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Impacts of Ambient Temperature and Humidity on Biochemical Traits in Male Rabbits Fed Dietary Protein and Selenium Supplementation

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Abstract: The deleterious effects of high ambient temperatures and undefined dietary protein supply to male rabbits, apparently continue to pose great challenges on efficient and productive performance in rabbit farming under tropical conditions. Forty eight male New Zealand White rabbits of 23 ± 1.414 weeks of age, weighing 2.8 ± 1.131kg, were randomly 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.Blood samples (2ml/sample) were drawn via the marginal ear vein using micro syringes. Significantly different values on the concentrations oftriidothyronine(R^2 = 0.17, P= 0.001), aspartate aminotransferase (R^2 = 0.13, P = 0.001), cholesterol (R^2 = 0.06, P= 0.003),cortisol (R^2 = 0.02, P= 0.049) and mean corpuscular volume (R^2 = 0.03, P = 0.027) between the periods. Significantly low values on cortisol (P= 0.037), Red blood cells (R^2 = 0.996, P= 0.028) and mean corpuscular volume (R^2 = 0.705, P= 0.251) were observed on 22g/100g protein groups as compared to other dietary protein treatments.There were significant differences in circulating alkaline phosphatase (P= 0.007)and platelets (P= 0.011) on dietary selenium levels with higher values on un-supplemented group. Most of the parameters were within normal range.Findings revealed the potentials of dietary protein and selenium supplements towards enhancingthe productive performance of the rabbits under tropical condition.

KEYWORDS*:* Ambient temperature, Biochemical indices, Dietary protein, Dietary selenium, Male rabbits

I. INTRODUCTION

The biochemical components of blood are considered as good indicators of physiological and biological functions of body organs and tissues in rabbits[1]. Seasonal changes has been found to affect the biochemical parameters in animals, in which some of them seems to increase while others decreases with high environmental temperature.The variation in climatic factors like temperature, humidity and solar radiations were shown to pose potential hazards in growth and production in all domestic animals[2] in most tropical and subtropical regions[3].Therefore exposure of animals to high environmental temperature, especially out of thermo-neutrality,perhaps couldresults in internal heat production with subsequent disruption in homeostasis as a consequent of physiological adjustment towards new adaptations. Alterations of physiological and metabolic activities in rabbits,with subsequentchanges in biochemical and hematological parameters had been reported[4].In addition, studies in rabbits have indicated higher mean values of plasma cholesterol and plasma total protein concentrations during the summer or high temperature periods[5]. In contrast,[6] have shown decreased in T_3,cholesterol and plasma protein in buffaloes exposed to elevated temperature above 35^{o}C, and attributed it to systematic way of preventing a rise in metabolic heat production as a consequent of reduced feed intake.Therefore, suggesting negative relation between these parameters and high environmental temperatures with subsequent effects on productivity. In contrast,[7] did not observe any effect of season on plasma protein in rams. High ambient temperatures has been shown to cause the release of cortisol into the circulation, which in turn increases the level of alkaline phosphatase (ALP) and aspartate amino transferase (AST), as consequences of oxidative stress on liver and muscle tissues due to hyperthermia[8]. Therefore[6], have shown that exposure of most animal species to elevated environmental temperature is associated withdecrease in dry matter intake, insulin secretion and nitrogen retention, as

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well as increase in catecholamines and adrenocorticotropic hormones in circulation with subsequent fat degradation and protein catabolism respectively.

It has been shown that the levels of WBC, RBC, PCV and Hb as well as total body weight and cellular oxygen decreased in New Zealand White rabbits exposed to ambient temperature of 36^{o}C as comparable to other rabbits in control group[4]. Thus haematological indices seem to be a good biomarker of rabbit's physiological response to elevated temperatures. A significant increasing effect of season on blood plasma testosterone was observed in NZW rabbits in Egypt with higher value during the summer as against lower value during spring [9]. Similarly[10], observed higher testosterone levels in goats during the summer and attributed it to influence of photoperiod or day length on hypothalamus pituitary axis. However in terms of nutrition,[11] have indicated that changes in testosterone levels in rams was not correlated to dietary protein treatment levels.

Basically, biochemical indices were shown to be sensitive to biological alterations and or nutritional inadequacy of the diets fed to animals[12]. As such, the biochemical blood components could be directly or indirectly be influenced by the quantity and quality of feed. Thus, were implicated as indicators of protein quality in feeds, since proteins are feed constituents that could affect the formation of blood and or plasma constituents. As it has been shown that the blood composition of amino acids would depend on quality and quantity of protein consumed/ingested and this is related to anabolic process in rabbits [13].

In addition, dietary Selenium supplementation has been shown to be important for selenoenzyme activity, since it enhances the antioxidant actions of certain enzymes such as glutathione peroxidase, catalase and superoxide dismutase[14]. However, the relationship between blood chemistry profiles and nutrients intakemay indicate the limits of benefitiallevels of such nutrients and their consequent dose effects.Literally, the biochemical response to change in ambient temperature may depend on individual animal tolerance ability, species, physiological status, period of blood sampling and duration of exposure. Therefore, the aim of the study was to evaluate the effects of dietary protein and selenium supplementation on biochemical parameters in New Zealand White male rabbits under tropical conditions.

II. MATERIALS AND METHODS

The research was carried out atthe 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) with an annual rainfall of 1100mm, which falls from April to October. Meteorological data (ambient temperature and relative humidity) of the study area was recorded throughout the experimental period (April 2012 to September 2012) using a digital thermo-hygrometer (Mextech TM-1, China).The temperature and relative humidity was measured at two different times (8.00hr and 14.00hr) daily, within the building and outside, and monthly average was computed using basic statistics (table 1).

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

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

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The temperature-humidity index (THI) was considered as an explanatory factor and computed (table 1) using the standard formula by[15], as shown:
THI = t - {(0.31 - 0.31RH)(t - 14.4)}
t – temperature (^{o}C) in Celsius
RH - relative humidity (%) in percentage/100

Study design
The design of the experiment was 3x 2 factorial in a complete randomized form(CRB). This comprised three levels of dietary protein and two levels of dietary selenium.

Experimental diet
The experimental diets (Table2) were formulated based on [16]. All ingredients were procured/purchased in livestock feed industry/shops, then assembled, ground, mixed and formulated in a standard feed mill and bagged at before placing/ distributing to the animals in their respective treatment groups. Six experimental diets were prepared which constitute three isocaloric dietary treatments with background Se (0.4mg/kg DM) and the other threeisocaloric dietary groups with additional organic Se at 0.3mg/kg DM.

Table 2: Ingredient composition of the experimental diet

	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.

Selenium inclusion (0.3 mg/kg DM) was determined by experimental treatment.

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The proximate chemical analyses (Table 3) of the experimental diets (A[140g/kg], B[180g/kg]& C[220g/kg]) were done according to[17] methods. The metabolisable energy was calculated using [18] method. This analysis was conducted prior to the commencement of the feeding trial, in order to determine the specific nutritive value of the experimental diets.

Table 3 : Proximate/Chemical Analysis of the Experimental Diets

	Dietary treatments		
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

Values are means of three determinations ± SD

Experimental animals and design

A total of 48 male New Zealand White [NZW] rabbits, with mean age of 23±1.414 weeks and weighing 2.8±1.131 kg were used for the experiment. The animals were blocked by initial live-weight and randomly allocated to one of six iso-caloric diets (Figure 1). Animals were housed individually in conventional rabbit cages with floor dimension of 1.2m x 0.8m, each equipped with feeder and drinkerin a naturally well ventilated building.

Blood Sample Collection

Blood samples were taken at monthly intervals from four bucks in each dietary treatment group. Prior to bleeding a cotton swab soaked in 70% ethanol/ xylene was used to dilate the ear vein and to prevent infection. Each blood sample comprised of 2ml blood taken between 11.00-13.00hr at every sampling. Blood samples were taken from the marginal ear vein by venipuncture using micro syringes (2ml) fitted with needle, and sterile cotton was used to cover the site following collection. Samples for biochemistry were collected into tubes without anticoagulant, and samples for haematology were collected into tubes treated with either disodium salt of ethylene di-amine tetra-acetic acid (EDTA) or with lithium heparin. Sampling was done between April and September,at 6 sequential monthly time points over the period of the study. Immediately after collection samples were taken to the laboratory for analyses.

Biochemical analyses

Blood samples were taken to the laboratory within 2hrs of collection and centrifuged at 3000 rpm for 10 minutes to separate serum/ plasma from the cells. Following centrifugationthe plasma fraction was decanted, stored that is

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refrigerated at 2-8^{o}C for maximum period of five days or stored at -20^{o}C for up to 30 days until analysis, using routine clinical methods[19].

The concentrationsof plasma hormones Testosterone(TST),Thyroxine (T_4), Triiodothyronine (T_3) and Cortisol (CORT) were determined by Enzyme Link Immuno Assay (ELISA) kit (MonobindIncLake Forest, CA 92630 USA). This is a quantitative determination of hormonal concentration in serum or plasma by using a Microplate Enzyme Immunoassay (Type 5). The principle of the test is based on competition between the specific amount of enzyme-antigen conjugate/ horseradish peroxidase and serum native antigen/ native hormone sampleantigen, for a limited number of antibody combining/ binding sites on the well[20].

The Elitech clinical analyser (SEPPIM S.A.S-Zone Industrielle 61500 SEES FRANCE) was used for determination of Total protein (TP), Albumin(ALB), Alkaline phosphatase(ALP), Cholesterol(CHOL),Malondialdehyde (MDA), Aspartate aminotransferase(AST) and Glutathione peroxidase(GSH-Px).

Haematological analyses

Haematocrit (HCT) was determined using Wintrobes micro-haematocrit.
Red Blood Cells (RBC) counts and White blood cells (WBC) count were determined using improved Neubauer haemocytometer.
Haemoglobin (Hb) concentration was determined using cyano-methemoglobin method.
White blood cell differential counts monocytes (Mc), lymphocytes(Lt) and neutrophils (Nt) were determined according to method by [21, 19], based on percentage as a fraction of the WBC.
Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were computed according to standard formulae[21].

Data analysis

The data obtained were analysed by ANOVA using Minitab GLM procedure. This was conducted to determine the main effects (3 levels of dietary proteins & 2 levels of dietary selenium) and 6 periods of time, as well as their respective interactive effects. Differences between treatment means were determined by Tukey pairwise comparison.

III. RESULTS

The results of period on biochemical and haematological parameters are shown in tables 4 and 5 respectively.

Effects of THI / period

Circulating concentrations of AST were significantly different between the months (P=0.001), such that values increases with advancing time up to the third month. However regression analysis indicated that these changes were not affected by THI (R^2= 0.13, P = 0.001).
Similarly there were significant differences between months in the concentration of T_3. These values were seen to decline with each successive month (P = 0.001) and regression analysis indicated that they were not related to changes in THI (R^2 = 0.17, P= 0.001). Therefore these changes are most probably due to temporal changes rather than temperature dependent changes.

Plasma concentrations of CHOL were seen to be significantly different between different periods (P = 0.001), and regression analysis indicated no relationship with THI (R^2 = 0.06, P= 0.003).
There were significant differences in CORT concentrations between months (P=0.011), but contrary to expectation these variations could not be explained by THI effects (R^2 = 0.02, P= 0.05) implying other unknown factors.
There were differences in GSHPx concentrations between months (P=0.022), but changes were unrelated to changes in THI. Other biochemical parameters were not affected by THI.
There were significant differences in the levels of MCV between the months (P=0.001), with highest value in the second month which decreased linearly until the fourth month .However, regression analysis indicated that these changes in MCV could not be attributed to THI as (R^2= 0.068, P= 0.027). There were no other effects of month on any other haematological parameter and all values were within normal range.

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Table 4: Effects of period on biochemical traits in male rabbits fed dietary protein and selenium supplements

Parameters	Months(Period) 1	2	3	4	5	6	SEM	P value
AST(IU/L)	15.96^{c}	16.54^{c}	19.13^{abc}	16.67^{bc}	19.75^{ab}	21.16^{a}	0.783	**0.001**
ALT(IU/L)	23.83	31.50	34.79	45.46	36.58	35.69	6.635	0.344
ALP(IU/L)	62.75	66.33	66.88	69.46	66.04	66.49	1.648	0.137
TP(g/L)	70.08	69.75	68.58	70.88	68.25	68.67	1.111	0.510
ALB(g/L)	40.54	40.83	39.50	40.71	39.46	39.06	0.783	0.455
T_3(µg/dl)	1.53^{a}	1.47^{a}	1.37^{ab}	1.30^{ab}	1.19^{b}	1.13^{b}	0.065	**0.001**
T_4(ng/dl)	72.58	66.83	71.54	70.25	94.42	70.02	10.946	0.517
CHOL(mmol/L)	2.25^{b}	2.37^{ab}	2.59^{a}	2.37^{ab}	2.47^{ab}	2.55^{a}	0.059	**0.001**
TST(µg/dl)	7.22	7.46	7.62	8.03	7.20	7.01	0.345	0.348
CORT(nmol/L)	132.7^{b}	144.0^{ab}	150.4^{a}	140.0^{ab}	143.2^{ab}	149.1^{a}	3.654	**0.011**
GSHPx(IU/L)	47.54^{ab}	50.25^{a}	50.12^{ab}	49.21^{ab}	46.21^{b}	49.29^{ab}	0.964	**0.022**
MDA(IU/L)	1.38	1.39	1.37	1.43	1.46	1.35	0.044	0.491
SOD(IU/L)	2.13	2.17	2.11	2.22	2.28	2.26	0.160	0.301

^{ab}Means with different superscripts in the same row are significantly different (P<0.05)

Table 5 Effects of period on haematological traits in male rabbits fed protein and selenium supplements

Parameters	Months(Periods) 1	2	3	4	5	6	SEM	P value
HCT(%)	40.21	39.41	38.85	39.30	38.79	39.44	0.573	0.542
Hb(g/dl)	12.62	12.92	12.75	12.86	12.67	12.74	0.184	0.858
MCHC(%)	32.57	32.60	33.28	32.65	32.80	37.01	1.788	0.467
RBC(x10^{6}/µl)	5.73	5.71	5.86	5.80	5.84	5.81	0.107	0.915
MCH((g/dl)	23.38	23.07	22.80	22.80	23.21	23.14	0.195	0.224
MCV((1x10^{-15})	70.16^{a}	70.35^{a}	69.47^{a}	65.71^{b}	68.83^{a}	68.84^{a}	0.735	**0.001**
PLTS(x10^{3}/µl)	218.5	187.9	247.3	231.3	237.6	202.2	17.485	0.149
WBC(x10^{3}/µl)	8.95	9.33	8.78	8.95	9.21	8.28	0.373	0.442
Lc(%)	72.03	70.61	70.75	69.83	69.66	70.63	1.105	0.711
Nt(%)	16.35	16.35	15.81	15.77	14.53	15.71	0.665	0.417
Mc(%)	11.13	11.12	10.49	11.37	10.20	9.99	0.502	0.277

^{ab}Means with different superscripts in the same row are significantly different (P<0.00

Effects of protein

The results on effect of protein on biochemical and haematological parameters are presented in tables 6 and 7.

There were significant differences in circulating CORT concentrations between dietary protein levels (P=0.037), However the values on 22% protein level were relatively lower, but there was no distinguishable pattern..

There were no statistical differences in the circulating TST concentrations between dietary protein levels. However, TST concentrations are numerically lower in the 14% protein diet (P= 0.072) when compared to the two higher protein levels.

Although no statistical differences were observed in concentrations of SOD between dietary protein levels , marginal differences in values were noted such that values were lower in the 14% protein diet (P= 0.078).

MCV differed significantly between dietary protein levels (P=0.006), with values decreasing linearly from the low dietary protein level to the high dietary protein level. These changes was confirmed by regression analysis (R^2= 0.705, P = 0.251)..

There were significant differences in the percentage of neutrophils (Nt) between dietary protein levels (P=0.005); the highest Nt value was seen at 18% protein and was lower but similar between 22 and 14% protein levels. This difference

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may not be connected to dietary protein as indicated by regression analysis (R^2 = 0.062, P= 0.112), but probably as a consequence of other unidentified factors that could not have been controlled.

Although the evidence was marginal, RBC counts were seen to increase linearly with increased dietary protein concentrations (P=0.058), suggesting that the RBC were partially affected by dietary protein. As this was confirmed by regression analysis (R^2=0.996, P= 0.028). Other biochemical and haematological parameters were not affected by dietary protein.

Effects of selenium

There were differences in the circulating concentrations of ALP between the dietary treatment levels, where ALP values were higher (P= 0.007) in the non-supplemented group when compared to the Se supplemented group. Circulating concentrations of CHOL were numerically lower in the Se supplemented group when compared to the non-supplemented group. However this difference failed to achieve statistical significance (P=0.098).

There were differences between the Se supplemented and unsupplemented groups such that platelets (PLTS) concentrations were higher in the unsupplemented group when compared to the Se supplemented group (P=0.011). Other biochemical and haematological parameters observed were not affected by the dietary Se.

Table 6: The effect of either dietary protein or supplementary selenium on the biochemical traits of male rabbits

	Protein concentration (g/100g)					**Selenium**			
Parameters	**14**	**18**	**22**	**SEM**	**P val.**	**NSe**	**Se**	**SEM**	**P value**
AST(IU/L)	18.46	18.33	17.81	0.554	0.683	18.63	17.77	0.452	0.186
ALT(IU/L)	39.46	33.26	33.21	4.691	0.432	37.82	31.42	3.831	0.243
ALP(IU/L)	65.15	66.89	66.94	1.165	0.465	68.17[a]	64.48[b]	0.951	**0.007**
TP(g/L)	68.65	69.44	70.02	0.785	0.462	69.67	69.07	0.641	0.511
ALB(g/L)	39.60	40.03	40.42	0.554	0.583	40.46	39.58	0.452	0.170
T_3(µg/dl)	1.31	1.37	1.32	0.046	0.563	1.34	1.33	0.037	0.955
T_4(ng/dl)	69.73	82.84	70.25	7.740	0.404	70.26	78.28	6.320	0.371
CHOL(mmol/L)	2.42	2.47	2.41	0.042	0.641	2.48	2.40	0.034	**0.080**
TST(µg/dl)	6.96	7.77	7.52	0.243	**0.072**	7.51	7.33	0.199	0.532
CORT(nmol/L)	144.5[ab]	147.2[a]	138.0[b]	2.584	**0.037**	144.9	141.6	2.110	0.267
GSHPx(IU/L)	49.12	48.27	48.92	0.681	0.653	48.88	48.66	0.556	0.789
MDA(IU/L)	1.39	1.41	1.39	0.0311	0.797	1.39	1.40	0.025	0.852
SOD(IU/L)	2.11	2.25	2.21	0.045	**0.078**	2.23	2.15	0.037	0.114

[ab]Means with different superscripts in the same row are significantly different (P<0.05)

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Table 7 The effect of dietary protein or supplementary selenium on the haematological traits of male rabbits

	Protein concentration (g/100g)					Selenium			
Parameters	**14**	**18**	**22**	**SEM**	**P value**	**NSe**	**Se**	**SEM**	**P value**
HCT(%)	39.19	39.86	38.94	0.405	0.261	39.13	39.53	0.331	0.389
Hb(g/dl)	12.71	12.98	12.58	0.130	0.095	12.67	12.85	0.106	0.233
MCHC(%)	35.19	32.66	32.60	1.265	0.258	32.63	34.34	1.032	0.246
RBC($x10^6$/µl)	5.66^b	5.80^{ab}	5.92^a	0.076	**0.058**	5.81	5.77	0.062	0.589
MCH(/dl)	23.01	23.20	22.99	0.138	0.497	23.09	23.05	0.1126	0.794
MCV($1x10^{-15}$)	69.74^a	69.43^a	67.52^b	0.520	**0.006**	69.33	68.46	0.425	0.152
PLTS($x10^3$/µl)	214.8	229.2	218.4	12.36	0.693	239.3^a	202.3^b	10.095	**0.011**
WBC($x10^3$/µl)	8.54	9.35	8.86	0.264	0.096	9.04	8.79	0.216	0.404
Lc(%)	70.91	70.27	70.58	0.781	0.848	70.04	71.13	0.638	0.228

[ab]Means with different superscripts in the same row are significantly different (P<0.05)

IV.DISCUSSION

Effects of period

Alkaline phosphatase

The significant effect of period on AST concentration indicated variations across different months. AST is an enzyme that catalyses the transamination of amino acids, and higher levels in the blood can be indicative of changes in liver function, probably due to physiological alterations or rather adjustments. The changes in AST may not be attributed to changes in THI, but some physiological disorder due to probably blood collection timing, enzymatic and metabolic functions.[1]has shown that quality of protein in diets may be responsible for changes in AST. In addition slight mouldin diets due to aflatoxins when the humidity is high has been shown to alter physiological functions in domestic animals including rabbits[22].Since, it was indicated that low protein diets could elicit certain physiological stress, due to metabolic demand for amino acids to maintain nitrogen balance for the body system [23].In contrast, changes in AST had been shown to be considered as a consequent action of cortisol on gluconeogenesis in the liver[3].Generally changes in serum enzymes activities seems to be correlated to biological functions of metabolically active organs and also levels of plasma proteins.

Triiodothyronine

Circulating concentrations of Triiodothyronine (T_3)declined with advancing period. Triiodothyronine is an iodine containing hormone that regulates growth, metabolism and body heat. The gradual decrease in T_3 concentration in this study was not related to changes in THI, suggesting other effects of other unidentified environmental and physiological factors. However the changes in T_3 observed in this study falls within normal range.Similarly, [3] have shown that no effect of high ambient temperature on T_3concentrationin ruminants, rather changes in T_3 may be associated with carbohydrate or protein content of the diet fed. Conversely, several scholars implicated T_3 in regulating body metabolism[3 ,24] indicating that T_3 is known as metabolic and thermogenic hormone, composition of diet and or changes in body temperature has important effect on its functional activities.

Cholesterol

Cholesterol (CHOL) is a component of cell membranes that stabilizes the phospholipid, and is alsoinvolved in the synthesis of all steroids hormones. The values were seen to change as time advanced. However these changes observed in CHOL as time advanced even though indicated significant differences between months, could not be accounted for by THI as indicated by regression analysis. Possibly,physiological adjustments due to advancing age,and or other metabolic alterations such as changes in total body fluid and cortisol as well as acetate concentration. Considering the fact that, the values observed were slightly above normal range (2.1mmol/L).

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Cortisol

Cortisol (CORT) is an anti-anabolic hormone produce by the adrenal gland and the level in blood can be elevated during stress response. Cortisol concentration varied significantly as time advanced. Contrary to expectations, the level of cortisol observed seems not be related to THI, as indicated in the regression analysis. Similarly it has been shown that CORT concentration was not affected THI when rams were exposed to high ambient temperature in Egypt[3].Therefore this variation may be as a result of environmental and other physiological alterations that were not identified such as high humidity, dietary metabolism and neuroendocrine functions.In addition the elevation in CORT during the first month may be attributed to fright as a consequence of initial blood sampling process. Therefore, cortisol could elicit physiological adjustments that may enable the animal to tolerate abnormalconditions. As such the activation or stimulation of hypothalamic-pituitary-adrenal axis and consequent changes in plasma cortisol and other blood metabolites are some of the inevitable responses of animals to stressful conditions.

Mean corpuscular volume

The significant effect of period on MCV indicated a particular shortfall at the fourth month as compared to all other periods. Although, the lower concentration observed at the fourth month fell within normal range, all other periods indicated values slightly above normal range (67 $x10^{-15}$) (Merck, 2012). However, these changes were not attributed to THI, but possibly due to some other environmental factor. According to[5], lower MCV valueswere observe in rabbits during winter and spring, but noted increased MCV values during summer when the temperatures were high, and attributed it to decreased salt content within the body fluid, as a consequence of dehydration, possibly due to rapid respiration. Hence blood metabolites may change, due to changes in body electrolytes, possibly as a result of increased respiratory rate. Hence, haematological parameters could be considered as good biomarkers of rabbit's physiological state/response to elevated ambient temperatures, although[3] did not observe any effects of season or changes in ambient temperature on haematological indices of sheep. Marai attributed the lack of any effect to be most likely due to species variations, the intensity of stress as well as the duration of exposure to heat stress. In addition, other factors like nutrition, age and depth of hair coat could be indicators on the expression of activities of these cells on the haematological indices in the body.

Effects of protein

Cortisol

Cortisol concentrations differed significantly between the three dietary proteins with high level at 18g/100g as compared to other dietary levels. Although no particular trend observed. However, it has been shown that low protein diets could elicit certain physiological stress in both human and animals, due to metabolic demand for amino acids to maintain nitrogen balance for the body system [23], and sustenance of normal body functions. As such, this biological response could be responsible for the increased cortisol levels when the level of dietary protein is low, and vice versa. In addition, it has been shown that adequate protein intake and utilization may enhance normal hormonal and enzymatic secretions or functions, with consequent optimum metabolic functions in rabbits[5]. Hence, there may be very low levels of cortisol in body circulation of group of animal under high protein dietary treatment, due to absence of stress. However, cortisol concentration was also affected by period, but there may be possible increase in metabolic heat in the body, as dietary protein level has been shown to enhance the production of metabolic heat in the body[26]. Therefore feeding protein diets at appropriate levels may be an important aspect of dietary modification in controlling stressful conditions in animals.

Testosterone

Testosterone is a steroid hormone produced in the leydig cells of the testis and serves as a reproductive and anabolic hormone. Testosterone concentrations were unaffected by dietary protein. However, values indicated slight variations, with low concentrations at 14g/100g dietary protein level, when compared to 18g and 22 g/100. These marginal differences in testosterone concentrations may be attributed to the anabolic functions of testosterone which may be involved in protein utilization[27]. Since it has been shown that, the production and secretions of testosterone as well as its specific functions could be influenced by several factors such as level/type of nutrition, season and age[28]. Hence, protein quantity and quality of feed has been shown to possibly have a direct or indirect influence on body biochemical components[13].

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Superoxide dismutase

Concentrations of SOD seem to change with varying dietary protein concentrations, with marginal variations. However, these changes could not be accounted for by the dietary treatments, but followed similar fashion with changes in cortisol and testosterone concentrations observed in this study. SOD has been implicated in antioxidant functions in Bufallos[2] andtherefore may require adequate protein concentrations in diets, to flow in body circulation its normal functions. Since,it had been implicated in protecting the internal cellular constituents from oxidative damage[15].

Neutrophils

There were significant effects of dietary protein treatment on the concentration of neutrophils. Neutrophils are types of WBC classified as granulocytes that functions as defence agents in the body against infections and or inflammations due to stress or injury. Higher concentration of Nt was observed in rabbits receiving 18g/100g dietary protein level, when compared to other dietary levels, whereas 22g/100g may probably increase response to alkaline condition due to possible accumulation of ammonia in the caecum, when excess dietary protein are fed to rabbits. Since ammonia was shown to be the major end product of protein metabolism in the caecum, and which contributes to elevation of caecal pH (Fraga, 1998). However, the levels of Nt observed in this study were fractionally below the normal range (20-75% x 10^3 µl)[25]. This reduced value of Nt, may be an indication of absence of damage/ inflammation to tissues or organs. Since high concentrations of Nt in body circulation, had been shown to be associated to stressful conditions as a response to inflammation and or infection [8].

Mean corpuscular volume

The MCV were significantly affected by dietary protein level as MCV concentrations decreased with ascending dietary protein. However, whereas MCV values on 22g/100g dietary protein treatment group were within the normal range. The other two groups had MCV concentrations marginally above the upper published limit of 67 $x10^{-15}$ [25]. However, these small differences are not indicative of any adverse pathology (i.e. anaemia) suggesting that protein levels were at an appropriate level to meet the requirements of the animal.

In addition, changes in MCV volume observed on respective dietary protein treatment groups, seems to be inversely proportional to the changes in red blood cells concentrations, as the dietary protein concentrations increased. Since these two parameters are inherently related..

Red blood cells

The red blood cells counts were significantly affected by dietary protein level; counts increased with ascending dietary protein level, although all values fell within normal ranges[25, 19]. Hence the levels of dietary protein supplemented, seems to provide optimum performance devoid of any adverse effects on red blood cells concentration.

Effects of selenium

Alkaline phosphatase

ALP concentrations were affected by dietary Se treatment, decreasing as dietary Se concentration decreased. ALP is a blood metabolite widely distributed in the liver, intestinal epithelium and kidneys [7], hence may corresponds to high activity of alkaline pH in body system. However, the values observed in this study are within normal range. Though it has been shown that,there was an induction of antioxidant effect of Se via SOD particularly in the liver[15]. In addition the significant reduction in ALP concentrations as reflected in Se supplemented group of the rabbits, could be an indication of growing process. As ALP is always elevated in young animals that have yet reach maturity, hence it is likely this was affecting concentrations more than the Se. Since the rabbits used in this study are within their growing phase.

Cholesterol

The concentration of cholesterol decreased marginally,with lower values on Se supplemented group, although not statistically significant.

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Platelets

Platelets are indicators of blood clotting capability. ThePLT concentration were affected by dietary Se treatment; values decreased as the level of dietary Se increased. However values on both groups fell within normal published range (112-795 x 10^3/µL). This would suggest that, lower levels at higher Se concentrations might not have any adverse effect on the animals.

V. CONCLUSION

Temporal effects on AST, CHOL and CORT as time advanced were eminent, even though these effects or changes were not significant. However, the changes seen between successive months followed no discernible pattern and seemed to be unrelated to changes in THI. This would indicate that other physiological and environmental factors may have played a role. The effects of protein and/ or selenium supplementation on most aspects of biochemistry were limited, although mild dietary protein influence was evident on concentrations of CHOL, CORT and MCV. Whereas no effect of dietary Se on all the parameters. In addition the changes in T_3 relayed a linear trend possibly as regulatory mechanism on some biological functions, since this plasma hormone was not affected by dietary treatments. However, there were numerical differencesmost of the parameters were within normal range. Therefore the unsupplemented or rather background Se (0.3mgSe/kg diet) and 22g/100g dietary protein level is adequate for optimum performance of the male rabbits in sub-humid tropical climate.

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