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PHYSIOLOGY AND BROMATOLOGY OF *Oryctolagus cunicullus* L. 1758 FED BROWSE LEGUME WITH ENZYME LEVELS

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ABSTRACT

The objective of this experiment was to determine the effects of feeding rabbits with Maxigrain® (M) enzyme supplemented Gliricidia sepium leaf meal (GLM) on their physiology, performance characteristics, and nutrients digestibility. Twenty weaned rabbits of mixed sexes, 5-6 weeks old, were randomly allotted to five dietary treatments including 0 g M which was soybean without M (control) and GLM supplemented with M at 50, 100, 150 and 200 g M per kilogramme of GLM. There were four rabbits per treatment and one rabbit as replicate in a completely randomized design. There were no significant ($p > 0.05$) treatment effects in all physiological and performance indices as well as those of crude fat, fibre and NFE digestibilities. There were variations ($p < 0.05$) due to the treatment effects on dry matter, crude protein and ash digestiblities. This implies that the feeding of soft faeces directly from the caecum called coprophagy mechanism in rabbits has its concomitant nutritional benefits. This advantageous benefit can enable rabbit to effectively and efficiently utilize forage or forage-based diet with or without enzyme supplementation.

Key words: Gliricidia, maxigrain enzyme, New Zealand red, utilization

INTRODUCTION

Egbo *et al.* (2001) reported that rabbits are efficient converters of feed to meat and utilize up to 30% fibre as against 10% by most poultry species. Thus, the daily weight gain of rabbit is high in proportion to the body weight which gives them a rapid growth rate before sexual maturity. Thus, they attain a high weight at sexual maturity 30% faster than other animals (Ajayi *et al.,* 2005). The rabbits *(Oryctolagus cuniculus*) are pseudo-ruminants, raised widely in developing countries. Its production contributes to improve nutrition and economy. The advantage of rabbit as a source of animal protein has been identified by Amaefule *et al.* (2004). Domestic rabbit is raised as a cheap source of meat due to its economic way of feeding, high prolificacy and small body size that makes it suitable for backyard rearing and easy consumption by a family, as well as extra income by sale of animals (Iyeghe-Erakpotobor *et al.,* 2013).

Rabbits are recommended as good alternative source of accessible animal protein source and seem to be suitable to solve a part of this lack of readily available, affordable and accessible quality animal proteins. Rabbits are highly prolific and have short generation intervals (John *et al.,* 2017). Rabbit meat has high biological value and contains with crude protein (21%), fat (10%), low cholesterol, low sodium (0.25 mg g^{-1}) and higher proportion of linoleic and linolenic fatty (John *et al*., 2017). Consequently, rabbit meat has been listed as an approved meat source for the hypertensive. The high and increasing prices for animal feeds have compelled researchers to direct their attention to non-conventional feed sources, with particular emphasis on protein substitutes (Gadzirayi *et al.,* 2012; Ogungbesan *et al*., 2013a). The use of leguminous multipurpose trees and shrubs has been suggested to be a viable alternative source of proteins, vitamins and minerals for poultry feeding. Plant leaves are commonly processed into leaf meals for use as poultry feed (Tijani *et al*., 2017). Examples of the leaf meals which have been widely used in feeding non-ruminant animals include *Leucaena leucocephala*, *Gliricidia sepium, Sesbania sesban*a nd *Manihot esculenta* (Ogungbesan *et al*., 2013a; Tijani *et al*., 2017).

The benefits of adding enzymes to diets of non-ruminant animals particularly rabbit have become more common in recent years (El-Katcha *et al.,* 2013). Current developments in this area include digestibility of starch and non-starch polysaccha-rides in forages. On the other hand, most of the trials that were performed during the last decade could not detect any significant effect of enzymes on rabbit's performances. The only exception was the decrease in mortality found with proteases and proteases + xylanases (probably reducing protein flow to the caecum). It is interesting to note that in some trial's enzymes improved fibre digestibility. Although rabbits are better able to digest phytic phosphorus than poultry, they are not equal to

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ruminants in this regard. In a trial of Gutiérrez *et al*. (2000), exogenous phytases improved not only the utilization of phosphorus but also increased nitrogen digestibility. According to these authors, phytases can be useful in rabbit diets. The digestive system of the rabbit is characterized by the relative importance of the caecum and colon when compared with other species (Blas and Wiseman, 2010). As a consequence, the microbial activity of enzymatic breakdown and fermentation ingesta of the caecum is of great importance for the processes of digestion and nutrient utilization, but also in the control of digestive pathologies (Blas and Wiseman, 2010).

Furthermore, caecotrophy, the behaviour of ingestion of soft faeces of caecal origin, makes microbial digestion in the caecum more important for the overall utilization of nutrients by the rabbit (Blas and Wiseman, 2010). This peculiar phenomenon in rabbits may make them to be less responsive than other animals to supplementation with exogenous enzymes. Therefore, the present research work was designed to investigate the effect of supplementation of multienzyme (Maxigrain®) with *Gliricidia sepium* on nutrient utilization, physiology and performance of growing rabbit.

MATERIALS AND METHODS

Site Description

The experimental site was at the Rabbit Unit of the Department of Animal Production, College of Agricultural Sciences, Olabisi Onabanjo University, Isa-Ope Campus, Ayetoro, located within 6º 55′ to and 7º 15′ N and 2º 45′ to 3º 5′E, and on an altitude 90-120 m asl. Climate/vegetation are of sub-humid forest mosaic savanna type with an annual rainfall of 1,945.3 mm. Rainy season is between early April and late October. Rainfall pattern is bimodal with two peaks in June and September. Maximum temperature varies at 29°C during the peak of the wet season and 34°C at the onset of the wet season and the mean relative humidity is 72.81% while evaporation is 1806.9 mm. According to FAO (2006) soil classification, the soil is of the Oxic Paleustalf, with sand, silt and clay contents of 75.16%, 9.25% and 15.57%, respectively (texture, sandy loam). Certain contents expressed in cmol kg^{-1} included Na^{+} 50, K^{+} 22, Ca^{++} 95, Mg^{++} 86, H^{+} 0.13, and CEC 2.67. Others were pH in H_2O 5.55, organic C 7.9 g kg^{-1}, total N 0.79 g kg^{-1}, P 6.86 mg kg^{-1}, and Zn 6.75 mg kg^{-1}, The entire area is made up of undulating surface, which is drained mainly by River Rori and River Ayinbo (Ogungbesan *et al.,* 2013b).

Feed Preparation

Fresh, young *Gliricidia sepium* leaves were harvested, air-dried for several days and then milled to obtain *Glricidia* leaf meal (GLM) and incorporated into five rabbit diets in which Maxigrain® was added at 0 (control), 50, 100, 150 and 200 g per tonne of diet 1, 2, 3, 4 and 5, respectively.

Hutches Preparation

Hutches with dimension 90 cm × 60 cm × 45 cm were used for the experiment and swept, dusted and fumigated with Dettol®. Two containers were provided for feed and water.

Experimental Animals

Twenty New Zealand red rabbits of mixed sexes, aged 5-6 weeks, were weighed individually, and randomly assigned to the five dietary treatments with four rabbits per treatment and one rabbit per replicate (Kurtong, 2014; Amata and Okorodudu, 2015). The initial weights of the rabbits ranged from 762 to 1065 g. The animals were managed according to the provisions of International Guideline Principles of Biomedical Research involving animals (CIOMS, 1985). Prior to the experiment, the rabbits were treated against internal and external parasites by subcutaneous injection of Ivomec at dosage rate of 0.2 ml per rabbit. Also, a broad spectrum antibiotics (Oxytetra- cycline LA - long acting) was administered at the rate of 0.2 ml per rabbit. The rabbits were housed singly in cages in two rows of hutches. The hutches were raised approximately 80 cm from the concrete floor. The stands were immersed in insecticidal solution to prevent crawling insects from getting to the animals. The rabbits were subjected to one week acclimation period, during which commercial growers mash and water were supplied *ad libitum*. The rabbits were provided with weighed amount of experimental diets (1-5) and clean water *ad libitum* for 12 weeks (Iyeghe-Erakpotobor *et al*., 2013).

Physiology

Physiological parameter measurements (rectal with ear temperature, respiratory and pulse rate) were taken at weekly intervals. Rectal temperature was measured by inserting a digital thermometer (Hartman-United Kingdom) to a depth of ca. 4 cm into the rectum for 1 minute. The ear temperature was measured by placing the digital thermometer in direct contact with the central area of the auricle. Respiratory rate was measured by visually counting the number of movements of the flanks of the rabbits in a resting position for 1 minute with a stopwatch while pulse rate was measured by counting the heart sounds for one minute using a stethoscope. (Iyeghe-Erakpotobor *et al*., 2013).

Performance Characteristics

Daily feed intake were collected by subtracting the left over from the feed given to each rabbit, while live weight changes were calculated as the difference between initial weight and the final weight for each week. Feed conversion ratio (FCR) was calculated as the ratio of the feed intake to the weight gain and the reverse for feed efficiency ratio (FER) (Ogungbesan *et al*., 2013a)

Table 1: Maxigrain Composition (1 g)

Content weight	Function
Cellulase, 10000 IU	Break down cellulose for more energy and release of locked nutrients
Beta-glucanase, 200 IU	Degrade the non-starch polysaccharides (NPS) in feed compensating for the anti-nutritive effect and increasing nutrient utilisation for maximum feed performance
Xylanase, 10000 IU	Degrade the non-starch polysaccharides (NPS) in feed compensating for the anti-nutritive effect and increasing nutrient utilisation for maximum feed performance
Phytase, 2500 IU	Efficient release of bound phosphorus from plant phytate and also liberates minerals and amino acids

Digestibility Trial

During the metabolism trial rabbits were housed in individual metabolism cage made of welded wire mesh fitted with feeders and arranged for quantitative collection of faeces and urine separately, total faeces and urine output were collected in the morning before feeding and watering. A 7-day collection period of faeces daily from each rabbit in addition to samples of feed offered were garnered for analysis of nutrients to be used in calculating the digestibility of the nutrients. The faeces were weighed fresh and 10% sample was taken from each animal daily, dried at 60°C for 48 h in a forced draught air over and bulked. A sub-sample was done, thoroughly mixed and milled to pass through a 0.60 mm sieve and stored in sealed hermetically labelled container until analysis. The urine was collected in a plastic tray attached under each cage, 10 mm of 10% concentrated sulphuric acid was added to the tray daily to prevent microbial colonization and contamination as well as preventing volatilization of NH_3 from the urine. The total output of urine per animal was measured and 10% aliquots were also saved in stoppered numbered plastics bottle and stored at –5°C before analysis (Ogungbesan *et al*., 2013a).

Table 2: *Gliricidia sepium* chemo-metric (% dry matter)

Variables	Percentage composition
Dry matter (% fresh matter)	37.21 ± 2.67
Crude protein	22.75 ± 2.45
Ether extract	5.46 ± 0.93
Crude fiber	21.28 ±1.32
Crude ash	9.84 ± 1.56
Nitrogen free extract	40.67 ± 3.82
Organic matter	90.16 ± 1.94
Neutral detergent soluble	56.92 ±.2.98
Neutral detergent fiber	43.08 ± 3.35
Hemi cellulose	13.97 ± 1.03
Acid detergent fiber	29.11 ± 2.54
Cellulose	20.85 ± 2.06
Acid detergent lignin	8.26 ± 1.55

Laboratory/Chemical Analysis

Ground samples of test ingredients and faeces were analysed for dry matter (DM) by drying samples at 105°C for 24 h in forced air oven. Crude ash content was measured after igniting samples in a muffle furnace at 550°C for 4 h. The crude protein (CP) was determined by Kjeldahl method (AOAC, 2000) crude fat (C fat) was determined by Soxhlet method (AOAC, 2000) and crude fibre according to the method of Weende (Yadilal *et al.,* 2017).

Table 3: Feed formulation

Ingredients	0M (Control)	50M	100M	150M	200M
Maize	41.00	41.00	41.00	41.00	41.00
SBM	20.00	20.00	20.00	20.00	20.00
GNC	8.00	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	10.00	10.00	10.00	10.00	10.00
Wheat offal	15.25	15.25	15.25	15.25	15.25
Bone meal	2.00	2.00	2.00	2.00	2.00
Vit. premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
GLM	-	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated chemical composition					
Crude protein (%)	21.59	20.65	20.65	20.65	20.65
Crude fat (%)	3.02	1.78	1.78	1.78	1.78
Crude ash (%)	16.26	12.48	12.48	12.48	12.48
NFE (%)	54.62	43.58	43.58	43.58	43.58
Energy (kCal kg^{-1})	2675.36	2553.53	2553.53	2553.53	2553.53
Calculated mineral components					
Phosphorus (%)	0.30	0.28	0.28	0.28	0.28
Calcium (%)	0.16	0.19	0.19	0.19	0.19
Sodium (%)	0.10	0.11	0.11	0.11	0.11
Magnesium (%)	2.06	1.92	1.92	1.92	1.92

GLM - *Gliricidia* leaf meal, SBM - soybean meal, M - maxigrain, GNC - groundnut cake, Vit. premix - vitamin mineral premix.

Statistical Analysis

Data obtained from chemical analysis of these ground samples of test ingredients and faeces were used to calculate the nutrients digestibility. The values obtained from the physiological trial and those generated from that of nutrients digestibility as well as performance characteristics were analysed using one-way analysis of variance. Also, simultaneous exposition of the data to inferential statistics was done to partition model sums of squares to show if results correlated to level of supplementation or not using the general linear model procedures. Sgnificantly different means were separated using Least Significance Differences at 0.05 level of probability as packaged in SAS (2002). The general linear model is as defined thus:

$$X_y = \mu + \alpha i + eij;$$

where X_y is individual data generated from the effects of the fixed treatments (ranging from 0 to 200 M per kg of GLM), μ is grand population mean, αi is the fixed treatments effects, and eij is the error (replicate) term within each treatment.

RESULTS AND DISCUSSION

As shown in Table 4, the temperature from ear (°C) ranged insignificantly ($p > 0.05$) from 0M (38.5°C) to 100M (39.0°C) and that from the rectum also ranged insignificantly from 100M (34.80°C) to 200M (35.30°C). Since the values are not different from that of the control, it implies that the test diets and/or its interaction with the supplemented enzymes do not have or potentiate any bioactive substances or plant secondary metabolites or phytochemicals, etc. (Abdullahi *et al*., 2014), that can neither cause abnormal diet induced low temperature (hypothermia) nor abnormal high temperature (hyperthermia). Moreover, that range of the recorded temperature values is comparable to that reported by Akinsola *et al.* (2013) and Iyeghe-Erakpotobor *et al*. (2013).

Table 4 also shows heart rate (beat per min. bpm) which was non-significantly ($p > 0.05$) lowest in 50 and 200 M having 56 to 100 M which was highest but with a similar value of 59 which scientifically presupposes that test diets with enzyme supplementation do not have ingredient of allelochemicals that abnormally increase in the heart rate (tacchycardia) or abnormally decrease in the rate (bradycardia) but rather eucardia which is normal rate that compares favourably to the chronicle of authors like that of Akinsola *et al.* (2013) and Iyeghe-Erakpotobor *et al*. (2013). Also in Table 4 is respiratory rate (no unit) was similar ($p > 0.05$) to 50 and 200M with 56 to 100M having mean value of 59. The respiratory rate was similar ($p > 0.05$) to the control which implies that the diets do not cause any clinical problems to the animals; so test diets with enzyme supplementation do not cause abnormally pathological increase respiratory rate (hyperpnea) or decrease rate (hypopnea).

The dry mater intake (DMI) was lowest in 0M (control) 5092 and highest in 150M 7671 (Table 5). There was a quadratic increase in DMI as observed also by El-Katcha *et al*. (2013). Weight changes and growth rate (g day^{-1}) increased linearly that is the was gradual increment in weight as the inclusion progressed from 0 to 200 M as reflected from 616 (0M) and 6 (0M) to 825 (200M) and 8 (200M) respectively though there was no significant statistical ($p > 0.05$) difference among them as this phenomenon of increment along inclusion has been also observed by El-Katcha *et al*. (2013). This is suggesting that with or without exogenous enzymatic supplementation, there might not be any difference in growth response while the growth rate is small compared with results of El-Katcha *et al*. (2013), this is because the breed used in this experiment is the red which are relatively smaller in weight and not the usual bigger New Zealand white. The FCR was unusually lowest in T2 (7.03) and highest in T3 (11.55) and not directly related to the increment levels of the enzymes rather it was even smallest with the highest level which is another pointer to the fact that neither enzyme supplementation nor its increment can categorically affect positively intake and even efficiency of utilization as we are going to witness in the FER (El-Katcha *et al*., 2013).

The FER which is a more effective means of measuring DM utilization than FCR was highest in T1 (control; 0.12) and lowest in treatments 2, 3 and 4 (0.09) that signifies a quadratic increased in FER. Quadratic increase typified by initial decrease in T2, 3 and 4 with 0.90 and higher value then the aforementioned 3 treatments but lower than that of T1 with value of 0.10. This is also alluding to the observed trend and fact that enzyme increment does not necessarily translates to increase and corresponding response. The relative to control (Rtc) values in all showed that not even any treatment was as low as 50 and as high as 200 in relation to control that is at first sight judging from the central or control which is 100. In essence, they are neither too low as to getting to half of control nor too high to the extent of getting to twice the control. This signifies that enzyme or its levels has no significant effect on the indices monitored as recorded by Marouneck *et al*. (1995) in that it is not unlikely that due to its peculiar digestive physiology, and in particular the fact that caecotrophy casts microbial enzymes along the whole length of the gut, rabbits may be less responsive than other animals to supplementation with exogenous enzymes and of course no visible or substantive harm came to the animals judging from the morbidity and mortality rates More so, all the values were not ($p > 0.05$) statistically different from one- another. The probability also showed that the inclusion level was quadratic indicating the non-effectiveness of supplementation of enzyme.

Table 4: Physiology of *Oryctolagus cunicullus* L 1758 fed *Gliricidia* with enzyme levels

Parameters	0M (Control)	50 M	100 M	150 M	200 M	Pooled SEM	p-values	L	Q
RT (°C)	38.50	38.70	39.00	38.90	38.60	0.06	0.687	NS	NS
Rtc	100.00	101.00	101.00	101.00	100.00				
ET (°C)	35.10	34.90	34.80	35.20	35.30	0.03	0.875		
Rtc	100.00	98.00	99.00	100.00	101.00				
HR	128 .00	129.00	127.00	130.00	128.00	1.15	0.910	NS	S
Rtc	100.00	101.00	99.00	102.00	100.00				
RR	58 .00	56.00	59 .00	57.00	56.00	2.00	0.796	NS	S
Rtc	100 .00	96 .00	102.00	98.00	96.00				

RT - rectal temperature, Rtc - relative to control, ET - ear temperature, HR - heart rate, RR - respiratory rates,
SEM - standard error of means, *L* - linear probability, *Q* - quadratic probability, NS - not significant, S - significant

Table 5: Performance characteristics of *Oryctolagus cunicullus* L. 1758 fed *Gliricidia* with enzyme levels

Parameters	0 M (Control)	50 M	100 M	150 M	200 M	Pooled SEM	p-values	*L*	*Q*
Initial weight (g)	1062.00	962.00	1065.00	987.00	1025.00	98.00			
Final weight (g)	1516.00	1654.00	1750.00	675.00	1850.00	73.00			
Weight changes (g)	616.00	691.00	637.00	687.00	825.00	123.00	0.874	NS	S
Rtc	100.00	112.00	103.00	111.00	134.00				
Dry matter intake (g)	5092.00	7476.00	7305.00	7671.00	7565.00	200 .00	0.796		
Rtc	100 .00	147.00	143.00	151.00	149.00				
Growth rate (g)	6.00	7.00	7.00	7.00	8.00	1.00	0.695	NS	S
Rtc	100.00	116.00	116.00	116.00	133.00				
FCR	8.25	7.03	11.55	11.15	9.20	1.50	0.853	NS	S
Rtc	100 .00	85.00	140 .00	135.00	112.00				
FER	0.12	0.09	0.09	0.09	0.10	0.01	0.852	NS	S
Rtc	100.00	75.00	75 .00	75.00	83.00				
Morbidity rate	0.00	0.00	0.00	0.00	0.00	0.00		NS	NS
Rtc	100.00	100.00	100.00	100.00	100.00				
Mortality rate	0.00	0.00	0.00	0.00	0.00	0.00		NS	NS
Rtc	100.00	100.00	100.00	100.00	100.00				

Rtc - relative to control, FCR - feed conversion ratio, FER - feed efficiency ratio,
SEM - standard error of means; *L* - Linear Probability, *Q* - Quadratic Probability, NS - not significant, S - significant

Table 6 shows the nutrient digestibility (%) of rabbits fed graded levels of maxigrain supplemented *Gliricidia sepium* leaf meal. The result showed that DM was highest ($p < 0.05$) in 50M that is 75.90 and lowest ($p < 0.05$) in 0M (control) 61.82. Crude protein of rabbits in 50M had the highest ($p < 0.05$) value (82.52) while the lowest (69.44) was recorded in 200M. Crude fiber in 100M (35.44) had the highest ($p < 0.05$) value while the lowest (31.40) was recorded in 0M. Crude ash values of the rabbit among various treatments had no significant difference ($p > 0.05$) the highest value was recorded in 50M (43.30) while the lowest was recorded in 200M (35.30). No significant difference ($p > 0.05$) were observed also in the digestibility of crude fat and nitrogen free extract, 100M (46.50) had the highest value of ether extract and lowest in 200M (41.35) and for nitrogen free extract 150M had the highest value and lowest in 50M (69.42). Crude ash had highest and similar digestibilities (%) in T2 with 43.30 (50M) and T3 (100M) with 42.48 were ($p < 0.05$) higher than T1 (0M) having 37.66, T4 (150M) and T5 (200M) with same value of 39.29. El-Katcha *et al.* (2013) observed the same irregular trend of significance of supplementation on nutrient digestibilities especially crude protein where no significance was observed. As conspicuously observed in nutrient digestibility of lack of response in terms of nutrients digestibility to enzymes levels. This also corroborates the assertion that with or without enzyme supplementation rabbit will utilize feed of plant origin like herbages.

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Table 6: Nutrients Digestibility (%) of *Oryctolagus cunicullus* L. 1758 fed *Gliricidia* with enzyme levels

Parameters	0 M (Control)	50 M	100 M	150 M	200 M	Pooled SEM	p-values	*L*	*Q*
Dry matter	61.82^{b}	75.90^{aa}	68.28^{b}	65.05^{b}	62.00^{b}	2.56	0.853	NS	S
Rtc	100.00	123.00	110.00	105.00	100.00				
Crude protein	71.68^{b}	82.52^{a}	76.48^{b}	72.54^{b}	69.44^{b}	2.90	0.796	NS	S
Rtc	100 .00	115.00	107 .00	101.00	97.00				
Crude fat	45.65	43.44	46.50	44.52	41.35	2.12	0.921	NS	S
Rtc	100.00	95.00	102.00	96.00	91.00				
Crude ash	37.66^{b}	43.30^{a}	42.48^{a}	39.29^{b}	39.29^{b}	2.15	0.851	NS	S
Rtc	100.00	115.00	113.00	104 .00	104.00				
Crude fiber	31.40	34.42	35.44	34.95	34.22	2.51	0.942	NS	NS
Rtc	100.00	110.00	113.00	111.00	109.00				
Nitrogen free extract	73.50	69.42	72.50	74.55	71.50	2.63	0.863	NS	NS
Rtc	100.00	94.00	97.00	101.00	97.00				

Rtc - relative to control, ^{a,b}represents treatment means within the same row bearing different superscripts are significantly ($p < 0.05$) different.
SEM - standard error of means, *L* - Linear Probability, *Q* - Quadratic Probability, NS - not significant, S - significant

CONCLUSION

Inclusion or non-inclusion of enzyme supplementation of *Gliricidia sepium* leaf meal in the diet of rabbit have no adverse effect on the performance, physiology and digestibility on the digestive make-up of the rabbits. Although, there is still need for further research in the areas of serology, reproductive physiology and product technology.

Authors' Contribution

Ogungbesan A.M. is the team leader, designer and coordination of the experiment. Fasina O.E. helped with the statistical analysis and some discussion in terms of the forage chemical utilization while Alagbe E.O. was in charge of the field experimental operation and Eniolorunda O.O. gave information concerning the experimental animal units.

Conflict of Interest

Authors have declared no conflict of interest exists.

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