may be moderately higher, possibly due its poor sensitivity to deficiency of this nutrient (9).

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