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Serum and haematological parameters of growing rabbits as affected by varying dietary inclusion levels of maggot (*Hermetia illucens*) meal as replacement for soybean meal.

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***Target Audience:** Nutritionists, Researchers and Rabbit farmers and Feed millers*

Abstract

This study investigated the effect of replacing soybean meal (SBM) with maggot meal (MM) in growing rabbit diets on the serum and haematological parameters. Sixty unsexed mixed breeds (New Zealand White x California) rabbits were allotted on a weight equalization basis into five dietary treatments, where the control diet (soybean meal-based) has no MM (MM0) while other diets contain 25% (MM25), 50% (MM50), 75% (MM75) and 100% (MM100) MM as replacement for soyabean meal. Each treatment comprises four replicates having three rabbits each (12 rabbits per treatment) and the feeding trial lasted for 56 days. Feed and water were offered to the rabbits' ad-libitum throughout the experimental period. Blood collection for the determination of serum and haematological parameters were done on the 56th day. All data obtained were subjected to one way analysis of variance SAS 2000 and significant means were separated using Duncan multiple range test. The feeding of MM100 diet to rabbits resulted in the highest (P<0.05) total serum protein (11.60 g/dl) and globulin (6.70 g/dl) while those fed MM0 diet had the lowest values for total serum protein (6.55 g/dl) and globulin (2.60 g/dl). The inclusion of MM in the diet of rabbits reduced (P<0.05) serum cholesterol. Haematological parameters were not significantly (P>0.05) influenced by varying inclusion levels of MM in the diet of rabbits. In conclusion, the replacement of soyabean meal with MM in the diet of rabbits up to 100% did not have any deleterious effect on the serum and haematological parameters.

***Key words:** Haematology, Serum, Maggot meal, Soyabean meal, Rabbit*

Description of Problem

The production of rabbits is becoming much more popular lately owing to its advantages nutritionally, prolificity and efficiency of production over other livestock (1). The major objective of this development is to provide alternative animal protein source to mutton, pork, beef and chicken to meet the demand of the growing populace (2). One of the major advantages of rabbit production to other categories of livestock includes its wide acceptance due to non-existing religious taboo against its consumption and the nutritive quality of the meat in terms of low cholesterol content. Unlike the pigs and poultry birds, rabbit can subsist majorly on forages with little additional concentrate for improved growth and better performance thus reducing the cost of production (3). However, the cost of the feed ingredients needed for the production of additional concentrate is on the increase (4) and if it is not judiciously managed it may overrun its production

advantages. As a known fact, about 60-70% of most livestock cost of production is expended on feed (5), which majorly evolves from the increasing prevailing cost of the ingredients making up their diets (6).

Amongst the most demanded feed ingredients by man and animal is maize and soyabean (7). Soybean is a household plant protein source that is used in soymilk production, as component of infant cereal meal, source of soyabean oil and the chaff is incorporated into livestock feed as soybean meal or cake. The demand pressure on soybean had caused escalation of its price than expected (8). Replacement of this hitherto expensive soybean meal with a cheaper suitable alternative may help to reduce the cost of animal protein to humans. Meanwhile, researchers have ventured into the search for farm and industrial waste as alternative feedstuff to animals (9,10, 11). Most of the research report have yielded profits as compared to the cost of the conventional ingredients. Previous work by Mafimidiwo *et al.,* (12) replaced soyabean meal with cotton seed cake in the diet of pigs without any side effect on the serum and haematology of the pigs. However, recently, cotton seed cake is scarce and sourcing for the ingredient is now competitive which calls for further investigation into other alternatives and one of the alternatives that can be explored in the nutrition of rabbits is maggot meal. Maggot meal possess a crude protein value of about 47-55%, 27.65% ether extract and 3955kcal/kg metabolizable energy according to Ahmad and Sayed (13). This nutrient composition is similar to that of soyabean meal and precisely the crude protein (CP) of soybean meal (45-54%) is comparable to that of maggot meal (14). It is interesting to note that maggot meal despite its source have been reported of not transmitting any known disease as a result of its inclusion in animal feed (15). Maggot meal have not been assayed of any known anti-nutritional factor unlike soybean with trypsin inhibitor (16). In addition, it is also fascinating to understand that the preparation of the maggot meal is less technical than some other livestock feed ingredients.

Positive reports have been documented on the use of maggot meal in the diet of fishes and poultry. Hwangbo *et al*., (17) reported improved weight gain of fish with dietary inclusion of maggot meal. Maggot meal have been successfully incorporated in the diet of broilers as replacement for fishmeal without adverse effect on carcass characteristics (18). The use of MM in the diet of broilers also improved weight gain and feed efficiency (15). However, research studies on the use of MM in the diet of rabbits is scarce and this further justify the relevance of this study. It is important to investigate the impact of dietary MM inclusion on the health status of rabbits by checking through haematological and serum parameters of the rabbits. The outcome of the analysis of blood parameters helps to provide information about the quality of the feed and the level of nutrients availability and utilization (19). It also helps to reveal any adverse effect or health implication that may arise due to the ingestion of feed materials (20). Therefore, this research work investigated the effect of replacing soybean meal with maggot meal on the haematological and serum parameters of grower rabbits.

Materials and methods

Experimental site

The experiment was carried out at the Teaching and Research Farm of the School of Agricultural Technology, Yaba College of Technology, Epe, Lagos. The protocol for the experiment was reviewed and approved by the Animal Use and Care Unit at Yaba College of Technology Lagos. The rabbit

house had average temperature and humidity of 30.10^{o}C and 75.60% respectively.

Animals, experimental diets, and management

Sixty (unsexed) mixed breed (New Zealand x California) rabbits weighing about 758±2.50 grams were procured from a reputable farm in Ibadan and were acclimatized for seven days at the Teaching and Research Farm of the School of Agricultural Technology, Yaba College of Technology, Epe, Lagos on Latitude 3. 58^{o}E and Longitude 6. 47^{o}N (21). They were offered feed and water *ad-libitum* and all pre-experimental medications were strictly adhered to. Maggot meal was procured from Magmeal Industries, Ora estate, Epe, Lagos, and other feed ingredients were purchased from a reputable feed mill at Epe, Lagos State. The proximate composition of soybean meal and maggot meal was determined using AOAC (22), calcium and phosphorus content were analysed using an atomic absorption spectrophotometer (Perkin Elmer Optima 4300DV ICP spectrophotometer, UK), and fibre fractions were conducted using the standard method by McCleary (23) (Table 1).

The rabbits were randomly allotted on a weight equalization basis into five dietary treatments with four replicates of three rabbits and the rabbits were housed in aluminum wire cages (60×45×50cm). The diets comprise a corn-soybean meal-based diet (Diet 1) (MM0= diet with 0% maggot meal), while diets 2, 3, 4, and 5 had the soybean meal in the feed replaced with maggot meal at 25% (MM25), 50% (MM50), 75% (MM75) and 100% (MM100) levels respectively (Table 2). The feeding trial lasted for 56 days while feed was offered liberally to the rabbits and they had unlimited access to cool clean drinking water. The humidity in the experimental pen was 65±10% and the temperature was 27± 2.5^{o}C. A cycle of 12h of light and 12h of dark was maintained throughout the feeding trial.

Table 1: Nutrient composition of soybean meal (SBM) and maggot meal (MM)

Item (% dry matter)	Soyabean meal	Maggot meal
Dry matter	89.00	95.00
Crude protein	44.43	55.56
Crude fibre	5.60	3.23
Ether extract	6.20	18.62
Calcium	0.31	0.45
Phosphorus	0.65	1.46
Acid detergent fibre	8.60	12.45
Neutral detergent fibre	13.50	28.56
Ash	6.50	9.21
NFE	33.30	19.62

Data Collection

Blood sample collection and analysis

On the 56th day, blood samples (2ml each) were collected from a rabbit per replicate (four per treatment) for serum and haematological analysis. Prior to bleeding, a cotton swab soaked in 70% ethanol was used to dilate the vein and to prevent infection. The blood samples were collected from the external ear vein of each rabbit using a 2ml sterilized disposable syringe and needle into labeled sample bottles.

Table 2: Ingredients and nutritional composition of experimental diets

Items	MM0	MM25	MM50	MM75	MM100
Ingredients (%)					
Maize	30.00	30.00	30.00	30.00	30.00
Maggot meal	0.00	3.75	7.50	11.25	15.00
Soybean meal	15.00	11.25	7.50	3.75	0.00
Wheat offal	38.00	38.00	38.00	38.00	38.00
Palm Kernel Cake	10.00	10.00	10.00	10.00	10.00
Molasses	2.50	2.50	2.50	2.50	2.50
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	2.00	2.00	2.00	2.00	2.00
Salt (NaCl)	0.35	0. 35	0.35	0. 35	0.35
Premix*	0.05	0.05	0.05	0.05	0.05
Lysine	0.05	0.05	0.05	0.05	0.05
Methionine	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100
Analyzed Nutrients[a]					
Dry matter (%)	90.12	89.22	87.95	88.45	87.84
Crude protein (%)	16.21	16.03	16.20	16.34	16.41
Crude fibre (%)	16.14	16.04	15.95	15.55	15.34
Ether extract	2.83	2.85	2.97	3.21	3.32
Ash	5.21	5.10	4.90	4.89	4.78
Gross energy (Kcal/kg)	3554.34	3563.45	3585.84	3601.88	3643.52
Metabolizable Energy (kg/kcal)[b]	2538	2553	2570	2584	2596

*Each 1 kg vitamin and mineral premix provides the following per kg diet: Vit. A 12000000 IU, Vit. D3 750000 IU, Vit. E 10000 mg, Vit. K 2000 mg, Vit B1 1000 mg, vit B2 4000 mg, Vit. B6 1500 mg, Vit B1210 mg, Pantothenic acid 10000 mg, Niacin 20000 mg, Biotine 50 mg, Folic acid 1000 mg, Choline chloride 500mg, selenium 100mg, Manganese 55 gm, Zinc 50 gm, Fe 60 gm, CU 2.5 gm, CO 6 mg and Iodine 1gm.

MM0= without maggot meal (Control), MM25= 25% maggot meal, MM50= 50% maggot meal, MM75= 75% maggot meal, MM100= 100% maggot meal.

a = dry matter basis, b = calculated using the Pauzenga equation: ME= (37 × CP % + 81.8 × CF % + 35.5 × NFE %) (24)

The blood samples collected into labeled sterile bottles without anticoagulant (allowed to clot) was used to determine the serum biochemical components. The blood samples were refrigerated for 6hrs and later spun in a centrifuge at 900rpm for five minutes to obtain serum free from cell debris for the biochemical analysis. The blood samples placed in bottles containing Ethylene-Diamine-Tetra-Acetic acid (EDTA) as anticoagulant was used for the determination of haematological parameters. The collected blood samples were taken to the laboratory to obtain the plasma for haemoglobin, red blood cells count, packed cell volume, white blood cells count and differentials.

Serum parameters

The serum total proteins and albumin were determined colorimetrically as described by Tietz (25) using commercial kits produced in USA by Stanbio Company with computerized spectrophotometer (Model Milton Roy 1201). The globulin values were obtained by deducting albumin values from their corresponding total

proteins values of the same sample. Serum creatinine was determined according to Bonsness and Taussky (26), Serum glucose was determined calorimetrically using Glucose-oxidase Peroxidase (GOD PAD) method (27), serum urea concentration was determined according to Wootton (28) while serum cholesterol was estimated using commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04).

Haematological parameters

The packed cell volume (PCV) was determined using the Wintrobes Microhaematocrit and Haemoglobin concentration (Hb) was determined using the Cyanomethaemoglobin methods as described by Dacie and Lewis (29). The red blood cell (RBCs) and white blood cell (WBCs) counts were determined using a hemocytometer according to Jain (30). Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) levels were calculated according to Bush (31).

Statistical analysis

Data collected were subjected to one-way analysis of variance using SAS (32) and the means were separated using Duncan multiple range test (33) and the level of significance was at 5%.

Result

Serum parameters

The serum parameters of grower rabbits fed diets containing MM as replacement for soybean meal is presented in Table 3. Total protein (TP) was highest ($P<0.05$) for rabbits fed diets with MM at 100% while those fed MM0 diet had the least TP. The globulin was highest ($P<0.05$) for rabbits fed MM100 diet but those fed MM0 diet had the lowest globulin. The rabbits fed MM25, MM50 and MM75 diet had intermediate TP and globulin content. Glucose content increased ($P<0.05$) for rabbits fed MM0 diet and it reduced for those fed MM100 diet. Rabbits fed MM0 diet had the highest ($P<0.05$) cholesterol while those fed MM25 and MM50 diets had the lowest cholesterol but those fed MM75 diet had intermediate cholesterol. Serum urea increased ($P<0.05$) for rabbits fed MM75 diet while those fed MM0 diet had reduced serum urea but those fed MM25, MM50 and MM100 diets had intermediate serum urea. Serum albumin and creatinine were not significantly ($P>0.05$) different.

Table 3: Serum parameters of growing rabbits fed diets containing maggot meal as a replacement for soybean meal

Parameters	MM0	MM25	MM50	MM75	MM100	Normal range¶	Pooled SEM	P-value
Total protein (g/dl)	6.55^{c}	8.15^{b}	8.80^{b}	8.05^{b}	11.60^{a}	5.40-7.50	0.49	0.001
Albumin (g/dl)	4.95	4.70	5.05	4.85	4.90	2.70-5.00	0.05	0.321
Globulin (g/dl)	2.60^{c}	3.45^{b}	3.75^{b}	3.20^{b}	6.70^{a}	1.50-2.70	0.48	0.000
Creatinine (mmol/L)	1.68	2.01	0.99	1.67	1.39	0.56-4.44	0.14	0.206
Glucose (mg/dl)	131.65^{a}	101.80^{bc}	115.75^{ab}	102.25^{bc}	88.20^{c}	75.00-155.00	4.49	0.003
Cholesterol (mg/dl)	94.85^{a}	73.65^{b}	74.25^{b}	80.85^{ab}	78.80^{b}	10.00-80.00	6.59	0.004
Urea (mg/dl)	22.85^{b}	32.35^{ab}	33.40^{ab}	43.75^{a}	33.90^{ab}	20.00-45.00	3.46	0.038

^{ab}Means within a row with different superscripts differ ($P<0.05$)

MM0= without maggot meal (Control), MM25= 25% maggot meal, MM50= 50% maggot meal, MM75= 75% maggot meal, MM100= 100% maggot meal.

SEM = Standard error of mean ¶Melillo (34)

Haematological parameters

Table 4 shows the haematological parameters of grower rabbits fed diets containing MM as replacement for soybean meal. The result shows no significant ($P<0.05$) effect MM inclusion on all haematological parameters determined.

Table 4: Haematological parameters of grower rabbits fed diets containing maggot meal as a replacement for soybean meal.

Parameters	MM0	MM25	MM50	MM75	MM100	*Normal range	Pooled SEM	P-value
Packed cell volume (%)	46.00	44.00	36.50	42.00	39.00	30.00-50.00	1.71	0.455
Haemoglobin (g/dl)	14.85	13.80	12.40	13.80	13.05	8.00-17.50	0.43	0.507
Red blood cell ($x10^{12}$/L)	7.60	7.83	6.45	7.35	6.85	4.00-8.00	0.73	0.359
White blood cell ($x10^{9}$/L)	9.60	9.30	8.10	9.35	8.90	4.68-5.90	0.32	0.673
Neutrophil (%)	29.50	30.50	29.50	28.5	31.00	36.00-50.00	0.51	0.641
Lymphocyte (%)	67.00	65.50	66.00	68.5	65.50	31.00-32.00	0.60	0.510
Eosinophil (%)	1.00	1.50	1.50	1.00	0.50	0.50-5.00	0.16	0.274
Basophil (%)	1.00	0.50	1.00	0.50	1.50	0.00-8.00	0.16	0.274
Monocyte (%)	1.50	2.00	2.00	1.50	1.50	4.00-13.50	0.11	0.274
MCV (fl)	60.63	57.38	56.63	57.19	56.89	58.73-60.14	0.71	0.413
MCH (pg)	19.57	18.25	19.22	18.83	25.47	19.67-20.04	1.29	0.426
MCHC (%)	32.28	32.00	33.95	32.91	33.60	33.10-33.30	0.33	0.281

MM0= without maggot meal (Control), MM25= 25% maggot meal, MM50= 50% maggot meal, MM75= 75% maggot meal, MM100= 100% maggot meal.
MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration
SEM = Standard error of mean
* Mitruka and Rawnsley (35)

Discussion

The result of serum parameters of grower rabbits fed diets with varying inclusion level of MM as replacement for soybean meal shows that rabbits fed MM100 diet had the highest total protein while those fed MM0 (control) diet had the lowest total protein. The increased total protein observed for the group of rabbits fed MM100 diet could be due partly to the high protein content of MM and partly due to level of inclusion. The increased serum total protein also implies that there was increased nutrient utilization particularly protein which will promote growth and development. According to Awosanya *et al*., (36), the dietary protein in terms of quantity and quality has direct influence on the blood protein. The observation in our study is contrary to the report of Hong *et al*., (37) who observed no influence of MM (Musa domestica) inclusion at 5% level of replacement for white fish meal on total protein of growing beagles. Shah (38) also reported no significant effect of MM (Musa domestica) inclusion up to 100% as replacement for soybean meal in the diet of leghorn layers on serum total protein. Furthermore, Mat *et al*., (39) reported reduced total protein for broilers fed diet containing 12% defatted black soldier fly larvae meal (BSFLM). The discrepancies in these reports could be due to differences in the source of MM used and processing methods. The serum globulin was also highest for rabbits fed diet containing MM at 100% as replacement for soybean meal while those fed diet without MM had the lowest

globulin content. It is worthy to note that the total serum protein and globulin of rabbits fed MM100 diet was higher than the upper limit of the normal range reported by Melillo (34) and this indicates the high amount of protein in circulation in the body of the animal which may increase the volume of nitrogen excretion. The serum glucose content was highest for rabbits fed MM0 diet and those fed MM100 diet had lowest serum glucose. The increased glucose observed for rabbits fed MM0 diet implies higher energy availability to the rabbits due to ingredient composition. However, the values obtained for other treatments were within the normal range. Increased cholesterol was obtained for rabbits fed MM0 diet while those fed MM25, MM50 and MM100 diets had reduced serum cholesterol. The reduced cholesterol observed for the groups of rabbits fed diet containing MM suggests that despite the high ether extract content of MM it did not result into high blood cholesterol. In addition, the values were within the normal range reported by Melillo (34). The glucose and cholesterol levels which are within the normal range with the inclusion of MM is an indication that abnormal conditions like diabetes, liver malfunction and under or over absorption of fat that are symptoms of abnormal glucose and cholesterol levels in the blood did not occur (31).

The urea content increased for rabbits fed MM75 diet while those fed MM0 diet had reduced urea content. The reduced urea content observed for rabbits fed MM0 diet may be due to the nitrogen content of the diet. Reduced blood urea has been associated with diseased liver or poor protein nutrition (31). The increased urea with the use of MM could be linked with the MM inclusion with higher crude protein. Although, the values across treatments were within the normal values and this clarifies that there is no existence of ill-health status of the rabbits. The albumin content was not significantly influenced by inclusion of MM in the diet of rabbits but the values were within the reference values even with 100% dietary replacement of soybean meal with MM. This observation suggests nutrient adequacy of the diets and specifically the dietary protein. Onifade and Tewe (40) stated that when normal protein utilization is altered, it results in abnormal serum albumin. The serum creatinine was also similar across treatments and the values were within the normal range which suggests absence of muscle wasting and this implies the rabbits were not surviving at the expense of their body reserves (41).

The haematological parameters determined were not significantly affected by replacement of soybean meal with MM irrespective of the inclusion levels. This observation indicates the suitability of MM inclusion in the diet of rabbits and this suggests that the dietary treatment did not pose the animal to any physiological discomfort (42). Hematological parameters are effective and important indices in determining the nutritional and health status of animal placed on feeding trial (43). This is in agreement with the report of Hatab *et al*., (44) who reported no significant effect of Spodoptera littoralis larvae meal inclusion at 50 and 100% inclusion levels in the diet of Japanese quail on PCV, WBC and haemoglobin. The findings of Schiavone *et al*., (43) are also in consonance with the outcome of this current study, the authors reported no significant effect of dietary inclusion of BSFLM on the blood parameters of broiler chickens. The result of our study is also similar to the report of Shah *et al.,* (38) who reported no significant effect of dietary inclusion of MM at 25-100% as replacement for soybean meal on RBC, PCV and WBC. The values obtained in this study for PCV, haemoglobin and RBC were within

the normal range and this confirms that the body of the animal is still functioning at the normal state. The RBCs are carriers of haemoglobin which reacts with oxygen to yield oxyhaemoglobin in respiration and they also help in transmitting oxygen round the body (45). It therefore means that reduced RBC count will result in reduction in circulating oxygen in the body which is not the case in this study. The WBCs and the differential were also not different across treatment which implies that the rabbits were not challenged with any form of disease organisms or dietary antimetabolites that may disrupt the defense mechanism. The WBCs and its differentials are known to be the factors of choice when immune responses of animal against diseases are to be examined. They help animals to defend the body against foreign agents causing diseases through the process of phagocytosis (46) and thus a situation of reduced of WBC count indicates the animal are at high risk of disease infection. Furthermore, the WBC count across treatments in this study were higher than the normal range which may not necessarily indicate incidence of disease or invasion of foreign agents but increased capacity to resist diseases. Soetan *et al*., (47) stated that animals with increased WBC count have the capacity to produce more antibodies thereby increasing disease resistance.

The MCV, MCH and MCHC also followed a similar trend with no significant difference across treatments. This observation reveals that there was no negative alteration in the blood parameters as these blood indices are important in determination of blood level in the body (48). The non-significant difference observed for MCV, MCH and MCHC is a reflection of the RBC values because RBC count is directly related to these parameters (39). In agreement with this study, Hong *et al*. (37) reported no significant effect of dietary inclusion of MM at 5% as replacement for fishmeal on MCV of growing beagles. Mat *et al*., (39) reported reduced MCH and MCHC for broilers with dietary inclusion of defatted black soldier fly larvae meal (BSFLM) at 4-12% compared to the control. The contrary report could be linked to the differences in the insect meal and the specie of animal used respectively. In addition, the values MCV, MCH and MCHC obtained in this study were within the normal range which further confirms that the use of MM in the diet of rabbits did not negatively influence their health status.

Conclusion and Applications

1. The use of MM at 100% as a replacement for soybean meal in the diet of grower rabbits increased serum protein, globulin and reduced cholesterol.
2. Maggot meal can be included in the diet of grower rabbits up to 100% as replacement for soybean meal without adverse effect on serum and haematological parameters.

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