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Effects of dietary replacement of fish meal with crayfish waste meal on the blood profile of rabbits

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Abstract

This study was conducted to evaluate the effect of the dietary replacement of fish meal (FM) with crayfish waste meal (CWM) on the blood profile of rabbits. A total of sixteen crossbred rabbits of about 10 weeks of age, with an average weight of 4.0±0.06kg were used for the experiment in a 90-day feeding trial. The diets used for the experiment contained graded levels of Crayfish waste meal (CWM)as replacement for fish meal (FM). The rabbits were assigned to four dietary treatments with four rabbits per treatment in a completely randomized design (CRD). Dietary treatments contained 0%, 50%, 75% and 100% crayfish waste meal for diets 1, 2, 3 and 4 respectively. Data collected were packed cell volume (PCV), Red blood cells (RBC), White blood cells (WBC), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), serum metabolites, total protein, albumin, aspartate amino transferase (AST), alanine amino transferase (ALT) and others. Data were analyzed using analysis of variance and the means were separated using Duncan multiple range test. There were no significant differences (P<0.05) in all the parameters measured for haematology across dietary treatments except in the PCV, RBC and MCV. Rabbits in T2 had higher PCV (25.50%) and RBC (6.61x10^{6} uL) than those in T1, (18.50% and 5.07x10^{6} uL, respectively). The blood biochemical composition showed no significant differences (P<0.05) in Albumin, ALT and creatinine across the dietary treatments. However, total serum protein, globulin, albumin-globulin ratio, AST, urea, glucose and cholesterol of the rabbits were significantly (P<0.05) influenced by the treatments with T4 having significantly higher total protein (12.75g/dl) and globulin (10.10g/dl) than the other treatments. Therefore, the values of haematological and biochemical parameters obtained in this study which were in the normal range and the good health status of the rabbit across the treatments is an indication that cray fish waste meal is nutritionally adequate without any deleterious effect on the animals.

Keywords: Crayfish waste meal, fish meal, haematological, biochemical.

Effets du remplacement alimentaire de la farine de poisson par de la farine de déchets d'écrevisses sur le profil sanguin des lapins

Résumé

Cette étude a été menée pour évaluer l'effet du remplacement alimentaire de la farine de poisson (FP) par de la farine de déchets d'écrevisses (FDE) sur le profil sanguin des lapins. Un total de seize lapins croisés d'environ 10 semaines d'âge, avec un poids moyen de 4,0 ± 0,06 kg ont été utilisés pour l'expérience dans un essai d'alimentation de 90 jours. Les régimes utilisés pour l'expérience contenaient des niveaux gradués de farine de déchets d'écrevisses (FDE) en remplacement de la farine de poisson (FP). Les lapins ont été assignés à quatre traitements diététiques avec quatre lapins par traitement dans une conception complètement randomisée (CCR). Les traitements diététiques contenaient 0 %, 50 %, 75 % et 100 % de farine de déchets d'écrevisses pour les régimes 1, 2, 3 et 4 respectivement. Les

données recueillies étaient l'hématocrite (H), les globules rouges (GB), les globules blancs (GB), le volume corpusculaire moyen (VCM), l'hémoglobine corpusculaire moyenne (HCM) et la concentration corpusculaire moyenne d'hémoglobine (CCMH), les métabolites sériques, la totale protéine, albumine, aspartate amino transférase (AST), alanine amino transférase (AAL) et autres. Les données ont été analysées à l'aide d'une analyse de variance et les moyennes ont été séparées à l'aide du test à plages multiples de Duncan. Il n'y avait pas de différences significatives (P < 0,05) dans tous les paramètres mesurés pour l'hématologie entre les traitements diététiques, sauf dans le H, GB et VCM. Les lapins en T2 avaient des H (25,50 %) et des GB (6,61 x 106 uL) plus élevés que ceux en T1 (18,50 % et 5,07 x 106 uL respectivement). La composition biochimique du sang n'a montré aucune différence significative (P<0,05) dans l'albumine, l'AAT et la créatinine entre les traitements diététiques. Cependant, les protéines sériques totales, la globuline, le rapport albumine-globuline, l'AAT, l'urée, le glucose et le cholestérol des lapins étaient significativement (P<0,05) influencés par les traitements avec T4 ayant des protéines totales significativement plus élevées (12,75 g/dl) et de la globuline (10.10g/dl) que les autres traitements. Par conséquent, les valeurs des paramètres hématologiques et biochimiques obtenues dans cette étude qui étaient dans la fourchette normale et le bon état de santé du lapin à travers les traitements indiquent que la farine de déchets d'écrevisses est nutritionnellement adéquate sans aucun effet délétère sur les animaux.

Mots-clés : Farine de déchets d'écrevisses, farine de poisson, hématologique, biochimique

Introduction

Traditionally, fishmeal is one of the major sources of protein in animal feeds. However, there is limitation in the use of this feed ingredient as a result of its relevance in human nutrition coupled with decline in productivity (IITA, 1990). Fish meal is a high-quality protein source considered in livestock feed, especially rabbit, because of its superior profile of essential amino acids and its value as an attractant. Moreover, the downturn in the economy of developing nations especially Nigeria has led to the rising cost of fish, owing to the high demand by its increasing population. As such, fishmeal has become less available with exorbitant price value placed on it where available. Many authors have therefore researched into alternative sources of animal protein to ameliorate the situation. The non-conventional feedstuffs of animal origin are high quality feed ingredients that could compare to some extent with the conventional types. These are cheaper by virtue of the fact that there isno competition for human consumption. Many scientists have reported the possible use of some alternative animal protein feedstuffs to fish meal such as Earthworm meal (Tacon and Jackson, 1985), Toad meal (Annune, 1990), Life maggot (Ayinla *et al*., 1994), Fermented fish silage (Faturoti *et al*., 1998), Maggot meal, Frozen maggot (Ugwumba *et al*., 2001 and Sogbesan *et al*. 2005), Poultry dung meal (Fasakin *et al*., 2000) and Garden snail meal (Sogbesan and Ugwumba, 2006). At present, research efforts had been geared towards theuse of locally available feedstuff such as agro-industrial by-products and farm waste that may bring about the expected reduction in feed cost. Crayfish waste meal is one of the potential feed resources that is a non-conventional protein source and can be fed to rabbits in place of fish meal because of its closeness in biological value. The use of crayfish waste meal may augment the problem of competitiveness and high cost of conventional protein ingredient like fish meal. However, measuring the blood metabolites and constituents of rabbits fed crayfish waste meal based-diets can be used

as basis for comparison to fish meal. Blood is an important index in determining the nutritional and health status of the animals. Hence, this study was conducted to evaluate the effects of substituting crayfish meal for fish meal on serum biochemical and haematological parameters of rabbits.

Materials and methods

Experimental site, design and management of animals

The experiment was conducted at the Rabbitary of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor-Plantation, Ibadan. Sixteen crossbred pre-pubertal male rabbits of 10 weeks, with average initial weight of 4kg, were used for the experiment in a 90-day feeding trial. The rabbits were allotted into four dietary treatments containing graded levels (0, 50, 75, and 100%) of crayfish waste meal as replacement for fish meal as shown in Table 1. The rabbits were randomly allocated to four treatment groups, each with four animals per treatments in a completely randomized design. The rabbits on arrival were exposed to preliminary feeding period of seven days for acclimatization and they were weighed and randomly allocated to the treatments. The rabbits were housed in a wire mesh well-cleaned and disinfected hutch measuring 60x40x40 cm containing a feeder and a drinker. Standard health and sanitation procedures were strictly observed during the experimental period. Rabbits were fed in the morning (8:00am - 9:15 am) and supplementary forage was also offered at 2% of their weekly body weight. Clean and cool water was offered *ad libitum* to the animals throughout the experimental period.

Experimental layout

T1= 0% Crayfish waste meal + 100% fish meal

T2= 50% Crayfish waste meal + 50% fish meal

T3= 75% Crayfish waste meal + 25% fish meal

T4= 100% Crayfish waste meal + 0% fish meal

Blood collection and evaluation

On day 80, two rabbits per treatment were selected and bled through cardiac puncture into two vacutainer tubes, one containing ethylene diamine tetra acetic acid (EDTA) for haematological study and the other sterile plasticine vaccutiner tubes without EDTA. The blood in the second set of tube was centrifuged and serum separated out, decanted, deep-frozen for serum biochemical analyses. Blood samples collected were analyzed for Packed cell volume, haemoglobin, red blood cell (RBC), white blood cell (WBC), platelets, lymphocyte, neutrophils, monocytes and eosinophils as outlined by Ewuola and Egbunike (2008).Blood corpuscular constants, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined using appropriate formulae (Jain, 1986). The blood samples collected for serum biochemical analysis were centrifuged at 3500 revolutions per minute (rpm) for 15 mins. The serum was collected and analyzed for the following parameters; aspartate amino-transferase (AST) and alanine amino-transferase (ALT), activities were determined using spectrophotometric methods (McComb *et al.*, 1988), Serum total protein was determined by Biuret method (Kohn and Allen, 1995), while albumin was determined using the BCG (Bromocresol green) method (Peter *et al*, 1982). The globulin concentration was obtained by subtracting albumin value from total protein, while albumin/globulin ratio was obtained by dividing the calculated albumin value by the calculated globulin value. Serum urea was determined by Urease method and creatinine by Foin-wu filtrate methods (Toro and Ackermann, 1975).

Table 1: Gross composition of experimental diets

Ingredients	Treatments			
	1	2	3	4
Maize	41.00	41.00	41.00	41.00
Corn bran	13.00	13.00	13.00	13.00
Fish meal	0.50	0.25	0.35	0.00
Crayfish meal	0.00	0.25	0.35	0.50
Wheat bran	16.00	16.00	16.00	16.00
Soya bean meal	5.00	5.00	5.00	5.00
Groundnut cake	5.25	5.25	5.25	5.25
Palm kernel cake	16.00	16.00	16.00	16.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100

T1 = 0% crayfish meal diet, T2 = 50%crayfish meal diet, T3 = 75% crayfish meal diet, T4=100 % crayfish meal diet

Statistical analysis

Data were subjected to analysis of variance and significant means were separated by Duncan multiple range tests using the procedure of SAS (2002).

Results

The result of proximate composition of test ingredients and diets are shown in Tables 2 and 3. The CWM contained 35.02, 12.90 and 3.85% CP, CF and EE respectively and 1454Kcal/kg ME, while the FM contained 65.1, 0.8 and 6.0% CP, CF and EE with corresponding ME of 2860Kcal/kg. The Metabolizable energy was calculated using the formulae of Guevara *et al*., 2008:

$$AME = \frac{100 - 100(Mdiet \times GE\ excreta)}{(M\ excreta \times GE\ diet)}$$

Where AME = Apparent metabolizable energy of the diets

M diet = Metabolizable energy of diet

GE diet = Gross Energy of diet

M excreta = Metabolizable energy of excreta

GE excreta = Gross Energy of excreta

The result of serum biochemical indices of rabbit fed graded levels of crayfish waste meal is presented in Table 4.There were significant ($p<0.05$)differences in the values obtained for total serum protein, globulin, albumin globulin (A/G) ratio, aspartate amino transferase, urea, glucose and cholesterol of rabbit fed experimental diet. However, the highest serum protein (12.75g/dL) was recorded in rabbits on 100% crayfish waste meal diet. The globulin and albumin/globulin (A/G) ratio of rabbits on experimental diets were influenced ($P<0.05$) by the dietary treatments. The globulin concentration of rabbits in treatment 4 (100% CWM) was the highest among the treatment groups and A/G ratio of T_2birds had higher value above $T_3$and T_4 rabbits. Similarly, significant differences ($P<0.05$) were observed in serum urea of rabbits fed experimental diets with the highest value (37.50mg/dL) observed in T_4rabbitswhich was not significantly different from rabbits on 100% fish meal diet (37.50mg/dL), significant differences ($P<0.05$) were also observed in glucose and cholesterol of the rabbits fed experimental diet with the highest value (4.50mg/dL) of glucose observed in rabbits on 100% fish meal and the higher values 2.90mg/dl, 2.30mg/dl and 2.55mg/dL of cholesterol observed in T_4, T_3 and T_1rabbits.

The results of haematological indices of rabbits fed graded levels of crayfish waste meal (CWM) as replacement for fish meal are shown in Table 5. The haematological

variables in this study showed that there were no significant (P>0.05)differences observed in the haemoglobin, white blood cell (WBC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocytes, eosinophil, neutrophils and monocytes of rabbits among the dietary treatments. The haemoglobin values ranged from 6.00g/dl-8.00g/dl in all the treatments. However, there were significant (P<0.05) differences observed in the Packed cell volume (PCV), Red blood cell (RBC) and Mean Corpuscular Volume (MCV). The overall highest PCV was obtained in T_2 rabbits above others. Similarly, values recorded for RBC in rabbits on 50% (T_2CWM) and 100% (T_4CWM)were not significantly different but were significantly higher than what was reported in T_1 and T_3 rabbits. The higher values of MCV (38.63μ^3) were observed in T_2 and T_1 rabbits compared to T_3 and T_4 rabbits. In all the treatments, eosinophils, neutrophils, lymphocytes and monocytes were not significantly(p>0.05) different.

Table 2: Proximate composition and energy value of crayfish waste meal and fish meal

Parameters	Crayfish waste meal	Fish meal
Dry matter (%)	81.30	81.60
Crude protein (%)	35.02	65.12
Crude fibre (%)	12.90	0.80
Ash (%)	11.56	23.45
Ether extract (%)	3.85	6.00
ME (Kcal/kg)	1454.00	2860.00

Table 3: Proximate composition of the experimental diets

Parameters	T_1(0% CWM)	T_2 (50% CWM)	T_3 (75% CWM)	T_4(100%CWM)
Dry matter (%)	94.96	93.48	94.79	93.72
Crude protein (%)	23.78	23.79	23.83	23.84
Crude fibre (%)	4.63	4.63	4.65	4.66
Ash (%)	4.85	4.79	4.76	4.73
Ether extract (%)	10.89	10.99	11.02	11.23
ME (Kcal/kg)	55.85	55.80	55.74	55.54

Table 4: Serum biochemistry of rabbits fed graded levels of crayfish waste meal

Parameters	T_1 (0%CWM)	T_2 (50%CWM)	T_3 (75%CWM)	T_4 (100%CWM)	P value	Ref. ranges
Totalprotein (g/dl)	8.30^{b}	8.30^{b}	9.35^{b}	12.75^{a}	<.0001	5.40-7.50
Albumin (g/dl)	2.60	2.70	2.75	2.90	0.2773	2.60-5.00
Globulin (g/dl)	5.70^{bc}	5.40^{c}	6.80^{b}	10.10^{a}	<.0001	1.50-2.70
Alb/Glo (g/dl)	0.46^{ab}	0.56^{a}	0.37^{bc}	0.26^{c}	0.0034	0.70-1.89
AST (IU/L)	10.00^{a}	9.00^{a}	5.00^{b}	10.00^{a}	0.0063	0.00-28.00
ALT (IU/L)	9.00	9.00	8.20	8.90	0.1861	0.00-61.00
Creatinine (mg/dl)	1.10	1.30	1.10	1.25	0.2966	0.50-2.60
Urea (mg/dl)	37.50^{a}	24.50^{bc}	20.00^{c}	37.50^{a}	0.0030	20.00-45.00
Glucose (mg/dl)	4.50^{a}	3.70^{ab}	3.55^{ab}	3.05^{b}	0.0491	4.20-8.90
Cholesterol (mg/dl)	2.55^{a}	1.50^{b}	2.30^{a}	2.90^{a}	0.0044	10.00-80.00

[ab=] Means on the same row with different superscripts are significantly (P<0.05) different.AST= aspartate amino transferase, ALT= alanine amino transferase, Alb/Glo= Albumin globulin ratio. T1= 0% Crayfish waste meal, T2= 50% Crayfish waste meal,T3= 75% Crayfish waste meal,T4= 100% Crayfish waste meal.
[*]Ref. ranges obtained from Medirabbit.com (http://www.medirabbit.com/EN/haematology/bloodchemistry.htm)

Discussion

The PCV and RBC counts were significantly influenced by dietary treatments. The RBC counts were within the reported normal physiological range of rabbit as reported by Mitruka and Rawnsley (1977), but there were lower PCV values below normal physiological range across the treatments. According to Togun *et al*. (2007), this can be an indicator of anaemia. Low haematological values could also be due to harmful effect of dietary content. Reports by Aletor and Egberongbe (1992) and Aletor (1989) indicated that the blood variables most consistently affected by dietary influence includes RBC, PCV and plasma protein as blood remains an important index of physiological, pathological and nutritional status in the organism (Ewuola *et al*., 2004; Olorode *et al*., 2007). The differences observed in RBC and MCV for animals in different treatment groups may be attributed to the various nutritional statuses of the animals. The normal values range of WBC observed in all treatment groups showed that WBC was not negatively influenced by the diets. Reilly (1993) opined that normal range of values for WBC is an indication that the animals are healthy because decrease in number of WBC below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism. The similarity of serum protein values in T4(100%) and T1 (0%) may be an index of similarity in their qualities. Levels of serum albumin and serum creatinine across treatments were not influenced. Ezekwesili (2005) reported that severe malnutrition decreases albumin fraction in the blood. Globulin combines with albumin in the blood to promote normal water retention. Iyayi and Tewe (1998) observed that globulin and albumin as well as urea concentration in the blood are indicators of the quality and quantity of proteins supplied in the diets. The utilization and synthesis of albumin was enhanced in the rabbits fed both fish meal and cray fish waste meal due to the presence of adequate level of available from the tests ingredients since albumin synthesis has been reported to be related to the amount of available protein in the diet (Iyayi and Tewe, 1998). The albumin values obtained for the rabbits were also comparable with the normal clinical range (2.70-5.00g/dL) for apparently healthy rabbits reported by Medirabbit (2001). This suggests a proper functioning of the liver of the rabbits fed experimental diets. Furthermore the globulin concentration values reported were higher than the range 2.50-4.50g/dL reported by Burke (1994) for rabbits. The elevation in serum globulin may be due to enhanced antibody secretion in response to an infection. The result also showed that A/G ratio, urea, glucose and cholesterol were significantly ($P<0.05$) different between dietary treatments. Kaneko (1997) has shown that serum biochemical indices vary and can be influenced by many biotic and abiotic factors such as water, temperature, seasonal pattern and feed. The serum urea levels of T1 (0%) and T4 (100%) that were similar and apparently higher than other treatment groups were within the range of 20-45mg/dl reported by Medirabbit (2011). Normal urea level in the blood portrayed adequate dietary protein quantity and quality. Elevated urea concentration in the blood above the normal range is an indication of excessive deamination of serum protein as a result of its poor quality in terms of its amino acid profile metabolism in the animal system. The glucose level reduced with an increase in the inclusion of CWM to 100%.

The similarity of the values of Cholesterol in treatments 1, 3 and 4 is suggestive of normal and safe concentration. Cholesterol test is relevant in the evaluation of the risk of heart disease. Aspertate transaminase (AST) that dropped at 75% dietary level

indicates that when deleterious toxic compounds become excessive, the cells of the organ are damaged and the enzymes which are contained in it leak into the general circulation. The liver remains the prime site for the destruction of toxic compounds and the serum concentration of the enzymes is therefore a function of hepatocellular integrity and functions. The similarity of the values for the serum enzymes from the experiment suggests that whatever toxic factor(s) contained in CWM were of mild concentrations and were adequately tolerated by the rabbits up to 100% dietary inclusion level. This observation agrees with the reports of several authors (Ologhobo *et al*., 1993; Onwukwe, 2000; Enemor *et al*., 2005; Owen *et al*., 2009) on serum or liver enzyme activities.

Conclusion

The values of haematological and biochemical parameters obtained in this study and the normal health status of the rabbit across the treatments indicated that crayfish waste meal was nutritionally adequate without any deleterious effect on the animals. The inclusion of CWM up to 100% did not elicit deleterious effects on the haematological and biochemical indices of the rabbits, but rather enhanced their blood profile.

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