ISSN 1682-8356
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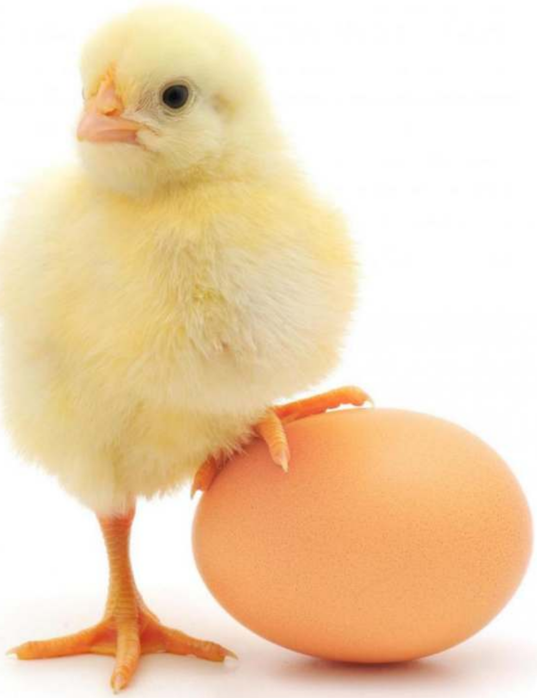

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 11 (1): 33-38, 2012
ISSN 1682-8356

Effects of Roxazyme G® and Maxigrain® on Performance, Egg Quality, Cost-Benefit and Haematological Parameters of Laying Hens Fed Wheat Offal, Corn Bran and Brewery Dry Grain Diets

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Abstract: The study examined effects of supplementing Roxazyme G® and Maxigrain® to wheat offal-, corn bran- and brewery dry grain-based diets on the performance, haematological parameters and economy of production in laying hens. Both enzymes were added to these 3 diets at 100 mg/kg, while control group was neither supplemented with Roxazyme G® nor Maxigrain®. The results showed that hens fed Roxazyme G® corn bran diets had the best feed conversion and maximum profit. Maxigrain® also significantly improved hen day production and egg weight of hens fed Brewery Dry Grain (BDG) than those fed Roxazyme G® BDG diet. Laying hens fed BDG diets had the lowest profits. Both enzymes failed to improve the performance and profits of hens fed Wheat Offal (WO) diet. Improvements in Red Blood Cells (RBC) for hens fed both enzyme supplemented diets were observed. Interactive effects of enzyme type and fibre source significantly ($p<0.01$) influence RBCs, PCV and white blood cells. The study revealed that Roxazyme G® and Maxigrain® should be added to respectively CB- and BDG- diets for improved laying performance. However, these enzymes failed to improve the laying performance of hens fed WO diet.

Key words: Corn bran, wheat offal, brewery dry grain, profit, performance

INTRODUCTION

Agro-Industrial By-Products (AIBPs) are cheap and abundant in Nigeria and they represent ready feedstuffs for feed formulation in animal production. Effects of AIBPs on performance and nutrient digestibility of animals have been reported. Iyayi *et al.* (2005) reported that 40% replacement of maize with each of brewery dry grain (BDG at 40%), corn bran (CB at 40%) and palm kernel meal (PKM at 40%) significantly reduced weight gain, nutrients digestibility, weights of carcass parts, impaired feed conversion and elongated duodenum, ileum, caecum, colon of broilers. These AIBPs are known to serve as a source of virtually all feed nutrients that are required by livestock (Onifade, 1993). Studies have shown that nutritive value of feedstuffs such as barley, wheat, rye, lupin, corn, soy bean meal have been improved using several exogenous enzymes (McCracken and Quintin, 2000; Preston *et al.*, 2000; Hughes *et al.*, 2000; Lazaro *et al.*, 2003 and Danicle *et al.*, 2003). Bekatorou *et al.* (2007) upgraded the nutritional value of Brewery Spent Grain (BSG) and Malt Spent Rootlets (MSR) through treatment with *Aspergillum oryzae*, *A. awamori* and *Phanerochaete chrysosporium* (white rot-fungus) for animal feed production. These authors also reported that the used BSG has a moisture content of about 60%. This is to allow cultured fungi to efficiently metabolize the BSG. However, limited efforts have been shown to improve the nutritional value of commonly available cheap agro-industrial by-products such as wheat offal, corn bran and brewery dry grain. Wheat Offal (WO) and Corn Bran (CB) are by-products of milling process for wheat and corn respectively, while Brewer Spent Grain (BSG) is produced in the mashing stage in beer production (Bekatorou *et al.*, 2007). When BSG is properly dry, it is known as Brewery Dry Grain (BDG). BSG consist of about (%w/w on dry matter) 16-25% cellulose, 12-28% lignin, 11-26% apparent starch (glucose, maltodextrins and residual starch), 15-25% crude protein, 15-20% crude fibre, 6-10% digestible fibre, 6-10% lipids and 3-5% total ash (Bekatorou *et al.*, 2007). Their composition depends on the barley variety as well as the malting and mashing programme (Bekatorou *et al.*, 2007). These three feedstuffs (WO, CB and BDG) are highly fibrous. The advent of exogenous enzyme preparations and their functions (in relation to specific enzyme activities) is necessary prerequisite for their application in diets and its expected maximum benefits for animal production. Efforts to ascertain their effectiveness to add value to animal nutrition (particularly poultry) using available AIBPs in feed formulation are therefore needed now. Hence, this study evaluated the performance,

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Table 1: Ingredient composition of experimental diets

Ingredients	Control	WO	CB	BDG
Maize	22.81	22.81	13.30	38.26
Soybean meal	14.39	14.39	18.90	14.04
Wheat offal	25.00	25.00	-	-
Rice bran	25.00	25.00	25.00	5.00
BDG	-	-	-	30.00
Corn bran	-	-	30.00	-
Methionine	0.15	0.15	0.15	0.10
Lysine	0.15	0.15	0.15	0.10
Fish meal	2.00	2.00	2.00	2.00
Bone meal	2.50	2.50	2.50	2.50
Oyster shell	7.50	7.50	7.50	7.50
Vitamin premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Calculated analysis				
Energy (kcal/kg)	2411.53	2411.53	2489.22	2487.13
Crude protein (%)	16.50	16.50	16.50	16.50
Crude fibre (%)	6.66	6.66	8.24	7.72
Calcium (%)	3.74	3.74	3.72	3.77
Phosphorus (%)	0.73	0.73	0.70	0.62
Methionine (%)	0.44	0.44	0.44	0.47
Lysine (%)	0.90	0.90	0.99	1.01

*Vitamin premix supplied the following vitamins and trace minerals per kg of feed: Vit. A. 6250 IU; Vit. D_3 1250 IU; Vit. E. 14.38 mg; Vit. K_3 1.25 mg; Vit. B_1 1.88 mg; Vit. B_2 3.75 mg; Niacin 31.25 mg; Calcium pantothenate 6.25 mg; Vit. B_6 3.13 mg; Vit B_{12} 0.02 mg; Choline chloride 250 mg; Folic acid 0.63 mg; Biotin 0.03 mg; Mn 75 mg; Fe 62.5 mg; Zn 50 mg; Cu 5.31 mg; I 0.94 mg; Co 0.19 mg; Se 0.08 mg and Antioxidant 75 mg

haematological parameters and productive benefits of laying hens fed wheat offal, corn bran and brewery dry diets supplemented with Roxazyme G® and Maxigrain®.

MATERIALS AND METHODS

Management of experimental animals: One hundred and sixty eight 37 weeks old Black Harco laying hens were randomly allotted to 7 experimental dietary groups. Each treatment group consist of 4 replicates with 24 hens. Feed and water were given to these hens *ad libitum*. Normal management practices were observed, while administration of medication and vaccination were given to the laying hens as at when due. A period of 10 weeks was observed for this study. The study was carried out at the Layer House, Teaching and Research Farm, LAUTECH, Ogbomoso, Nigeria.

Formulation of experimental diets: Seven diets were formulated for the study. Control diet contained majorly Wheat Offal (WO) at 25%. Three diets {consisting of 25%WO diet and two other diets containing majorly Corn Bran (CB) and Brewery Dry Grain (BDG) each at 30%} were formulated. Addition of Roxazyme G® and Maxigrain® to these diets resulted in 6 experimental diets (Table 1 and 2). Roxazyme G® and Maxigrain® were to these diets at 100 mg/kg. All the diets were iso-nitrogenous and they have similar calcium and phosphorus at 3.74%±0.02 and 0.70%±0.05 respectively. Each gram of Maxigrain® contains 10,000 IU cellulase, 200 IU beta-glucanase, 10,000 IU xylanase and 2500 FTU phytase. Each gram of Roxazyme G® consists of 8000 IU cellulase, 18000 IU beta-glucanase and 26000 IU xylanase.

Cost-benefit analysis of the study: This was carried out based on the value of current exchange of naira to a dollar (#160 to $1).

Chemical analysis: Blood samples (8 samples per treatment) were collected into EDTA bottles for estimation of haematological parameters using Ghai (1993) methods.

Statistical analysis: All data collected were analyzed by factorial ANOVA using General Linear Model (GLM) of SAS (1999) under completely randomized design. The factors were fibre source (WO, CB and BDG) and enzyme type (Roxazyme G® and Maxigrain®). Means were separated by Duncan option of the same software. A probability of 5% was considered significant.

RESULTS AND DISCUSSION

Effects of both Roxazyme G® and Maxigrain® on laying performance and cost-benefit effect are shown in Table 2. Hen Day Production (HDP) of hens fed CB-, BDG-diets supplemented with Maxigrain® were lower than hens fed WO diet supplemented with Maxigrain® (with reductions of 6.5% and 12.9%, relative to WO Maxigrain® group). Furthermore, the HDP of hens fed BDG diet containing Roxazyme G® was significantly reduced when compared to those fed WO and CB diets supplemented with Roxazyme G®. Addition of Roxazyme G® to CB diet produced significantly higher (about 11.2% increase) HDP than those fed Maxigrain® CB diet. However, hens fed Maxigrain® BDG diet had higher HDP and egg weight than those fed Roxazyme G® BDG diet. The feed conversion of hens fed Roxazyme G® added to CB diet was the best. The response of the laying hens fed enzyme supplemented diets showed that the type of Non-Starch Polysaccharide (NSP) degrading enzymes used is crucial to the performance of laying hens fed these different sources of dietary fibre. The chemical nature of polysaccharides present in these dietary fibres are varied, therefore NSP degrading enzymes type for the utilization of each fibre source will also be different. Maxigrain® enzymatic profile shows that it is cellulase and xylanase based enzyme preparation with equal activity whereas Roxazme G® is xylanase based enzyme preparation with relative better beta-glucanase activity than those present in Maxigrain®. Furthermore, it has been reported that Roxazyme G® is an enzyme complex derived from *Trichoderma viride* with glucanase and xylanase activity that has been developed to complement the digestive enzyme of poultry (Broz and Frigg, 1990; McNab and

Smithard, 1992) so that polysaccharides in cereal offal can be broken down into simpler molecules which can be digested and utilized (Tuleun *et al.*, 2009). Choct and Annison (1992) confirmed that it is the viscosity of the arabinoxylan of wheat that exerts their anti-nutritive activity. Soluble arabinoxylans present in wheat-based diets display considerable antinutritive properties for monogastric animals (Edwards *et al.*, 1988; Chesson, 1993 and Choct *et al.*, 1996). Hence, addition of xylanase based enzyme preparation to wheat (*Triticum aestivum*) and wheat by-products diet may enhance the nutritive value of this cereal and its by-products. Choct *et al.* (1995) reported that a commercial glycanase product (Avizyme TX) significantly increased the apparent Metabolizable Energy (ME) of the low ME wheat from 12.02-14.94 MJ/kg DM. However, in Durum wheat (*Triticum durum*) the presence of xylanase inhibitors considerably reduced the function of exogenous xylanase, during durum wheat semolina processing (Ingerlbrcht *et al.*, 2000). It is therefore, likely that protein inhibitors could affect the action of xylanases used to supplement wheat- and rye- based diet (Ponte *et al.*, 2004). These authors showed that the levels of soluble arabinoxylans are low in *T. durum* and the presence of significant proportions of the insoluble polysaccharide might have resulted in poor feed efficiency. Addition of cellulases and hemicellulases enhanced the nutritive value of cereals rich in soluble NSPs for poultry and pigs (Bedford and Classen, 1992). The efficacy of enzyme supplementation, particularly for wheat-based diets, is variable (Bedford, 2003). A range of factors has been advanced to explain the unpredictable response of animal performance to enzyme supplementation, which includes cereal type, growing conditions, age of animal, processing of the diet, nutrient density and enzyme dose (Ponte *et al.*, 2004). The addition of Maxigrain® and Roxazyme G® to wheat offal based diet slightly improved feed conversion. This implied that both Maxigrain® and Roxazyme G® could not completely hydrolyse the NSP present in WO diet probably due to low soluble arabinoxylan in wheat offal. Another factor may be the presence of xylanase inhibitors in wheat offal as was suggested in Durum wheat (*Triticum durum*). Feed intake of hens fed WO- and BDG-diets was influenced by the type of enzyme used in this study. Hens fed Roxazyme G® wheat offal- and brewery dry grain-diets consumed less feed than those fed Maxigrain®. This could be probably due to more availability of soluble carbohydrate from both WO and BDG when supplemented with xylanase based enzyme. Atteh (2001) reported 56% and 26% increase in Apparent Metabolizable Energy (AME) of wheat offal and BDG respectively when supplemented with xylanase enzyme. However, it has been reported that neither metabolizable energy nor enzyme affected feed intake when supplementing a high wheat middling-based diet with Avizyme 1300 (Wang *et al.*, 2005). Different enzyme activities in enzyme preparations and fibre source may account for the differential feed intake response. Laying hens fed Roxazyme G® corn bran diet had the lowest feed intake and this revealed that this diet was probably well digested and utilized by laying hens in this study.

The cost-benefit response of laying hens fed three sources of fibre diets supplemented with Maxigrain® and Roxazyme G® is shown in Table 2. Hens fed BDG diets had the highest feed cost and the lowest profit, whereas birds fed CB diet with Roxazyme G® had the lowest feed cost and highest profit. Supplementation of both enzymes to WO diets did not significantly improve feed cost and profit. Several studies have reported that enzymes supplementation in low quality diets improved the performance of poultry. Alam *et al.* (2003) reported that among three enzymatic diets (Alquerzim®, Roxazyme G® and Feedzyme® diets), broilers fed Roxazyme G® had the best performance in terms of final liveweight, weight gain, meat yield and maximum profit (Tk/kg broiler). They concluded that Roxazyme G® showed the highest performance due to more different enzyme combination than other two enzymes.

Agger *et al.* (2010) reported that enzymatic hydrolysis of arabinoxylan from pre-treated corn bran (190°C, 10 min) after treatment with mixture of monocomponent enzymes consisting of α-1-arabinofuranosidases, an endoxylanase and a β-xylosidase. The pretreatment divided the corn bran material 50:50 into soluble and insoluble fractions having arabinose:xylose ratio of 0.66 and 0.4 respectively. Superior performance of laying hens fed corn bran diet supplemented with Roxazyme G® indicated that xylanase-based enzyme preparation may best suited for the utilization of corn bran based diet. This may be probably due to abundance of soluble arabinoxylan in corn bran. Iyayi *et al.* (2005) observed that replacement of 40% maize by each of brewery dry grain (40%), palm kernel meal (40%) and corn bran (40%) in broiler diet significantly reduced nutrient digestibility and inhibited performance of broilers. This suggested that the utilization of these dietary fibre sources by broilers were impaired. Hence, supplementation of diets based on WO, CB and BDG with appropriate NSP degrading enzyme preparation is a necessity for improved poultry performance. This study also revealed that Maxigrain® could be best used for brewery dry grain based diet rather than Roxazyme G®. This could be due to the effect of more cellulase activity in Maxigrain whereas Roxazyme G® has less cellulase activity. In support of this assertion, Mathlouthi *et al.* (2002) reported that the endosperm cell wall of wheat is mainly composed of arabinoxylans, whereas β-glucan is the major constituent of barley cell walls. It has been reported that the composition of brewery spent grain depends on the barley variety as well as the malting and mashing programme (Bekatorou *et al.*, 2007).

Table 2: Laying performance and cost-benefit effects of hens fed Wheat Offal (WO), Corn Bran (CB) and Brewery Spent Grain (BSG) supplemented with Roxazyme G® and Maxigrain®

		Maxigrain®			Roxazyme G®							
Parameters	Control	T1(WO)	T2(CB)	T3(BDG)	T4(WO)	T5(CB)	T6(BDG)	p-value	SEM	ENZ type	Fibre source	INT
HDP (%)	80.76^{a}	80.89^{a}	75.64^{b}	70.48^{c}	81.10^{a}	84.09^{a}	65.82^{d}	0.0001	1.34	NS	***	***
Egg weight (g/egg)	57.25^{b}	57.29^{b}	59.52^{ab}	60.62^{a}	58.47^{ab}	58.09^{b}	58.27^{b}	0.029	0.70	NS	***	***
Feed intake (g/hen/d)	205.77^{b}	199.72^{bc}	196.19^{cd}	212.38^{a}	191.06^{d}	190.24^{d}	199.17^{bc}	0.0001	2.20	***	NS	NS
Feed conversion (feed/egg)	4.57^{c}	4.38^{cd}	4.48^{cd}	5.18^{b}	4.20^{cd}	4.02^{d}	5.78^{a}	0.0001	0.16	NS	**	**
Feed cost												
($/kg egg)	1.45^{c}	1.40^{c}	1.43^{c}	1.85^{b}	1.34^{c}	1.30^{c}	2.05^{a}	0.0001	0.06	NS	***	**
($/tray)	2.45^{bc}	2.42^{bc}	2.50^{b}	3.31^{a}	2.34^{bc}	2.20^{c}	3.50^{a}	0.0001	0.08	NS	***	**
Income												
($/kg egg)	2.41^{a}	2.35^{ab}	2.33^{ab}	2.27^{b}	2.33^{ab}	2.39^{a}	2.37^{a}	0.032	0.03	*	NS	NS
Profit												
($/kg egg)	0.96^{ab}	0.95^{b}	0.89^{b}	0.42^{c}	0.99^{ab}	1.09^{a}	0.33^{c}	0.0001	0.04	NS	***	**
($/tray)	1.62^{ab}	1.64^{ab}	1.56^{b}	0.75^{c}	1.72^{ab}	1.85^{a}	0.56^{c}	0.0001	0.08	NS	***	**

^{abcd}Means along the same row with different superscripts are significantly different (**p<0.01, ***p<0.001) NS = Not significantly different

Table 3: Selected egg quality and haematological parameters of laying hens fed Wheat Offal (WO), Corn Bran (CB) and Brewery Dry Grain (BDG) supplemented with Roxazyme G® and Maxigrain®

		Maxigrain®			Roxazyme G®							
Parameters	Control	T1(WO)	T2(CB)	T3(BDG)	T4(WO)	T5(CB)	T6(BDG)	p-value	SEM	ENZ type	Fibre source	INT
Shell + membrane weight (%)	13.98	13.80	13.40	13.67	14.17	13.77	14.46	0.136	0.25	**	NS	NS
Shell thickness (mm)	33.37	34.14	33.07	33.06	33.37	33.34	33.94	0.815	0.60	NS	NS	NS
Haugh unit	82.17	84.37^{a}	84.02^{a}	82.25^{ab}	84.16^{a}	81.76^{ab}	80.62^{b}	0.049	0.89	NS	**	NS
PCV (%)	32.25^{b}	35.25^{a}	37.25^{a}	31.25^{b}	35.00^{a}	35.25^{a}	35.00^{a}	0.0001	0.83	NS	***	**
Hb (g/dl)	10.50^{d}	11.55^{ab}	12.23^{a}	10.65^{cd}	11.48^{ab}	11.50^{ab}	11.38^{bc}	0.0007	0.27	NS	**	NS
RBC x10^{6} (/mm^{3})	5.68^{bc}	6.30^{ab}	6.70^{a}	5.38^{c}	6.23^{ab}	6.70^{a}	6.45^{a}	0.0009	0.24	**	**	**
WBC x10^{3} (/mm^{3})	10.06^{c}	14.15^{a}	12.76^{ab}	12.84^{ab}	11.88^{bc}	13.52^{ab}	14.49^{a}	0.0012	7.14	NS	NS	**
Lymphocytes (%)	55.25^{ab}	53.00^{b}	55.38^{ab}	57.00^{a}	53.38^{b}	54.00^{b}	53.75^{b}	0.057	0.95	NS	NS	NS
Neutrophils (%)	38.00^{b}	39.25^{ab}	38.00^{b}	35.00^{c}	41.00^{a}	39.75^{ab}	39.50^{ab}	0.0001	0.70	***	***	NS
Monocytes (%)	5.25	5.50	5.75	6.00	5.25	5.00	5.75	0.368	0.34	NS	NS	NS

^{abcd}Means along the same row with different superscripts are significantly different (**p<0.01, ***p<0.001) NS = Not significantly different

Consequently, NSP degrading enzyme containing the enough cellulase activity could assist in the proper hydrolysis of brewery dry grain based-diet for maximum laying performance. Maxigrain® enzyme may have fulfilled this required cellulase activity for the hydrolysis of BDG.

The RBCs of hens fed Roxazyme G® and Maxigrain® added to CB diet were richer than the control group (Table 3). Furthermore, hens fed BDG-Maxigrain® diet had the lowest RBCs which shows that BDG was not efficiently utilized by the laying hens. Laying hens fed enzyme supplemented diets had significantly ($p<0.0012$) higher WBCs than the control. This showed enhanced immune-competence of the hens. This study revealed that Roxazyme G® (a xylanase based enzyme preparation) was the best enzyme supplement for CB diet whereas supplementation of Maxigrain® (a cellulase and xylanase based enzyme preparation) to BDG diet did not fully hydrolyse non Starch Polysaccharides (NSPs) of BDG diet. This is reflected in reduced HDP but it was better than Roxazyme G® BDG diet. Improved feed conversion, PCV, Hb and RBCs for hens fed Roxazyme G® and Maxigrain® were observed when compared to the control. Finally, both enzymes slightly enhanced feed conversion without significant improvement in profit of hens fed WO diet. This revealed that both enzymes could not improve the profitability accrued from hens fed wheat offal based diet.

Conclusively, this study specified the beneficial effects of Roxazyme G® and Maxigrain® on the performance, egg quality and haematological parameters of hens fed WO-, CB- and BDG- diets. Roxazyme G® and Maxigrain® significantly improved HDP, FC and profits of hens fed CB- and BDG-diets respectively. Both enzymes failed to significantly enhance the performance and profits of hens fed WO diet.

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