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Biochemical and Oxidative Stress Parameters of Broilers Fed Meal and Protein Isolate of *Mucuna pruriens* Seeds

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Abstract

Mucuna pruriens (velvet bean) represents an interesting source of protein poorly studied. The effect of dietary inclusion of meal and protein isolate of *Mucuna* seeds on biochemical and oxidative stress parameter of broilers (135-one-day $Cobb_{500}$ chickens) was investigated. Three isonitrogenous diets were formulated from soya bean meal (Control group: RTS), *Mucuna* meal (coded RFM) and *Mucuna* protein isolate (coded RIM). Each of the dietary treatments was triplicated with 15 birds per replicate in a completely randomized design. The birds were offered feed and water *ad libitum*. The results revealed significant ($p < 0.05$) effect of N source on the organ total proteins with treatment RFM and RIM exhibiting lower but comparable levels in the Liver (2.01 and 1.98 g/dL), Heart (1.95 and 1.89 g/dL) and Kidney (1.92 and 1.91 g/dL). Triglycerides contents were significantly ($p < 0.05$) higher in the liver of broilers fed RIM and RFM (2.49 and 2.36 mg/dL), in the Kidney of chicks fed RIM and RTS (2.27 and 2.34 mg/dL) and in the Heart of birds fed RTS and RFM (1.90 and 1.87 mg/dL). Broilers fed RFM presented the highest ($p < 0.05$) Liver total cholesterol (1.61 mg/dL) and ALAT contents but with similar values with birds fed RTS (36.43 and 35.50 UI/L respectively). ASAT level was significantly high ($p < 0.05$) in the Liver and Plasma (265.50 and 264.50 UI/L respectively) of broilers of RFM diet. In all the organs, MDA content was highest ($p < 0.05$) in chicks of RIM batch. In the Heart and Plasma, chicks of RFM (3.23 and 5.05 µl/mg respectively) and RIM (5.45 and 5.35 µl/mg respectively) diets registered elevated rate of CAT. In view of these results, investigations remain to be carried out on the impact of the inclusion of

meal and protein isolate of *M. pruriens* seeds in broilers diet during the growth-finishing phase.

Keywords

Mucuna pruriens seeds, Serum Indices, Starter Phase, Broilers

1. Introduction

The high costs of animal feedstuff particularly of protein origin tend to suggest that alternative protein sources are explored for poultry feed in order to achieve favorable economic returns [1] [2] [3] Feed is an important factor for broiler production. Among feed ingredients, protein costs are higher than others *i.e.* it involves about 15% of the total feed cost [4]. Protein enhances muscle building and is very critical in animal diet formulation because it is the most limiting and expensive nutrient and the best indicator of diet quality [5]. Dietary protein is a major source of body protein. Poor quality or imbalanced protein can create metabolic stress which reduced growth performance [4]. There is, therefore, the need to explore the use of high-quality protein sources but cheap and non-conventional feeding stuff like *M. pruriens*.

In addition to its agronomic potential as a cover crop and for replenishing soil fertility, *Mucuna* is high in protein with a range of 25% - 36% [6]. The use of *Mucuna pruriens* seeds as a source of plant protein for non-ruminant animals remained limited because of the presence of certain anti-nutritional compounds like total free phenolics, tannins, L-Dopa, phytic acid, lectins, oligosaccharides, trypsin, and chymotrypsin inhibitors and α-amylase inhibitors [7] [8]. Severe inhibitions in feed intake, growth rate, poor egg production and incidence of high mortality in broiler chicks fed raw *Mucuna* beans have been reported [6]. Various processing methods applied on *Mucuna* seeds reported to improve their nutritional quality such as soaking, cooking, dehulling, roasting, fermentation, sprouting, toasting have not always been effective in the elimination of anti-nutritional factors present in its seeds [8] [9]. The best way to exploit the full potential of this nonconventional legume as food and feed will be mapped both by looking back at ways in which it has been used traditionally and by exploring the potential of modern processing methods to identify and reduce the toxic substances [8]. In this regard, special technics such as isolating protein technic and water extraction method under acidic or alkaline conditions have been reported to be effective in the elimination of anti-nutrients [10] [11] [12] [13]. However, informations on the use of such technics on legume seeds are still scarce. The present study was carried out to produce meal and protein isolate of Mucuna seeds treated according to the aforementioned methods and to assess their effect on biochemical and oxidative stress parameters of broilers during starter phase.

2. Materials and Methods

2.1. Site and Period of the Study

The study was conducted at the animal store of the University of Ngaoundere, capital of the Adamawa region in Cameroon. This town is located between the 6th and 8th degrees of north latitude and between the 11th and 15th degrees of East longitude on the Adamawa ridge. Ngaoundere is a transition zone between the northern lowlands and the southern Cameroon plateau. This position gives it a Sudano-Guinean climate with a rainy season of 8 months, from April to November and a dry season of 4 months, from December to March. The plant cover consists of Sudano-Guinean shrub savannah. The annual rainfall varies between 900 and 1500 mm. Average temperatures vary between 23˚C and 25˚C. The region of Adamaoua, thanks to its climate and its vegetation cover, is a zone of strong potentialities.

2.2. Preparation of Meal and Protein Isolate of *Mucuna* Seeds

Processing *M. pruriens* seeds meal following Rakotomanana *et al.* [14] method with some modifications: mature seeds of *M. pruriens* var. Cochinchinensis were manually rid of infested seeds and impurities. They were soaked in tap water (1:10, w/v) for 48 h. After dehulling manually, they were requenched in a solution of sodium bicarbonate ($NaHCO_3$) at a concentration of 0.8% for 24 hours. The seeds were boiled in clean water for 30 minutes and sundried for 2 days, after which they were milled and ground to a particle size of 1.00 - 1.70 mm using a commercial milling machine and the meal was stored in plastic bags for incorporation into the experimental diet.

Processing protein isolate of *M. pruriens* seeds according to the associated methods of Kom *et al.* [15] and Rakotomanana *et al.* [14] with some modifications, the seeds were soaked in a volume of water so that the seeds were completely submerged for 48 h, the water was changed after 24 hours. Then, they were rinsed successively 3 times with drilling water. These seeds were ground using a wheel mill and the resulting paw was collected in a 13L clear white bucket to which was added 1:4 ratio water (w/v). The pH was adjusted between 8 and 8.5 with 2N NaOH and the whole was then homogenized at 120 rpm for three hours using a PROLABO brand arm shaker. The mixture was allowed to stand for 24 hours. The supernatant was collected and set aside. The residue was extracted again according to the same protocol but the mixture was homogenized for one hour and left to stand for two hours, then filtered. The two supernatants obtained were mixed and the pH was adjusted between 4 and 4.5 with 2N acetic acid by homogenizing the solution, then leave to stand for 16 hours. This allowed the precipitation of the proteins and the isoelectric precipitate obtained was filtered, drained and finally dried in the sun for 12 hours. Protein isolate meal obtained was then stored in plastic bags for incorporation into the experimental diet.

2.3. Experimental Diets and Animal Management

Three (3) isonitrogenous broiler starter diets (20% - 22% CP) were formulated in which were incorporated the soya bean (RTS). *Mucuna* seed Meal (RFM) and *Mucuna* seed protein isolate (RIM) as principal protein sources respectively. Table 1 shows the ingredients and nutrient composition of the experimental diets. Proximate analysis was carried out on the respective experimental diets [16].

One hundred and thirty-five (135) one-day-old $Cobb_{500}$ broiler chicks birds of comparable weights were randomly allocated to the three treatment batches (RTS, RFM and RIM) each with three replicates in a completely randomized experimental design. Each treatment had a total of 45 birds distributed into 3 replicates, and each replicate had 15 birds and reared in an electrically heated cage (size 80 × 50 × 60 cm) on wood shavings. Feed and water were given *ad libitum* on a daily basis during the three weeks of the trial. The chicks received timely and adequate vaccination against common poultry viral, bacterial and protozoal diseases [17].

Table 1. Centesimal and chemical composition of experimental diets.

Ingredients	RTS	RFM	RIM
Maize	50	45	56
Corn bran	18	14	14
Soya bean meal	**07**	**00**	00
Mucuna meal	**00**	**16**	00
Protein isolate of *Mucuna*	**00**	**00**	05
Fish meal	14	14	14
Cotton cake	05	05	05
Bone meal	01	01	01
MNVC 5%	05	05	05
Total	100	100	100
Calculated chemical composition			
Metabolisable Energy (Kcal/Kg DM)	3144	3172	3196
Crude proteins (% DM)	21.2	21.4	21.7
Energy/Proteins	148.3	148.2	147.2
Fats (%DM)	5.4	4.8	4.4
Crude fibres (%DM)	5.0	6.1	4.3

CMAV 5%: Mineral Nitrogen and Vitamin Complex: PB = 40%; Calcium = 8%; Phosphore = 2.05%; Lysne = 3.3%; Methionine = 2.40%; EM = 2078 kcal/kg; DM = Dry Matter; RTS = Meal-based diet of soya bean; RFM = Meal-based diet of *M. pruriens*; RIM = Meal-based diet of protein isolate of *M. pruriens*.

2.4. Organs Evaluation and Organ Homogenates Preparation

At the end of the trial, after 12 h of feed depravation, three chickens per treatment replicates were randomly selected and bled by severing the jugular vein. Vital organs (heart, liver and kidney) were collected. They were washed in 0.9% sodium chloride (NaCl) solution, dewatered and weighed. The weight of each organ was standardized to 100 g body weight of each animal. These organs were then ground separately in a mortar containing fine sand. Then 1 volume of the ground material was homogenized in 9 volumes of phosphate buffer (0.1 M, pH 7.4). The organ homogenates obtained were stored in dry tubes at a temperature of −20˚C for the determination of biochemical and oxidative stress parameters.

2.5. Biochemical Indices

Blood samples collected from sacrificed birds in the sterile glass test tubes were allowed to coagulate at room temperature for 30 min and were subsequently centrifuged at 3000 g for 10 min. Serum was removed and stored frozen at (−4˚C) until required analysis. Estimation of biochemical parameters in serum and homogenates such as Total cholesterol [18], Triglycerides [19] and Total proteins [20] were measured. Activity of transaminases, Aspartate aminotransferase (ASAT) and Alanine Aminotransferase (ALAT) [21] was determined. The amount of Malondialdehyde (MDA) [22] and Catalase (CAT) activity [23] were determined.

2.6. Statistical Analysis

Data collected were subjected to Analysis of variance (ANOVA) and significant differences were observed between replicates of treatment and between treatments. The means were compared using Duncan's Multiple Range Test [24].

3. Results and Discussion

3.1. Effect of Meal and Protein Isolate of *M. pruriens* on Organs of Broilers in Starter Phase

No significant difference was observed between the relative masses of the organs (liver and heart), except at kidney's level whose mass was heavier ($p < 0.05$) in the chicks of the test groups (RFM and RIM) compared to those of the control group (RTS) (Table 2).

Table 2. Characteristics of organs of chicks in the start-up phase according to experimental diets.

	RTS	RFM	RIM	p
Heart	0.64 ± 0.88	0.74 ± 0.12	0.77 ± 0.09	0.09
Liver	2.78 ± 1.12	2.84 ± 0.26	2.98 ± 0.29	0.33
Kidney	0.30 ± 0.04^{b}	0.41 ± 0.03^{a}	0.40 ± 0.02^{a}	0.02

^{a,b}: Averages with the same letters on the same line are not significantly different at the 5% level; RTS: Meal-based diet of soya bean; RFM: Meal-based diet of *M. pruriens*; RIM: Meal-based diet of protein isolate of *M. pruriens*; p = probability.

3.2. Effect of Meal and Protein Isolate of *M. pruriens* on Biochemical Parameters of Broilers in Starter Phase

The comparison of biochemical values between the experimental diets showed that their contents in Total proteins and Triglycerides were similar in the Plasma whatever the diet consumed while in Total Cholesterol, Plasma level in the one hand and Liver level in the other hand were significantly ($p < 0.05$) high in chicks fed RFM (Table 3). Compared with the control diet, the birds fed RFM and RIM diets had comparable and lower ($p < 0.05$) total protein levels in the Liver, heart and kidney. Triglyceride levels were found to be significantly high in chicks ($p < 0.05$) of RIM in the Liver and the kidney though the value in the kidney was comparable to that in birds of the control with these animals having a higher ($p < 0.05$) but similar content in the heart with chicks in RFM.

Table 3. Some biochemical characteristics in some organs and plasma of broilers in starter phase according to experimental diets.

	Organs	RTS	RFM	RIM	*P*
Total proteins (g/dL)	Liver	3.56 ± 0.06^{a}	2.01± 0.03^{b}	1.98 ± 0.02^{b}	0.00
	Heart	2.84 ± 0.01^{a}	1.95 ± 0.01^{b}	1.89 ± 0.03^{b}	0.00
	Kidney	2.97 ± 0.04^{a}	1.92 ± 0.04^{b}	1.91 ± 0.02^{b}	0.00
	Plasma	3.31 ± 0.04	3.24 ± 1.14	3.32 ± 0.09	0.59
Triglycerides (mg/dL)	Liver	2.07 ± 0.02^{b}	2.36 ± 1.15^{ab}	2.49 ± 0,20^{a}	0.03
	Heart	1.90 ± 0.01^{a}	1.87 ± 0.02^{ab}	1.83 ± 0.02^{b}	0.03
	Kidney	2.34 ± 0.01^{a}	2.13 ± 0.04^{b}	2.27 ± 0.04^{a}	0.00
	Plasma	1.94 ± 0.04	1.92 ± 0.01	1.99 ± 0.02	0.12
Total Cholesterol (mg/dL)	Liver	1.37 ± 0.03^{b}	1.61± 0.10^{a}	1.41 ± 0.05^{b}	0.01
	Heart	1.22 ± 0.01	1.18 ± 0.01	1.20 ± 0.04	0.39
	Kidney	1.33 ± 0.04	1.38 ± 0.02	1.31 ± 0.04	0.14
	Plasma	1.68 ± 0.02^{c}	2.07 ± 0.06^{a}	1.48 ± 0.02^{b}	0.00

^{a,b,c}: Averages with the same letters on the same line are not significantly different at the 5% level; RTS: meal-based diet of soya bean; RFM: meal-based diet of *M. pruriens*; RIM: meal-based diet of protein isolate of *M. pruriens*; p = probability.

3.3. Effect of Meal and Protein Isolate of *M. pruriens* on Some Blood Toxicity Parameters of Broilers in Starter Phase

The dietary treatments affect significantly blood toxicity parameters. Except in the Liver, chicks fed RFM and RTS diets presented significantly highest ($p < 0.05$) but comparable Alanine aminotransferase (ALAT) contents in the plasma (Table 4). Broilers fed RFM diet recorded the highest ($p < 0.05$) Aspartate aminotransferase (ASAT) content both in the Liver and the Plasma.

Table 4. Some biochemical characteristics in the Liver and the Plasma of broilers in starter phase according to experimental diets.

	Organs	RTS	RFM	RIM	*p*
ALAT (UI/L)	Liver	43.00 ± 2.00	42.00 ± 0.50	44.33 ± 1.04	0.18
	Plasma	35.50 ± 0.50^{a}	36.43 ± 0.57^{a}	33.00 ± 1.00^{b}	0.00
ASAT (UI/L)	Liver	170.16 ± 0.76^{c}	265.50 ± 2.00^{a}	179.66 ± 1.52^{b}	0.00
	Plasma	208.66 ± 3.32^{c}	264.50 ± 1.80^{a}	239.16 ± 2.02^{b}	0.00

^{a,b,c}: Averages with the same letters on the same line are not significantly different at the 5% level; RTS: meal-based diet of soya bean; RFM: meal-based diet of *M. pruriens*; RIM: meal-based diet of protein isolate of *M. pruriens*; p = probability.

3.4. Effect of Meal and Protein Isolate of *M. pruriens* on Oxidative Stress Parameters of Broilers in Starter Phase

The effect of including different processed *M. pruriens* seeds in broiler diets on activities of antioxidant enzymes is presented in Table 5. Compared with the control, the activity of MDA in birds fed on RIM diet significantly ($p < 0.05$) increased in the Liver, Heart and Kidney, while CAT activity increased significantly ($p < 0.05$) but similarly in chickens fed RFM and RIM diets in the Heart and Plasma. Broilers fed RFM and RIM diets registered the highest ($p < 0.05$) CAT activity in the Kidney and in the Liver respectively compared to other treatments.

Table 5. Some stress oxidative characteristics in some organs and Plasma of broilers in starter phase according to experimental diets.

	Organs	RTS	RFM	RIM	*p*
Malondialdehyde (µM/mg)	Liver	1.03 ± 0.01^{b}	1.14 ± 0.03^{b}	1.60 ± 0.09^{a}	0.00
	Heart	1.39 ± 0.06^{c}	1.69 ± 0.09^{b}	1.93 ± 0.03^{a}	0.00
	Kidney	1.98 ± 0.01^{b}	2.12 ± 0.09^{b}	2.53 ± 0.12^{a}	0.00
	Plasma	1.21 ± 0.09	1.11 ± 0.05	1.25 ± 0.09	0.19
Catalase (µl/mg)	Liver	4.27 ± 0.06^{c}	5.05 ± 0.04^{b}	5.45 ± 0.16^{a}	0.00
	Heart	2.75 ± 0.08^{b}	3.23 ± 0.24^{a}	3.52 ± 0.11^{a}	0.00
	Kidney	4.13 ± 0.25^{b}	5.06 ± 0.05^{a}	4.22 ± 0.13^{b}	0.00
	Plasma	4.19 ± 0.17^{b}	5.05 ± 0.05^{a}	5.35 ± 0.13^{a}	0.00

^{a,b,c}: Averages with the same letters on the same line are not significantly different at the 5% level; RTS: meal-based diet of soya bean; RFM: meal-based diet of *M. pruriens*; RIM: meal-based diet of protein isolate of *M. pruriens*; p = probability.

3.5. Principal Component Analysis of Serological Parameters of the Experimental Diets

Biochemical, toxicity and stress oxidative parameters of the broilers were submitted to principal component analysis (PCA) and the results are presented in Figure 1 and Figure 2. Figure 1 presents the correlation circle of the variables

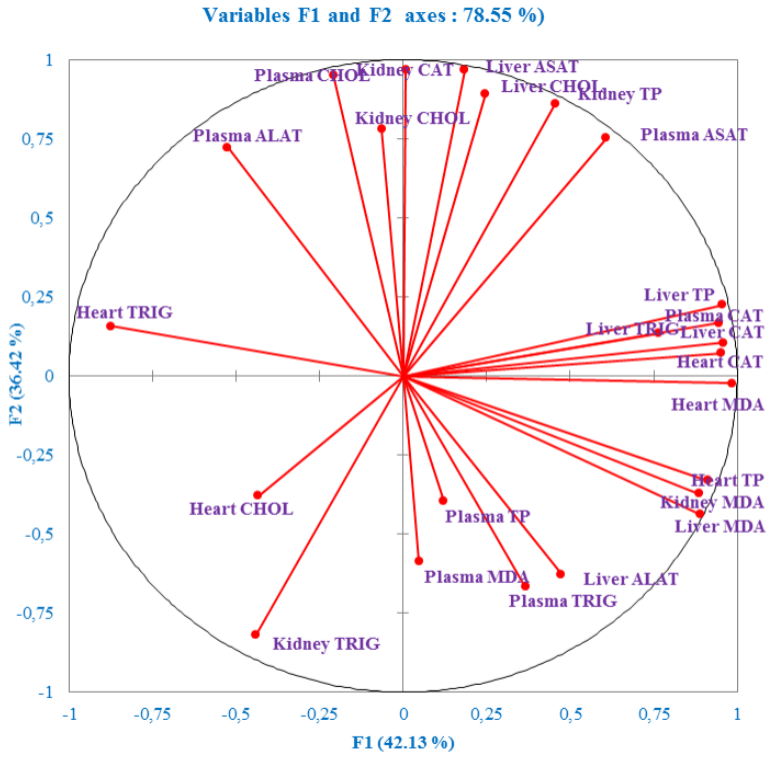

Figure 1. Representation of the variables of biochemical, toxicity and stress parameters of broilers on the principal components F1 and F2. TP: Total proteins; TRIG: Triglycerides; CHOL: Cholesterol; MDA: Malondialdehyde; CAT: Catalase.

under investigation on the PC1 and PC2 axes. The principal component 1 (PC1) and 2 (PC2) respectively explained 42.13% and 36.42% variations among serological parameters, allowing PCA to explain a total variation of 78.55%. All serological characteristics (biochemical, toxicity and stress oxidative) highly contributed to the PC1 and PC2 axes. The variables in PC1 that contributed much to discriminate the serum parameters were Heart TP, Kidney MDA, Liver MDA and Heart MDA followed by Kidney TRIG; In PC2, those which much discriminate were Heart CAT, Liver CAT, Plasma CAT, Liver TP, Plasma ASAT, Kidney TP, Liver CHOL, Liver ASAT, Kidney CAT. Highly significant correlations were found ($r > 0.95$; $p < 0.001$) between Liver MDA and Heart TP, Liver CAT and Liver TP, Plasma CAT and Liver CAT, Plasma CAT and Liver TP, Liver CAT and Liver TP as attested by their proximity in the correlation circle of PCA (Figure 1).

The representation of experimental diets on the PC1 x PC2 plot revealed a net separation of the experimental diets (Figure 2). Generally, a diet with *M. pruriens* meal (RFM 1, RFM 2 and RFM 3) was diametrically opposed to soya bean meal (RTS 1, RTS 2 and RTS 3) which was positioned at the left frame down of the PC1 × PC2 reference alongside with diet containing protein isolate of *M. pruriens* (RIM1, RIM2 and RIM3) positioned just at the right frame down of the PC1 × PC2 reference. Diet with *M. pruriens* meal (RFM 1, RFM 2 and RFM 3) was characterized by their high value in Kidney CAT, Liver ASAT, Liver CHOL,

Plasma ASAT followed by lower levels in Liver TP, Plasma CAT, Liver CAT, and Heart CAT along with low values in Heart CHOL and Kidney TRIG. Similarly, diet containing protein isolate of *M. pruriens* (RIM 1, RIM 2 and RIM 3) were associated with high Kidney MDA, Liver MDA levels and relatively low levels in Plasma TRIG, Liver ALAT, Plasma MDA. High values of Heart CHOL and Kidney TRIG characterize soya bean meal (RTS 1, RTS 2 and RTS 3) diet compared to the supplemented diets.

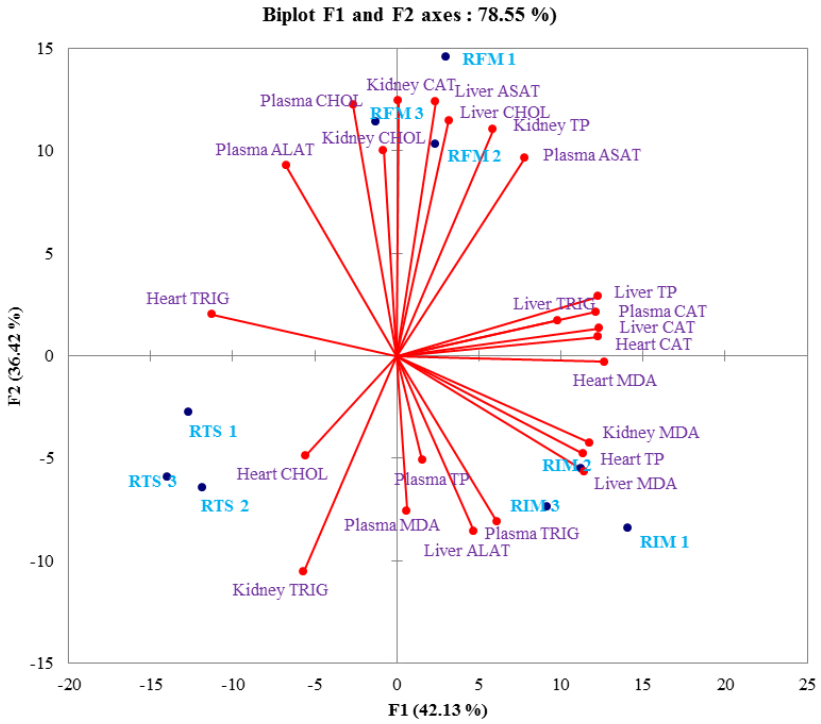

Figure 2. Representation of the experimental diets on the principal components F1 and F2. RTS1, RTS2 and RTS3: Meal-based diets of soya bean; RFM1, RFM2, RFM3: Meal-based diets of *M. pruriens*; RIM1, RIM2, RIM3: Meal-based diets of protein isolate of *M. pruriens*.

4. Discussion

The increase or decrease in the relative mass of organs in animals after consumption of a substance means that the substance is toxic [25]. In this case, the observed increase in the kidney's weight of broilers fed RFM and RIM diets may be related to toxic material still present in meal and protein isolate of *M. pruriens* which has not been eliminated or destroyed after treatments. Our findings are in agreement with the findings of Mang *et al.* [26] who reported a significant increase in kidneys of rats supplemented with vegetable milk prepared from whole and dehulled *Mucuna* bean flours when compared to the control.

Blood total proteins are strongly associated with feeding regimes and reflect their fluctuation occurring during the protein metabolism [27]. In the present study, the lower ($p < 0.05$) total protein concentrations registered in broilers fed RFM and RIM diets (Kidney, Liver and Heart) reflected low protein metabolism

in the experimental broiler chickens. RFM and RIM diets led to hypoproteinemia which may be caused by insufficient protein production in the event of liver damage [28].

Total cholesterol and Triglycerides are of particular importance for cardiovascular disease, especially coronary artery disease [29]. As reported by Arija *et al.* [30], the increase of Triglycerides in the Liver of birds fed RIM and RFM diets, in the Heart of birds fed RTS and RFM diets and in the Kidney of chicks RTS and RIM diets, and the increase in Cholesterol observed in the liver and the Plasma of broilers fed RFM diets could be related to physicochemical modification of nutrients (amino acids, lipids, Non-Starch Polysaccharides, phytosterols) in the experimental diets. According to Martins *et al.* [31], these changes could affect the nutrient digestibility or modify the intestinal microflora and the enterohepatic metabolism of steroids. Our results corroborate those of Arija *et al.* [30] in Cholesterol and Triglycerides of broiler chick fed raw kidney bean and extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto).

The significant increase in blood ASAT and ALAT enzymes of birds fed RFM and RIM diets act as hepatocellular damage indicators [32] [33]. Our results supported the findings of Marijani *et al.* [34] who found an increase in ALAT level of *Oreochromis niloticus* exposed to aflatoxin B1 diet. These results with ALAT suggest that there was liver damage in chicks fed protein isolate of *Mucuna* compared to control as also supported by Carew *et al.* [32]. The increase in ASAT level in plasma of chicks of RIM batch is in agreement with results of Raza *et al.* [35] in different swiss albino mice submitted on prolonged vigabatrin treatment and contrasts with those of Ngatchic *et al.* [36] in rats fed on *M. pruriens* flour and protein-rich *Mucuna* product.

Malondialdehyde (MDA) a marker of oxidative stress, qualified as an indicator of lipid peroxidation increased ($p < 0.05$) in the organs and in the plasma of supplemented broilers (RFM and RIM diets) suggesting a high lipid peroxidation [37] [38]. This increase in MDA content means that these animals were under stress. This finding is in disagreement with those of El-bahr *et al.* [37] in broilers fed dietary microalgae and in agreement with previous works of He *et al.* [38] in broilers fed on dietary fumacic acid, Mirzaie *et al.* [39] in broiler chickens fed on dietary Spirulina and Ngatchic *et al.* [36] in rats fed on *M. pruriens* flour and protein-rich *Mucuna* product.

The first level of defense is based on the activity of specific enzymes such as catalase (CAT) which, together with metal-binding proteins, are responsible for prevention of free radical formation and keep this process under control [40]. Dietary supplementation of meal and protein isolate of *Mucuna* increased the activity of CAT in organs and plasma of birds, implying that free radical formation could be prevented in broilers by dietary meal and protein isolate supplementation. This observation suggests that dietary meal and protein isolate of Mucuna could contain substances that can act either by stimulating the synthesis of antioxidant enzymes or by preventing their denaturation or their inhibition by free radicals [41].

Otherwise, in addition to the significant increase in MDA and CAT activity in the organs and in the plasma at the same time, our results imply that the activation of anti-oxidative enzymes cannot prevent the oxidative injury induced by dietary exposure. It should be noted that oxidative stress is associated not only with the changes in the scavenging capacity of antioxidant systems but also with the elevated production of free radicals [38]. The increased MDA level and activity of anti-oxidative enzyme (CAT) imply that the balance between the production and scavenging of hydroxyl radicals is disrupted, resulting in their compensatory increase and an adaptive mechanism underlying the increase in oxidative stress [38].

The ability of a protein source to be efficient for the supply depends not only on its chemical composition but also on the digestibility of its protein [42]. The three experimental diets served to the animals presented three different profiles trough the dispositions observed at the PCA level. While the control diet (RTS) was characterized by an increase in the content of Cholesterol and Triglycerides, high CAT activity and ASAT level were observed with the *Mucuna* flour diet (RFM) and a high MDA content with the *Mucuna* proteins isolate diet (RIM). This shows that, although being isonitrogenous, these diets were not digested in the same way. A possible explanation of this effect could be related to the low digestibility of protein and amino acids in birds fed *Mucuna* compared with the control diet. Similar results have been published in previous studies with raw and extruded kidney beans suggesting that their low nutritional value is due to the antinutritional factors (ANF) present in the seed, mainly trypsin inhibitors [30]. Pugalenthi *et al.* [8], Arija *et al.* [30], Emiola *et al.* [9] and Iyayi and Taiwo [6] also attributed this antinutritional effect in broilers to the inefficient use of *Mucuna* proteins and excessive secretion of endogenous nitrogen. This means that the proteins should end up in the feces. However, in this study, this aspect was not evaluated. Another possible explanation would be that *Mucuna* proteins may have been all ingested in the same way, but the presence of ANF once more prevents their assimilation. The results of this study underline the harmful role of residues of antinutritional factors still present in the *Mucuna* regardless of the treatment applied. Our findings confirmed those of Ngatchic *et al.* [10] [36] who also demonstrated in rat systems that when ingested, *Mucuna* proteins still exhibit toxic biological activity dependent on the extraction method. The processing methods used in this study confirmed this assertion. These residual ANF may be the reasons that stand behind the observed lower growth performance recently reported in broilers fed meal and protein isolate of *M. pruriens* seeds by Mweugang *et al.* [43]. As reported by several authors, *Mucuna* contains numerous ANF such as tannins, lectins, phytic acid, cyanogens, trypsin inhibitors, and L-Dopa (3,4-dihydroxy-L-phenylalanine), which is prominent among these factors [1] [7] [42]. It is therefore very likely that the level of L-Dopa was still very high in meal and protein isolate of *Mucuna* after processing and would have reduced the digestibility of proteins in the RFM and RIM diets. However, L-Dopa

content was not determined in this study. On the other hand, Hou *et al.* [44] reported that ANF and other toxins that enter the animal's body through the diet have been suggested to induce oxidative stress in animals. This study revealed that broilers fed RFM and RIM diets were under stress with positive signs of oxidative stress expressed through an increase in plasma MDA and higher CAT activity in the birds. The increase of cholesterol and triglycerides concentrations in plasma could be related to physicochemical modification of nutrients (amino acids, lipids, Non Starch Polysaccharides, phytosterols) in the control diet. These changes could affect the nutrient digestibilities or modify the intestinal microflora and the enterohepatic metabolism of steroids [31].

5. Conclusion

The complete substitution of soya bean by meal and protein isolate in broilers diet caused a negative effect on the biochemical parameters with the consequence of increased oxidative stress in broilers. The residues of ANF still present in the seeds even after the different processing technics used in this study could explain these results. Hence, further toxicity studies of meal and protein isolate of *M. pruriens* seeds need to be investigated.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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