COMPARISON OF EFFECTS OF ANTIBIOTICS AND ENZYME INCLUSION IN DIETS OF LAYING BIRDS

COMPARACIÓN DE LOS EFECTOS DE LA INCLUSIÓN DE ANTIBIÓTICOS Y ENZIMAS EN DIETAS PARA PONEDORAS

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ADDITIONAL KEYWORDS

Productive performance. Egg quality.

PALABRAS CLAVE ADICIONALES

Eficacia productiva. Calidad de huevos.

SUMMARY

A study was conducted to compare the effects of enzyme and antibiotic inclusion in diets for laying birds. The inclusion of enzyme alone or enzyme alternated with antibiotic (enzyme/antibiotic) in a standard diet for laying birds had the best egg productive performance in terms of eggs produced/hen day and feed/kg egg. Body weight was highest in the diets where enzyme/antibiotic was given. Egg quality in terms of egg weight and Haugh units were highest in the hens given diets containing either enzyme alone or enzyme/antibiotic. The egg quality indices such as egg shape index, egg composition values, yolk colour and blood spots were not affected by the diets. Antibiotic residues were recorded in eggs from laying birds fed with diets containing antibiotics alone and enzyme/antibiotic diet.

RESUMEN

Se realizó un estudio para comparar los efectos de la inclusión de enzimas y antibióticos en la dieta de ponedoras. La inclusión de enzima solo o enzima alternado con antibiótico (enzima/antibiótico) en una dieta estándar para ponedoras tiene el mejor efecto sobre la producción de huevos por gallina día y pienso por kg de huevos. El peso corporal fue mayor en las dietas donde se administró enzima/antibiótico. La calidad de los huevos, en términos de peso o unidades Haugh, fue mayor en las gallinas que recibieron enzima solo o enzima/antibiótico. Los índices de calidad del huevo como la forma, composición, color de la yema y manchas de sangre, no fueron afectados por las dietas. Se registraron residuos de antibióticos en los huevos de las gallinas alimentadas con dietas que contenían antibióticos solo o enzima/antibiótico.

INTRODUCTION

Feed additives are included in diets for poultry in order to increase production by improving nutrient availability. Antibiotics as feed additives act as growth promoters by preventing disease occurrence and the treatment of existing diseases, thus improving the efficiency of animal production. Of recent, the use of antibiotics as growth promoter in diets for layers is being discouraged. In the European Union (as in many developed countries), inclusion of antibiotics in animal feed is forbidden. This is because residues of these drugs may appear in eggs constituting potential health hazards to the consumers. The major health risks associated with these groups of drugs are the development of drug resistance in the exposed individuals, hypersensitivity reactions and the development of resistant micro-organisms to antibiotics in human beings (Schothrost *et al*., 1978, DuPont and Steele, 1987).

Enzymes have also been found to be useful feed additives in poultry production.

In layer diets, corn serves as the major energy source, while ingredients such as soybean meal and groundnut meal serve as the major sources of plant protein. However, there is a great deal of variation in the composition of these ingredients. Changes in the composition will influence the performance of the birds (Aman and Graham, 1990). Addition of enzymes such as amylase and xylanase are useful in the utilization of the non-starch polysaccharide component of the ingredients, while others such as proteases may enhance the utilization of protein compounds. All in all, enzymes increase the effectiveness of nutrient utilization resulting in improved performance (Acamovic, 2001).

This study was conducted to compare the effects of inclusion of enzymes and antibiotics as feed additives in diets of laying birds on performance characteristics and egg quality parameters.

MATERIALS AND METHODS

EXPERIMENTAL BIRDS

The study was carried out at the Teaching and Research Farm, University of Agriculture Abeokuta Ogun State Nigeria (Latitude 7° 5.5' - 3° 2' and Longitude 3° 11.2' - 3° 2.5' and altitude 76 MASL), situated in the rain forest vegetation zone. The area has a humid climate with mean annual rainfall and temperature of 1037mm and 34.7°C respectively and average relative humidity of 82 percent. A total of One hundred and eighty (180) 30-week old Nera pullets (14 weeks in lay) were used for the study. The birds were divided into 4 groups of 45 birds each. Each group was further divided into three sub-groups of 15 birds each, these serve as replicates. Four experimental diets were fed to each of the 4 groups of birds. The basic diet given in **table I** represents a typical layer diet commonly used in the South-Western part of Nigeria. This contains corn and wheat offal as the major energy source, with soybean meal, groundnut meal and palm kernel meal serving as the protein sources.

The basic diet was then supplemented with enzymes and tetracycline antibiotic as the treatment groups. The first treatment (T_1) was given to the group one birds which serve as the control for the study. This treatment is made up of the basic diet that was neither supplemented with enzyme or antibiotic. In treatment 2, (T_2) the diet

Table I. *Composition of experimental diet (g/kg).* (Composición de la dieta experimental (g/kg)).

Ingredients	Experimental Diet			
	T_1	T_2	T_3	T_4
Corn	450.0	450.0	450.0	450.0
Wheat Offal	145.0	145.0	145.0	145.0
Soybean meal	100.0	100.0	100.0	100.0
Groundnut cake	100.0	100.0	100.0	100.0
Palm kernel cake	100.0	100.0	100.0	100.0
Fish meal	10.0	10.0	10.0	10.0
Bone meal	10.0	10.0	10.0	10.0
Oyster shell	80.0	80.0	80.0	80.0
Vit/Min Premix[1]	2.5	2.5	2.5	2.5
Sodium chloride	2.5	2.5	2.5	2.5
Enzyme (Avizyme 1500; 200mg/kg)	-	-	+	-
Tetracycline Antibiotic (200mg/kg)	-	+	-	-
Enzyme/Antibiotic	-	-	-	+
Calculated Nutrient Content				
Crude protein (g/kg)	165			
Calcium (g/kg)	34.4			
Phosphorus (g/kg)	5.2			
Lysine (g/kg)	8.4			
Methionine (g/kg)	2.9			
Metaboliable energy (MJ/kg)	11.01			

[1]Vitamin mineral premix contains the following (per kg diet): retinal acetate 1.65 mg, cholicalceferol 27.5 μg/, dl α tocopherol acetate 10 mg, riboflavin 4.4 mg, calcium pantothenate 12 mg, nicotinic acid 44 mg, choline chloride 220 mg, cyanocobalamin 6.6 μg, pyridoxine 2.2 mg, menadione 1.1 mg, folic acid 0.55 mg, d-biotin 0.11 mg, thiamine mononitrate 2.2 mg, ethoxyquin 125 mg, manganese sulphate 60 mg, zinc oxide 50 mg, iron 30 mg, sodium selenate 0.1 mg, copper carbonate 1.5 mg.

was supplemented with feed-grade tetracycline antibiotic at a level of 200 mg/kg. In treatment 3 (T_3), the diet was supplemented with enzyme (Avizyme 1500®) at a level of 200 mg/kg. The enzyme which is a mixed enzyme contained α-amylase (EC 3.2.1.1, 400 U/g) xylanase (EC 3.2.1.8, 300 U/g), protease (EC 3.4.21.62, 400 U/g) and pectinase (EC 3.2.1.25, 25 U/g) (Finfeeds International Ltd.). In treatment 4, tetracycline antibiotic and enzyme were added to the basic diet alternately on a weekly basis. This was done to see the effect of each of these additives in the bird after one week of administration. The study lasted for a total of 28 laying weeks. The birds were all raised in standard battery cages with feed and water given *ad libitum*. All birds were subjected to standard management practices. Birds were weighed at the commencement of the study and at the end of the study. Records of egg production were kept on a daily basis while those of feed intake were taken on a weekly basis.

Determination of EGG Quality

A total of 36 eggs were collected weekly for measurement of external and internal egg quality parameters. For external parameters the eggs were weighed within 24 hrs of lay, thereafter the length and breadth of each egg were measured to the nearest 0.01 cm using vernier callipers. For internal egg quality parameters, the eggs were broken onto a flat glass plate and the height of the albumen determined using a spherometer. Haugh unit values were calculated using the formula of Haugh (Vadehra, 1974). Yolk colour was determined using a Hoffman la – Roche colour fan. Blood spots were recorded by scoring the size of the blood spots from 0 – 5, whereby 0 was for no blood spots and 5 for a large area of blood spots (5 mm radius). The yolk was separated from the albumen with the aid of an egg separator and yolk weight determined. The thickness of the shell was measured by the use of a micrometer screw gauge. The shell was then air-dried to constant weight before the weight of the shell determined.

Determination of antibiotic residues in EGG

A total of 36 eggs, comprising of 3 eggs from each replicate were sampled on a weekly basis for 8 weeks for the determination of antibiotic residues. The residues of tetracycline antibiotic in the eggs were assayed by micro-biological assay method (diffusion agar technique). A modification of the assay method described by Katz and Fassbender (1972) was used for the study. Eggs were broken and pooled, the egg mixture was then weighed. Thereafter, a solution of 0.75 percent Tween 20 (Polyoxyethene sorbitan monolaurete) in phosphate buffer (pH

Table II. *Egg production of layers on experimental diets (eggs produced/hen d).* (Producción de huevos/gallina día con las dietas experimentales).

Period in lay (weeks)	Experimental Diet T_1	T_2	T_3	T_4	SEM
4 – 8	0.658	0.686	0.712	0.670	0.013
8 – 12	0.676^{c}	0.757^{ab}	0.780^{a}	0.722^{bc}	0.014
12 – 16	0.563^{b}	0.642^{a}	0.643^{a}	0.634^{a}	0.012
16 – 20	0.615	0.679	0.700	0.684	0.013
20 – 24	0.646	0.627	0.611	0.641	0.009
24 – 28	0.555^{ab}	0.535^{b}	0.595^{a}	0.603^{a}	0.010
28 – 32	0.571	0.559	0.590	0.608	0.008
Overall Average	0.612^{b}	0.641^{ab}	0.662^{a}	0.652^{a}	0.007

$^{a-c}$All values within rows with the same superscript or no superscript are not significantly different (p>0.05).

4.5) double the weight of the eggs was added. The solution was homogenized in a blender at high speed for 30 sec. The homogenate was thereafter heated in a water bath at 85°C for 5 min, and centrifuged at 4000 rpm for 10 min. The supernatant (1 ml) was thereafter inoculated into wells bored in prepared antibiotic medium 2 agar seeded with *Bacillus subtilis* (ATCC 6633) organism. The inoculated plates were incubated at 37°C overnight. Presence of residues of tetracycline antibiotic in the egg samples were signified by clear zones of bacteria growth inhibition around the inoculated wells. These zones were measured with vernier callipers and the concentrations of the antibiotic residues were determined by extrapolation from the standard curve drawn for the tetracycline antibiotic.

STATISTICAL ANALYSIS

Data collected for performance and egg quality were subjected to statistical analysis using analysis of variance procedure (Minitab, 1989) and means separated using Duncan Multiple Range Test (Duncan 1955), while those for antibiotic residues were determined using T-test (Minitab, 1989).

RESULTS AND DISCUSSION

Table II gives the laying performance of the hens given the different experimental diets. Birds fed experimental diets supplemented with enzyme and antibiotic (T_2-T_4) had higher egg production values than those given the control diet (T_1), however between wk 24 & 28 the lowest production was recorded in the diet containing antibiotic alone (T_2). Significant differences were obtained in weeks 8-12, 12-16 and 24-28. Brufau *et al.* (1994) and Pan *et al.* (1998) have shown that enzyme inclusion improved egg production by utilization of fibrous material. Chesson (2000) also observed that maize and sorghum provide targets for NSP enzymes and has potential to improve nutrient availability. The improved nutrient availability may have been responsible for the improved egg production obtained in these weeks. The overall average production also revealed that diets T_3 and T_4 which had enzyme alone and enzyme/antibiotic inclusion respectively had a significantly higher production than that of the control diet and diet with antibiotic alone. In the past, most enzyme supplements have been used commercially in the presence of dietary antibiotic growth promoters. Antibiotics reduce the intestinal bacterial load, gut thickness and indirectly result in increased alkaline phosphatase levels with beneficial effects on absorption and subsequent improvement in performance of birds (Mandal *et al.,* 2000). The feed conversion ratio values reported in **table III** shows significant differences during all the periods recorded except for the 4-8 week period.. Overall feed conversion values also showed lowest values in diets T_3 and T_4 indicating that supplementation either with enzyme or enzyme/antibiotic resulted in efficient feed utilization. Oloffs *et al.* (1999) have shown improved utilization of starch, fat and protein digestibility in birds fed enzyme supplemented with wheat and barley-based diets. The improved releases of the energy from the diets due to the

Table III. *Feed conversion ratio of layers fed experimental diet (g feed : g egg mass).* (Índice de conversión de pienso de las ponedoras que consumían la dieta experimental (g de pienso:g de huevo).

Period in lay (weeks)	Experimental Diet T_1	T_2	T_3	T_4	SEM
4 – 8	4.71	4.12	3.83	4.15	0.02
8 – 12	4.31^{a}	3.21^{b}	3.18^{b}	3.71^{ab}	0.03
12 – 16	6.45^{a}	4.81^{b}	4.84^{b}	5.18^{b}	0.05
16 – 20	6.08^{a}	4.68^{b}	4.53^{b}	4.49^{b}	0.04
20 – 24	5.50^{a}	5.42^{a}	5.60^{a}	4.88^{b}	0.22
24 – 28	6.95^{a}	7.38^{a}	5.76^{b}	5.64^{b}	0.05
28 – 32	6.15^{a}	6.64^{a}	5.83^{b}	5.35^{b}	0.10
Overall Average	5.69^{a}	5.04^{a}	4.70^{b}	4.74^{b}	0.05

$^{a-b}$All values within rows with the same superscript or no superscript are not significantly different (p>0.05).

breakdown of NSP in the ingredients such as soybeans and corn kernel may be responsible for the improved feed conversion values observed. Mandal *et al.* (2000) in their study with broilers showed that alternating antibiotics and probiotics in the diets of these birds resulted in improved FCR and body weight gains.

The results for body weights of the experimental birds given in **table IV**, showed that the final weight recorded at the end of the study for the birds fed diet T_4 (enzyme/antibiotic supplemented diet) was significantly higher ($p<0.05$) than the weights of the birds given the other 3 experimental diets. The same is also observed in the weight gain values recorded in the table. This shows that the birds placed on the enzyme/antibiotic supplemented diet had improved feed utilization in

Table IV. *Body weights of layers fed experimental diets (kg).* (Peso corporal (kg) de las ponedoras alimentadas con las dietas experimentales).

Body weight	Experimental Diet T_1	T_2	T_3	T_4	SEM
Initial weight	1.35	1.37	1.34	1.36	0.01
Final weight	1.88^{b}	1.80^{c}	1.88^{b}	1.96^{a}	0.05
Weight gain	0.53^{b}	0.43^{c}	0.54	0.60^{a}	0.04

$^{a-c}$All values within rows with no superscript or the same superscript are not significantly different (p>0.05).

comparison with the birds in the other groups.

Among the egg quality parameters shown in **table V**, egg weights were highest in birds given diets T_3 and T_4 and were significantly different ($p<0.05$) from those of diet T_1. This shows that the inclusion of enzymes alone and enzyme/antibiotic did have an effect on egg size. The enzymes may have caused improved utilization of the proteins responsible for egg formation in the birds thereby resulting in the bigger egg size recorded in the study. Haugh units were also highest in the eggs produced from birds on diets T_3 and T_4. Egg shape index was however, uniform in the eggs across all the treatment groups. This shows that the treatments had no effect on the formation of egg shape. The **table V** also shows that the diets given to the birds had no significant effects on the albumen, yolk and shell percentages of the experimental birds. As all the diets had the same basal ingredients, and since no colouring agent was added, the yolk colours recorded were justified. It can also be deduced that the supplementation of the diets with enzymes and antibiotic did not affect the mineralization of the shell of the eggs produced by the birds given the experimental diets.

The antibiotic residue profile of the sampled eggs is given in **table VI**. Residues of tetracycline antibiotic were not detected in the egg samples from the birds given the control diet (T_1) and those from the birds given enzyme supplemented diet (T_3). This can be expected because no antibiotic was administered to these birds. The contrary is however the case for the egg samples from the other two groups (diets T_2 and T_4). This shows that the

Table V. *Overall effect of experimental diets on egg quality parameters.* (Efecto general de las dietas experimentales sobre los parámetros de calidad del huevo).

Parameters	Experimental Diet T_1	T_2	T_3	T_4	SEM
Egg weight (g)	58.28^{c}	59.11^{bc}	60.53^{ab}	61.19^{a}	0.33
Egg mass (g/day)	35.67^{c}	37.89^{b}	40.07^{a}	39.90^{a}	0.11
Egg shape index	0.76	0.76	0.76	0.76	0.004
Haugh units	62.38^{b}	58.00^{b}	67.22^{ab}	75.46^{a}	1.77
Albumen (percent)	65.84	66.09	66.08	66.69	0.23
Yolk (percent)	24.27	24.38	24.18	23.61	0.18
Shell (percent)	9.89	9.53	9.74	9.70	0.17
Yolk colour	1.32	1.42	1.42	1.15	0.05
Shell thickness (mm)	0.48	0.46	0.47	0.46	0.01
Blood spots	0.93	0.80	0.95	1.11	0.10

$^{a-c}$All values within rows with the same superscript or no superscript are not significantly different ($p>0.05$).

Table VI. *Mean concentration of residues of tetracycline antibiotic in eggs (μg).* (Concentraciones medias (μg) del antibiótico tetraciclina en los huevos).

Week	Experimental Diet			
	T_1	T_2	T_3	T_4
1	ND	0.017	ND	ND*
2	ND	0.008	ND	0.007**
3	ND	0.013	ND	0.005*
4	ND	0.014	ND	0.005*
5	ND	0.003	ND	ND
6	ND	0.010	ND	0.008**
7	ND	0.006	ND	0.003*
8	ND	0.012	ND	ND

ND: Tetracycline antibiotic not detected in the samples.
*enzyme supplementation.
**antibiotic supplementation.

tetracycline antibiotic given to the birds is continually released in the eggs, while the metabolism of the drug continued in the body of the layer. The birds given antibiotic supplemented diet (T_2) produced eggs with tetracycline residue levels of between 0.003 μg/g and 0.017 μg/g. Residues of the tetracycline antibiotic was recorded in the eggs of these birds throughout the experimental period, since the antibiotic was fed continuously to the bird. The concentration of the tetracycline residues recorded in the egg samples from birds fed enzyme/antibiotic supplemented diet was between 0.003 μg/g and 0.008 μg/g, there are however no significant differences ($p>0.05$) in the concentration of the residues in the egg samples. The result showed that residues of tetracycline were not detected in the eggs during the first week of the study, when the birds were fed enzymes only. Residues of the antibiotic started appearing in the egg samples from the second week when the birds in this group were fed tetracycline-supplemented diet. By the third week when enzyme was again fed to the birds, residues of tetracycline antibiotic still appeared in the egg samples. This shows that tetracycline antibiotic will continue to appear in the egg of a laying bird even after one week of medication.

The Codex's recommended maximum residue level (MRL) for tetracycline antibiotic in eggs is 0.02 μg/g (Crosby, 1991). The concentration levels of between 0.003 μg/g and 0.017 μg/g obtained in this study are lower than this recommendation. However, in view of the health risks associated with residues of antibiotics in human food, the zero residue level obtained in Japan (Cronin, 1998) is the most desirable. While adherence to withdrawal periods of antibiotics might prevent residue deposition in other food products, this may not be feasible in laying birds. This is because it will be difficult for the poultry producer (especially large scale producers) to dispose of eggs during medication. The use of antibiotics as feed additive in laying birds should therefore be discouraged in Nigeria as in most developed countries.

The study showed that the addition of antibiotic to layer diets on a weekly alternate basis had the best results in terms of egg production, feed conversion, body weight and egg quality. This is in agreement with Mandal *et al.* (2000). This could be due to the effect of the antibiotics in reducing gut thickness and consequently improving the absorption of nutrients after enzyme inclusion. Since the inclusion of enzymes also gave similar results, use of enzymes as feed additive over antibiotics is thus desirable, this is in view of the antibiotic residue deposition observed with its attendant health hazards to the consumers.

In conclusion, the use of antibiotics in laying birds for the purpose of growth promotion should be discouraged this can be replaced with enzymes.

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