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Full Length Research

Studies on the haemato-biochemical changes in growing broilers after being fed on *Icacina oliviformis* (false yam) tuber meal

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ABSTRACT: Haemato-biochemical alterations were studied from feeding and slaughter experiments in growing broilers to investigate the effects of *Icacina oliviformis* (false yam) tuber meal on haemato-biochemical changes. Diets contained: raw seed meal without any treatment (30 g/kg diet), tuber meal treated without aqueous ethanol (70% alcohol; 60 g/kg), tuber meal treated with aqueous ethanol (70% alcohol; 60 g/ kg), soaked, dried tuber meal (90 g/kg), and standard commercial corn, soybean and fishmeal based feed as control diet. Both control and treatment groups allocated 42 g diet/day (restricted feeding) and were pair-fed up to the end of the experiment. The cellular components and parameters of blood and serum were subjected to detailed studies. Cellular elements were found to be little affected. Nevertheless, values recorded on the effects of test substances on hematological indices of packed cell volume (PCV), hemoglobin concentration (HBC) and red blood cell (RBC) were found to be significantly ($p < 0.05$) different among indices measured. PCV recorded was highest ($p < 0.05$) in groups fed 6% raw tuber meal treated with 70% aqueous ethanol. HBC value was highest ($p < 0.05$) for birds fed the 9% soaked tuber meal (8.58 g%) as compared to those of others. The significant ($p<0.05$) lowering effect of total cholesterol was observed in birds fed false yam meal at different concentrations compared to those of birds fed on standard control diet. The water-soaked false yam meal which was incorporated at the level of 90 g/kg diet lowered total serum cholesterol by 36% compared with that value for the standard commercial feed. However, the present experiments could not adequately explain the underlying reasons for lowering effects which is related to lipid metabolism. The results suggest that false yam protein and or fiber may be involved in reducing cholesterol and may exert long-term effects of this material on the consumers through unknown mechanisms. It is highly likely that our preliminary investigation has revealed hitherto unknown cholesterol-lowering natural product from false yam and thus, further work is warranted.

Keywords: Broilers, cholesterol, false yam, feed.

INTRODUCTION

Being known as Takwara in Ghana (Kay, 1987), false yam (*Icacina oliviformis*) is a small perennial shrub and is drought-resistant and grows as erect leafy shoots from a large underground fleshy tuber. It is seldom cultivated. Nonetheless, from Senegal, it is reported to be propagated by pieces of tuber, before the wet season. People truly enjoy the fruits as well as the seeds, which represent a permanent, reliable and very tasty food if properly processed. The tubers which resemble large turnips or beetroots is such a great source of emergency moisture and food energy to the plant that it can survive at least four years without rain. Thus, as long as false yam is around, food is always available for people (NRCNAP, 2008).

Although it produces nutritious food and is found throughout Africa – the false yam remains one of the crops most neglected by science. Isolated as a “poor man’s crop”, the plant has never been accorded a large scale breeding, improvement and research programme. Yet empirical evidence and a recent investigation suggest that it is indeed a crop with great promise. Incorporation of false yam meal to improve the performances of birds (Aning, 2006) and animals have been going on for the last couple of years in West Africa. Osei et al. (2015) reported no apparent effects on birds’ health when they fed false yam (*Icacina oliviformis*) tuber meal as a feed ingredient for broiler chicken. Regarding haemato-biochemical changes, Okosun et al. (2018) reported that Albino rats after being fed on oven-dried false yam tuber meal at varying replacement levels for maize showed enhanced blood quality of the experimental animals. Likewise, in Wister rats, the inclusion of graded levels of flour from soaked and dried or boiled false yam tuber resulted in significant improvements in the nutritional status and red blood cell indices of the animals such as PCV, MCH, MCV and MCHC compared to those obtained from the control (David-Oku et al., 2018). This indicated an improvement in volume, size, and concentration of haemoglobin in the red blood cells. However, there was a reduction in the levels of white blood cells and platelets, contrary to what was reported in broiler chicken fed false yam tuber meal (Dei et al., 2011). It was agreed that in poultry, haematological parameters are affected by diurnal fluctuations or changes in daily physical and metabolic activities (Sanni et al., 2000; Piccione et al., 2001, 2005). Factors, which affect the RBC counts, include breed, sex and the nutrition supplied to the bird (Sturkie, 1965). Packed Cell Volume (PCV), haemoglobin concentration and red blood cell count had been reported to increase with age in chickens (Islam et al., 2004). Although haematological information on Ghanaian chickens have been published and available in the literature, however, records on the effects of lipid parameters in chickens had rarely been carried out and have been reported from outside of Ghana which has had different kinds of climate (Mia et al., 2021). Thus, the present study was designed to partly rectify this deficiency and to provide baseline data on haemato-biochemical parameters of growing broilers fed diets based on Icacina oliviformis (false yam) using restricted feeding techniques.

MATERIALS AND METHODS

False yam meal preparation

False yam tubers used in this trial was obtained from Professor Dr Herbert Dei of the University for Development Studies, Tamale, Ghana. The yam tuber meal was prepared in his laboratory following established techniques. The basal diet was a practical corn-based diet formulated to contain all nutrients to fulfill or exceed the nutritional requirements of broiler chickens (NRC, 1994). *Icacina oliviformis* false yam) tuber meal were used as the primary protein source in the dietary treatments. Composition of dietary treatments and nutrient contents are shown in Table 1. All diets were isocaloric and isonitrogenous. Each diet was offered *ad libitum* to a group of 24 (six birds per group) day-old male broiler chicks for 15 days. The raw tuber meal was extracted with 70% ethanol/water at 5°C with a view to remove anti-nutrional factors. These pre-treated false yam tuber meals were incorporated into diets for broilers to their amounts in the untreated meal and the diet was prepared following Table 1. Maize starch, corn oil, soybean meal, amino acids, minerals and vitamins were bought locally and were of general-purpose grade. Test and control diets were formulated by substitution of maize starch.

Pre-experimental period

Twenty-four (24) day-old Cobb 500 broiler chicks were obtained from local hatchery through their local agent in Mymensingh and were housed on rice husk until 15 days of age. They were fed a commercial broiler starter diet *ad libitum* and reared on husk floor. Water was supplied *ad libitum* in plastic water trough. The body weight was monitored daily.

Housing

Adaptation: 24 male Cob-500 broilers, 18 days of age, were housed in a four (six birds/group) and fed 42 g of control diet for 3 days, while water was given *ad libitum*. Only those birds which had a regular food intake and gained weight at a similar rate during this 3 day adaptation period were subsequently used in the experiment.

Bird husbandry and sample collection

A total of 24 growing broilers, six birds in each group were

Table 1. The composition of diets: (g/ kg diet).

Ingredients	Control	6% raw tuber without ethanol treatment	6% raw tuber with ethanol treatment	9% soaked tuber meal
Maize	465	310	405	375
False yam tuber meal	0	30	60	90
Fish meal	110.40	120.40	110.40	110.40
Soybean meal	149.30	159.30	149.30	149.30
Wheat bran	115.0	125.0	115.0	115.0
Oyster shell	10.30	10.30	10.30	10.30
Di-calcium phosphate	50.0	50.0	50.0	50.0
Vitamin-mineral mix	50.0	50.0	50.0	50.0
Salt	50.0	50.0	50.0	50.0
Total (g)	1000	1000	1000	1000
Calculated nutritive value				
Crude protein (%)	21,01	20.81	20.81	20.71
Lysine (%)	1.2	1.1	1.1	1.1
Methionine (%)	0.427	0.418	0.418	0.413
Methionine + cystine (%)	0.766	0.744	0.744	0.744
ME (Kcal/ Kg)	2967	ND	ND	ND

ND-Not determined.

weighed on day 16 weighing 525 ± 18.4 g and randomly allocated to cages. The experiment was conducted in open rooms under constant lighting. The chickens were housed in 84 cm by 46 cm metal cages with raised metal wire floor. Two broiler birds were placed per cage. Water was provided ad libitum. The birds were monitored three times per day for overt signs of toxicity and depressive behavior. There were a total of three experimental and control diet-fed groups. Diets were fed *ad libitum* while water was made available *ad libitum* throughout the study, via a plastic water trough. After having a three-day adaptation period from day 16th, the feeding trials started from day 19th and continued for 10 days to attain conventional slaughter weight. Bodyweight, feed intake, feed refusal and fecal weights were recorded every day. Cumulative feed efficiency per bird was calculated as the ratio of weight gained to feed consumed. The dry matter of the droppings was determined from the last 10 days of feeding trial by drying aliquots of every day's droppings for 6 hours at 105°C. The number of chicks with sticky droppings adhered to the cloacal area was noted on the same days of excreta collection. At the termination of the experiment, birds were euthanised by a deep, swift cut severing carotid and jugular on both sides of the neck was imperative to allow a quick blood loss resulting in loss of consciousness

Analytical methods

Blood samples were collected in tightly rubber stoppered polystyrene test tubes containing EDTA as an anticoagulant, and the following were estimated: Total red and white blood cells, hemoglobin, total platelets, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration. These were done on an automatic Blood Analyzer (Baker System 9000 Hematology Analyzer, Baker Instruments, Bethlehem, PA). Baker standards (Haem QC plus normal and Haem QC plus abnormal Hi/Lo) were used as control. Aliquots of these blood samples were centrifuged at 3,000 X g for 15 minutes in a bench-top centrifuge. Clear plasma samples were analyzed for glucose (Neidle and Dunlop, 1990), cholesterol (Hashimoto et al., 1993), and triglycerides (D'Aniello et al., 1996). Blood samples were allowed to clot, and serum samples were analyzed for serum aspartic acid transaminase (AST) and serum alanine transaminase (ALT) by enzymatic methods.

RESULTS

Table 2 shows the cellular components and parameters of blood from broilers fed various false tuber meals (soaked tuber, raw tuber, raw tuber alcohol-treated) and control. Values recorded on the effects of test substances on hematological indices of broiler PCV, hemoglobin concentration (HBC) and red blood cell (RBC) values were found to be significantly ($p < 0.05$) different among indices measured. PCV recorded was highest ($p < 0.05$) in groups fed 6% raw tuber meal treated with 70% aqueous ethanol as opposed to the value in groups fed 6% raw tuber meal treated without alcohol and 9% soaked tuber as dietary inclusions and as well as the standard control respectively. HBC value was highest ($p < 0.05$) for birds fed the 9%

Table 2. Effects of *Icacina oliviformis* raw and treated tuber meal incorporated at different concentrations on haematological parameters of growing broilers.

Blood values (6 birds/group)	MEAN±SD			
	Control	6% raw tuber alcohol treated	6% raw tuber without alcohol treated	9% soaked tuber
Hb (g%)	7.80± 0.32^{b}	7.54± 0.21^{b}	8.34± 0.24^{a}	8.58± 0.24^{a}
TEC (million/mm^3)	3.68± 0.07^{c}	3.69± 0.06^{bc}	3.80± 0.05^{ab}	3.89± 0.04^{a}
PCV (%)	21.40± 2.07^{bc}	25.00± 1.58^{a}	22.80± 1.92^{abc}	24.60± 2.41^{ab}
MCV (fl)	58.20± 5.38^{bc}	67.66±3.79^{a}	60.00± 4.44^{abc}	63.14±5.73^{ab}
MCHC (%)	36.75± 4.17^{a}	30.24± 1.65^{b}	36.81±3.70^{a}	35.16± 3.69^{ab}
MCH (pg)	21.22± 0.72^{ab}	20.41±0.51^{b}	21.96±0.65^{a}	22.04± 0.63^{a}

Note: Values expressed as a mean ± SD of birds in parentheses. a, b, c, d; Values in a row with distinct superscript differes significantly at least to a level of 95% confidence (P = 0.5). For details of the ingredients used to prepare diets, see Table 1.

Table 3. Effects on serum biochemical parameters of different groups of broilers fed different levels of *Icacina oliviformis* (False yam).

Bio-chemical parameters	Mean±S.D			
	Control	6% raw tuber alcohol treated	6%raw tuber without alcohol treated	9% soaked tuber
Alanine aminotransferase [ALT (IU/L)]	11.75± 0.83^{a}	6.70± 0.74^{c}	6.35±0.76^{c}	8.49±0.87^{b}
Aspartate aminotransferase [AST (IU/L)]	108.67±4.75^{d}	135.12±5.32^{b}	123.73±4.59^{c}	144.84±4.77^{a}
Triglyceride (mg/dl)	162.11± 5.85^{a}	145.68±5.39^{b}	106.84± 3.17^{cd}	105.49±3.99^{cd}
BUN (mg/dl)	0.43±0.04^{b}	0.59±0.07^{a}	0.24±0.03^{cd}	0.17±0.04^{d}
Glucose (mg/dl	162.37±5.25^{a}	143.17±3.77^{b}	120.65±2.71^{cd}	113.63±5.60^{d}
Cholesterol (mg/dl)	165.97±5.85^{a}	152.54±4.82^{b}	113.39±4.57^{cd}	107.07±3.49^{d}

Note: Values expressed as a mean ± SD of birds in parentheses. a, b, c, d; Values in a row with distinct superscript differes significantly at least to a level of 95% confidence (P = 0.5). For details of the ingredients used to prepare diets, see Table 1.

soaked tuber meal (8.58 g%) as compared to those of other groups. The latter again showed the highest level of erythrocytes (TEC million/mm^3). The standard control also maintained its trend with a red blood cell count of 3.68 TEC million/mm^3 which was similar to other groups.

Like PCV, mean corpuscular volume (fl) was significantly different ($p < 0.05$) in birds that received 6% raw tuber that was treated with alcohol and similar results were also obtained from other two experimental groups; contrasting documented values for birds fed standard commercial group. Also, the mean corpuscular hemoglobin (MCH) in pg concentration was found significantly lower than other experimental groups, however, similar to those of standard control.

Table 3 shows that birds fed different diets had no effects on ALT level as compared to those of control. However, there was a significant increase in AST in birds fed 9% soaked tuber meal than those values obtained from other groups fed false yam based diet. Feeding alcohol-treated meals caused an increase of AST which was not significantly different ($p <0.5$) from the 6% raw tuber meal without alcohol treatment. There was a tendency to show depressing effect on the level of plasma glucose and blood urea nitrogen (BUN) (p<0.5) in all the birds fed experimental diets, however, the value was found to be more pronounced in birds fed 9% soaked tuber meal. Birds that received 6% alcohol-treated tuber meal showed a significant increase ($p < 0.05$) of BUN as compared to those values obtained from the standard control. The overall tendency show a significant increase in plasma urea in birds fed 6% alcohol-treated false yam tuber meal diet when compared to those values for the standard corn-soy–fish meal based commercial diet fed birds. The group that fed on 6% tuber meal which was treated with 70% ethanol-water (v/v) showed a significant increase ($p<0.5$) in plasma urea level. There was very consistent depression of glucose level in all the birds, however, it was more pronounced in birds fed on 9% soaked tuber meal.

Plasma triglycerides concentrations in false yam fed birds showed a tendency to reduce significantly while the 9% soaked tuber meal fed were significantly lower ($p<0.05$) than any other experimental groups and as well as those in the control birds. The highest triglyceride (TG) level was recorded in control group and the lowest was in 9% soaked tuber treated group.

Birds fed on experimental diets, all groups developed hypo-lipidemia which was marked by a significant ($p<0.05$) decrease in plasma triglycerides and total cholesterol (TC) as compared with birds fed standard control feed. It appeared that false yam meal is hypocholesterolaemic, however, the most pronounced hypo-cholesterolaemic effect was recorded from the birds fed 9% soaked tuber meal and was highly significantly (p<0.05) lower than those recorded from the standard control. Incorporation of 9% soaked false yam tuber meal resulted in 35.1% reduction in total cholesterol reduction in 15 days.

DISCUSSION

The feeding with meal extracted with aqueous –ethanol showed a modest improvement in Hb and as well as TEC when compared to those of standard control and other two groups. Nonetheless, values of PCV and MCV increased as result, MCH level is increased significantly (p<0.05).

It is apparent that our extraction of false yam tuber meal with cold (5°C) ethanol/water [70% (v/v) has successfully eliminated most of the alkaloids or flatus glycosides (Peterson and Young, 1968). They appeared to be soluble in aqueous ethanol and as a result, they ought to have been removed by such treatments. Since it was a restricted feeding experiment, all the birds were offered same amount of feed. However, there was about four times increase of BUN in the above birds than those values obtained from all other groups including 9% soaked tuber meal and as well standard control diet-fed birds. This high level of BUN could have been derived partly from undigested dietary protein since false yam contains high fibre (28.61%), which may prevent digestible materials from being hydrolysed by digestive enzymes (Dei et al., 2011). Alternatively, it could have been derived from endogenous sources such as digestive enzymes, mucus, shedding of gut cells, bacteria and leakage of serum proteins into the gut lumen. Nearly four-fold increase of BUN may have been linked to several anti-nutritional factors (Pusztai, 1987). Alternatively, it may have been due to a change in systemic intermediary metabolism which led to a higher rate of catabolism of amino acids and hence a higher level of BUN. Thus, BUN is used as an indicator of muscle catabolism or breakdown as well, because tissue degradation increases BUN.

Qaid and Maged (2021) showed that uric acid excretion is increased in stressed poultry owing to corticosterone-driven gluconeogenesis. Other indicators or measures of protein breakdown (muscle catabolism) include increased plasma and thus, the increased concentration of cystatin-C in blood and serum samples is highly sensitive to assess renal impairment and compared with creatinine. It is a very potent indicator of acute renal injuries because of its shorter half-life (Herget-Rosenthal et al., 2004), this investigation could not have been carried out from the present experiment.

Alanine aminotransferase (ALT) was found not to be significantly influenced in birds fed false yam based diet with high values recorded for birds in the standard control group. At the level of 6% inclusion in the diet, ALT level showed a decline and reduced to about 50% lesser than those obtained from the control group. This may be a genetic condition of the chicks inherited from the parent stock. However, AST level was increased significantly (p<0.05) when birds received diet with 9% soaked tuber meal. ALT is more specific to the liver and can be an indicator to detecting liver injury. However, ALT is still of poor diagnostic value in birds due to its existence in many tissues (Perelman, 1999; Harr, 2002). Thus, since no mortality was recorded during the experimental period, it may be right to assume that the extent of liver damage was tolerable and false yam unequivocally supports life. All dietary inclusion of false yam meal significantly decreased ALT values than the corresponding standard commercial control group, pointing to the ability of test ingredients to possibly treat damage to the liver (Akram et al., 2010).

Effects of feeding false yam as a source of dietary ingredient on serum indices of growing broilers showed that plasma triacylglycerol by incorporation of false yam tuber meal without having treated with aqueous ethanol was best (p < 0.05) suppressed and never elevated in any group fed as compared to those values obtained from feeding standard diet.

Marked hypocholesterolaemic effects of dietary false yam meal and 6% ethanol-treated meal were demonstrated in feeding trials with growing broilers. Birds receiving the 9% soaked tuber meal showed a decrease of 35% in 10 days compared with a control group fed standard commercial diet. This result is similar to those of feeding gamma conglutin that resulting in a 34% reduction of total plasma cholesterol in rats in 10 days. In comparison, human volunteers taking 900 mg/day garlic powder for 12 weeks (standardized to 1.3% alliin, Kawai', Lichtwer Pharma GmbH, Berlin, Germany) showed a reduction of 6% in plasma cholesterol level relative to that level of the corresponding placebo group. Furthermore, the cholesterol-lowering effect of feeding 9% soaked tuber meal found in this experiments was greater than any reported previously in feeding studies with plant proteins in rats (Rahman et al., 1996; Rahman, 2005). Although it remains to be known as to how total cholesterol gets reduced to this scale, the cholesterol removed from the blood is likely excreted rather than being accumulated in liver tissue. Thus, it is also possible that cholesterol concentrations in the livers of the treated birds were also decreased as compared with those in the control group (Noseda et al., 1980).

The amino acid profile of the sun-dried sample showed that it had low concentrations of most essential amino acids except arginine. The least concentrated amino acid in the meal was methionine (600 mg/100 g protein) and that was followed by cysteine and tryptophan. The calculated value for amino acid from the protein showed that the most abundant amino acids in the meal are aspartic acid which accounted for 52.6g/100 g protein followed by arginine and glutamic acid.

L-arginine accounted for 14.7% of false yam protein. The body can change L-arginine to nitric oxide, a substance known to widen blood vessels. Some people take L-arginine supplements to relax and open arteries, which

Table 4. Total protein and amino acid composition of sun-dried false yam tuber meal (SFYTM on DM basis Composition (% of DM).

Components	Amino acids (g AA / 100 g protein)
DM	86.46
CP	5.41
ARG	14.7
GLY	1.73
HIS	2.12
ILE	1.46
LEU	2.29
LYS	3.54
PHE	0.77
MET	0.06
THR	1.42
TRY	0.40
VAL	1.88
ALA	2.84
ASP	52.6
CYS	0.41
GLU	7.52
PRO	3.43
SER	3.49
Arginine to Lysine ratio	4.15

Note: The concentrations of amino acids in the sun-dried sample were determined by Eurofins Laboratories Ltd. (Wolverhampton, UK). The sample was analyzed for all amino acids except methionine, cystine, and tryptophan after acid hydrolysis with hydrochloric acid (method D1004; European Commission, 2009; Dei et al., 2011). DM = Dry matter; CP = Crude protein; ARG = Arginine; GLY = Glycine; HIS = Histidine; ILE = Iso-leucine; Leu = Leucine; LYS = Lysine; PHE = Phenylalanine; MET = Methionine; THR = Threonine; ; TRY = Tryptophane; VAL = Valine ; ALA = Alanine; ASP = Aspartic acid; CYS = Cystine ; GLU = Glutamine; PRO = Proline ; SER = Serine.

might help lower blood pressure. However, L-arginine supplements are rarely necessary and may only benefit people who have a true deficiency. Although research on L-arginine has had mixed results.

False yam tuber has been found to contain 52.6 g aspartic acid (Table 4). It may be mentioned here that the present assessment of aspartic acid gave an accurate value in 100g protein for false yam. Thus, compared to that of 100 g of soy protein isolate which contains 10.23 of aspartic acid, the level of this amino acid in false yam is 52.6 g per 100 g of protein. This level is around five times higher than the corresponding soy protein isolate and highest among the known aspartic acid rich foods. In order to increase or decrease the amount of aspartic acid in the diet, false yam may act as a novel source that hitherto is unknown to the scientific community.

It is not clear from the present investigation whether non-protein amino acids present in false yam tuber may have contributed to 52.6 g aspartic acid/100g protein. Non-protein amino acids function as mimics of 20 protein amino acids and can mistakenly be incorporated in protein in the place of the corresponding protein amino acids similar in structure, thereby leading to the production of unnatural proteins that cannot function properly. However, they serve as a defense against mammals and insects in general. In addition, aspartic acid has been found to be used as a key element for the improvement of sperm quality. The use of sodium D-aspartate has been found to improve the number and the motility of the spermatozoa and consequently improve the rate of pregnancies of their partners (D’Aniello et al., 2012).

Conclusion

In conclusion, blood values of the growing broilers were significantly influenced by fed false yam meal or its solvent treated meal as compared with those obtained from birds on the commercial control diet. False yam water-soaked meal had shown to have marked hypocholesterolaemic effect when fed to growing broilers, and that 9% of the water-soaked meal after being incorporated was particularly effective (Ueda and Fukui 1996). This reduction in plasma cholesterol levels does not appear to be caused by any specific protein and or fibers and or non-protein material, and or may have been related to the high arginine : lysine and/or cystine : methionine ratio in the proteins. It has been known for some time that vegetable proteins are hypocholestero-lemic, and as false yam meal has emerged as the most potent in this respect, they provide a useful source of material for further study of the mechanism of action of this effect. It appeared that the proteins of false yam is not only less nutritious than the standard control diet but also appears to cause changes in body metabolism, for reasons as yet not clear. In contrast, it is apparent that although false yam contains several potentially active ingredients of nonreactive phytochemicals, however, it is important that its metabolic fate needs to be studied in further detail. Furthermore, besides hypocholesterolaemic effects, false yam tuber may be useful in treating sub-fertile patients due to its high aspartic acid content.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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