



HAEMATOLOGICAL AND SERUM BIOCHEMICAL RESPONSES OF RABBIT BUCKS TO QUANTITATIVE FEED RESTRICTION AND FEEDING TIME

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Abstract

The experiment was carried out to evaluate the effects of feed restriction, feeding time and their interaction on haematological and serum profile in rabbit bucks. Eighteen (18) rabbit bucks were used for the study. The rabbit bucks were fed 75.00, 67.50 and 60.00g commercial growers' pellets to represent 100, 90 and 80% daily ration respectively. The eighteen rabbit bucks were divided into two groups of nine (9) bucks each. The first group was fed in the morning while the second group was fed their daily ration in the evening. Bucks fed 100% daily ration served as control. The study adopted a 2 x 3 factorial arrangement in completely randomized design (CRD). At the end of the 4th week of the experiment, blood samples were collected from each replicate for determination of hematological and serum biochemical indices. Data obtained were subjected to General linear model (GLM) procedure in a completely randomized design, using IBM Statistical Package for Social Science (SPSS) version 21. The result showed that feed restriction affected ($p < 0.05$) PCV, WBC, neutrophils, lymphocytes and platelets of rabbit bucks in the study. Feeding time significantly influenced ($p < 0.05$) PCV, neutrophils and lymphocytes. Restricted feeding significantly affected ($p < 0.05$) total protein, creatinine and serum enzymes. There was significant interaction between feed restriction and time of feeding on AST and ALT of the bucks fed 90% daily ration. In conclusion, feeding rabbit bucks 90% of their daily ration in the evening improves most haematological and serum biochemical parameters without compromising the health of the animals.

Keywords: feed restriction, feeding time, serum biochemistry, haematology

Introduction

Rabbits are small animals that can be produced optimally by using mixture of forage and formulated feeds, and according to Adewole et al. (2018), can survive on all forage diet. Empirical study by Chodova et al. (2016), has shown that feed restriction in growing rabbits influences the growth of rabbits by enhancing feed efficiency and reducing carcass fat (Adewole et al. 2018). Chodova et al. (2016) in their study also noted that the effect of feed restriction explicitly hinge on timing of its beginning, its duration and its intensity. The physiology of farm animals is affected by several factors, one of which is nutrition (Ajao et al. 2013). Nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes and can be determined by either or combinations of chemical, anthropometric, biochemical, or dietary methods as stated by Bamishaiye et al. (2009). Additionally, Chodova et al. (2014), stated that feeding regime is one of the major factors that



influence meat quality of farm animals. Addass et al. (2012), in their study also stated that nutrition affects blood values of animals: Earlier report by Dukes in Isaac et al. (2013), also documented that haematological values of farm animals are influenced by nutritional status. An evaluations of haematological profile according to Ihedioha et al. (2004), furnishes vital information on the nutrients contained in the diets fed to animals. As stated by Onwujiariri (2020), the liver biochemical parameters of a species serve as a marker in monitoring health status of the animal.

This study was therefore designed to evaluate the effect of quantitative feed restriction and feeding time on haematological and serum biochemical indices in rabbit bucks.

Materials and Method

Experimental Site

The study was carried out at the Rabbitry unit of Teaching and Research Farm of the Department of Animal Science, University of Uyo, Uyo, Akwa-Ibom State. Uyo is located at 50 1'N; 70 35'E with a mean annual temperature of between 26°C and 28°C while the mean annual rainfall ranges from 2000mm – 3000mm (Solomon and Udoh 2017).

Experimental Animals and Management

Eighteen (18) clinically sound mongrel rabbit bucks were purchased and used for this study. The rabbit bucks were allowed for two weeks of acclimatization period during which they were fed with commercial growers' pellets and forages (*Calopogonium*) ad-libitum. Before the commencement of the experiment, the rabbits were treated against internal and external parasites with ivermectin injection and a broad spectrum antibiotic (Oxytetracycline L.A). The rabbits were managed intensively in a wired rabbit hutch. The experimental period was 28 days (4 weeks).

Experimental Diets

The rabbit bucks were fed 75.00, 67.50 and 60.00g commercial growers' pellets to represent 100, 90 and 80% daily ration respectively. *Calopogonium mucunoides* was given to the bucks ad-libitum after wilting for 24 hours, since rabbits are basically foragers.

Table 1. Nutrient Composition of the Commercial Growers pellet fed to the bucks

Ingredients	Composition
Crude protein (%)	15.00
Carbohydrates	5.00
Crude fibre (%)	8.00
Calcium (%)	1.00
Available phosphorus (%)	0.35
Lysine (%)	0.60
Methionine (%)	0.35
Metabolizable Energy (Kcal/Kg)	2600

Experimental Design

The eighteen rabbit bucks were divided into two groups of nine (9) bucks each. The first group were fed in the morning while the second group were fed their daily ration in the evening. The bucks were randomly assigned to the three (3) different levels of feed restriction, and replicated three (3) times in both morning and evening group respectively. Bucks fed 100% daily ration served as control in this study. The study adopted a 2 x 3 factorial arrangement in completely randomized design (CRD).

Blood collection and Analysis

Haematological Profile

At the end of the 4th week of the experiment, blood samples were collected from each replicate for determination of hematological profile. A total of 2.0ml of blood was collected through the external ear veins of the bucks into labeled sterile universal bottle containing 1.0mg/ml ethyl diamine tetraacetic acid (EDTA) as anticoagulant to determine the haematological component in the blood. Haematological parameters analyzed were packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell counts (WBC), red blood cell counts (RBC), neutrophil, lymphocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Serum Biochemistry

Another 3ml blood was collected into labeled sterile sample bottles without anticoagulant to determine the biochemical components. All blood samples were collected in the morning before feeding (between 7.00 am to 9.00 am) and transported immediately to the laboratory for analysis. For serum biochemical analysis, blood samples collected into plain tubes without anticoagulant were centrifuged at 3000 rpm for 10 min, the serum was collected and kept at 20°C until analysis.

Proximate Composition

Proximate analysis of the experimental diets was carried out according to the method of AOAC (2000) to determine the dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract (NFE) contents.

Statistical Analysis

The experimental data were subjected to General linear model (GLM) procedure in a completely randomized design, using IBM Statistical Package for Social Science (SPSS) version 21. Differences between treatment means were separated using Duncan multiple Range Test.

Results and discussions

Effect of Restricted Feeding on Haematological Profile of Rabbit Bucks

The effect of restricted feeding on haematological profile of male rabbits is presented on Table 2. The result showed that feed restriction affected ($p < 0.05$) PCV, WBC, neutrophils, lymphocytes and platelets of rabbit bucks in this study while other parameters such as RBC, haemoglobin (HB), MCV, MCH and MCHC were not significantly affected ($p > 0.05$) by feed restriction. Etim et al. (2014), assessed the effects of nutrition on haematology of rabbits and stated that the physiology of farm animals is influenced by several factors, one of which is nutrition. Packed cell volume (PCV), was significantly higher in bucks fed 90% of their daily ration when compared with bucks fed 100 and 80% of their daily ration. According to Adeyemi (2014), PCV is the ratio of red blood cells to the whole blood volume expressed as a percentage. PCV values observed in this study were 35.30, 36.00 and 33.00% for bucks fed 100, 90 and 80% of their respective daily ration. These values are within the range of 30.00 – 50.00 g/dl recommended by Mitruka and Rawnsley (1977) and 9-19.4(g/dl) by Burke (1994). The PCV is an easily obtained measure for detecting anaemia or polycythemia and can be useful in estimating changes in haemodilution or haemoconcentration (Brian et al. 2000). This result

hence, imply that the bucks did not suffer from anaemia, dehydration nor polycythemia in the course of the study.

Red blood cells (RBC) were not statistically significant ($p > 0.05$) in the study. The respective similar values of 5.30, 5.45 and 4.75 $\times 10^{12}/L$ for 100, 90 and 80% restrictions were recorded in the study. According to Adeyemi (2014), all energy in RBCs that is devoted to maintaining cell shape, membrane structure, enzymatic functions, reduced iron in haemoglobin and other functions do so to optimize oxygen delivery to tissues. She added that RBCs have no nuclei and no organelles, and thereby no ability to synthesize proteins, they are cells full of haemoglobin and made in the bone marrow. Reduction in the haemoglobin may be accompanied by a fall in the RBC as stated by Akinmutimi et al. (2004). As reported by Adejumo (2004), haematological traits, especially, PCV and Hb were correlated with the nutritional status of the animal.

Table 2. Effect of feed restriction and feeding time on haematological profile in rabbit bucks

Factors	PCV (%)	RBC ($10^{12}/L$)	WBC ($10^9/L$)	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	NEUT (%)	LYM (%)	Platelets ($10^9/L$)
Feed Restriction										
100.00%	35.50 ^{ab}	5.30	7.00 ^{ab}	12.2	67.00	23.00	34.50	35.50 ^b	60.50 ^a	149.50 ^c
90.00%	36.50 ^a	5.45	8.60 ^a	12.45	67.50	23.00	34.50	38.00 ^{ab}	58.50 ^{ab}	188.50 ^b
80.00%	33.00 ^b	4.75	5.35 ^b	11.35	69.50	23.50	34.50	39.00 ^a	57.00 ^b	212.50 ^a
SEM	2.79	0.79	0.26	1.82	2.43	1.82	1.76	3.92	3.69	44.51
P-Value	0.02	0.81	0.05	0.62	0.11	0.88	1.00	0.03	0.03	0.00
Feeding Time										
Morning	33.67 ^b	4.83	7.53	11.60	69.67 ^a	23.67	34.67	35.33 ^b	60.33 ^a	192.33 ^a
Evening	36.33 ^a	5.50	6.43	12.40	66.33 ^b	22.67	34.33	39.67 ^a	57.00 ^b	174.67 ^b
SEM	0.67	0.33	0.21	0.94	0.67	2.21	0.34	4.34	0.54	5.88
P-Value	0.015	0.493	0.266	0.413	0.004	0.310	0.730	0.001	0.004	0.000
Interaction										
Feed Restriction X Feeding time	0.237	0.887	0.811	0.853	0.032*	0.695	0.619	0.002*	0.001*	0.000*

^{a,b,c} Mean in the same column with different subscripts are statistically significant ($p < 0.05$); SEM – Standard error of mean; PCV – packed cell volume, RBC – red blood cells, WBC – White blood cells, HB – haemoglobin, MCV – Mean cell volume, MCH – Mean cell haemoglobin, MCHC – Mean cell haemoglobin concentration, NEUT – neutrophils, LYM – lymphocytes

WBC was significantly higher ($p < 0.05$) in bucks fed 90% ($8.60 \times 10^9/L$) while 100 and 80% had similar ($p > 0.05$) WBC values of $7.00 \times 10^9/L$ and $5.35 \times 10^9/L$ respectively. The higher WBC observed in bucks fed 90% feed restriction suggests better immunity (Adeyemi 2014). Robert et al. (2003), earlier reported that the higher the value of WBC, the better the ability of the animal to fight diseases. There are five basic white blood cell types: Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes (Teske 2010). Animals with low WBC are exposed to high risk of infection while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan et al. 2013).

Haemoglobin, MCV, MCH and MCHC were not significantly influenced ($p > 0.05$) in this study. Adeyemi (2014), noted that haemoglobin has a central role in physiology by binding, transporting, and delivering oxygen to tissues. The values in this study were within the normal range of 9-19.4(g/dl) recommended by Burke (1994). Thus, the oxygen binding, transport and final delivery to tissues was not compromised by feed restriction. MCV values recorded in the course of the study were 6.00, 67.50 and 69.50fl while MCH and MCHC were 23.00, 23.00 and 23.50pg; and 34.50, 34.50 and 34.50g/dl respectively for bucks fed 100, 90 and 80% of their

daily intake. The average MCH and MCHC values of the bucks were all within the recommendations of Mitruka and Rawnsley (1977). This therefore signify no anaemic condition in the bucks during the study.

The study observed significant increase ($p < 0.05$) in neutrophils in bucks fed 80% (39.00) daily ration while 100 and 90% restriction levels were the same statistically ($p > 0.05$), with values of 35.50 and 38.00% respectively. Adeyemi (2014), noted that neutrophils are the most common of the WBCs and serve as the primary defense against infection. When neutrophil number in the blood reaches a critically low level, animals are highly susceptible to bacterial infection (Weiss et al. 2010). According to Adeyemi (2014), the typical response to infection or serious injury is an increased production of neutrophil, and further added that immature forms of neutrophils are seen early in the response to infection. The presence of these immature cells can be the earliest sign of a WBC response, even before the WBC becomes elevated (Adeyemi 2014). Values in this study were however, within the recommendation of Mitruka and Rawnsley (1977).

Lymphocytes on the other hand were significantly higher ($p < 0.05$) in bucks fed 100% (60.50%) than in bucks whose feed were reduced to 90% (58.50%) and 80% (57.00%) of their normal daily ration. Blood platelets decreased significantly ($p < 0.05$) as the level of daily ration decreases. Platelet was higher (212.50) in 80% restriction and lower (149.50) in 100% restriction, while 90% restriction was 188.50.

Effect of Feeding Time on Haematological Profile of Rabbit Bucks

Feeding time significantly influenced ($p < 0.05$) PCV, MCV, neutrophils, lymphocytes and platelets while, RBC, WBC, HB, MCV, MCH, and MCHC were not affected. Rabbit bucks fed in the morning (33.67%) had lower PCV than those fed in the evening (36.67%). These values are however, within the normal range suggested by Mitruka and Rawnsley (1977), and showed that the bucks were not anaemic nor suffered polycythemia in the course of the study. The increase in PCV in bucks fed in the evening suggests improved protein intake and tissue synthesis because a decline in PCV has been associated with protein synthesis inhibition and suppressed immune system (Ebegbulem 2018). The RBC, WBC and HB of bucks fed in the morning were 4.83 and 5.50 x 10¹²/L; 7.53 and 6.43 x 10⁹/L; and 11.60 and 12.40g/dl respectively for bucks fed in the morning and evening respectively. The MCV of the bucks varied significantly ($p < 0.05$) and was higher (69.67fl) in bucks fed in the morning than those fed in the evening (66.33fl). According to Isaac *et al.* (2013), RBC is involved in the transport of oxygen and carbon dioxide in the body implying that, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs and vice versa when RBC count increases. MCH and MCHC were not affected by time of feeding. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) indicate blood level conditions (Chineke et al. 2006).

Neutrophils was lower in bucks fed in the morning while lymphocytes and platelet on the order hand, were higher in bucks fed in the morning. Adeyemi (2014), reported that increased numbers of lymphocytes are seen in most viral infections, some bacterial infections, and some cancers while decreased numbers of lymphocytes are seen in steroid exposure, some cancers, immunodeficiency and renal failure. Despite these variations, all values were within normal ranges recommended by Burke (1994) and Mitruka and Rawnsley (1977). An increase in WBC count and differentials suggest anaemia, infection and leukemia (Holland 2013). Both the WBC count and WBC differential can provide clues as to why farm animals have elevated or low white blood cell numbers.

Effect of Interaction between Restricted Feeding and Feeding Time on Haematological Profile of Rabbit Bucks

There was interaction between feed restriction and time of feeding on MCV, neutrophils, lymphocytes and platelets in the study. Bucks fed 80% of their daily ration showed higher MCV than those fed other levels of restriction and time. Neutrophils was increased significantly in bucks fed 80% daily ration in the evening. The values in this study were 39.00 and 39.67 for 80% restriction and feeding in the evening. Lymphocytes were 60.33 and 60.50% for bucks fed 100% ration in the morning. Blood platelets were significantly higher ($p < 0.05$) in bucks fed 80% (212.50) daily ration in the morning (192.33).

Effect of feed restriction on serum biochemistry in Rabbit Bucks

The result on effect of feed restriction on serum biochemistry in rabbit bucks is presented on Table 3. Restricted feeding significantly affected ($p < 0.05$) total protein, creatinine, aspartate alanine transferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT). Blood glucose and urea were not significantly ($p > 0.05$) affected by feed restriction. Blood glucose recorded in the study were 4.50, 5.30 and 6.60mg/dL for 100, 90 and 80% feed restriction levels respectively. Rabbit bucks fed 90% daily ration recorded significantly higher ($p < 0.05$) total protein than those fed 100 and 80% respectively. Bucks on 90% restriction had 56.50g/L total protein while bucks on 100 and 80% recorded values 50.50 and 49.00g/L. Serum creatinine was significantly elevated ($p < 0.05$) in bucks fed 100% (94.00 $\mu\text{mol/L}$) daily ration when compared to bucks fed 90% (90.50 $\mu\text{mol/L}$) and 80% (89.00 $\mu\text{mol/L}$) restriction levels. Blood urea was statistically the same ($p > 0.05$) in all restriction levels. The presence of these enzymes at a high level in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood (Adeyemi 2014). Rabbit bucks fed 90% daily ration recorded the highest AST, ALP and ALT. Values in the study were 50.50 iu/L, 33.50 iu/L and 24.00 iu/L respectively. The values were within the normal range recommended by Mitruka and Rawnsley (1981). The activities of both aspartate amino transferase (AST) and alanine amino transferase (ALT) are high in tissues especially liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood (Srivastava and Chosdol 2007).

Effect of feeding time on serum biochemistry in Rabbit Bucks

Feeding time significantly affected ($p < 0.05$) blood glucose, total protein, creatinine and urea. Serum enzymes, AST and ALT were significantly affected by time of feeding. The higher total protein observed in bucks fed in the evening suggests a better utilization of protein in the diet offered irrespective of the restriction level (Iyaode et al. 2020), which further implies higher levels of digestion, assimilation and utilization of the feed offered by the bucks. Iyaode et al. (2020), added that normal levels of blood protein and glucose indicate adequate nutritional status and normal systemic protein function. Blood glucose was higher ($p < 0.05$) in bucks fed in the evening (6.70mg/dL) than in those fed in the morning (4.23mg/dL). Rabbit bucks fed in the morning had lower total protein those fed in the evening. The values observed were 50.67 and 53.33g/L for bucks fed in the morning and evening respectively.

Serum creatinine was significantly increased ($p < 0.05$) in bucks fed in the evening (95.00) than in those fed in the morning (87.33 $\mu\text{mol/L}$). Blood urea was higher ($p < 0.05$) in bucks fed in the evening (9.20mmol/L) than in the morning (6.03mmol/L). Serum AST was higher in bucks fed in the evening than in bucks fed in the morning. Rabbit bucks fed in the morning had the same ($p > 0.05$) ALP. Serum ALT was higher in bucks fed in the morning than in those fed in the evening.

Effect of Interaction between Feed Restriction and Feeding Time on Serum Biochemistry in Rabbit Bucks

There was significant interaction ($P < 0.05$) between feed restriction and time of feeding in his study on blood glucose, total protein, creatinine, AST, ALP and ALT. However, blood urea did not show significant interaction between feed restriction and time of feeding. Rabbit bucks fed 80% in the evening had higher blood glucose (6.60 and 6.70 mg/dL respectively). Total protein was higher in bucks fed 90% restriction in the evening (56.50 and 53.33). Low or high total protein is an indication of liver disorders and malnutrition (Augustine et al. 2020). Serum creatinine was observed to be higher in bucks fed 100% (94.00 $\mu\text{mol/L}$) daily ration in the evening (95.00 $\mu\text{mol/L}$). Urea did not show any significant interaction between feed restriction and time of feeding. This implies minimal damage to the kidney of bucks fed 100% daily ration in the morning, since urea and creatinine are important markers of kidney function (Goji et al. 2023).

There was significant interaction between feed restriction and time of feeding on AST and ALT of the bucks fed 90% daily ration. Bucks fed 90% daily ration in the morning had average AST values of (50.50 and 51.67 iu/L) and ALT values of (24.00 and 21.00 iu/L). According to Alagbe (2019), serum enzymes values are triggered by the presence of antinutrients or toxic substances in the feed of an animal. Burnett et al. (2006), reported that husbandry practices had effect on creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase and calcium levels.

Table 3. Effect of feed restriction and feeding time on serum biochemistry in rabbit bucks

Parameters	Glucose (mg/dL)	Total Protein (g/L)	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/L)	AST (iu/L)	ALP (iu/L)	ALT (iu/L)
Feed Restriction							
100.00%	4.50	50.50 ^b	94.00 ^a	7.80	42.50 ^b	32.50 ^a	21.00 ^b
90.00%	5.30	56.50 ^a	90.50 ^b	7.50	50.50 ^a	33.50 ^a	24.00 ^a
80.00%	6.60	49.00 ^b	89.00 ^b	7.55	29.50 ^c	25.50 ^b	12.00 ^c
SEM	1.55	2.84	6.94	1.81	3.03	2.00	3.29
P-Value	0.226	0.000	0.003	0.962	0.000	0.000	0.000
Feeding Time							
Morning	4.23 ^b	50.67 ^b	87.33 ^b	6.03 ^b	51.67 ^a	30.33	21.00 ^a
Evening	6.70 ^a	53.33 ^a	95.00 ^a	9.20 ^a	30.00 ^b	30.67	17.00 ^b
SEM	0.48	2.71	5.22	0.82	2.79	0.82	1.71
P-Value	0.023	0.015	0.000	0.006	0.000	0.730	0.001
Interaction							
Feed Restriction X Feeding time	0.051*	0.000***	0.000***	0.861	0.000***	0.012**	0.035*

^{a,b,c} Mean in the same column with different subscripts are statistically significant ($p < 0.05$); SEM – Standard error of mean; AST – Aspartate aminotransferase, ALP – Alkaline phosphatase, ALT – Alanine aminotransferase, * = significant; ** = very significant; *** = highly significant

Conclusions

The result in this study showed that feed restriction and feeding time can significantly affect some blood parameters such as PCV, WBC, neutrophils, lymphocytes, total protein, creatinine and liver function enzymes. There was significant interaction between quantitative feed restriction and feeding time on both haematological and serum biochemical indices in rabbit

bucks. Hence, feeding rabbit bucks 90% of their daily ration in the evening can improve most haematological and serum biochemical parameters without compromising the health of the animals.

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