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Research Article

Evaluation of processed *Mucuna pruriens* seeds as a feed ingredient in poultry (*Gallus domesticus*) nutrition: Zootechnical performance and haematology

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Abstract – *Mucuna pruriens* seeds, commonly known as velvet beans, are rich in protein but underutilized in poultry feed due to anti-nutritional factors. This study examined the proximate composition of pretreated *M. pruriens* seed meal and its effects on zootechnical and haematological performance in broiler chickens. Seeds were soaked in bicarbonate of soda, dehulled, dried, roasted, and ground. Proximate composition was analyzed following AOAC guidelines. Protein digestibility was assessed via *in vitro* trypsin inhibitory activity using four seed meal concentrations (0%, 5%, 10%, and 15%), which corresponded to dietary inclusion levels of 0, 50, 100, and 150 g/kg (T1–T4). Thirty-six Cobb 500 broilers were assigned to these diets from day 29 to 42 in a completely randomized design (3 reps × 4 diets). The pretreated seed meal contained a high crude protein level of 328.7 ± 10.6 g/kg. Trypsin inhibition increased with seed meal concentration, highest at 15%. Significant differences ($P < 0.05$) were observed in average daily gain (ADG), average daily feed intake, and dressed weight. Birds fed the 100 g/kg diet (T3) showed the best performance with an ADG of 129.45 ± 1.53 g/kg, FCR of 1.74 ± 0.04, and dressed weight of 2058.66 ± 0.13 g/kg. Haematological data showed significant variation in packed cell volume, white blood cell count, mean corpuscular volume, and mean cell haemoglobin concentration. Neutrophil and

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lymphocyte counts varied across treatments. The 100 g/kg inclusion level (T3) proved optimal, indicating the potential of pretreated *M. pruriens* seed meal as a safe, effective protein source in poultry feed.

Keywords: *Mucuna pruriens*, poultry, pretreatment, haematology, proximate composition

1. Introduction

The use of prebiotics, probiotics, synbiotics, and antibiotics as growth promoters has been proposed and used in livestock production for years. However, their use as growth promoters has raised concerns due to antimicrobial resistance and the subsequent effect on the health of consumers. Negative effects have been reported due to the incorporation of antibiotics as growth promoters in livestock feeds (Barton, 2000). Modern-day consumers are increasingly conscious of the quality of meat they consume. Medical recommendations restricting red meat consumption in favour of white meat, especially poultry, have been one of the major stimuli and drive towards sustainable broiler production (Font-i-Furnols, 2023). A consistent increase in demand for energy and protein sources led to an increase in grain prices (Sarmiento-Franco et al., 2019). It is therefore imperative to find effective alternatives to replace antibiotics as feed additives due to rising global plans to reduce antibiotics and provide food that is safe and healthy. Interestingly, phytogens such as thyme, oregano, cinnamon, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin, and coriander, among others have been proposed for considered as alternatives in poultry production in recent times (Cheng et al., 2014; Flees et al., 2020).

The seeds of *Mucuna pruriens*, a leguminous plant found in tropical countries, have been reported to be one of the promising phytogenic feed additives due to their nutritional value as a rich source of protein (23-35%) (Ayodele et al., 2021). *M. pruriens* is also known as velvetbean because the seed pods are covered in non-stinging silky hairs that give the pods a smooth, soft appearance. In a bid to reduce the amount of soybean meal utilized in livestock feed formulation, *M. pruriens* seeds are a viable source of protein and are rich in lysine which is an important limiting amino acid, especially in poultry diets (Mthana et al., 2022). The phytic acid content of *M. pruriens* has been reported to possess antioxidant, anticarcinogenic, and hypoglycaemic activities (Sowdhanya et al., 2024). The availability of some bioactive substances such as anti-nutrients, however, limits the use of raw *M. pruriens* seeds in animal feeding. Anti-nutritional factors reduce animal feed intake as well as nutrient utilization. These anti-nutrients and toxic compounds arise from secondary metabolism in plants and include trypsin inhibitors, chymotrypsin inhibitors, polyphenols, nicotine, phytostigmine, serotonin, and phytates (Condori & de Camargo, 2023).

Due to their attractive phytochemical composition and nutrient profile, *M. pruriens* seeds have the potential to be used as one of the ingredients in poultry feed. This study was therefore conducted to determine the proximate composition of *M. pruriens* seed meal pretreated by soaking the seeds in bicarbonate of soda, dehulling, drying, roasting and grinding, followed by evaluation of the effects of the pretreatment on zootechnical performance and haematological parameters when included in the diet of broiler chickens.

2. Materials and methods

A mass of 50 kg *M. pruriens* seeds used in this current study was procured from small-scale farmers in Karoi (Latitude: 16° 48′ 36′ S, Longitude: 29° 42' 32.89 E) located in agro-ecological region 2 of Zimbabwe,

with an average temperature of 28 °C and rainfall ranging from 600 mm-800 mm.,

2.1 Processing of *M. pruriens* seeds

Masses of 1 kg of *M. pruriens* seed samples were used in triplicate during this research. The seeds were soaked in sodium bicarbonate for 72 hours in a ratio of 1 kg: 3 litres, and the final pH was adjusted with phosphate buffer to between 6.4 and 8. The seeds in the sodium bicarbonate solution were placed in a water bath at 50 °C for 72 hours and the sodium bicarbonate solution was then removed. To increase the digestibility of the seeds, they were then dehulled (removing the seed coat and splitting the cotyledons) using fingers whilst moist. The dehulled seeds were then oven-dried and roasted in a pan for 15 minutes until the whitish seeds turned to brown colour. The seeds were ground to fine powder using a blender and stored at room temperature.

2.2 Proximate analysis of *M. pruriens seed* meal

Ground fine granules of *M. pruriens* seeds were analysed for proximate composition according to the Association of Official Analytical Chemists (AOAC, 1990). All the samples were used in triplicate to determine the content of moisture, crude protein, nitrogen-free extractives, crude fibre, ash and ether extract (fat).

2.3 Determination of trypsin inhibitor activity

To determine the concentrations required for the formulation of chicken feed, the inhibitory effect of *M. pruriens* seed meal extract on the activity of trypsin was first investigated. This was done *in vitro* using caseinolytic activity with commercial trypsin and casein as a substrate according to Nizkii and Dildina (2020). *M. pruriens* seed meal was defatted using petroleum ether (boiling point 60 – 70 °C). A volume of 19 mL of water was used to suspend a gram of the seed flour and the pH adjusted to 7.6. Mechanical shaking was done for 60 minutes and the suspension was centrifuged. The supernatant was collected and 1 mL was mixed with 50 mL phosphate buffer, set aside at room temperature for later use as seed extract. Trypsin stock solution was prepared by adding 5 mg crystalline, salt-free trypsin into 100 mL 0.001 M HCl. Triplicate sets of test tubes for each treatment were prepared and 0.5 mL of the trypsin stock solution was pipetted into each test tube. The final volume of each tube was adjusted to 2 mL with phosphate buffer and test tubes were placed in a water bath set at 37 °C. A volume of 2 mL of 5 % trichloroacetic acid was added to one triplicate set of test tubes and served as a blank. A 2 % casein solution was prepared by suspending 2 g of casein in 80 mL phosphate buffer, completely dissolving by heating on a steam bath for 15 minutes and then making up to 100 mL with phosphate buffer. The solution was then cooled and stored at 4 °C. The 2 % casein solution was brought at 37 °C and 2 mL were added to each test tube and incubated at 37 °C for 20 minutes. The reaction was terminated by adding 6 mL of 5 % trichloroacetic acid to all test tubes. The suspension was allowed to settle for 1 hour at 25 °C and the suspension was filtered, and the absorbance of the filtrate measured at 280 nm. Trypsin inhibitor activity was determined by pipetting 0 %, 5 %, 10 % and 15 % aliquots of *M. pruriens* seed extract of the total volume in each test tube (one triplicate set for each extract concentration). For each specified percent aliquot, the volume of the extract was brought to 1 mL using phosphate buffer. A 1 mL trypsin stock solution was added and allowed to stand in a water bath at 37 °C for 20 minutes. A blank solution was prepared using the same procedure as described above for the trypsin-casein activity assay. A volume of 2 mL of casein that had been brought at 37 °C was added and test tubes were allowed to stand at 37 °C for 20 minutes. The reaction was stopped by adding 6 mL of 5 % trichloroacetic acid to test tubes and allowed to stand for

1 hour at 25 °C until the suspension was filtered. The absorbance of the filtrate was measured at 280 nm to calculate trypsin units inhibited.

2.4 Formulation of experimental diets and feeding trials

The feeding trial was conducted in Chinhoyi (Latitude: 17°20′59.0″ S; Longitude: 30°11′40.0 E), agro-ecological region 2 with an average temperature of 28 °C and rainfall ranges from 600 mm-800 mm. A total of 36 Cobb 500 strain broiler day-old chicks were fed with commercial starter from day 1 to day 14 and a grower diet from day 15 to day 28. From day 29, the *M. pruriens* seed meal was added to the feed formulation to give four treatment compositions of 0 g/kg, 50 g/kg, 100 g/kg and 150 g/kg. The treatments were named T1, T2, T3, and T4, respectively. The diets were formulated according to Nutrient Requirements for Animals 1994 (NRC, 1994). A Pearson square method was used to come up with a 3-phase broiler diet consisting of the starter (21 % crude protein), grower (19.5 % crude protein) and broiler finisher (18 % crude protein). Feed was provided at ad libtum with light provided in pens for 23 hours every day. From day one to day seven, temperature was controlled and maintained at 32±2℃. Newcastle disease vaccination was done on day 10 and day 21 during the feeding trial. From day 28, feed intake per replicate was measured daily to come up with the feed intake per bird per day. On day 42, birds were deprived of feed overnight followed by measuring the final live weight. Birds were slaughtered by exsanguination and the carcass weight was determined.

2.5 Haematological parameters

At the end of the feeding trial, one bird from every replicate was used as a representative sample for blood analysis (differential red and white blood cells). A volume of 5 mL of blood was collected aseptically from the jugular vein of each bird and stored in EDTA bottles. After collection, blood was transferred to an EDTA anticoagulant vial to prevent clotting. Blood was centrifuged at 200 rpm using a microhaematocrit centrifuge and stored at room temperature for 2 hours. A haemocytometer (Neubauer improved method) was used to measure the red blood cell and white blood cell counts. A volume of 20 μL of blood was placed in the haemocytometer counting chamber and ×40 objective lens on a microscope was used to manually count in the Neubauer chamber. The method by Jain (1986) was used for the determination of the mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). The packed cell volume was calculated by filling heparinized capillary tubes and sealing at one end. The capillary tubes were centrifuged at 12000 rpm for 5 minutes. Red blood cells (RBC) were determined as RBC × 10^{12}/L. After the sedimentation of blood cells, the cells were read using a micro-haematocrit reader. Differential white blood cells were determined using a slide smear of methyl alcohol fixed blood drop and the blood-stained Leishman stain. The microscope was focused and counted using ×10 and × 40 objective lenses, respectively. Thus, the number of each type of white blood cell was calculated as the percentage of total white blood cell type counted x10 total White Blood Cells / 100 = absolute number (%). Determination of haemoglobin concentration was done using Drabkin's solution, dispensed into a sample tube and allowed to stay for 5 minutes to attain (2 °C). Blood was aspirated using a micropipette into the sample tubes. Diluted blood was decanted into cuvettes and a spectrophotometer was used to measure absorbance at 540 nm. Ammonia solution was used as a blank to tare the spectrophotometer. Methods by Erhabor et al. (2021) were used to determine other variables as follows:

Mean cell volume (fL) = Packed cell volume (PCV) × 10/ RBC × 10,

Mean cell haemoglobin (pg) = Hb × 10/RBC (10^6),

Mean cell haemoglobin concentration (%) = Hb × 100/PCV

2.6 Differential leukocyte count

Blood smears were prepared from broilers subjected to graded levels of pre-treated *M. pruriens* seed meal and stained by Leishman's stain, and manual differential leukocyte count was done on dried blood smears. The Battlement method was used to count the 100 cells with the aid of a microscope using a cell counter. Analysis was performed using SPSS version 10. The mean and standard deviation of continuous variables were calculated. Paired sample t-test was used to compare the means. A'P' value of < 0.05 was considered statistically significant.

2.7 Data analysis

Statistical analysis was conducted using GraphPad Prism version 8.0.1. Data collected was analysed for variance (ANOVA) and a completely randomized design (CRD) was used to allocate experimental treatments. Significant differences among treatments were calculated using pairwise differences at $P < 0.05$ using multiple t-tests. A P-value of 0.05 or less indicated that the difference between the two samples is statistically significant.

3. Results

3.1 Nutritional composition of pretreated seeds of *M. pruriens*

Table 1 shows the proximate composition of the pretreated *M. pruriens* seed meal. The results showed that the seed meal had a high crude protein level of 328.7 g/kg.

Table 1. Proximate composition of pretreated *M. pruriens* seed meal

Parameter	Quantity (g/kg Dry matter)
Crude Protein	328.7±16.4
Crude Fibre	53.6±4.2
Ether Extract	64.3±3.1
Moisture	79.6±1.5
Ash	32.3±1.5
Nitrogen Free Extractives	441.3±1.3

Note: Means with ± SEM of triplicate set of determinations

3.2 Trypsin inhibitor activity

Figure 1 shows the effect of the pretreated *M. pruriens* seed meal on trypsin enzyme inhibition. Treatments were measured in 3 replicates with absorbance versus seed meal extract inclusion level. The results showed that inhibitory activity increases with an increase in seed extract concentration. There was a significant difference among the means of trypsin inhibitor activity ($P < 0.025$).

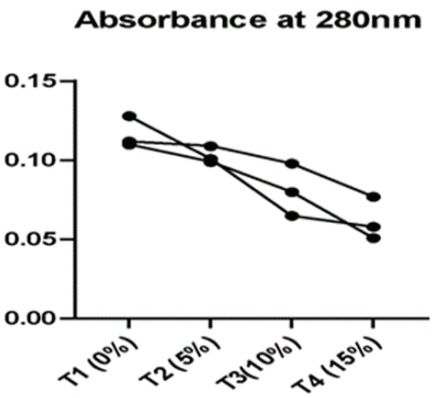

Figure 1. Rate of enzyme activity in the presence of pretreated *M. pruriens* seed meal extract.

3.3 Zootechnical parameters

Birds subjected to *M. pruriens*-supplemented diets had a lower feed intake value than the control treatment (T1) without any *M. pruriens* seed meal infusion (Table 2). Average daily gains were also influenced ($P < 0.05$) by *M. pruriens* inclusion such that birds on 100 g/kg *M. pruriens* seed meal diet (T3) had the highest average daily gain compared to the rest of the treatments. The control diet (T1) presented the lowest average daily gain weight. The inclusion of *M. pruriens* seed meal in experimental diets had no significant effect on the feed conversion ratio ($P > 0.05$). However, there was a progressive decline in feed conversion ratio as supplementation levels with *M. pruriens* seed meal increased (Table 2). There were significant ($P < 0.05$) reductions in live and dressed weights of chickens with incremental inclusion levels of *M. pruriens* seed meal (Table 2). Birds on the 50 g/kg *M. pruriens* diet had the highest live weights compared to those on the 100 g/kg and 150 g/kg and the converse was true for dressed weights.

Table 2. Zootechnical parameters of birds fed graded levels *M. pruriens* seed meal

Performance parameter	Treatments				SEM	CV
	M. pruriens inclusion					
	0 g/kg	50 g/kg	100 g/kg	150 g/kg		
Feed intake (g/bird/day	178.4^{a}	169.1^{ab}	157.8^{b}	156.6^{b}	5.14	0.62
Average Daily gain (g/bird/day)	96.90^{a}	97.49^{a}	129.45^{ab}	79.21^{b}	29.9	1.53
Feed conversion ratio	1.85^{a}	1.86^{a}	1.74^{a}	1.98^{a}	0.04	0.52
Live weight (g/bird)	2777^{a}	2984^{b}	2762^{b}	2648^{ab}	254	0.19
Dressed weight (g/bird)	1817^{ab}	1530^{b}	2058^{a}	2007^{a}	1332	0.13

N=9

3.4 Haematological parameters

Haematological indices were determined to assess the effect of the *M. pruriens* seed meal on the immunology and physiology of the broiler chickens. Table 3 shows haematological indices of broilers on a diet supplemented with *M. pruriens* seed meal. There was an overall significant difference in blood parameters except in RBC and platelets when broiler birds were fed with a *M. pruriens* seed meal-based diet. From the differential blood count, birds subjected to a 50 g/kg (T2) inclusion diet

presented the highest packed cell volume among all other treatments ($P < 0.05$). The control diet (T1) resulted in birds that had a high haemoglobin concentration compared to all other treatments ($P < 0.05$). The mean cell volume of broilers from the 150 g/kg (T4) was 195.39 ± 62.40 being the highest among other treatments ($P < 0.05$). Furthermore, the mean cell haemoglobin concentration in blood from birds that were fed on a control diet of 0 g/kg (T1) was 44.17 ± 8.06 and it was the highest among all treatments ($P < 0.05$). A significant difference was noted in the white blood cell count of birds that had received the experimental diets. T1 had the highest white blood cell count followed by the 50 g/kg (T2) inclusion level whilst the 150 g/kg (T4) inclusion level had the least white blood cells ($P < 0.05$). Platelet count was similar across treatments. Despite the high haemoglobin content observed in birds fed with the control diet (T1), birds fed 150 g/kg (T4) diet had a higher mean cell haemoglobin concentration of 62.02 ± 19.42 ($P < 0.05$).

Table 3. Haematological indices of broilers on *M. pruriens* seed meal-supplemented diet.

Indices	0 g/kg (T1)	50 g/kg (T2)	100 g/kg (T3)	150 g/kg (T4)	P- value	CV
PCV (%)	30.43±0.40^{a}	31.35±0.51^{b}	29.20±0.18^{ab}	25.68±0.62^{b}	0.006	7.108
RBC ($\times 10^{6}$/mm)	2.18±0.11	2.19±0.07	2.35±0.02	1.48±0.58	0.100	18.951
Hb (g/dL)	9.63±0.26^{a}	9.51±0.31^{b}	8.45±0.04^{a}	8.46±0.23^{ab}	0.004	6.435
MCV (fL)	139.65±7.76^{a}	143.26±5.84^{ab}	124.31±1.46^{a}	195.39±62.40^{b}	0.042	20.760
MCHC (%)	44.17±8.06^{a}	30.98±0.32^{ab}	28.98±0.09b	31.82±0.31^{a}	0.002	10.919
WBC ($\times 10^{3}$/mm)	24.75±0.25^{b}	24.33±0.72^{ab}	23.37±0.13^{a}	22.15±0.63^{a}	0.002	4.582
Platelets ($\times 10^{3}/\mu L$)	1.75±0.14	1.75±0.43	1.75±0.25	1.67±0.29	0.640	2.816
MCH (pg)	40.87±2.86^{ab}	43.82±0.61^{ab}	35.36±1.32^{a}	62.02±19.42^{b}	0.003	25.365

Note: Means followed with a different superscript are significantly different ($P < 0.05$). % CV = coefficient of variance

3.5 Differential leukocyte count

The differential leucocyte count was done to specifically assess the effect of the graded levels of *M. pruriens* seed meal on the immunology of the broiler chickens. A significant difference was observed between manual counts of neutrophils and lymphocytes as shown in Table 4.

Table 4. White blood cell profile of broilers subjected to graded levels of pre-treated *M. pruriens* seed meal

Indices (% Differential counts)	Treatments				SEM
	0 g/kg (T1)	50 g/kg (T2)	100 g/kg (T3)	150 g/kg (T4)	
Neutrophils	1.50^{b}	1.67^{b}	4.47^{ab}	4.53^{ab}	0.45
Monocytes	0.00	0.00	0.00	0.00	0.00
Eosinophils	0.00	0.00	0.00	0.00	0.00
Basophils	0.00	0.00	0.00	0.00	0.00
Lymphocytes	98.50a	98.33^{a}	95.53^{ab}	95.47^{ab}	0.46

Note: Means in the same row followed by a different subscript are significantly different ($P < 0.05$). SEM = Standard error of means.

4. Discussion

In the present study, the crude protein of *M. pruriens* is higher than that of most consumed legumes, such as *Cicer arietinum, Pisum sativum, Phaseolus vulgaris, Cajanus cajan* and *Lens culinaris* that range from 18.5 to 22 % for raw grains (Sherasia et al., 2017). This could be attributed to the interaction between the genetic makeup and agroecology (Mugendi et al., 2010). In this study, *M. pruriens* seeds contained higher crude fat content (6.43 ± 0.31 %) than other legumes. The observed crude fibre content of 5.36 % was lower than reported by other researchers. Jadhav et al. (2022) reported neutral detergent fibre of 13.91 ± 0.12 % and acid detergent fibre of 6.45 ± 0.10 %. The reduction in crude fibre levels could be due to the removal of the seed coat which contains considerable amounts of fibre material such as lignin as well as other polysaccharides. Boiling and roasting reduced the crude fibre levels of *M. pruriens* seeds compared to raw ones (Adepo et al., 2016). The nitrogen-free extractives found were 44.134 ± 1.332 % and were composed of sugars and small amounts of other materials such as hemicellulose and pectins. The ash content of 3.23 ± 0.15 % was within the documented 3.02 – 3.82 % range for other *Mucuna* varieties (Ezegbe et al., 2023).

The highest trypsin inhibition activity was observed in the 15 % *M. pruriens* seed extract (0.062 ± 0.013), whilst the lowest inhibition was in treatment without *M. pruriens* extract. This implies that the rate of enzyme activity and protein digestion was lower in broiler birds fed on an *M. pruriens*-based diet. Enzyme inhibitors (protease inhibitors), for example, trypsin inhibitors in animal diets result in the formation of non-reversible inhibitor-enzyme complexes. This causes a decline in trypsin enzymes in the gastrointestinal tract and subsequently affects protein digestibility (Pugalenthi et al., 2005). Raffinose and stachyose cause flatulence due to the presence of microflora in the large intestines that utilize carbohydrates, producing flatus gases such as hydrogen, carbon dioxide and small amounts of methane gas. The fact that trypsin inhibitor activity deviates from linearity at high

levels of inhibitor concentration has been attributed to the partial dissociation of the trypsin-inhibitor complex (Kakade et al., 1969). Kakade et al. (1969) also reported the presence of inhibitors other than the Kunitz inhibitor in the crude extract that could influence the kinetic picture one would observe with such a complex system. This current study showed that the processing methods used were effective in reducing protease inhibitors, but the level of inhibition increases with higher than 10 % *M. pruriens* seed meal doses.

The average daily gain obtained among the treatments was high in the 100 g/kg seed meal inclusion (T3) diet (129.45 g) whilst the 150 g/kg seed meal inclusion (T4) diet resulted in a low average daily gain (79.21 g). Significant differences in average daily gain (ADG) and dressed weight (DW) could be attributed to various factors including nutrient availability and enzyme inhibitory activity. The results obtained are not consistent with findings reported by Aboh et al. (2011) who noted that broiler chickens fed on processed *M. pruriens* seeds at 12.5, 18.75 and 30 % as a replacement for soybean meal resulted in growth depression due to the residues of anti-nutritional and toxic factor components such as tannins, hydrocyanic acid, L-DOPA, phenolic compounds, phytates, lectins. However, in another finding, broilers fed with *M. pruriens* powder presented a heavier live weight than birds without *M. pruriens* bean (Sarmento-Franco et al., 2019). In the current study, *M. pruriens* seed meal inclusion levels exceeding 100 g/kg resulted in a decline in the average daily gain (ADG) and the decrease in average daily gain recorded in animals receiving experimental diets suggests that the remaining anti-nutritional factors reduced the bioavailability of nutrients and enzyme inhibition during digestion. These anti-nutritional factors act either by complexing nutrients and preventing their absorption along the gastrointestinal tract, by inhibiting the activity of enzymes responsible for their hydrolysis, or by inducing toxicity at high doses (Mang et al., 2016). The findings elaborated that *M. pruriens* seed meal is capable of accelerating the growth rate until a specific level is reached (100 g/kg) after which the growth rate starts to decline. The results obtained in this study agree with the findings of Sese et al. (2013) who reported that processing tends to have a positive impact in the reduction of the anti-nutritional factor concentrations in the seeds or leaves of *M. pruriens* so as to improve broilers' performances, it does not entirely eliminate them. Feed composition with no *M. pruriens* seed meal showed the highest feed intake among the treatment means (178.460 g), whilst the 150 g/kg seed meal inclusion (T4) diet resulted in the lowest feed intake (156.687 g). The reduced feed intake in broilers fed *M. pruriens* seed meal-based diet can be due to the presence of residual anti-nutritional factors that affect animal voluntary feed intake such as tannins. Tannins are reported to affect the palatability of the diet due to their astringent properties and as a result, they bind with protein in saliva and mucous membranes of the gastrointestinal tract (Dohouda et al., 2009).

The highest packed cell volume (PVC) was noted in the 50 g/kg seed meal inclusion diet group with 31.35 ± 0.51 mm3. All the PCV values were within the normal range of 25 to 45 % reported by Ikhimioya et al. (2000), who stated that, despite the processing method, all the diets were nutritionally high enough to provide a sound plane of nutrition. No significant differences were observed in the red blood cell count but the, 100 g/kg seed meal inclusion group had the highest red blood cells of 2.35 ± 0.02 mm3. The haematocrit values represent the number of red blood cells as a percentage in the blood (Etim et al., 2014). The increase in the rate of haematocrit level in broiler birds fed graded levels of processed *M. pruriens* seed meal could be attributed to the increase in the bioavailability of the nutrients responsible for the erythrocyte synthesis. Similar results were reported

by Nebedam et al. (2010) who reported an increase in red blood cells, haemoglobin and PCV implying that the extracts increase the red blood cell population produced from the bone marrow. The differences in mean corpuscular volume and mean corpuscular haemoglobin concentration could be due to the effects of graded levels of pretreated *M. pruriens* seed meal on animal physiology. However, Ayo et al. (2023) reported no significant $P > 0.05$ difference in white blood cells, mean corpuscular haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration, lymphocyte count and neutrophil levels. The haemoglobin concentration is also a good measure of meat quality; therefore, *M. pruriens* seed meal supplementation produced adequate haemoglobin in broiler birds. Zoraunye et al. (2023) reported meat colour as one of the proxy indicators of meat quality, being influenced by haemoglobin and myoglobin formation, thus the potential to alter meat colour. The mean cell volume and mean cell haemoglobin values obtained in all treatment groups fall within the normal range of 90 to 100 fL and 34 to 51 pg, respectively (Yavorkovsky, 2021). The results are consistent with the findings of Adenkola et al. (2011), who reported that nutrients are an important factor in haemopoiesis. Broiler chickens fed with T1 diet had the highest mean cell haemoglobin concentration of 44.17 ± 8.06 %, but no significant differences ($P > 0.05$) were noted in the platelet count. Moreover, among the four treatment groups, none was found to be anaemic. Haemoglobin concentration ranged from 8.45 to 9.63 g/100 mL in this study. Haemoglobin concentration values obtained in this study were within the normally accepted range of 7.0 – 13.0 g/dl for broiler chickens (Anon, 1980). This shows that all the experimental birds could withstand respiratory stress since haemoglobin is the oxygen carrier in red blood cells.

Graded levels of soaked, dehulled, dried and roasted *M. pruriens* seed meal had a significant ($P < 0.05$) effect on the white blood cells (WBCs). The number of WBCs is influenced by several factors, including nutrition (Oliveira et al., 2014). Neutrophil count was high in the 150 g/kg seed meal inclusion diet group. Monocytes and neutrophils are important components of white blood cells that are capable of oxygen-dependent and oxygen-non-dependent metabolism for protection against viruses and bacteria. Treatment with the 0 g/kg seed meal inclusion diet presented birds with a higher lymphocyte count of 98.50 % which is more than 40 to 48 % reported by Bhatti et al. (2010). Therefore, all the experimental diets had adequate nutrition to produce an effective immune response status. It was noted from the lymphocyte levels that *M. pruriens* seed meal, as a feed ingredient, is capable of activating lymphocyte production as well as CD4+ and CD8+ cells. The results obtained prove that all the diets were sufficient in protecting broiler chickens from infection. Eosinophils, monocytes and basophils were not found in all the birds from experimental treatments. Thus, there was no inflammation encountered in all experimental birds. Favourably, the lack of mortality among all experimental birds shows that the techniques of processing the *M. pruriens* seeds were effective in reducing anti-nutritional factors (ANFs). Another way in which *M. pruriens* seed meal as a phytogen possibly influences animal health is through amelioration of oxidative stress (Gupta et al., 2006). This is also in line with Nathalie et al. (2020), who reported a lack of mortality cases which suggests effective methods of processing *M. pruriens* seeds for broiler feeding.

According to Willems et al. (2013), a desirable feed conversion ratio in poultry should be below 2. In this study, the diet with the 100 g/kg *M. pruriens* bean meal (T3) inclusion diet resulted in birds that had a better feed conversion efficiency (1.743) whilst the 150 g/kg *M. pruriens* bean meal-based (T4) diet presented a poor feed conversion ratio (1.982). Nwani et al. (2017) reported similar results and noted a

significant increment in weight difference from week 1 to week 7 showing that birds were gaining more weight as they grew.

The lowest feed conversion ratio value of 1.743 registered in experimental broilers fed 100 g/kg *M. pruriens* diet showed that at this level, there was a lower feed intake and the broiler chickens were gaining more, therefore, this indicates a good feed utilization efficiency. The improvement in feed conversion efficiency registered in the 100 g/kg seed meal-based diet was a result of efficient utilization of minerals, protein, energy and vitamins.

5. Conclusion

The present research findings revealed that pretreating *M. pruriens* seeds by soaking, dehulling, drying and roasting, is effective in improving the legume's nutritional quality without side effects at 100 g/kg inclusion on broiler chickens (*Gallus domesticus*). The differential count of leukocytes presented a positive effect of processed *M. pruriens* seed meal in boosting the immune status. These results support the potential use of *M. pruriens* seed meal as a phytogen and an alternative source of protein in broiler diets.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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