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EFFECTS OF ENZYME (XZYME) SUPPLEMENTATION ON THE PERFORMANCE OF LAYING HENS FED DIETS CONTAINING DIFFERENT LEVELS OF CASSAVA (*Manihot esculenta, Crantz*) LEAF MEAL

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ORIGINAL ARTICLE

ABSTRACT: *An eight-week feeding trial was conducted to determine the additive effect of enzyme (Xzyme) and cassava leaf meal on the performance of laying hens. The cassava leaf meal (CLM) replaced different levels of fishmeal at levels of 0, 5, and 10% CLM in three iso-nitrogenous diets. One hundred and twenty Lohman strains of layers at thirty-weeks of age were randomly assigned to the three dietary treatments in a completely randomized design (CRD). Each treatment had forty birds, with ten birds per replicate. The initial average liveweight of birds from each replicate was 1.7 kg. Feed and water were offered ad libitum. Data collected included feed intake, liveweight gain, egg production, carcass characteristics, hematology and serum biochemistry. Economics of production was also calculated. Feed intake of birds on the treatment groups with CLM + xzyme was not different from those on the control. Final weight gain of birds on diet with 10% CLM +xzyme was lower than their counterparts on the other diets. Carcass weight of birds was not affected by dietary treatments. There was no difference in egg production, egg weight, shell thickness, Haugh unit and egg mass between dietary treatments. The rate of utilization of feed was reduced with inclusion of CLM in diet, with birds on 5% CLM+xzyme based diet recording the least feed conversion ratio (FCR). Except for heart, kidney, full proventriculus, full and empty gizzard, the other organs showed differences in their weights. All hematological parameters investigated except Mean Corpuscular Hemoglobin (MCH) showed difference in their mean values. The total serum protein (TSP), albumin, globulin and albumin/globulin ratio showed differences. Cost benefit analysis indicated that profit derived from the incorporation of CLM+xzyme in the diets was the same as control diet.*

Key words: *Egg Production, Hematology, Layer Diets, Leafmeal, Serum Biochemistry*

INTRODUCTION

Feed supply remains a major constraint in animal production due to high cost of conventional feedstuffs and the competition between man and animal for the same food (Amaefule et al., 2001). Efforts to lower the cost of production have elicited current interest in the search and use of non-conventional feedstuffs that are cheap, readily available and less competed for by man and industry. One of such novel feed resources so far under exploited is the leaves of cassava.

Cassava (*Manihot esculenta, Crantz*) is an all–season crop in several parts of Africa, Asia and Latin America (Ukuchukwu, 2008). In areas where cassava is grown, it is often planted for its tuberous root, leaving the leaves to wither. Meanwhile, leaf meal could be prepared from cassava leaves as a component of livestock feed (Fasuyi, 2005). Plant protein is perhaps the most naturally abundant and the cheapest potential source of protein. Natural resources are available for the synthesis and polymerization of amino acids into less mobile forms and stored as such in plant leaves.

The protein content and nutritive value of cassava leaves are well documented. Cassava leaf contains between 16.7-39.9% crude protein (Yousuf et al., 2007) with almost 85% of the crude protein fraction as true protein (Ravindran, 1991). Results of analyses by Ukanwoko and Ukandu (2011) revealed that cassava leaf meal contains 88.29, 25.81, 16.28, 3.64, 5.74, and 36.82% of dry matter, crude protein, crude fiber, ash, ether extract and nitrogen free extract respectively. Apart from the highly balanced amino acid profile (Wanapat, 2001), it also contains high level of potassium, iron, calcium, sodium, vitamin B1, B2, B6, C and carotenes (Bokanga, 1994).

However, the build-up of the amino acids in cassava leaves is accompanied by some limitations that render it less nutritious for consumption by livestock. Such factors are the high fiber content and anti-nutritional factors such

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as cyanide, tannin and phytin (Aletor and Adeogun, 1995). Stage of maturity is the major factor contributing to the variability in fiber content.

Benefits can however be realized from cassava leaf meal by supplementing poultry diets containing such leaf meals with exogenous enzymes like carbohydrases and other cellulases and proteolytic enzymes as found in Xzyme to improve the nutrient digestibility (Adeola and Olukosi, 2009). Interest in applying biotechnology for enzyme production has led to the production of enzyme preparation for poultry feeds which can improve the utilization of high fiber containing feedstuffs in poultry diets (Aderolu et al., 2007). There has been considerable success in the use of enzymes to improve nutrient utilization (Shivaram and Devegowda, 2004).

The present study was undertaken to evaluate the effects of enzyme supplementation on the performance of laying hens fed diets containing different levels of cassava (*Manihot esculenta, Crantz*) leaf meal.

MATERIALS AND METHODS

Experimental diets and preparation of cassava leaf meal (CLM)

Succulent leaves of cassava (a month old) were harvested from a-month-old cassava stands on a cassava farm near the College of Agriculture, University of Education, Winneba, Mampong-Ashanti, Ghana. The branches were cut and spread out on a clean concrete floor of well ventilated room for a period of 3-4 days until they became crispy. The leaves were separated from the twigs and milled in a hammer mill to obtain the leaf meal.

Chemical analysis of CLM and experimental diets

Samples of the CLM and experimental diets were subjected to proximate analysis using standard methods (AOAC 1990).

Experimental design and statistical analysis

The experiment was conducted with one hundred and twenty thirty-week old Lohman strains of layers for 56 days. Three experimental diets were formulated. CLM was included in the diets at various levels of 0, 5, and 10%. There were three replicates per each treatment, with 10 birds per replicate in a Completely Randomized Design (CRD). The birds were allocated in such a way as to ensure that the average bird weight per replicate was 1.7 kg. Feed and water were provided *ad libitum* and all required managerial practices were the same for each treatment group.

Parameters studied were feed consumption, egg production (hen-day and hen-housed egg production), egg weight, egg quality, yolk colour score and egg shell thickness. Economics of production was also calculated at the end of the study. The data were analyzed using the analysis of variance (ANOVA) technique and differences among means were separated by means of Duncan Multiple Range Test. Statistical significance was determined at P=0.05. The computations were performed using the general linear models procedures of the Statistical Analysis System Institute Inc (1999).

Hematology and blood serum biochemistry analysis

Blood samples were obtained from two birds per replicate making a total of six birds per treatment at the end of the experiment by inserting a new sterilized needle into the wing vein of the birds and collecting 2 ml of blood in labeled sterile test tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA). The blood samples were shaken to mix with the EDTA in order to prevent coagulation. The samples were then analyzed for Red Blood Cells (RBC), Packed Cell Volume (PCV), Hemoglobin (Hb), White Blood Cells (WBC), Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular hemoglobin (MCH) and Lymphocytes (LYM) using the Abbott Diagnostics Cell Dyn 3500 (Abbott Diagnostics, Abbott Park, IL) automated hematology analyzer.

Again, blood samples were obtained from each bird by the same procedure into vacuumed capillary tubes to determine the blood cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels, coronary risk, total protein and glucose. After coagulation, blood samples were centrifuged and then serum was collected for analysis. Blood serum biochemistry was determined by using Cobas integral 400 plus chemistry analyzer (Roche Diagnostics Ltd., Switzerland).

Carcass evaluation

Two birds were randomly selected from each replicate at the end of the study. They were weighed and killed by severing the carotic arteries. They were bled and immersed in hot water for 5 minutes to loosen feathers. The defeathered carcass was weighed. After dressing, the following weights were taken: carcass weight, dressed weight, gizzard, liver, heart, neck, shanks, and intestine.

RESULTS

The chemical composition of cassava leaf meal is shown in Table 1 while the nutrient composition of the experimental diets is presented in Table 2. Data on the performance of experimental birds on the various dietary levels of CLM + enzyme are captured in Table 3. Feed intake by birds on the treatment groups with CLM + enzyme was not different from those on the control diet. Final weight gains of birds on CLM + enzyme-based diets were lower than their counterparts on the control. Based on the overall egg weight, egg mass, egg shell thickness, haugh unit and egg production, it was apparent that there was no effect of CLM or Xzyme on these parameters. The results of the study also showed that the overall efficiency of feed utilization (FCR) was higher (3.9) in layers on control diet lower (4.3) for birds on diet with 5% of CLM+Xzyme. Carcass weight of birds was not affected by dietary treatments. Records on carcass and organ traits are shown in Table 4.

Except for heart, kidney, full proventriculus, full and empty gizzard, the other organs measured exhibited differences. The weight of both full and empty intestine (small and large intestines, duodenum and ileum) decreased with the inclusion of CLM + enzyme. The hematological and serum indices are summarized in Tables 5 and 6 respectively. All hematological parameters investigated except MCH showed difference in their mean values. Among the serum parameters measured, total serum protein (TSP), albumin, globulin and albumin/globulin ratio showed significant ($P<0.05$) differences.

Table 1 - Proximate composition of cassava leaf meal

Composition	Level (%)
Dry matter	85.0
Crude protein	22.8
Ether extract	5.5
Crude fibre	12.7
Ash content	4.5
NFE	39.5

Table 2 - Composition of experimental diets

Ingredients	Level of dietary CLM (%)		
	0%	5%	10%
Maize	53	53	53
Fish meal (62% CP)	4	4	4
Fish meal (54% CP)	7	6	4
Soya bean meal	8	4	2
Wheat bran	20	20	20
Cassava leaf meal	0	5	10
Oyster shell	7.5	7.5	7.5
Vitamin/mineral premix	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5
Xzyme	0	0.05	0.05
Analyzed composition (%):			
Dry matter	87	87	88
Crude protein	16.4	16.5	16.4
Ether extract	3.0	3.5	3.5
Crude fibre	4.1	4.7	5.2
Ash	13	15.5	14
NFE	53	53	53
Calculated composition (%)			
Calcium	1.2	1.3	1.2
Available phosphorus	0.4	0.38	0.37
Lysine	0.2	0.2	0.2
Methionine	0.2	0.2	0.2
ME (k cal/kg)	2500	2489	2546

**Composition of vitamin/mineral premix per kg: Vitamin E, 25mg; Vitamin A, 6250 IU; Vitamin D3, 1250 IU; Vitamin K3, 25mg; Vitamin B1, 25mg; Vitamin B2, 60mg; Vitamin B6, 40mg; Vitamin B12, 2mg; Elemental calcium, 25mg; Elemental phosphorus, 9mg; Elemental magnesium, 300mg; Iron, 400mg; Selenium 1.0mg, Iodine 20mg, Copper 60mg, Magnesium 100mg, cobalt 10mg, Zink, 150mg; Sodium Chloride, 1.5mg; Choline Chloride, 500mg; Live Lactobaccillus spore, 0.2 million cfu; Niacin, 40mg; Folic Acid, 10mg; d-Biotin, 5mcg*

DISCUSSION

Proximate analysis of cassava leaf meal revealed a crude protein level of 22.8% which is lower than those obtained by Iheukwumere et al. (2008). They obtained 25.10% crude protein in their study. The crude fibre obtained in the present study was however slightly higher than theirs (12.70%). According to NRC (1994), the proximate composition of plant feedstuffs can vary due to various factors such as crop variety, processing methods and soil conditions. The result of feed intake during the experimental period revealed that Xzyme supplementation did not

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stimulate feed intake of birds on diets containing cassava leaf meal. The present study corroborates earlier findings by Abbas et al. (1998) and Naqvi (1996) who noticed no difference in feed consumption among birds fed diets with or without supplementation of enzymes. The results of this study vary with work by Marck and Splitek (1990) and Arora et al. (1991) who observed increased body weight gain in broiler chicks when cellulolytic enzymes was added to their diet containing a higher level of fibre. In another experiment conducted by Iheukwumere et al. (2008) involving 5-week old Anak broilers, daily body weight gain and feed conversion ratio were similar for birds fed diet without cassava leaf meal (control) and those fed diet containing 5% cassava leaf meal. But these parameters reduced at 10% and 15% inclusion levels. The depression in growth at 10% inclusion level of cassava leaf meal as was also the case in this experiment has been reported by many researchers (Ash and Akoh-Detaia 1992; Opara 1996).

Table 3 - Effect of CLM on the performance of experimental birds

Parameter	Level of dietary CLM (%)				
	0	5	10	SEM	Prob.
Mean initial body weight (kg)	1.7	1.7	1.7	-	0.45
Mean final body weight (kg)	1.7[a]	1.7[a]	1.6[b]	0.04	0.01
Mean body weight gain (kg)	0.0	0.0	0.1	0.15	0.40
Mean feed consumption (g)	150	151	151	1.0	0.19
Hen – day egg production (%)	67.0	62.3	62.5	2.68	0.20
Hen – house egg production (%)	67.0	62.3	62.5	2.68	0.20
Mean egg weight (g)	57.3	57.3	59.0	1.58	0.47
Yolk colour score	1.0[a]	5.5[b]	7.0[c]	0.24	0.00
Egg shell thickness (mm)	0.4	0.4	0.4	0.02	0.32
Haugh unit score	88.5	80.0	81.5	5.3	0.28
Egg mass/day (g/hen/day)	38.3	35.6	36.9	1.41	0.21
Feed conversion ratio (kg feed/kg egg)	3.9[a]	4.3[b]	4.1[c]	0.03	0.00
Mortality (%)	0	0	0	-	-
Feed cost (kg/GH¢)	1.00	1.00	1.00	-	-
Total cost of feed over the period (GH¢)	344.00	324.00	301.00	-	-
Price per kg eggs (GH¢)	3.50	3.50	3.50	-	-
Value of egg (GH¢)	0.20	0.20	0.20	-	-
Feed cost/bird/day (GH¢)	0.20	0.20	0.20	-	-
Net revenue (GH¢)	0.00	0.00	0.10	-	-

SEM = Standard error of mean, *Significant difference at P<0.05, (a,b,c) Treatment means with different superscripts within the same row are significantly different at p<0.05.

NOTE: US $ 1.0 = GH¢1.5

Table 4 - Effect of CLM on organ weights of experimental birds

Variable	Level of dietary CLM (%)				
	0	5	10	SEM	Prob.
Liver (g)	39.5[a]	30.5[b]	33.5[b]	3.15	0.05
Kidney (g)	1.0	1.0	1.0	–	–
Heart (g)	8.0	9.0	8.5	0.53	0.22
Crop (Full), g	14.0[a]	25[b]	47.5[c]	7.5	0.01
Crop (Empty), g	9[a]	6.5[b]	8.5[c]	0.58	0.01
Proventriculus (Full), g	16.5	12.5	16.6	3.57	0.01
Proventriculus (Empty), g	10.5[a]	9.0[c]	7.5[b]	0.75	0.30
Gizzard (Full), g	41	42.0	42.5	2.51	0.83
Gizzard (Empty), g	26.5	28.5	28	2.29	0.67
Intestine (full), g	125[a]	93.5[b]	97.0[c]	1.67	0.01
Intestine (Empty), g	102[a]	45.5[b]	49[b]	2.22	0.01
Carcass weight (g)	1560	1381	1429	1293	0.40

SEM = Standard error of mean, *Significant difference at P<0.05, (a,b,c) Treatment means with different superscripts within the same row are significantly different at p<0.05

Data gathered from production performance parameters showed that the laying rate of hens fed CLM+Xzyme based diets led to reduction in egg production compared to those on control diet. This suggests that the amount of Xzyme supplementation in the different levels of CLM+Xzyme diets did not have a noticeable effect on the performance of laying hens. Egg production, egg weight, egg mass, haugh unit, and egg shell thickness were not increased significantly with incorporation of CLM+Xzyme. The results of yolk colour index agree with report by

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Okeke and Oluremi (2003), that incorporation of leaf meal in diets pigments egg yolk. This is as a result of xanthophylls (a pigment that impart a yellow colour to egg yolk) in green leaves.

The findings of the study are at variance with reports that the inclusion of leaf meals in diets of poultry result in increased weight of intestines and gizzard (Borin et al., 2006). According to them, birds fed high fibre diet containing cassava leaf meal recorded high gizzard and intestinal weight but this was reversed when enzyme was added to the diet. Similar observation was also made in this study. In the present study, enzyme supplementation decreased the relative size of liver, crop (empty), proventriculus (empty) and intestines (full and empty). Hajati et al., (2009) in an earlier study recorded higher gizzard and intestines weight when they fed broiler chickens corn-soybean meal-wheat diets supplemented with enzyme. Birds on 0% (control) and 5% CLM recorded higher total serum protein, albumin and globulin. Serum proteins are divided into albumin and globulin. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, proteins help balance the osmotic pressure of the blood tissue. Total serum protein has been reported as an indication of the protein retained in the animal's body (Esonu et al., 2001), and it depends on the quantity and quality of dietary protein (Iyayi, 1998). This observation is surprising since the experimental diets were formulated to be iso-nitrogenous. The results of hematological variables in this study (except MCH), suggest that the test diets did not precipitate adverse effects on the health status of laying hens. Blood represents a means of assessing clinical and nutritional health status of animals in feeding trial (Adeyemi et al., 2000).

Table 5 - Effect of CLM on hematological parameters

Parameter	Level of dietary CLM (%)				
	0	5	10	SEM	Prob.
WBC ($\times10^3$/ul)	235^a	242^b	228^c	3.79	0.01
RBC ($\times10^6$/ul)	2.3^a	2.4^{ab}	2^c	0.09	0.01
HGB (g/dl)	9.4^a	9.6^{ab}	7.7^c	0.28	0.01
HCT (%)	30^a	30.3^{ab}	26.4^c	0.99	0.01
MCV (fl)	128^a	126^{ab}	131^c	2.11	0.07
MCH (Pg)	40	40.1	38.1	0.94	0.02
MCHC (g/dl)	31.2^a	31.8^{ab}	29.2^c	0.78	0.02

SEM = Standard error of mean, *Significant difference at P<0.05, (a,b,c) Treatment means with different superscripts within the same row are significantly different at p<0.05

Table 6 - Effect of CLM on serum parameters

Parameter	Level of dietary CLM (%)				
	0	5	10	SEM	Prob.
Total cholesterol (mmol/L)	2.2	3.1	2.7	0.42	0.16
Total protein (g/l)	52.5^a	52.1^{ab}	46.7^c	3.45	0.21
Albumen (g/l)	17.8^a	18^{ab}	15.5^c	0.95	0.05
Globulin (g/l)	34.8^a	34.6^{ab}	31.2^c	2.74	0.38
Albumin:globulin	0.5	0.5	0.5	-	-
Magnesium (mmol/L)	1.2	1.2	1.2	0.10	0.63
Phosphate (mmol/L)	1.6^a	0.0^b	0.0^b	0.45	0.01
Uric acid (mmol/L)	139	133	109	26.07	0. 52

SEM = Standard error of mean, *Significant difference at P<0.05
(a,b,c) Treatment means with different superscripts within the same row are significantly different at p<0.05

CONCLUSION

The findings of this study showed that CLM could be included up to 10% in the diet of laying hens (30-38 wks old) without any deleterious effects on production performance. Supplementation of diets with Xzyme at 5 and 10% inclusion levels of CLM did not significantly improve egg production.

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