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INFLUENCE OF DIETARY CRUDE PROTEIN ON TESTIS SIZE, HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN RABBIT BUCKS EXPOSED TO TRANSIENT NEONATAL GOITROGEN

*Hammed, Opeyemi Oladipupo[1]; Amao, Olajide Abraham[2]; Olugbade Babatunde Oluwaseun[2]; Imran, Tesleem Omotayo[3]; Popoola, Abimbola Deborah[1]; Rom-Kalilu, Fiwasade Adejoke[1]; Togun, Mary Eniola[2]; Okanlawon, Eden Olusegun[1]

[1]Animal Production and Health Department, Ladoke Akintola University of Technology (LAUTECH), P.M.B. 4000, Ogbomoso, Nigeria.

[2]Animal Nutrition and Biotechnology Department, Ladoke Akintola University of Technology (LAUTECH), P.M.B. 4000, Ogbomoso, Nigeria.

[3]Physiology Department, College Medicine, University of Lagos, Lagos, Nigeria.

*Corresponding Author

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ABSTRACT

An experiment was conducted to investigate the influence of dietary crude protein on testicular morphometrics, haematological and serum biochemical parameters of growing male rabbits exposed to transient neonatal goitrogen. Sixty-three (63) male rabbit neonates were transiently exposed to goitrogen through suckling of their mothers' milk. The neonates were weaned after 5 weeks and 10 rabbits were randomly assigned to each of three dietary treatments (T1-16% CP (Control); T2-14% CP (Low) and T3-18% (High) crude protein) in a Completely Randomized Design. Animals were fed with the experimental diets from the 5th to 13th weeks of age. Five animals per treatment were humanely sacrificed on the 13th week. Blood samples were collected for haematological and serum biochemical parameters. Testes were harvested for testicular morphometric evaluation (Testes weight, length, width and volume). The data were analyzed using one-way analysis of variance. Rabbits fed with high crude protein diets had significantly ($p<0.05$) higher test is weight, length and width than those fed lower dietary crude protein. The haematological and serum biochemical variables fell within the normal physiological range for

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male rabbits. In conclusion, 18% dietary crude protein increased testicular morphometrics in rabbits exposed to transient neonatal goitrogen without posing health risk to the animals.

Keywords: Testis, Haematology, Serum, Rabbit, Bucks, Neonates, Goitrogen, Crude-protein.

1.0 INTRODUCTION

There is the need to evaluated and explore viable options in order to maximize food production and meet the protein requirements in Nigeria and world at large [1]. Among such alternatives is the use of livestock species that are yet to play a major role in animal production, especially in the developing countries of the world. Fast-growing livestock such as rabbits possess a number of features that might be of advantage in the small holder, subsistence and integrated farming. Nigeria and other developing countries are faced with myriad of problems, which have resulted in a gross shortage of meat in order to meet up their ever-growing population [2].

Reproductive inefficiency is one of the most limiting constraint to efficient rabbit production in the tropics [3]. The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs, and chemicals [4]. Gage and Freckleton [5] reported that the testis size is directly related to the age of the animal and testicular weight is related to sperm production.

Transient neonatal goitrogen treatments in rat pups, induced by adding a reversible goitrogen to the mothers' water from birth to day 25, resulted in adult testis size and sperm production of about 80% and 140%, respectively [6, 7, 8, 9]. This goitrogen treatment is effective only when begun during neonatal life, suggesting that the treatment alters an early postnatal process to eventually produce the observed increase in testis size and sperm production [8,10]. Furthermore, rabbits have been found to give high reproductive performance when fed concentrate feeds [11]. The major nutritional requirements in tropical rabbit production are protein and energy. Protein however, plays a very significant role in the animal body, therefore must be appropriately provided for in the diet. All living cells have protein as one of their principal constituents. Protein has an essential association with reproduction processes [12].

There are limited of information on the manipulation of testes morphometric which will invariably enhance sperm production rates in farm animals. One of the interventions is the manipulation of the nutrient status of animals especially the dietary crude protein level as it has been reported to play a vital role in cell tissue growth and reproduction processes of rabbits [12].

This research work was necessitated to establish a relationship between crude protein level in rabbit feeds and the testicular morphometrics. Also, to provide some basic technical information

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on the effect of dietary crude protein on testis size in rabbits exposed to transient neonatal goitrogen.

2.0 MATERIALS AND METHODS

The experiment was carried out at the Rabbit Production and Research unit, Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso.

Forty-five (45) mature rabbit does (with average age of 9 months) were procured from a reputable breeding farm in Ogbomoso, Oyo State, Nigeria. The does were separately housed in wooden cages for a period of two weeks for physiological adjustment. They were dewormed using Ivermectin injection (administered subcutaneously at 0.15 ml per kilogram body weight of rabbit) against potential ecto and endo-parasites. The does were afterward mated repeatedly (twice in a day per doe) at a mating ratio of 1bucks to 3 does.

2.1 Experimental Procedure

Preliminary study

Animals were fed with concentrate diet appropriate for pregnant does (i.e. diet containing 18% crude protein and 2500 kcal/kg metabolizable energy in the morning, while forage comprising *Tridaxprocumbens* was offered in the evening until kindling. After first week of kindling, goitrogenic compound (Carbimazole) was administered to forty two of the does for the next 21 days (i.e. these does received a constant daily oral dose of 5mg/1kg bodyweight /day of antithyroid drug (Carbimazole) dissolved in 120cl of their drinking water from the 7^{th} to 28^{th} day of lactation) while the remaining three does were not introduced to goitrogen treatment. Kits were sexed in both groups and the experimental schedule commenced on the male kits.

Five male kits were randomly picked, from both litter groups .i.e. the goitrogen treated does group and the group of does without goitrogen treatment. They were dosed with chloroform and blood samples were collected through cardiac puncture into bottles free from any anticoagulant. They were centrifuged at 1000 r.p.m for 10 minutes to obtain the plasma. Glass beads were introduced into the plasma to coagulate the fibrinogen to give the serum. The sera were assayed for Thyroid Stimulating Hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) so as to confirm the establishment of goitrogen treatment.

2.2 Experimental treatments

Sixty-three (63) weaned male rabbits with prior-goitrogen treatment were selected and randomly divided into 3 groups of twenty one (21) rabbits each in a completely randomized design. Group A (T1) which was the control group were fed pelletized diet containing 16% crude protein.

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Group B (T2), Group C (T3) were fed pelletized diets of 14 and 18% crude protein (CP), respectively. The 3 experimental diets were restricted to 100 g/rabbit/ day.

2.3 Data Collection

Three rabbits were randomly selected and humanely sacrificed from each group at the end of the12-week feeding trial. Testes were carefully dissected from the sacrificed animals and trimmed off adhering tissues. Testis length, testis width and testis volume were measured. The testis length and testis width were measured with the aid of a pair of vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle [13].

2.4 Data Collection

Data obtained were analyzed by One-way analysis of variance using SAS (2003). Separation of mean was accomplished by Duncan's Multiple Range Test of the same statistical software.

Table 1: Gross Composition of experimental diet

Feed Ingredients	T1 Control-16% CP	T2 14% CP	T3 18% CP
Maize	48.61	54.70	42.55
SBM	16.39	10.34	22.46
BDG	15.00	15.00	15.00
Rice husk	15.00	15.00	15.00
Fish meal (72%)	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25
Vitamin premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Lysine	0.15	0.15	0.15
Methionine	0.10	0.10	0.10
TOTAL	**100.00**	**100.00**	**100.00**

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Determined Nutrients			
CF (%)	9.74	9.47	10.01
CP (%)	16.02	14.04	18.01
Metabolizable Energy (Kcal/kg)**	3190.83	3236.58	3146.59

***Vitamin premix**: Supply per kg diet: 2 000 000 iuvit. A; 400 000 iu D_3; 8.0 g vit. E; 4 g vit. b_1; 1.0 g vit. B_2; 0.6 g vit.; 0.4 mg vit. B_{12}; 24.0 g Niacin; 0.2 g Folic acid; 8.0 g Biotin; 48.0 g Choline; 320.0 g BHT; 16.0 g Manganese; 8.0 g iron; 7.2 g] Zinc; 0.32 copper; 0.25 iodine; 36. 0 mg cobalt; 16.0 mg selenium. ** Metabolizable Energy calculated using Pauzenga, (1985)

3.0 RESULTS

Testicular morphometrics of rabbits fed dietary crude protein after exposure to transient neonatal goitrogen is shown on Table 1. All the testicular morphometrics were significantly ($p<0.05$) influenced. Left and right testis weights were significantly ($P<0.05$) higher for T3 than the T2 which were also significantly ($P<0.05$) higher than T1. Similarly, a significant ($P<0.05$) variation was observed in both the left and right testis length among the treatments. Left and right testis width was significantly ($P<0.05$) higher at T3 while it was significantly ($P<0.05$) lower at T2 compared with the control treatment. The result further revealed a significant ($P<0.05$) decrease in the left and right testicular volume of animals at T2 while a significant ($P<0.05$) increase was observed at T3 when compared with the control treatment.

Table 2: Effect of dietary crude protein on the overall testicular morphometrics of rabbits exposed to transient neonatal goitrogen.

Parameters	T1(Control-16% CP)	T2- (14% CP)	T3- (18% CP)	±SEM
Weight (g)				
Left testis	0.35^b	0.29^c	1.07^a	0.13
Right testis	0.35^b	0.30^c	1.08^a	0.12
Length (cm)				
Left testis	3.09^b	2.51^c	3.50^a	0.46
Right testis	3.26^b	2.65^c	3.64^a	0.46
Width (cm)				
Left testis	0.42^b	0.35^c	0.53^a	0.09
Right testis	0.50^b	0.40^c	0.60^a	0.05
Volume (ml)				
Left testis	1.07^a	0.96^b	1.11^a	0.22
Right testis	1.09^a	0.98^b	1.12^a	0.22

abc: Means on same row with different superscripts differ significantly ($P<0.05$)
SEM: Standard Error of Mean

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The haematological parameters of rabbits fed varied dietary crude protein after exposure to transient neonatal goitrogen are shown in Table 2. The results showed no significant (P>0.05) influence in Red blood cell, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red blood cells distribution width coefficient of variation (RDW-cv), Red blood cells distribution width standard deviation (RDW-sd), Mean Platelet Volume (MPV) and Platelet Distribution width (PDW). Significant (P<0.05) decrease was however observed at T2 in Haematocrit (HCT), Haemoglobin (Hb) and White Blood Cell (WBC). Significant (P<0.05) decrease was also observed at both T2 and T3 in Platelets (PLT), Lymphocyte, Minimum Inhibitory Dilution (MID), Plateletcrit (PCT).

Table 3: Haematological parameters of rabbits fed dietary crude protein after exposure to transient neonatal goitrogen.

Parameters	T1 (Control-16% CP)	T2- (14% CP)	T3- (18% CP)	±SEM
HCT (%)	35.10^{a}	30.03^{b}	37.67^{a}	1.19
Red blood cell (10^9/l)	3.73	3.98	4.83	0.24
Hb (g/dl)	10.90^{ab}	9.87^{b}	11.73^{a}	0.32
White blood cell (10^9/l)	4.20^{b}	4.67^{b}	7.03^{a}	0.47
PLT (10^9/l)	226.67^{a}	169.67^{b}	103.33^{c}	7.23
Lymphocyte (%)	68.33^{a}	60.33^{b}	59.16^{b}	1.50
MID (%)	10.97^{a}	6.70^{c}	8.40^{b}	0.66
Granulocyte (%)	32.70	33.77	32.43	0.69
MCV (μ^3)	87.20	88.97	88.60	1.38
MCH (µµg)	28.40	29.97	28.9	0.44
MCHC (%)	31.93	32.23	31.13	0.34
RDW-cv (%)	13.50	13.40	14.17	0.27
RDW-sd (fL)	42.17	42.10	42.77	0.84
MPV(fL)	8.63	8.67	8.90	0.08
PDW (%)	15.77	16.00	15.70	0.08
PCT (%)	0.20^{a}	0.14^{c}	0.19^{b}	0.09

abc: Means on same row with different superscripts differ significantly (P<0.05) SEM: Standard Error of Mean; Mean corpuscular volume (MCV); Mean corpuscular haemoglobin (MCH) Mean corpuscular haemoglobin concentration (MCHC); Red blood cells distribution width coefficient of variation (RDW-cv); Red blood cells distribution width standard deviation (RDW-sd); Haematocrit (HCT); Haemoglobin (Hb); Mean platelet volume (MPV); Platelet distribution width (PDW); Platelets (PLT), Lymphocyte; Minimum inhibitory dilution (MID); Plateletcrit (PCT)

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The serum biochemistry of rabbits fed varied dietary crude protein after exposure to transient neonatal goitrogen is shown in Table 3. Alanine aminotransferase (ALT), Albumin, Serum protein and Creatinine were not significantly (P>0.05) influenced. Significant (P<0.05) decrease was however observed at T2 in Aspartate aminotransferase (AST) and urea.

Table 4: Serum Biochemistry of Rabbits fed Varied Dietary Crude Protein after Transient Neonatal Goitrogen Exposure.

Parameters	T1 (Control-16% CP)	T2- (14% CP)	T3- (18% CP)	±SEM
AST (IU/I)	40.00[a]	34.50[b]	48.77[a]	2.55
ALT (IU/I)	17.43	15.73	15.54	0.63
Urea (mg/dl)	8.78[a]	6.59[ab]	5.78[b]	0.53
Albumin (g/dl)	4.10	4.10	4.31	0.06
Serum protein (g/dl)	5.31	5.36	4.50	0.20
Globulin (g/dl)	2.49	2.41	2.40	0.08
Creatinine (mg/dl)	81.84	71.21	69.08	3.90

ab: means on same row with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

4.0 DISCUSSION

The results obtained from this study indicate that 18% CP had an optimal effect on the testicular morphometrics of rabbits exposed to transient neonatal goitrogen. In the same light, 14% CP had a sub-optimal effect in the overall testicular morphometrics of the rabbits exposed to transient neonatal goitrogen. The optimal performance of rabbits at T3 could possibly be due to the influence of transient neonatal goitrogen on testicular size. Goa *et al.* [7] have suggested that when rodents were neonatally induced into hypothyroid condition and were allowed to recover back to euthyroid state, a significant increase in testis size and daily sperm production would be observed in adulthood. The reason for the sub-optimal influence of 14% CP on the overall testicular morphometrics could be linked to the report of Akande, [12] that lower dietary protein adversely affects reproductive processes in rabbits.

Blood is an important index of physiological, pathological and nutritional status in the organism [14, 15]. Egberongbe [16] indicated that Red Blood Cell (RBC) is most constantly affected by dietary influence. In this study, RBC values across the treatments were still within the normal physiological range reported by Moore *et al.* [17]. The insignificant influence of dietary CP in majority of the haematological parameters shows that dietary crude protein after exposure to

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transient neonatal goitrogen did not affect the blood parameters in male rabbits. Moreover, all the parameters were still within the normal physiological range for rabbits [18, 19, 20].

The AST mean values obtained from this study were within the normal physiological range of (17-98 IU/l) as reported by [21]. Archetti *et al*. [21]. Kaneko *et al.* [22] stated that aminotransferases are usually intracellular enzymes with low levels found in the plasma representing the release of cellular content during normal cell turnover. The ALT and AST levels according to Kaneko *et al.* [22], are elevated in nearly all liver diseases and particularly high in conditions that can cause extensive cell necrosis indicating severe hepatitis or toxic injury. The values for the serum urea and globulin obtained for all the treatments are also within the normal physiological range of (9.1-25.5 mmol/l) and (1.5-3.3g/dl), respectively as reported by [18, 19, and 20].

5.0 CONCLUSION

The results obtained from this study revealed that 18% dietary crude protein optimally increased testicular morphometrics in rabbits exposed to transient neonatal goitrogen. It also stabilized the haematological and serum biochemical parameters of the rabbits. It can therefore be inferred from this study that dietary crude protein significantly influenced the reproductive traits in rabbits exposed to transient neonatal goitrogen with 18% CP having an optimal impact without posing health threat, since the haematological and serum biochemical parameters determined were within the normal physiological range for rabbits.

REFERENCES

[1] Owen, O.J., Alawa, J.P., Wekhe S.N., Isirimah N.O., Chukuigwe E.C., Aniebo, A.O., Ngodigha, E.M. and Amakiri A.O. (2008): Incorporating poultry litter in animal feed: a solid waste management strategy. *Egyptian Journal of Animal Production* (In press)

[2] Nworgu, F.C. (2007). Economic importance and growth rate of broiler chicken served fluted pumpkin (*Telfariaoccidentalis*) *African Journal of Biotechnology*, 2:6 34-39.

[3] Gbadamosi, A.J. and Egbunike, G.N. (1999). Studies on cock semen (1) Effects of frequency of ejaculation and breed on physical characteristics. *Tropical Journal of Animal Science*.1: 157-164.

[4] Togun, V.A. andEgbunike, G.N. (2006). Seasonal variations in the sperm production characteristics of Zebu (white Fulani) cattle genitalia in the humid tropical environment. *Middle-East Journal of Science and Research,* 1: 87-95.

[5] Gage, M.J.G. and Freckleton, R.P. (2003). Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm

length. *Proceedings of the Royal Society London. B.*270:625–632. http://doi.org/10.1098/rspb.2002.2258

[6] Rijntjes, E., Swarts, H. J., Anand-Ivell, R., and Teerds, K. J. (2009). Prenatal induced chronic dietary hypothyroidism delays but does not block adult-type Leydig cell development. *Annual Journal of Physiology, Endocrinology and Metabolism.* 296: 305–314. https://doi:10.1152/ajpendo.90750.2008

[7] Gao, Y., Lee, W. M., and Cheng, C. Y. (2014). Thyroid hormone function in the rat testis. *Front. Endocrinol.* 5:188. doi: 10.3389/fendo.2014.00188

[8] Picut, C. A., Remick, A. K., De Rijk, E. P., Simons, M. L., Stump, D. G., and Parker, G. A. (2015). Postnatal development of the testis in the rat: morphologic study and correlation of morphology to neuroendocrine parameters. *Toxicol. Pathol.* 43: 326–342. https://doi:10.1177/0192623314547279

[9] O'Donnell, L., Smith, L.B. and Rebourcet, D. (2022). Sertoli cells as key drivers of testes formation. *Seminars in Cells and Developmental Biology*. 121:2-9. https://doi.org/10.1016/j.semcdb.2021.060

[10] Tharwat, E.E., sabra, M.M., Hussein, A.F. and Hanna, H.M. (2015). Impact of propythiouracil on testes function and semen characteristics of New Zealand White rabbits. *Egyptian Journal of Animal Production*. 52(2): 135-143.

[11] Obinne, J.I. (2002). Energy and protein requirements of rabbits in the humid tropics of southern Nigeria. PhD thesis presented to Department of Animal Science, University of Nigeria, Nsukka.

[12] Akande, K.E. (2015).The Requirements of Protein and Amino Acids in Rabbit Nutrition and Production. *Case Studies Journal*, 4(4): 34-38

[13] Adu, O.A and Egbunike, G.N. (2010). Fertility, Semen quality and Reproductive organ weights of boars fed dietary copper. *Journal of Applied Agricultural Research*, 2: 61-76

[14] Olorode, B.R, Adeniran, R.A and Abiola, J.O (2007). Effect of graded levels of Moringa olifera seed meal on haematological values and organ weight of broiler chicken. *Tropical Journal of Animal Science.* 10: 63-67

[15] Ewuola E.O, Egbunike G.N. (2008). Hematological and serum biochemical response of growing rabbits bucks fed different levels of dietary fumonisin B_1*AfricanJournal of Biotechnology*. 7, 4304-4309

[16] Egberonge O, Aletor V.A. (1992). Feeding differently processed soya bean. Part 2: An assessment of haematological responses in chicken. *Die Nahrung*. (36): 364-369.

[17] Moore, M.D., Zimmerman, K. and Smith, A.S. (2015). Haematological assessment of pet rabbits: Blood sample collection and blood cell identification, Veterinary and Clinicals in Exotic Animals, 18: 9-19. http://dx.doi.org/10.1016ij.cvex.2014.09.003

ISSN: 2455-6939

Volume: 10, Issue: 02 "March-April 2024"

[18] Mitruka, B.M. and Rawnsley, H.M (1981). Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals. Masson Pub. USA Inc., N.Y. pp 104-106.

[19] Kerr, M (1989). Veterinary Laboratory Medicine. Clinical Biochemistry and Haematology. Blackwell Scientfic Publications. 22: Pg. 11-13

[20] Jones, R.T. (2005). Normal values for some biochemical constituents in rabbits. *Laboratory Animal*. 9(2): 143-147

[21] Archetti, I., Tittarelli, C., Cerioli, M., Brivio, R., Grilli, G. and Lavazza, A.(2008). Serum chemistry and hematology values in commercial rabbits: preliminary data from industrialfarms in northern italy. In: *Proceedings of the 9th World Rabbit Congress*, 10-13 June 2008, Verona, Italy. Brescia: Edito a Cura Della Fondazione Iniziative Zooprofilattiche e Zootecniche, pp. 1147-1152.

[22] Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (2008). *Clinical biochemistry of domestic animals*. 6th ed. Orlando: Academic Press.

