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Full Length Research Paper

Haematological and biochemical profile of weaner rabbits fed raw or processed pigeon pea seed meal based diets

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Twenty four weaner rabbits of 6-8 weeks of age and averaging 550 g in weight were divided into four groups of six (6) rabbits per group and used in a 60 - day feeding trial for blood chemistry and haematological studies. The rabbits were fed raw or processed pigeon pea seed based diets in a Completely Randomized Design. Diet A contained raw (control), Diet B boiled, Diet C toasted and Diet D soaked pigeon pea seed meal (PSM) incorporated at 20% level in weaner rations. The haematological parameters investigated were haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), neutrophil (N), lymphocyte (L) and eosinophil (E), while the biochemical components were urea, serum creatinine, bilirubin (total and conjugated), total protein, globulin, serum glutamic transaminase (SGPT) and serum glutamic oxalo acetic transaminase (SGOT). Results showed that white blood cells, lymphocytes, globulin and PCV values were influenced ($P<0.05$) by dietary treatments among the experimental groups. The values were 6.30, 6.03, 6.0, 6.80 (x 10^3) WBC; 17.0, 33.0, 36.6, 33.0 (%) lymphocytes; 2.70, 1.10, 1.76, 1.26 (md/l) globulin and 15.3, 19.6, 24.6, 25.3 (%) PVC, for diets A, B, C, and D, respectively. Haemoglobin, neutrophil and eosinophil values were nevertheless similar ($P>0.05$) for all groups. Not affected ($P>0.05$) by diets were blood urea, creatinine, bilirubin, total protein, SGOT and SGPT concentrations. Most haematological and biochemical values obtained were out of normal range for rabbits. Raw or processed pigeon pea did not support remarkable changes in haematological and biochemical profile for weaner rabbits at 20% dietary level of inclusion.

Key words: Haematological, biochemical, blood profile, weaner rabbit, pigeon pea.

INTRODUCTION

Nigeria's rapidly growing population has informed the need to increase livestock production to satisfy her animal protein requirement. Contributions of beef and poultry products to this national dilemma has been indeed marginal, providing succor to only a select few who mostly are urban and peri-urban dwellers, while leaving about 90% of the populace who reside in the hinterlands on consumption of less than 10g as against recommended 35 g (Ahamefule et al., 2000) animal protein per day. This wide nutritional gap has fuelled the need to intensify the production of some livestock species to address the low per capita animal protein intake by Nigerians. Among the livestock of interest is rabbit, a caprophagous herbivore whose production before now has been low. What inspired the interest in rabbit are her short generation interval and good meat quality.

Intensive approach to rabbit production would however entail the use of alternative plant protein sources other than the conventional ones to enable 'keepers' produce meat at affordable price. Such alternative plant protein sources are currently under investigation in Nigeria; they are being evaluated for nutritiveness, availability, acceptability and affordability. It is in light of the above that pigeon pea, a relatively unexploited legume, is being assayed for its feed value. Of importance however, is the

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fact that most of these non-conventional legumes (even conventional) used in animal nutrition contain some anti-nutritional properties which affect their utilization in the raw (unprocessed) state by livestock.

Pigeon pea (*Cajanus cajan*) contains trypsin inhibitor and haemaglutinin which affect growth and performance of livestock in the raw state (Amaefule, 2002). Boiling, toasting and soaking are some of the processing methods which had been used (Amaefule, 2000) to inactivate these anti-nutritional factors (ANF). Akinmutimi (2004) had observed that most processing methods employed in improving the feed value of non conventional or alternative feedstuffs do not completely eliminate ANF substances, but only reduce their concentrations to tolerable levels in feedstuffs. Quantities of ANF present in formulated rations therefore will depend on the concentration (remnants) of these substances in feedstuffs which invariably is determined by processing methods. Processing methods therefore influence quality of compounded rations or diets and thereby affect blood biochemical and haematological properties.

Biochemical components of blood are sensitive to elements of toxicity in feeds. They can also be used to monitor protein quality of feeds. Haematological components are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998).

This study examines the effect of methods of pigeon pea processing on the haematological and biochemical profile of rabbits.

MATERIALS AND METHODS

Experimental site

This study was conducted at livestock unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike. Umudike is geographically located in Abia state, Nigeria on latitude 05°29° North, longitude 33° East at altitude of 122 meters or 400 feet above sea level. It falls within the rain forest zone of West Africa which is characterized by long duration of rainfall (April-October) and short period of dry season (November-March). Average rainfall is 2169.8 mm in 148 – 155 rain days. Average temperature is 26°C with maximum of 32°C and minimum of 22°C. Relative humidity ranges from 50 – 95%.

Experimental animals and management

Twenty four (24) New Zealand White x Chinchilla weaner bucks, averaging 0.55 kg in weight and aged between 6 - 8 weeks were randomly divided into four groups of six animals per group. Each animal was housed in a standard hutch of 120 by 150 cm and raised 120cm from the ground in a three-tier hutch system. The animals were each provided with a feeder and drinker. Each animal was vaccinated against prevalent undercurrent diseases. They were also dewormed and given acaricide bath prior to the experiment.

Processing of pigeon pea seeds

Forty kilograms of pigeon pea seeds (white variety) was purchased from the local market, divided into 4 lots and subsequently processed as follows:

Raw: The first lot (10 kg) of raw pigeon pea seed was subjected to milling and the product used in this study as raw pigeon pea seed meal.

Boiled: The second lot (10 kg), in a mammoth pot, was subjected to boiling at 100°C for 30 min. The boiled seeds were sun-dried for 3 days to reduce moisture content (10 - 15%) before being milled and used as boiled pigeon pea seed meal.

Toasted: Another 10 kg of raw pigeon pea was subjected to toasting for 20 min at 110°C. The resultant product was cooled, milled and subsequently used as toasted pigeon pea seed meal.

Soaked: The fourth lot (10 kg) of raw pigeon pea was soaked in a large basin containing clean water. After 24 h water was decanted. The soaked seeds were sun-dried for 3 days, milled and used as soaked pigeon pea seed meal.

Experimental diets

Four diets, A, B, C, and D were formulated from maize, wheat offals, soya bean meal, blood meal, oyster shell, bone meal, vitamin premix and common salts to contain 20% raw, boiled, toasted and soaked pigeon pea seed meals, respectively. The compositions and the proximate constituents of the experimental diets are given in Table 1.

Experimental design

The four treatment groups were assigned the four experimental diets in a Completely Randomized Design. Each treatment was replicated thrice and there were 2 rabbits per replicate. Each rabbit received an assigned diet for 60 days.

Blood sampling

Blood samples were collected from each replicate on weeks 6, 7 and 8 of the study. Method of Uko et al. (2000) was used and this was achieved by puncturing the jugular vein and allowing free flow of blood into labeled sterile universal bottles. Pooled samples per treatment group were divided into two. An initial 10 ml was collected over labelled sterile universal bottles containing 1.0 mg/ml ethyldiamine tetracetic acid (EDTA) and 0.1mg/ml Heparin. This was used to determine the haematological component according to the method of Ajagbonna et al. (1999) and Uko et al. (2000). Another 10ml was collected over labelled sterile sample bottles without coagulant and used to determine the biochemical components (Doumal, 1972; Sigma 1985; Ajagbonna et al., 1999; Spencer and Price, 1997; Uko et al., 2000).

Parameters determined

Haematological – This included haemaglobin, white blood cell, packed cell volume, lymphocyte, eosinophil and neutrophil. Biochemical – This included urea, serum creatinine, bilirubin (total and conjugated), total protein, globulin, SGPT and SGOT.

Table1. The composition and proximate constituents of the experimental diets.

Ingredient (%)	A	B	C	D
	RP	**BP**	**TP**	**SP**
Maize	38	38	38	38
Wheat offal	22	22	22	22
Pigeon pea	20	20	20	20
Soya bean meal	15	15	15	15
Blood meal	1.5	1.5	1.5	1.5
Oyster shell meal	1.0	1.0	1.0	1.0
Bone meal	2.0	2.0	2.0	2.0
Salt	0.25	0.25	0.25	0.25
Vitamin mineral premix*	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated contents (%)				
Crude protein	13.25	18.41	14.40	16.55
ME (Kcal DE/Kg DM)	2651.6	2688.5	2759.3	2654.1
Analyzed Contents (%)				
Dry matter	90.34	91.04	89.70	90.50
Crude protein	13.19	18.31	14.35	16.48
Crude fibre	10.90	11.75	10.95	11.30
Ether extract	3.70	3.60	2.85	3.15
Nitrogen free extract	52.10	47.53	51.65	49.87
Ash	10.45	9.85	9.00	9.70
ME (Kcal DE/Kg DM)	2634.8	2678.4	2710.4	2638.5

*To provide the following per kg diet: Vit, A, 1500IU; Vit E, 11.0mg; Riboflavin, 9.0mg; Biotin, 0.25; Pantothenic acid, 11.0mg; Vit k3, 3.0 mg; B2, 2.5 mg; B6, 0.3 mg; B12, 8.0 mg; Nicotinic acid, 8.0 mg; Fe, 5.0mg; Mn, 10.0mg; Zn, 4.5mg; Co,0.2mg; Se,0.01mg
ME = metabolizable energy, RP = raw PSM based diet, BP = boiled PSM based diet, TP = toasted PSM based diet, ST = soaked PSM based diet.

Data analysis

All data were subjected to analysis of variance (ANOVA) applicable to a Completely Randomised Design (Steel and Torrie, 1980). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The haematological and biochemical values obtained for weaner rabbits fed raw or processed pigeon pea based diets are presented in Table 2. Haemoglobin (Hb) values (g/100ml) varied but not significantly ($P>0.05$) among treatment groups. Values obtained were generally low and did not fall within normal range (9.4 -17.4) for rabbits (Mitruka and Rawnsley, 1997; Ross et al., 1979). The low level Hb of the treatment diets imply that the dietary proteins were not of high quality (Abu et al., 1998), probably due to traces of ANF's in the treatment diets. This agrees with the observation of Akinmutimi (2004) that processing methods reduce but do not completely eliminate all traces of ANF's in feedstuffs. Diets containing poor quality protein would usually influence poor transportation of oxygen from the respiratory organs to the peripheral tissues (Robert et al., 2000).

The PCV values (%) for the treatment groups were also below normal range for rabbits (33.0-50.0), even though significant differences ($P<0.05$) existed among treatment groups. PCV is an index of toxicity and its distribution vary with breeds. Reduction in the concentration of PCV in the blood usually would suggest presence of a toxic factor (e.g. haemagglutinin) which had adverse effect on blood formation (Oyawole and Ogunkunle, 1998). Raw pigeon pea had been reported to contain haemagglutinin (Amaefule, 2002). It is possible that traces of this ANF still abiding in the treatment diets may perhaps have been responsible for the abnormal values. This demonstrated also that the processing methods were not able to completely remove the ANF's in the diets. The PCV values recorded for various treatment groups could be viewed as coefficient of efficiency of the various processing methods in reducing the ANF's substances; the higher the value, the more capable the method. This

Table 2. Haematological and biochemical components of rabbits fed diets containing raw, boiled, toasted and soaked pigeon pea seed.

Parameters	RP	BP	TP	SP	SEM
Haematological					
Haemoglobin(g/100 ml)	6.53	6.93	8.40	8.40	0.50
PCV (%)	28.5^{a}	19.6^{c}	24.6^{b}	25.3^{b}	1.75
WBC x 10^3	6.80^{a}	6.03^{ab}	5.00^{b}	6.30^{a}	0.25
Neutrophil (%)	72.6	55.3	56.3	56.0	3.07
Eosinophil (%)	10.3	10.0	8.33	11.0	0.44
Lymphocyte (%)	37.0^{a}	33.6^{b}	33.6^{b}	33.0^{b}	3.02
Biochemical					
Urea (mg/dl)	49.3	65.0	63.3	70.6	5.26
Creatinine (mg/dl)	0.70	0.60	0.66	0.36	0.06
Total Protein (g/dl)	5.30	4.73	3.93	2.90	0.43
TB (mg/dl)	0.60	0.56	0.66	0.63	0.03
CB (mg/dl)	0.20	0.20	0.23	0.23	0.02
SGOT (mg/ml)	6.00	7.33	7.33	6.67	0.36
SGPT (mg/ml)	4.33	5.33	5.00	4.00	0.28
Globulin (g/l)	1.10^{b}	2.70^{a}	1.76^{ab}	1.26^{b}	1.25

^{abc}Mean values with different superscripts are significantly different ($p< 0.0.05$)
RP = raw PSM based diet, BP = boiled PSM based diet,
TP = toasted PSM based diet, ST = soaked PSM based diet.
CB = conjugated bilirubin, TB = total bilirubin
SGOT = serum glutamic oxalo acetic transaminase
SGPT = serum glutamic transaminase

implied therefore that for pigeon pea, toasting and soaking processing methods were better of than boiling in removing the haemagglutinins. This finding however runs contrary to the report of Odoemelam, 2007 who observed that moist heat (boiling) was the best processing method for *Canavalia plagiosperma*, another non conventional plant protein.

White blood cells (x10^3) differed significantly ($P<0.05$) among treatment groups. The values obtained were within normal range (5 - 8 x 10^3) for rabbits. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system.

The neutrophil (%) values obtained were similar ($P>0.05$) for all treatment means, values however exceeded the normal range for rabbits (35.02 - 43.2) (Mitruka and Rawnsley, 1997). Conversely, the eosinophil concentration, except for rabbits fed toasted pigeon pea based diet, were within normal range (10-12.5%) (Ahamefule et al., 2006) for rabbits in the other experimental groups.

Lymphocyte (%) was influenced ($P<0.05$) by dietary treatments. The values obtained were however below the stipulated range (53.5-65.8) for rabbit (Mitruka and Rawnsley, 1997). The observed values suggest that the processing methods were quite ineffective in removing completely the anti-nutritional elements in the pigeon pea as observed by Akinmutimi (2004).

Blood urea concentrations (mg/dl), even though similar ($P>0.05$) for all groups, were higher than the normal range (30.0 - 37.3). High blood urea levels are associated with poor protein quality (Eggun, 1970) or excess tissue catabolism associated with protein deficiency (Oduye and Adadevoh, 1976). The latter was not the case in this study because all the rabbits within each treatment group maintained positive weight balance; none of the animals lost weight during the course of study.

Total protein (g/dl) and serum creatinine values (mg/dl) did not show any significant differences ($P>0.05$) in values obtained for all treatment groups. However, the values for the latter were also out of normal range (12.0 – 18.0) for rabbits, suggesting that there was muscle wastage in the rabbits and that the animals survived at the expense of body reserves. This inference could not however be substantiated in this study as there was no evidence of loss of weight within each treatment group. It is however possible that the dietary proteins were not fully utilized by the animals within each treatment group probably because the processing methods did not facilitate total protein availability (Eggun, 1970; Ross et al., 1979). Perhaps if the study had spanned much longer, the dietary proteins would not have been able to sustain the maintenance requirements of the animals and this eventually would have led to catabolism.

The globulin values (g/l) influenced by the test diets were significantly different (P <0.05) from one another; this indicates the difference in strength or the ability of rabbits subsisting on the different dietary treatments to fight against diseases (Robert et al., 2000). The values obtained were higher for rabbits fed processed pigeon pea (diets B, C, D) than the control group (A). Rabbits fed diet containing boiled pigeon pea had superior advantage all over others.

Values for conjugated and total bilirubin (mg/dl) did not show significant differences (P> 0.05) among rabbits of different treatment groups. Total and conjugated bilirubins are indicators of protein adequacy. The non-significant values suggest that each of these detoxify-cation or processing methods employed for pigeon pea seeds was capable of releasing sufficient nutrients, enough to support basic maintenance and metabolic functions in the rabbits (Ologhobo et al., 1992).

Even though the SGOT and SGPT serum concen-trations (mg/ml) of rabbits had similar (P>0.05) values for all treatment groups, their values were above the normal ranges (12.0 – 18.0 and 9.0 – 15.0, respectively) stipulated for weaner rabbits (Eggun, 1970; Ross et al., 1979). An increase in serum glutamic oxalo-acetic transaminase (SGOT) and serum glutamic pyruvic trans-aminase (SGPT) signal necrosis and myocardial infarct-tions, which are indicators of poor quality protein of diets fed (Fasina et al., 1999).

In conclusion, the processing methods could not possibly eliminate all traces of anti-nutritional properties in pigeon pea seeds. Even though rabbits fed both the raw and processed pigeon pea seeds appeared generally healthy, most haematological and biochemical para-meters were out of normal range for rabbits. At 20% level of inclusion, there were no outstanding differences in blood profile between rabbits fed raw and processed (boiled, toasted, soaked) pigeon pea seed based diets. Raw or processed pigeon pea did not support remarkable changes in haematological and biochemical blood profile for weaner rabbits at 20% dietary level of inclusion.

REFERENCES

Abu OA, Rabo JS, Onifade AA, Danny CB (1998). Blood composition and histological changes in the gastro-Intestinal tract of rabbit fed untreated rice husk based diets. Proceedings of annual Conference of Nigerian Society for Animal Production. 23: 144-147.

Ahamefule FO, Edouk GA, Usman A, Amaefule KU, Oguike SA (2006). Blood biochemistry and haematology of weaner rabbits fed sun-dried, ensiled and fermented cassava peel based diets. Pakistan J. Nutr. 5 (3): 248-253.

Ahamefule FO, Ejiofor CA, Ibeawuchi JA (2000). A comparative study of the constituents of cattle, sheep and goat milk in a hot humid environment. Disc. and Innov. 15 (1/2): 64-68.

Ajagbonna OP, Onifade KI, Suleman U (1999). Haematological and biochemical changes in rats given extracts of *Calotropis procera.* Sokoto J. Vet. Sci., 1: 36-42

Akinmutimi AH (2004). Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resources for broiler chickens . Ph.D Thesis. Department of Non-ruminant Animal Production, Michael Okpara University of Agriculture, Umudike, Nigeria.

Amaefule KU (2002). Evaluation of Pigeon pea seeds (*Cajanus cajan*) as protein source for pullets. Ph.D Thesis, Department of Animal Science, University of Nigeria, Nsukka.

Doumal E (1972). Standard Method of Clinical Chemistry. Publ. Academy press. pp. 5-88

Duncan DB (1955). Multiple range and multiple F- test. Biometrics. 11:1-42

Eggun BO (1970). Blood Urea- measurement as a technique for assessing Protein quality. Brit. J. Nutr. 24. 985 -988.

Fasina OE, Ologhobo AD, Ayoade GO, Adenira GA, Adeyemi OA (1999). Nutritional and toxicological assessment of various Amyadalin tears in nutrition of broilers chicks: Effect on Performance, haematological and biochemical indices. Proceedings of annual Conference of Animal Science Association of Nigeria 4: 19-22.

Mitruka BM, Rawsley HM (1997). Clinical, biochemical and haematological reference value in normal experimental animal. Mason publishing company New York. pp. 35 – 50.

Odoemelam VU (2007). Proximate composition, anti-nutritional properties and mineral assay of raw and processed *Canavalia plagiosperma* seed. M. Sc Thesis, Department of Animal Production and Management, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria, 2007.

Oduye OO, Adadevoh BK (1976). Biochemical values of apparently normal Nigerian sheep. Nig. Vet. J. 5 (1): 43 -50.

Ologhobo AD, Apata DF, Oyejide A, Akinpelu RO (1992). Toxicity of raw lima beans (*Phaseolus lunatus*) and lima bean fractions for growing chicks.Brit. Poultry. Sci. 34: 505-522

Oyawoye EO, Ogunkunle M (1998). Physiological and biochemical effects of raw jack beans on broilers. Proceedings of annual Conference of Nigerian Society of Animal Production. 23: 141-142.

Roberts KM, Daryl KG, Peter AM, Victor WR (2000). Mayer's Biochemistry. 25th edition, Mc Grawhill, New York. 25: 763 – 765.

Ross JG, Christies G, Halliday WG, Jones RM (1979). Haematological and blood chemistry comparison values for clinical pathology in poultry. Vet. Record 102: 29 -31.

Sigma diagnostic (1985). Transaminase (ACT/GPT) and AST/GOT. Quantitative colorimeter determination in serum plasma or cerebrospinal fluid procedure No. 505.

Spencer K, Price CP (1977). Chemical analysis of bilirubin in biological fluids. Annal of Clinical Biochemistry 14: 105-115.

Steele RGD, Torrie JH (1980). Principles and Procedures of Statistics. McGraw Hill Book, Co., New YorK. pp.66-94

Uko OJ, Ataja AM, Tanko HB (2000). Weight gain, haematology and blood chemistry of rabbits fed cereal offals. Sokoto J. Vet. Sci. 2: (2) 18-26.