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The Possible Role of DL-Methionine in the Detoxification of Gliricidia Leaf Anti-nutrients in Rabbit Nutrition

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Abstract— **A feeding trial was performed to investigate the potential of methionine as detoxifying agent of secondary metabolites present in *Gliricidia sepium* leaf. The leaves were dried, milled, and included in rabbit diets at 0 (D0, control), 10 (D10) and 15% (D15) raw and at 10 (D_{SM}10) and 15% (D_{SM}15) with 0.3% DL-methionine supplementation. 60-weaned rabbits (of 7-weeks old) averaging 1066±16.3 g/rabbit were used and fed the 5-diets (*ad libitum*) over a feeding trial lasting 6-weeks. At the end of the feeding trial, 6 rabbits were sampled per treatment for digestibility tests and 6 other for carcass characteristics. The experiment was designed as a one way classification. Proximate analysis and quantification of GLM phytochemicals revealed that *G. sepium* leaves are potentially rich in nutrients but contain substantial amount of antinutrients namely tannins, phytic and oxalic acids, alkaloids and saponins. Dietary performance traits gave no significant differences (p>0.05) in feed consumption, body weight gain and feed conversion ratio on the test diets relative to the control diet. The digestibility of dry matter, organic matter and crude protein decreased with increasing level of GLM. Supplementation of methionine to the feeds containing GLM had slightly improved the digestibility rates though the improvement was not significant (p>0.05). Feeding cost and economic feed efficiency were better in rabbits fed the diet D10 but not significant (P>0.05). Carcass characteristics were not significantly (p>0.05) affected by diets. Conclusively GLM at 10 or 15% inclusion in diets with or without methionine supplementation elicited no untoward effects on dietary performance traits and carcass characteristics of the rabbits. Methionine supplementation for detoxification should be carried out along with other chemical detoxicants to enable the inclusion of the leaf meal at higher levels.**

Index Terms— **Bio-economic parameters, metabolic detoxification, *Oryctolagus cuniculus*, secondary metabolites, sulfur amino acid.**

I. Introduction

Short generation interval animals like poultry, rabbit, rodent, insect, fish etc., occupy prominent positions in providing animal protein, contributing significantly to the rural households in developing countries and in meeting the rapidly growing demand for animal products [1]. Rabbit meat is well appreciated by Beninese and the animal production is drawing more attention than ever before. In 1992, more than 64% of the population has once consumed rabbit meat and 95% of them delighted it [2]. However rabbit industry is also facing the problem of increasing cost of feeding which account for about 60-65% of its production expenses [3]. This situation is due to the competition that exists among humans, animals and industries for the available grains. Moreover, increased industrial use of grains has led to a sharp rise in the prices of feeds/foodstuffs. To tackle this problem, livestock industry could strive on the use of nonconventional feedstuffs in animal diets [4].

One possible source of available and cheap feedstuffs is the leaf meals of some tropical legume browse plants of appreciable primary metabolites (protein, carbohydrate, fat, etc.). However, the constraints militating against the enhanced utilisation of leaf meals reside chiefly in the presence of secondary metabolite compounds (tannins, saponins, alkaloids, phytates, etc.) that elicit toxic and/or physiological effects on the animal body when consumed in amounts above the normal threshold [5], [6]. Therefore, if their feeding value is improved, many unconventional feedstuffs could alternatively be fed at cheaper cost to monogastric animals like rabbits.

In view of the forth going, *Gliricidia sepium* leaf meal appears to be an ideal plant material to be evaluated. That why this study aims at evaluating the detoxification potential of synthetic methionine on the dietary leaf meal in rabbit nutrition. Hence methionine has been reported to overcome in some cases, the dietary effects of some anti-nutrients by metabolic detoxification [7], [8], [9].

II. Materials and Methods

A. Experimental site

The field work for this study was performed on the Research farm of the Faculty of Agriculture (FSA/UAC, Republic of Benin).

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B. Preparation of the test feedstuff

Fresh Gliricidia leaves have been harvested in the vicinity of the University of Abomey-Calvi, and processed as describe by [10].

C. Experimental diets, animals and feeding trial

60-local breeds of rabbit kits (49±2 days old) with an average of 1066±16.3 g/rabbit were used for the assay. The rabbits were randomly assigned to 5-dietary treatments with 12-rabbits per diet and each diet made of 4-replicates of 3-rabbits each. Each replicate of the experimental rabbits being accommodated in a hutch seized: 80×50×30 cm3 (length×width×height).

The experimental diets were similar in nutrient content. They contain 0 (D0, control), 10 (D10) and 15% (D15) of raw GLM, and 10 ($D_{SM}10$) and 15% ($D_{SM}15$) of GLM supplemented with 0.3% DL-methionine. The feeding trial lasted 6-weeks and the composition and nutrient content of the experimental diets is presented on Table 1.

Table 1: Composition and nutrient content of the experimental diets

Ingredients (%)	D0[1]	D10	$D_{SM}10$[2]	D15	$D_{SM}15$
GLM	0.00	10.00	10.00+Met	15.00	15.00+Met
Maize	20.00	18.00	18.00	16.00	16.00
Palm kernel meal	29.00	25.00	25.00	25.00	25.00
Cottonseed meal	7.00	7.00	7.00	7.00	7.00
Soybean meal	9.00	7.00	7.00	7.00	7.00
Wheat offal	30.70	28.70	28.40	25.70	25.40
Palm oil	1.00	1.00	1.00	1.00	1.00
Oyster shell	1.70	1.70	1.70	1.70	1.70
NaCl (Salt)	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	-	-	0.30	-	0.30
Premix[3]	0.20	0.20	0.20	0.20	0.20
Di-calcium Phosphate	1.00	1.00	1.00	1.00	1.00
Total	**100**	**100**	**100**	**100**	**100**
Feed price (F CFA/kg)[4]	232.31	215.51	228.53	209.21	222.23
Nutrient content of the diets					
Digestible Energy (Kcal/Kg)	2688.07	2745.67	2756.32	2777.52	2795.12
Crude Protein (%)	17.47	17.16	17.29	17.18	17.52
Crude Fibre (%)	9.31	9.73	9.70	10.06	10.01

[1] D0, D10, and D15 are diets containing 0, 10, and 15% of raw Gliricidia Leaf Meal (GLM), respectively.
[2] $D_{SM}10$, and $D_{SM}15$ are diets containing respectively 10, and 15% of Gliricidia Leaf Meal, supplemented with Methionine.
[3] 800000 UI, E 2000 mg, K 800 mg, B1 600 mg, B2 2000 mg, niacin 3600 mg, B6 1200 mg, B12 4mg, choline chloride 80000 mg; Minerals: Cu 8000 mg, Mn 64000 mg, Zn 40 000 mg, Fe 32000 mg, and Se 160 mg;
[4] Republic of Benin Currency: 1€ = 655.9 F CFA.

D. Digestibility study

At the end of the feeding trial, six rabbits were randomly selected per dietary treatments. The rabbits (similar in weight p>0.05) were randomly penned in individual digestibility cage and accommodated for seven days. During the accommodation period, the experimental animals were fed *ad libitum* their respective diets. To empty their digestive tract, the rabbits were fasted for 24 hours prior the startup of the digestibility assay. Throughout the fasting period, water and vitamin was delivered as anti-stress. During seven days, feed intake was recorded on each rabbit. Besides, faeces excreted were collected daily and oven dried to determine moisture content. Then proximate analysis of the experimental diets and representative faecal samples was performed. Finally, apparent digestibility coefficient of dry matter (dDM), organic matter (dOM) and crude protein (dCP) was calculated as following:

$$dDM(\%) = \frac{\text{DM intake} - \text{DM exceted in faeces}}{\text{DM intake}} \times 100$$

$$dOM(\%) = \frac{\text{OM intake} - \text{OM exceted in faeces}}{\text{OM intake}} \times 100$$

$$dCP(\%) = \frac{\text{CP intake} - \text{CP exceted in faeces}}{\text{CP intake}} \times 100$$

E. Carcass characteristics

For the examination of carcass quality, six rabbits were selected per treatment. The rabbits selected were closest to the treatment mean weight of the replicate. The selected rabbits were deprived from feed for 24 hours. During the fasting period, they were served water and vitamins as anti-stress. Thereafter the animals were stunned by neck hitting and bled by severing the jugular vein. The skin was removed by flaying and the rabbits eviscerated. Then the full digestive tract, hot carcass, and abdominal fat were weighted and expressed as percentage of live body weight according to Blasco and Ouhayoun [11] standards.

F. Chemical analyses

Proximate analysis of GLM was carried out using the methods described by AOAC [12]. Total Nitrogen (N) was assessed by the micro-Kjeldahl method [13] while the crude protein was computed by multiplying % N with a factor of 6.25. Soluble carbohydrate (NFE) was obtained by subtracting the sum of the percentage of crude protein, ether extracts (EE), crude fibre (CF) mineral matter from dry matter (DM). Anti-nutrients determination of tannins, phytic acid, total oxalate, saponins and alkaloids was conducted by the methods of [14], [15], [16], [17] modified by [18], [19] respectively.

G. Statistical analysis

Data on chemical composition of raw GLM samples were interpreted by descriptive statistics while all the rest of the data were subjected to analysis of variance (ANOVA) using general linear model (GLM) in R version 3.0.2. The performances of rabbits were compared using each cage of three rabbits as replication. Replication effect and interaction between diets and replications were not significant (P > 0.05). Thus, analyses were performed according to the model as follows:

$$Yi = \mu + Fi + \varepsilon i$$

Where:
Yi is the observation for dependent variables;
μ the general mean;
Fi the fixed effect of the feed and
εi the residual error.

III. RESULTS

A. Chemical composition of Gliricidia Leaf Meal (GLM)

Table 2 gives data on proximate composition of Gliricidia Leaf Meal (GLM). GLM is observed to contain valuable nutrients namely soluble carbohydrate, crude protein, fat, fibre, mineral matter and dry matter in the range of 44.49; 16.34; 4.73; 15.31; 10.46 and 91.33% respectively.

Table 2: Proximate Analysis of GLM

Nutrient	% Content
Dry matter (DM)	91.33
Crude protein (CP)	16.34
Crude fibre (CF)	15.31
Ether extract (EE)	04.73
Mineral matter (Total ash)	10.46
Soluble carbohydrate (NFE)	44.49

NB: Proximate values are determined from an average of two (2) determinations for each nutrient.

The phytochemical quantification of some of the toxins (anti-nutrients) in the leaf of *G. sepium* is presented on table 3. The leaf is found to contain high levels of tannins (23.78%), alkaloids (5.77%) and saponins (2.04%) and moderate level of anti-metals (Phytic acid, 16mg/100g and oxalic acid, 25.42mg/100g).

Table 3: Quantification of GLM anti-nutrients

Phytochemical	Concentration
Tannins (g/100g)	23.78±0.01
Phytic acid (mg/100g)	16.00±0.005
Oxalic acid (mg/100g)	25.42±0.0015
Saponins (g/100g)	02.04±0.15
Akaloids (g/100g)	05.77±0.25

NB: Values of the phytochemical concentration are taken from average of three (3) determinations for each anti-nutrient.

B. Feed intake of growing rabbits

During the experiment, the daily feed intake of rabbits fed with the five diets (Figure 1) was not significantly different (P>0.05). However the lowest feed intake registered was in the control diet (77.47±7.41g/d) while the experimental diets consumption was 87.34±6.46 g/d; 86.31±1.51 g/d; 84.42±5.69 g/d; and 81.31±8.47 g/d respectively for rabbits fed the diets D10, $D_{SM}10$, D15, $D_{SM}15$. Then the average feed consumption, despite disparities observed during the experiment, was higher with diets containing 10% of GLM supplemented or not.

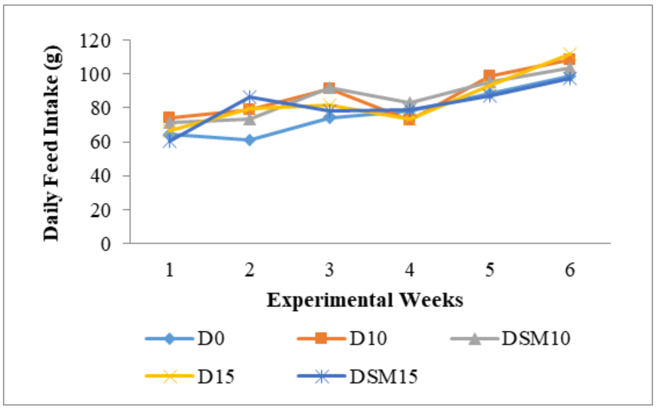

Figure 1: Average daily feed intake of growing rabbits fed diets containing GLM supplemented with or without methionine.

C. Digestibility of diets

Incorporation of GLM in the rabbit diets had significantly reduced (P<0.05) the digestibility of dry matter (dDM), organic matter (dOM) and crude protein (dCP) (Table 4). Besides, supplementation of methionine to the feed containing GLM had slightly improved the digestibility rate of those parameters. However, that improvement of the apparent digestibility was not significant (p>0.05).

Table 4: Apparent Digestibility (%) of dry matter, organic matter and crude protein for rabbits fed diets containing GLM treated with or without Methionine

	D0[1]	D10	$D_{SM}10$[2]	D15	$D_{SM}15$	SEM[3]	[4]P
dDM (%)	77.71±3.41a	70.33±1.14b	72.91±1.73b	68.54±3.34b	70.22±2.15b	1.601	*0.0011*
dOM (%)	80.32±3.02a	74.15±0.99b	75.10±1.60b	70.57±3.12b	71.85±2.04b	1.685	0.0003
dCP (%)	80.03±3.06a	74.96±0.96b	76.85±1.48ab	74.64±2.69b	74.84±1.82b	1.022	0.0133

dDM: digestibility of dry matter; dOM: digestibility of organic matter; dCP: digestibility of crude protein
[1] D0, D10, and D15 are diets containing 0, 10, and 15% of non-supplemented Gliricidia Leaf Meal (GLM), respectively.
[2] $D_{SM}10$, and $D_{SM}15$ are diets containing respectively 10, and 15% of Gliricidia Leaf Meal, supplemented with Methionine.
[3]SEM: Standard error of mean, P: probability

D. Growth Performance of Rabbits

The growth of rabbits was regular during the experiment (Figure 2). The daily body weight gains (Table 5) were similar between diets (P > 0.05). Consequently, the final body weights between rabbits fed the diets D0 (1999.17 g), D10 (2106.67 g), $D_{SM}10$ (2052.5 g), D15 (1978.75 g) and $D_{SM}15$ (1980.56 g) were statistically similar (P > 0.05).

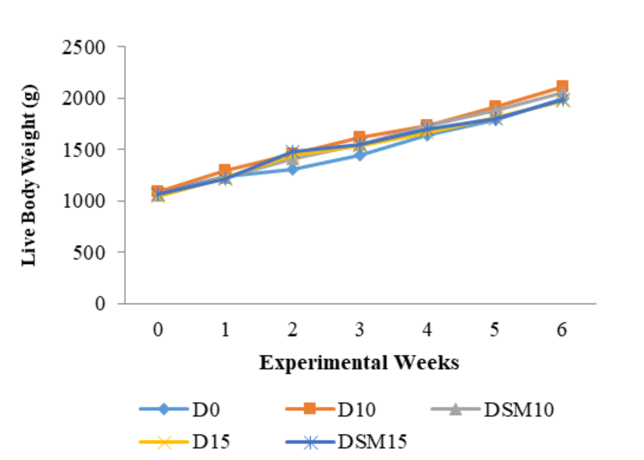

Figure 2: Growth curve of rabbits fed diets containing GLM supplemented with or without methionine.

E. Efficiency of Feeds

The feed conversion ratio, feeding cost and economic feed efficiency (Table 5) was not statistically affected by dietary treatment. However, the lowest feed conversion ratio was recorded on rabbits fed the $D_{SM}10$ diet while the lowest Feeding cost was registered on rabbits fed the diet D10. The economic feed efficiency that reports the revenue from the live body weight gain comparatively to the feeding cost, was highest in the diet D10, followed by the diet D15.

F. Carcass characteristics

The carcass yields of rabbits were similar (P > 0.05) between dietary treatments (Table 6). Additionally, the relative weight of digestive tract and abdominal fat expressed as percentage of live weight were not significantly influenced by dietary treatment (p>0.05).

Table 5: Daily weight gain (g), feed conversion ratio (g feed/g BWG1), feeding cost (FCFA2feed/kg BWG), and economic feed efficiency (FCFA BWG/FCFA feed) of rabbits fed diets containing GLM supplemented with or without Methionine

	D0[1]	D10	$D_{SM}10$[2]	D15	$D_{SM}15$	SEM[3]	[4]P
DWG (g)	22.08±1.94	24.25±3.60	23.71±3.72	22.23±2.74	20.40±2.22	0.678	0.4071
FCR	4.78±1.06	4.03±0.97	3.97±1.01	4.86±1.15	6.05±1.58	0.376	0.1376
FC	1111±247	866±209	906±232	1017±241	1346±352	85.60	0.1243
EFE	2.09±0.27	2.24±0.5	2.10±0.34	2.20±0.21	1.88±0.33	0.062	0.6321

DWG: Daily weight gain, FCR: Feed conversion ratio, FC: Feeding cost, economic feed efficiency, BWG: Live body weight gain, Currency: 1€ = 655.9 FCFA.

Table 6: Carcass yield, full digestive tract and abdominal fat weight of growing rabbits expressed as percentage of live weight

	D0[1]	D10	$D_{SM}10$[2]	D15	$D_{SM}15$	SEM[3]	[4]P
Carcass Yield (%)	66.61±1.65	66.02±2.20	66.22±2.33	65.24±1.83	64.3±2.58	0.411	0.102
Full digestive tract (%)	15.5±2.07	15.25±2.56	15.73±1.91	15.77±3.06	18.59±3.40	0.612	0.411
Abdominal fat (%)	1.26±0.33	1.36±0.30	1.10±0.18	0.71±0.52	0.88±0.29	0.119	0.093

[1] D0, D10, and D15 are diets containing 0, 10, and 15% of unsupplemented Gliricidia Leaf Meal (GLM), respectively.
[2] $D_{SM}10$, and $D_{SM}15$ are diets containing respectively 10, and 15% of Gliricidia Leaf Meal, supplemented with Methionine.
[3]SEM: Standard error of mean, [4]P: probability

IV. Discussion

A. Chemical composition of Gliricidia Leaf Meal (GLM)

Data on Proximate composition of GLM shows that the leaf contains valuable nutrients. These values, except the protein content fell within the range of nutrients early reported for *G. sepium* by [20]. Actually, its protein content is lower than values observed by [20], [21], [22], [23], [24]; meanwhile it is higher than that obtains by [25] in Nigeria. So, if the constraints militating against the enhanced utilisation of the leaf of *G. sepium* are handled, that is its content of anti-nutritional factors, it may provide valuable unconventional alternative feedstuff for domestic animals especially monogastrics that exert high demand on nutrients particularly energy, protein and micronutrients. These could be of nutritional advantage to the fed animals when the nutritive value of the leaf is improved.

Quantitative determination of some of the toxic or anti-nutritional factors in GLM in this experiment revealed high levels of tannins, alkaloids and saponins while containing moderate level of phytic and oxalic acids. These contents of anti-nutrients in GLM agree with other reports which indicate presence of tannins, phytin, oxalate, alkaloids in the leaf [22], [23], [26]. Besides, GLM have been reported by [24] to contain cyanides. Cyanides derive from hydrolysis of cyanogens which suppress natural respiration and provoke cardiac arrest [27]. However no exact limit of toxicity of cyanide for monogastric animals has been reported yet and more studies are required to find out the cyanide level of tolerance for rabbit. *G. sepium* has also been implicated in the content of a non-protein amino acid and the possiblility of coumarin, a low molecular weight phenolic compound [28], [29].

Although the leaf of *G. sepium* is shown to contain valuable nutrients, feeding the leaves direct to domestic animals in diets or as sole feedstuff may not be beneficial to the fed animals due to the presence of anti-nutrients.

B. Digestibility study

The incorporation of GLM in the rabbit diets reduced the digestibility of dry matter (dDM), organic matter (dOM) and crude protein (dCP). The digestibility of dry matter (68.54 to 77.71%) observed is higher than values (62 to 67%) obtained by [30], [31] in rabbits, but lower than dry matter intake (77.62 to 85.99%) recorded by [32] [33].

The decrease of organic matter and crude protein digestibility with the inclusion of GLM in diet could be due to the presence of anti-nutritional factors which interfere with the utilisation of dietary nutrients in a various ways, including the reduction of protein digestibility, binding to various nutrients or damaging the gut wall [34] and thereby depressing digestive efficiency [35]. Previous researchers [36], [37], [38] reported that the presence of secondary metabolites ranging from tannins, oxalates, phytates and saponins may greatly reduce digestibility.

Tannins form complexes with carbohydrates and proteins in feeds, and with digestive enzymes. Consequently nutrient digestibility is lowered. Other effects of tannins include reduced feed consumption and absorption of some minerals, increased damage to the gut wall, [39], [40], [41].

Oxalates have been reported to: (*i*) form complexes with mineral (Na, K, Ca, Fe and Mg) thereby rendering them inaccessible to Animals; (*ii*) cause irritation of the gut and resulting in low feed consumption, (*iii*) inhibit energy and protein utilisation in broilers [40].

Phytic acid is reported to reduce the availability of a variety of minerals (calcium, cobalt, copper, magnesium, manganese, selenium, iron and zinc) through complexes binding. Phytic acid can also form complexes with basic residues of proteins and may interfere with the activity of endogenous enzymes and digestibility of nutrients other than minerals [34].

Beside the depressive effect of GLM on dDM, dOM and dCP, supplementation of methionine to the GLM containing feed had slightly improved the digestibility rate of those parameters. However, that improvement of the apparent digestibility was not significant. this finding is congruent with the works of [42] who found that the addition of 0.3% L-methionine and 0.3% choline chloride to black locust meal in the diet of New Zealand White rabbits increased ADF digestibility and gave a slight, although not significant, increased of protein digestibility. Also, [43] found that supplementation of methionine to Shea kernel cake in broilers' diets abolished or prevented the ill-effect caused by saponin in the cake. Dietary supplementation with methionine is shown to prevent the deleterious effects by inactivation of the saponins thereby stepping up the nutrient content of the saponin containing diet [44]. In addition to its effect on saponin, supplementation with L-methionine or its hydroxyl analog had been shown to overcome the detrimental effect of condensed tannins of sorghum in chicks [9], but not in rats [45] and Swine [46]. Although methionine supplementation was effective against tannic acid, this beneficial effect of methionine detoxification by donating methyl group was not evidence by a lack of effect of supplemental choline, another methyl donor [34].

C. Growth Performance of Rabbits and Efficiency of Feeds

Table on dietary performance of rabbits fed *G. sepium* leaf meal at 10 and 15% level treated with or without methionine gave no significant differences in feed consumption, body weight gain and feed conversion ratio suggesting that GLM in diets at 10 or 15% inclusion treated with or without methionine has no adverse effect on performance traits. The growth rates of the experimental rabbits (20.40 to 24.25 g/d) were close to values (15.72 to 23.1 g/j) reported in Benin in similar rabbit breed [33], [47], [48]. Compared to the reference diet D0 (22.08g/d), the diets D10 (24.25g/d) and $D_{SM}10$ (23.71g/d) did ameliorate the growth performance of rabbits without any deleterious effect on their survivalability. Likewise the final body weights were best in diets D10 (2106.67g) and $D_{SM}10$ (2052.5g) implying that at 10% of inclusion in diet, rabbits perfectly cope the anti-nutrient factors in GLM.

The lowest feed conversion ratio recorded in rabbits fed the diet $D_{SM}10$ indicate supplemental effect of methionine on GLM at 10% inclusion. But the lowest feeding cost register in rabbits fed the diet D10 reveal that beside the beneficial effect of methionine supplementation on feed utilisation in rabbits, no methionine supplementation at all is economically advantageous. Moreover values of economic feed efficiency confirm that tendency. Actually, the highest economic feed efficiency reported in diet D10 (2.24 FCFA weight gain/FCFA feed), followed by the diet D15 (2.20 FCFA weight gain/FCFA feed) demonstrate that GLM included raw in growing rabbit diet at 10 and 15% is more profitable than supplemented with methionine at 0.3%. This is due to the high cost of that amino-acid compared to other feed ingredients.

D. Carcass characteristics

The carcass yields (64.3 to 66.22%) in rabbits fed the experimental diets are lower than those fed the control diet (66.61%) though differences among dietary treatments are not significant (p>0.05). Also the carcass yield tends to decrease with increasing value of GLM in diets. This could be due to the increasing value of fibre intake by the experimental rabbits. However those carcass yield values observed are higher than values obtained by [48], [49] while similarly close to rates (64.8 to 66.6%) reported by [50]. GLM-Based diets did not significantly affect the relative weight of the rabbits' digestive tracts. Also, it does not statistically influence the proportion of abdominal fat though numerically lower in diets D15 and $D_{SM}15$ probably due to higher level of GLM incorporation. These findings show suitable impact GLM based diets on the quality of rabbit carcass.

V. Conclusion

This experiment indicated no adverse effect of GLM on Nutrients utilisation rate, performance and carcass traits of growing Rabbits. Researches are in progress to investigate the inclusions of methionine and choline chloride together to enable inclusion of GLM at levels higher than the 10 and 15% used in this study.

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