

Assay Of Detoxification Potential Of DL-Methionine On Dietary Gliricidia Leaf Meal In Rabbit Nutrition: Relative Organ Weight And Blood Indices

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Abstract: An investigation on the detoxification potential of DL-Methionine on Gliricidia Leaf Meal (GLM) was performed. Gliricidia sepium leaves were dried and milled into meal (GLM) and incorporated in diets at 0% (F0, control), 10% (F10) and 15% (F15) raw and at 10% (F_{SM}10) and 15% (F_{SM}15) with 0.3% DL-methionine supplementation. 60-weaned rabbits (of 49-days old) averaging 1066±16.3 g/rabbit were used as test animal models and fed the 5-diets to appetite over a feeding trial lasting 42-days. At the age of 91±2 days, 4-rabbits were sampled per treatment for internal organ weight and three (3) for haematological and biochemical studies. Dietary treatment gave no significant differences ($p>0.05$) for all the relative organ weights (liver, kidneys, heart and spleen) but the lungs ($p<0.05$). Haematological parameters such as PCV, RBC as well as MCH, MCV and MCHC were not significantly affected by the dietary treatment ($P>0.05$) while WBC counts decreased in diets F_{SM}10, F15 and F_{SM}15. The biochemical determinants (glucose, albumin, globulin, ALT, ALP, Na⁺, P, Mg²⁺, Cl⁻, HCO₃⁻) measured on the rabbits fed the test diets were comparable to those fed the conventional diets ($p>0.05$). Diet F15 significantly increased AST enzyme ($p<0.05$) while ALT value was significantly augmented ($p<0.05$) in all the experimental treatments compared to the control diet. Besides, methionine supplementation in diet F_{SM}10 and F_{SM}15 reduced ALT activities compared to methionine unsupplemented diets F10 and F15 respectively. However, this latter was not statistically significant ($p>0.05$). Dietary leaf meal of *G. sepium* with or without methionine treatment had no significant effect ($p>0.05$) on blood concentration of sodium (Na⁺), phosphorus (P), magnesium (Mg²⁺), chloride and bicarbonate ions (HCO₃⁻). Calcium electrolyte was increased ($p<0.05$) with dietary treatments compared to the conventional diet (F0). In summary, GLM at level up to 15% in diets had no adverse effects on internal organs status, haematological parameters, some biochemical determinants and electrolyte profile of growing rabbits. Elevation in serum ALT and AST suggested that antinutrients in GLM elicited mild tissue lesions in rabbit which is slightly corrected by methionine supplementation. It is recommended that in subsequent researches, methionine supplementation, to facilitate detoxification, should include other detoxicant like Choline chloride and Polyethylene Glycol (PEG).

Keywords: blood indices, detoxification, GLM, methionine, organ weight, rabbit.

1. Introduction

Gliricidia tree belongs to the family of leguminosae. Many species of the tree exist but Gliricidia sepium is the widely known and cultivated species. It is also one of the most widely-researched non-industrial tree species in the world in terms of number and variety of its uses in different farming systems. The major part/product of the tree used in animal nutrition is the leaf. The leaves are used as animal fodder and have high nutritive value especially for ruminants. However, problems posed to ruminants and other domestic animals when fed dietary *G. sepium* leaves are those of toxicities or acceptability. Past works encountered problems when *G. sepium* leaves were fed to non-ruminants such as rabbits [1], rodents [2], poultry [3]-[4] and horses [5]. Evaluation of the feeding value of *G. sepium* in terms of chemical composition and digestibility presented it as highly nutritious fodder. High in protein, digestibility, low in fibre content, *G. sepium* is however found to contain anti-nutritional or toxic factors in the leaf such as cyanide [6], tannins, phytin, phytic phosphorus and oxalic acid as well as alkaloids and saponins [7]. [8] Suspected the presence of a toxic non-protein amino

acid in *G. sepium* which was subsequently revealed by research [9] as a low molecular weight phenolic and volatile compound occurring in fresh *G. sepium* leaves at 0.7% levels on dry matter basis called coumarin. Coumarin is a secondary metabolite in *G. sepium* responsible for the characteristic smell of the leaves and cause of unpalatability due to bad odour. Farmed animals were shown to reject *G. sepium* on the basis of bad smell without tasting the leaves. [10] and [11] reported that lack of palatability is the main factor limiting or even preventing *G. sepium* use in nutrition of animals because of their reluctance to eat it. Coumarin is also a precursor of phytoestrogens which can cause infertility, abortion and other deleterious effects on animal reproduction [12]. Besides, [13] confirmed that inclusion of *G. sepium* leaf meal at 20% in diets for mature rabbit bucks caused mild depression in semen production and quality. In spite of the quoted side effects of *G. sepium* leaves in nutrition of domestic animals, attempts were made in this experiment to improve its nutritional value to serve as an alternative protein source for rabbit. Detoxification of the dietary leaf was attempted by supplementation with synthetic methionine (DL-methionine) hence methionine has been

reported to overcome the dietary effects of some anti-nutrient by metabolic detoxification. In addition to improving the biological value of a feedstuff, methionine serves as detoxifier by donating methyl group radicals into the system to effect detoxification [14] and to facilitate excretion of absorbed toxic principle(s) [15].

2. Materials and methods

Experimental site

This experiment was conducted on the Research farm of the Faculty of Agricultural Sciences, University of Abomey-Calavi, Republic of Benin (LaRAZE/FSA/UAC/RB).

Preparation of the test feedstuff

Fresh *G. sepium* leaves were obtained from trees present in the University environs. They were first shade-dried for several days before subsequent sun-drying so that sensitive nutrients in the leaves were not destroyed. Shade and sun-drying besides enabling the leaves to be easily milled,

brought about wilting of the leaves to reduce some of the anti-nutrients or toxic volatile compounds like Coumarin [16]. Leaves after drying were milled into meal to obtain *Gliricidia* Leaf Meal (GLM).

Experimental diets, animals and feeding trial

Sixty (60) young rabbits of both sexes averaging 1066 ± 16.3 g/rabbit obtained from a local breed aged 49 ± 2 days old were used for the experiment. They were randomly allotted to the five dietary treatments with twelve rabbits per diet and each diet made of four replicates containing three rabbits each. The experimental animals were accommodated in a cage size: $80 \times 50 \times 30$ cm³ (length \times width \times height). The rabbits were fed the iso-caloric and iso-nitrogenous diets containing 0, 10 and 15% GLM with or without 0.3% DL-methionine supplementation (Table 1). Pellets feed and drinking water were supplied to appetite (*ad libitum*). The feeding trial lasted 42 days and the composition of the experimental diets on as fed basis is presented on Table 1.

Table 1: Composition of the experimental diets

| Ingredients (%) | F0 ¹ | F10 | F _{SM} 10 ² | F15 | F _{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 | 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 F0, F10, and F15 are diets containing 0, 10, and 15% of raw *Gliricidia* Leaf Meal, respectively.

² F_{SM}10, and F_{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 FCFA.

Assessments of internal organs

After the 42-days feeding trial, four rabbits were selected per treatment (one per replicate) for internal organ weight assessment. The rabbits selected were closest to the treatment mean weight of the replicate. The selected rabbits were starved for 24 hours and thereafter bled by severing the jugular vein. The skin was removed by flaying. Then each rabbit was eviscerated, the internal organs collected, weighed separately and expressed as a percentage of live weight.

Blood sample collection

At the end of the 6-weeks feeding trial, the animals were starved of feed for 24 hours and blood samples collected. Three rabbits per treatment were randomly taken and blood samples collected for the analysis of serum chemistry and haematological profile. The blood samples were collected into Vials from each rabbit from the external ear vein using a sterilized disposable syringe and needle. One set of Vials

without anti-coagulant was used to collect blood for biochemical parameters while the other set of sterile universal bottles containing Ethylene Diamine Tetra-Acetic acid (EDTA) as an anticoagulant were used to collect blood for haematological parameters.

Blood analyses

Haematological parameters (RBC, WBC, Hb and PCV) were determined using the Automated Haematological Analyser (AHA) while MCV, MCH and MCHC were calculated as outlined by [17]. Biochemical indices were determined using a commercial Kit. Determination of the measured electrolytes Sodium (Na⁺), Phosphorus (P), Potassium (K⁺), Bicarbonate (HCO₃⁻), Chloride ion (Cl⁻), Calcium (Ca²⁺), and Magnesium (Mg²⁺) was carried out using the appropriate analytical apparatus. Sodium and potassium were analysed by using flame photometer while chloride and bicarbonates were analysed by the methods of [18] and [19] respectively.

Statistical analysis

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 rabbit as replicate. Replication effect and interaction between diets and replications were not significant ($P>0.05$). Thus, analyses were performed according to the model as follows:

$$Y_i = \mu + F_i + \epsilon_i$$

Where:

Y_i is the observation for dependent variables; μ the general mean; F_i the fixed effect of the feed; and ϵ_i the residual error.

3. Results

Relative organ weight

Values of internal organs expressed as percentage of live weight were shown on table 2. There was no significant ($p>0.05$) difference for all the parameters considered except for the lungs ($p<0.05$).

Table 2: Relative organs' weight of growing rabbits expressed as percentage of live weight

| Relative organ weight (%) | F0 ¹ | F10 | F _{SM} 10 ² | F15 | F _{SM} 15 | SEM ³ | P value |
|---------------------------|-----------------|-------------|---------------------------------|-------------|--------------------|------------------|---------|
| Lungs | 0.45±0.06b | 0.52±0.04ab | 0.66±0.14a | 0.58±0.01ab | 0.50±0.13ab | 0.037 | 0.0357 |
| heart | 0.31±0.09 | 0.30±0.71 | 0.3±0.11 | 0.3±0.09 | 0.32±0.09 | 0.004 | 0.9976 |
| Liver | 2.41±0.21 | 2.91±0.26 | 2.72±0.42 | 2.76±0.43 | 2.77±0.47 | 0.083 | 0.4435 |
| Spleen | 0.04±0.02 | 0.04±0.008 | 0.05±0.018 | 0.053±0.019 | 0.048±0.017 | 0.003 | 0.7661 |
| Kidney | 0.57±0.03 | 0.56±0.07 | 0.58±0.09 | 0.63±0.03 | 0.57±0.05 | 0.013 | 0.4371 |

¹ F0, F10, and F15 are diets containing 0, 10, and 15% of raw *Gliricidia* Leaf Meal (GLM), respectively.

² F_{SM}10, and F_{SM}15 are diets containing respectively 10, and 15% of *Gliricidia* Leaf Meal, supplemented with Methionine.

³ SEM: Standard error of mean, P: probability

^{a,b,c} Means with unlike superscripts in the same row differ significantly ($P < 0.05$).

Haematological parameters

Influence of *G. sepium* leaf meal (GLM) in diets treated with or without DL-methionine at 0.3% inclusion on blood composition in the fed rabbits is shown on table 3. GLM in diets at 10 or 15% inclusion with or without methionine

supplementation did not influence PCV, RBC as well as MCH, MCV and MCHC ($P>0.05$). The two levels of methionine supplemented diets (F_{SM}10 and F_{SM}15) and the diet F15 containing 15% of GLM non-supplemented with methionine reduced WBC counts ($P<0.05$).

Table 3: Influence of GLM in diets treated with or without DL-methionine on haematological parameters in rabbits

| | F0 ¹ | F10 | F _{SM} 10 ² | F15 | F _{SM} 15 | SEM ³ | P value |
|----------------------------|-------------------------|--------------------------|---------------------------------|-------------------------|-------------------------|------------------|---------|
| PCV (%) | 35.70±0.66 | 40.03±1.12 | 36.00±3.56 | 42.03±0.25 | 36.67±5.28 | 1.254 | 0.086 |
| Hb (g/dl) | 11.33±0.25 ^b | 12.67±0.25 ^{ab} | 11.00±1.80 ^b | 13.67±0.31 ^a | 11.10±1.31 ^b | 0.523 | 0.035 |
| RBC ($\times 10^{12}/L$) | 4.10±0.10 | 4.67±0.15 | 4.67±0.81 | 5.00±0.20 | 4.93±0.59 | 0.159 | 0.212 |
| WBC ($\times 10^9/L$) | 9.00±0.53 ^a | 9.33±0.46 ^a | 5.90±0.30 ^c | 7.07±1.03 ^b | 7.33±0.46 ^b | 0.638 | 0.0002 |
| MCH (Pg) | 27.66±1.06 | 27.16±0.95 | 23.75±3.17 | 27.38±1.71 | 22.69±3.62 | 1.041 | 0.079 |
| MCV (fl) | 87.12±3.32 | 85.87±4.59 | 78.38±12.24 | 84.16±3.58 | 74.40±8.03 | 2.417 | 0.228 |
| MCHC (%) | 31.75±0.20 | 31.65±0.58 | 30.44±2.37 | 32.51±0.71 | 30.45±2.90 | 0.402 | 0.545 |

¹ F0, F10, and F15 are diets containing 0, 10, and 15% of raw *Gliricidia* Leaf Meal (GLM), respectively.

² F_{SM}10, and F_{SM}15 are diets containing respectively 10, and 15% of *Gliricidia* Leaf Meal, supplemented with Methionine.

³ SEM: Standard error of mean, P: probability.

^{a,b,c} Means with unlike superscripts in the same row differ significantly ($P < 0.05$).

Serum Biochemistry

Serum biochemistry parameters in rabbits fed 10 or 15% GLM with or without DL-methionine supplementation are shown on table 4. GLM in diets had no significant effects ($P>0.05$) on blood glucose level, Total protein, serum albumin, and the activity of ALP enzyme. As for globulin concentration, and AST and ALT enzymes, they were statistically influenced ($p<0.05$) by dietary treatment. AST

was statistically increased in diet F15 while ALT values were significantly augmented in all the experimental treatments compared to the control diet. Moreover, methionine supplementation reduced ALT activities compared to methionine unsupplemented diets F10 and F15 respectively. However, this latter was not statistically significant ($p>0.05$).

Table 4: Dietary impact of GLM treated with or without DL-methionine on some biochemical indices in serum of the fed rabbits

| | F0 ¹ | F10 | F _{SM} 10 ² | F15 | F _{SM} 15 | SEM ³ | P value |
|----------------------|--------------------------|--------------------------|---------------------------------|-------------------------|-------------------------|------------------|---------|
| Glucose (mg/dl) | 45±9.54 | 51±11.53 | 39±7.81 | 47±10.82 | 40±5.57 | 2.227 | 0.520 |
| Total protein (g/dl) | 6.45±0.55 | 6.80±0.14 | 7.19±0.20 | 6.28±0.91 | 6.89±0.21 | 0.162 | 0.247 |
| Albumin (g/dl) | 4.95±0.59 | 5.50±0.49 | 5.52±0.17 | 4.98±0.81 | 5.57±0.19 | 0.139 | 0.314 |
| Globulin (g/dl) | 1.50±0.10 ^b | 1.30±0.10 ^c | 1.67±0.03 ^a | 1.30±0.10 ^c | 1.32±0.02 ^c | 0.074 | 0.0006 |
| AST | 54.33±10.50 ^b | 71.00±10.54 ^b | 59.00±7.55 ^b | 88.00±7.55 ^a | 64.33±8.02 ^b | 5.865 | 0.0078 |
| ALT | 67.00±7.00 ^b | 107.00±7.00 ^a | 92.00±3.00 ^a | 108.0±27.0 ^a | 100.0±4.00 ^a | 7.519 | 0.0181 |
| ALP | 50.00±3.00 | 58.33±3.51 | 55.00±3.61 | 54.00±4.58 | 56.00±7.00 | 1.370 | 0.318 |

¹ F0, F10, and F15 are diets containing 0, 10, and 15% of raw *Gliricidia* Leaf Meal (GLM), respectively.

² F_{SM}10, and F_{SM}15 are diets containing respectively 10, and 15% of *Gliricidia* Leaf Meal, supplemented with Methionine.

³ SEM: Standard error of mean, P: probability

^{a,b,c} Means with unlike superscripts in the same row differ significantly ($P < 0.05$).

Table 5 presents the effect of dietary GLM treated with or without DL-methionine on electrolytes profile in the experimental animals. Dietary leaf meal of *G. sepium* with or without methionine treatment had no significant effect ($p>0.05$) on blood concentration of sodium (Na^+), phosphorus (P), magnesium (Mg^{2+}), chloride and bicarbonate

ions (HCO_3^-). Meanwhile, dietary treatment reduced ($p<0.05$) the level of potassium ion (K^+) in rabbit fed the diet F10 relative to the F_{SM10} . Calcium electrolyte however was increased ($p<0.05$) on the GLM based diets with or without DL-methionine supplementation compared to the conventional diet (F0).

Table 5: Dietary response of rabbits fed GLM treated with or without DL-methionine to blood electrolyte levels

| | F0 ¹ | F10 | F_{SM10} ² | F15 | F_{SM15} | SEM ³ | P value |
|--------------------------|-----------------|------------|-------------------------|-------------|-------------|------------------|---------|
| Na^+ mEq/l | 134±9.00 | 144±3.61 | 140±3.61 | 137±8.00 | 141±8.54 | 1.115 | 0.498 |
| P (mg/l) | 209±8.54 | 196±5.57 | 198±22.52 | 204±5.00 | 215±37.00 | 3.501 | 0.765 |
| K^+ (mEq/l) | 5.63±0.57ab | 3.80±0.10b | 6.30±1.45a | 4.40±0.85ab | 5.00±0.44ab | 0.441 | 0.0274 |
| Ca^{2+} (mg/l) | 133±6.00c | 144±9.00bc | 154±7.55ab | 163±3.61a | 165±6.56a | 5.995 | 0.0009 |
| Mg^{2+} (mg/l) | 34.00±1.00 | 34.00±0.00 | 31.00±3.00 | 33.00±1.00 | 34.67±0.58 | 0.642 | 0.0906 |
| Cl ⁻ (mEq/l) | 117±0.00 | 113±3.61 | 116±5.00 | 113±6.56 | 118±1.00 | 1.030 | 0.466 |
| HCO_3^- (mg/dl) | 0.10±0.01 | 0.1±0.03 | 0.10±0.05 | 0.11±0.01 | 0.16±0.01 | 0.012 | 0.554 |

¹ F0, F10, and F15 are diets containing, 0, 10, and 15% of raw *Gliricidia Leaf Meal*, respectively.

² F_{SM10} , and F_{SM15} are diets containing respectively 10, and 15% of *Gliricidia Leaf Meal*, supplemented with Methionine.

³ SEM: Standard error of mean

^{a,b,c} Means with unlike superscripts in the same row differ significantly ($P<0.05$).

4. Discussion

Relative organ weight

In nutritional Biochemistry and toxicology studies, Analysis of organ weight is an important endpoint for identification of potentially harmful effects of anti-nutritional or toxic factors. Differences in organ weight between treatment groups are often followed by differences in body weight between these groups, making interpretation of organ weight differences more difficult [20]-[21]. Based on [20] findings, absolute organ weights cannot be an optimal endpoint for the evaluation of organ weight changes in the presence of body weight differences between the groups. Toxicity evaluation with organ-body weight ratio is best predicted for liver, kidneys, heart and spleen and also for most of the organs [20]-[21]-[22]. Though histopathology is supposed to be the gold standard for identifying a treatment related effect on any organ [23]; incidences are reported where relative organ weights give a better understanding of mechanism of toxicity instead of histopathology. For example, minimal increment in liver weight without any microscopic lesions can be correlated with enzyme induction [20]. Consideration should also be given to the residual blood that may remain in organs such as the spleen, heart, lungs, liver and kidneys, which may vary between animal to animal due to the method of exsanguinations [24]. In this study, no significant differences was found for all the relative organ weights (liver, kidneys, heart and spleen) but lungs while this latter is not of major importance in toxicological assessment. Hence, suggestion could be made that anti-nutritional factors in GLM-based diet did apparently not affect health status of rabbits at levels up to 15% of inclusion.

Haematological parameters

GLM based diets with or without methionine supplementation did not influence PCV, RBC as well as MCH, MCV and MCHC, indicating that the levels of inclusion (10 and 15%) of the leaf meal whether supplemented or not had no adverse effect on the composition of the mentioned haematological parameters. Hemoglobin (Hb) values in rabbits fed the supplemented diets (F_{SM10} and F_{SM15}) are similar to those fed the reference diet (F0) while slightly lower than rabbits fed

unsupplemented diets (F10 and F15). This result showed no untoward effect of dietary treatment on haemoglobin concentration in rabbits. Animals fed the diets containing 10% of supplemented leaf and 15% of GLM with or without methionine supplementation showed reduction in white blood cells (WBC) counts. Reduction of WBC counts may render the animals vulnerable to attack by pathogenic agents following reduction in their immune system. Extraction from herbs shows a reduction in WBC as extract dosage increase, [25]-[26]. However the WBC values obtained in this study were similar for both rabbits fed 10% GLM unsupplemented and those fed the control diet meanwhile different from those fed 10% supplemented GLM which showed the lowest value of WBC counts. Besides rabbits fed 15% supplemented (F_{SM15}) and non-supplemented (F15) GLM diets displayed similar WBC counts, indicating that methionine supplementation showed no deleterious effect on the WBC parameter. Hence, this finding suggest that the differences observed in the WBC counts might neither be in connection with the GLM inclusion nor the methionine supplementation in rabbit diet. Moreover, values obtain for all haematological parameters (RBC, WBC, PCV, Hb, MCH, MCV, MCHC) were in the range of normal values defined for these parameters by previous studies [27]-[28]-[29]-[30]-[31]-[32]-[33] in rabbits, demonstrating that dietary treatment had no adverse effect on haematological parameters.

Serum Biochemistry

The analysis of serum biochemical parameters provides important information about visceral organ damage in rabbits, particularly for the liver and the kidneys [31]-[33]-[34]. An increase in ALT and AST is an indicator of tissue damage; it is frequently used as an indicator of liver disease. According to [35]-[36], serum ALT and AST levels are elevated in nearly all liver and Kidney damages and are particularly high in conditions that cause extensive cell necrosis, including severe viral hepatitis or toxic liver injury. However, [37] stated that serum ALT increment is a consequence of cell damage, although the augmentation degree does not correlate with the severity of hepatic disease and is not a prognostic indicator. In the present study, it is observed that dietary GLM induce AST elevation at 15% of incorporation and ALT increase at both 10 and 15% level of

inclusion. This is meant to suggest that anti-nutrients in *Gliricidia* leaf meal elicited mild tissue damage in rabbits. The finding of this study also showed methionine supplementation effect in AST and ALT reduction. This might be constructed to mean that methionine serve as detoxifier, donating methyl group radicals into a system to effect detoxification [14] and both methionine and choline Chloride have been reported to act as methyl donors to facilitate the excretion of absorbed anti-nutrients [15]. Potassium is an important ion in the maintenance of membrane potential. About 95% of the total body potassium is intracellular, so measurement of extracellular potassium in blood samples does not give a true reflection of the potassium status of the animal material. However, abnormally high or low blood potassium concentrations can have life-threatening consequences due to impaired electrical activity of cells [38]. High blood potassium concentrations can result in cardiac arrest [38]. Alterations in blood potassium levels could be due to the presence of anti-nutrient or toxic factor which can cause alteration in dietary intake and excretion, or redistribution across cell membranes. In this study, though blood Electrolytes concentration of rabbits showed reduction in potassium ion level at 10% unsupplemented GLM, this reduced value according to [39] finding is still in the normal range (3.5-7 mmol/l) of potassium ion for rabbit. As for calcium, it is an essential element that is involved in many body systems. Hypocalcaemia is a life-threatening condition and a total serum calcium concentration is a reflection of dietary calcium intake [40]-[41]. In this assay, serum calcium concentration in rabbit was improved with increasing level of Dietary GLM conjugated with methionine supplementation. This finding shows the good utilization of the rich minerals in the leaf meal by the experimental animal models.

5. Conclusion

This research work using *G. sepium* leaf meal at 10 or 15% in diets and supplemented with or without methionine showed that inclusion at the two levels had no adverse effects on internal organs status, haematological parameters, some biochemical determinants and electrolyte profile of the rabbits. Elevation in serum ALT and AST suggested that Antinutrients in GLM elicited mild tissue lesions in rabbit which is slightly corrected by methionine supplementation. It is recommended that in subsequent researches, methionine supplementation, to facilitate detoxification, should include other detoxicant like Choline chloride, Polyethylene Glycol (PEG) or Polyvinylpyrrolidone (PVP). This could enable the inclusion of the leaf meal at higher levels.

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