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Effect of Enzymes Supplementation (*Rovabio* and *Kemin*) on some Blood Biochemical Parameters, Performance and Carcass Characterizes in Broiler Chickens

Research Article

S. Goli[1] and H. Aghdam Shahryar[1*]

[1] Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

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*Correspondence E-mail: ha_shahryar@iaushab.ac.ir

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ABSTRACT

An experiment was conducted to evaluate the efficacy of exogenous enzymes supplementation (ES) on performance, carcass traits and some blood parameters of broilers chickens. One hundred and eighty day old chicks with 40 ± 2 g initial body weight in a completely randomized design with 3 treatments and 4 replications were fed experimental diets included, 1) basal diet no enzymes (control), 2) basal diet + Rovabio® multi enzymes and 3) basal diet + Kemin® multi enzyme. The diets of all groups were formulated in an isonitrogeous and isoenergetic diet based on corn and soybean. Data on feed consumption, weight gain, feed conversion ratio, carcass characters, high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol and triglyceride levels were collected for 3 periods starter (1-21 d), grower (22-42 d), finisher (43-49 d) and total (1-49 d). Results showed significant differences ($P<0.05$) among different experimental diets for weight gain, feed intake and feed conversion ratio. The lowest feed consumption and weight gain were observed in control group while the highest were obtained in groups that fed with multi enzyme supplementation. Also, poor feed conversion ratio (FCR) was observed in control group and best FCR (lowest) were in groups that were fed multi enzymes. In carcass traits no differences ($P>0.05$) between groups were observed. Adding enzyme significantly increased the concentration of blood HDL and decreased triglyceride, cholesterol and LDL at 21, 42 and 49 d of age ($P<0.05$). Results from this experiment suggest that multi enzyme supplementation can improve broiler performance.

KEY WORDS biochemical parameters, broiler, multi enzymes, performance.

INTRODUCTION

Use of non starch polysaccharides (NSP) degrading enzymes in poultry diets has increased considerably in recent years. However, the effects of exogenous enzymes can be variable and are dependent on a large number of factors such as the age of the bird and the quality and type of diet (Bedford, 2000). Neither corn nor soybean meal is regarded as viscous feedstuffs even though they do contain appreciable amounts of NSP's. Corn contains approximately 0.9% soluble NSP and 6% insoluble NSP, whereas soybean meal contains approximately 6% soluble NSP and 18-21% insoluble NSP (Bach Knudsen, 1997). Noy and Sklan (1994) reported that ileal digestibility of corn starch rarely exceeds 85% in broilers between 4 and 21 d of age, indicating opportunities to further improve the digestibility of resistant starch in the jejunum and ileum through amylase supplementation. Furthermore, proteases could potentially degrade such soybean proteins as glycinin and conglycinin and some anti-nutritional factors (lectin and trypsin-inhibitor) in inadequately processed soybean meal (Thrope and Beal, 2001). Legume NSP are much more complex in structure than those present in cereals and therefore, the use of "classical" NSP-degrading enzyme products tends to provide limited and inconsistent responses (Broz and Ward, 2007). Wheat is an important source of energy in broiler diets because of its high starch (ST) and crude protein (CP) content and is often the only cereal in grower and finisher diets (Gutierrez Del Alamo *et al.* 2008). In a survey of 18 wheat cultivars, Kim *et al.* (2003) reported that ST content ranged between 58.5 and 73.7%, CP between 9.7 and 19.1%, NSP between 7.8% and 11% (on dry matter basis). When added to relevant poultry diets, NSP-degrading enzymes usually result in numerous beneficial effects, such as increased utilization of nutrients (e.g., fat and protein), improved apparent metabolisable energy (AME) values, increased growth rate, improved feed to gain ratio, decreased viscosity of intestinal digesta, reduced incidence of sticky excreta, improved litter conditions and reduced environmental pollution due to a decreased output of manure and gases such as ammonia (Guilherme Perazzo Costa *et al.* 2008). Rovabio excel is a stabilized multi-enzyme combination, naturally produced by the non-genetically modified fungus *Penicillium funicullosum*. It contains xylanases, ß-glucanases and cellulases. Similarly, hamecozyme-II is also multi-enzyme product (Hameco Agro B.V.Z wolle, Holand), which contains protease, amylase, xylanase, ß-glucanase and cellulose (Khan *et al.* 2006). This study was conducted to determine the effect of supplementation (Rovabio® and Kemin®) on some blood biochemical parameters, performance and carcass characteristics in broiler chickens.

MATERIALS AND METHODS

Animals and diets

In this study, 180 one day-old Ross chicks with 40 ± 2 g initial body weight were used. The chicks were divided into 3 groups and 4 replications, 15 chicks in each. Each group was housed separately in individual cages. The chicks were fed standard starter (from 1 to 21 days), grower (from 22 to 42 days) and finisher (from 43 to 49 days) diets. Groups were randomly assigned to following treatment groups, 1) basal diet-no enzymes (control), 2) basal diet + Rovabio ® multi enzymes, included (xylanase 2200 u/g, ß-glucanase 200 u/g, cellolase 100 u/g and pectinase 100 u/g) and 3) basal diet + Kemin® multi enzyme, included (xylanase 1800 u/g, ß-glucanase 2600 u/g and cellolase 500 u/g). The experimental diets were formulated to meet NRC (1994), nutrient requirements for broiler, in particular the recommendations for Ross 308 strain.

Experimental procedure

Each experimental group was fed *ad-libitum* with its own diet for 49 d. Feed intake and gain weight was determined in each period. At the end of the study period (day 49), 3 broilers were randomly selected from each replicate and were slaughtered determine to carcass characters. Also, chicken was collected to blood measure serum biochemical parameters with biochemical kit (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran). Experimental procedure was in according to recommendations of Islamic Azad university-veterinary department animal ethics committee.

Statistical analysis

The data obtained from the experiment analyzed by SAS (2001) with a general linear models procedure for ANOVA. Differences between means were analyzed with Duncan's multiple tests. The significant difference statements were based on the possibility (P<0.05).

RESULTS AND DISCUSSION

Blood parameters

Present study showed that adding Rovabio and Kemin multi enzyme to broilers diet significantly increased the concentration of blood total HDL at d 21, 42 and 49 d (P<0.05). The concentration of blood LDL, cholesterol and triglyceride was decreased by enzyme addition at 21, 42 and 49 d (P<0.05). Studies with animal models have shown that high level of dietary cholesterol, saturated fatty acids and an increased small intestinal uptake of these components due to, for example, a low dietary fiber concentration or enzyme supplementation of the diet may increase plasma cholesterol levels. LDL and HDL-cholesterol is formed when cholesterol and fats get together in circulatory system. With changing the physico-chemical properties of intestinal chyme due to the presence of soluble NSPs in wheat and the known interaction effects of them with saturated fatty acids (Kussaibati *et al.* 1982) and the effect of NSP-degrading enzymes might explain some of these results. Adding enzyme may alleviate the limitations present for the function of bile salts and the emulsifying properties of them in intestinal chyme and therefore it might be a reason for increasing total fat in blood. It is reported that the digestion of big molecules of carbohydrates with pentosanase (xylanase) can change the viscouse nature of intestinal chyme and therefore improves fat digestibility (Van Der Klis *et al.* 1995).

Performance parameters

The effects of enzyme supplementation on broiler performance at 21, 42 and 49 d are shown in Table 2. Body weight gain (BWG), feed conversion ratio (FCR), relative growth significantly improved (P<0.05) by enzyme supplementation. Among different experimental diets for weight gain, feed intake and feed conversion ratio were significant (P<0.05).

Table 1 Composition experimental diets in different periods of the experiment

Ingredients (%)	Starter (1-21 d)			Grower (22-42 d)			Finisher (43-49 d)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Corn	57.84	57.84	57.84	58.69	58.69	58.69	61.69	61.69	61.69
Soybean meal (44% CP)	36.65	36.65	36.65	35.30	35.30	35.30	31.48	31.48	31.48
Fat	1.60	1.60	1.60	2.20	2.20	2.20	3.17	3.17	3.17
DCP	1.56	1.56	1.56	1.30	1.30	1.30	1.15	1.15	1.15
$CaCO_3$	1.26	1.26	1.26	1.24	1.24	1.24	1.09	1.09	1.09
Salt	0.20	0.20	0.20	0.25	0.25	0.25	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.25	0.25	0.25	0.30	0.30	0.30
Lysine	0.04	0.04	0.04	0.07	0.07	0.07	0.07	0.07	0.07
Mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium bi carbonate	0.10	0.10	0.10	0.15	0.15	0.15	0.20	0.20	0.20
Enzyme supplementaion[3]	-	0.05	0.05	-	0.05	0.05	-	0.05	0.05
Sand	0.05	-	-	0.05	-	-	0.05	-	-
Analysis results									
Metabolizable energy (kcal kg^{-1})	2900	2900	2900	2950	2950	2950	3050	3050	3050
Crude protein (%)	21	21	21	20.50	20.50	20.50	19	19	19
Calcium (%)	0.94	0.94	0.94	0.87	0.87	0.87	0.78	0.78	0.78
Methionine (%)	0.52	0.52	0.52	0.48	0.48	0.48	0.50	0.50	0.50
Lysine (%)	1.30	1.30	1.30	1.10	1.10	1.10	1.10	1.10	1.10

[1] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate): 6050 µg; vitamin D_3: 55 µg; vitamin E (α-tocopheryl acetate): 22.05 µg; vitamin K_3: 2 mg; vitamin B_1: 5 mg; vitamin B_2: 6 mg; vitamin B_3: 60 mg; vitamin B_6: 4 mg; vitamin B_{12}: 0.02 mg; Pantothenic acid: 10.0 mg; Folic acid: 6 mg; Biotin: 0.15 mg and Ethoxyquin: 0.625 mg.

[2] Supplied per kilogram of diet: CaCO: 500 mg; Fe: 80 mg; Zn: 80 mg; Mn: 80 mg; Cu: 10 mg; I: 0.8 mg and Se: 0.3 mg.

T1: control group; T2: includes Rovabio (xylanase 2200 u/g, ß-glucanase 200 u/g, cellolase 100 u/g and pectinase 100 u/g) and T3: includes Kemin (xylanase 1800 u/g, ß-glucanase 2600 u/g and cellolase 500 u/g).

Table 2 Effects of enzyme supplementation on some of biochemical blood characters in broiler chickens (mg/dL)

Items	T1	T2	T3	SEM	P-value
21 d					
HDL	54.42^{b}	78.22^{a}	78.02^{a}	3.53	< 0.0001
LDL	34.95^{a}	21.17^{b}	21.42^{b}	1.97	< 0.0001
Triglyceride	118.20^{a}	99.90^{b}	98.30^{b}	2.98	< 0.0001
Cholesterol	88.23	94.6	90.68	7.76	0.954
42 d					
HDL	62.65^{b}	80.85^{a}	80.22^{a}	2.60	< 0.0001
LDL	36.07^{a}	24.65^{b}	24.82^{b}	1.63	< 0.0001
Triglyceride	126.25^{a}	103.01^{b}	99.35^{b}	3.88	0.0002
Cholesterol	129.42^{a}	98.70^{b}	90.32^{c}	5.19	< 0.0001
49 d					
HDL	66.75^{b}	84.22^{a}	82.55^{a}	2.78	0.002
LDL	37.02^{a}	23.57^{b}	24.29^{b}	2.04	0.0008
Triglyceride	149.7^{a}	117.72^{b}	115.2^{b}	4.94	< 0.0001
Cholesterol	139.32^{a}	106.8^{b}	105.07^{b}	5.06	< 0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05) and (P>0.01).

T1: control group; T2: includes Rovabio (xylanase 2200 u/g, ß-glucanase 200 u/g, cellolase 100 u/g and pectinase100 u/g) and T3: includes Kemin (xylanase 1800 u/g, ß-glucanase 2600 u/g and cellolase 500 u/g).

HDL: high density lipoprotein and LDL: low density lipoprotein.

SEM: standard error of the means.

Table 3 Effects of enzyme supplementation on performance in broiler chickens from 1-49 days (g)

Trait		Treatment			
		T1	T2	T3	SEM
FI	Starter	890.25	892.75	849.25	10.49
	Grower	1352.70^{b}	1443.10^{a}	1469.50^{a}	18.93
	Finisher	1839.70^{b}	2018.90^{a}	2019.20^{a}	26.86
	Total	4082.70^{b}	4354.70^{a}	4338.00^{a}	40.13
WG	Starter	510.00^{c}	574.50^{a}	544.00^{b}	9.22
	Grower	651.20^{b}	822.50^{a}	775.20^{a}	23.75
	Finisher	829.70^{b}	1097.30^{a}	1073.20^{a}	38.24
	Total	1991.00^{c}	2494.30^{a}	2392.50^{b}	66.81
FCR	Starter	1.74^{a}	1.55^{b}	1.57^{b}	0.03
	Grower	2.07^{a}	1.71^{c}	1.86^{b}	0.05
	Finisher	2.21^{a}	1.84^{b}	1.83^{b}	0.06
	Total	2.05^{a}	1.75^{b}	1.81^{b}	0.04

The means within the same row with at least one common letter, do not have significant difference (P>0.05) and (P>0.01).

T1: control group; T2: includes Rovabio (xylanase 2200 u/g, ß-glucanase 200 u/g, cellolase 100 u/g and pectinase100 u/g) and T3: includes Kemin (xylanase 1800 u/g, ß-glucanase 2600 u/g and cellolase 500 u/g).

SEM: standard error of the means.

The poorest feed consumption and weight gain were observed in control group while the best were obtained in groups that fed with multi enzyme supplementation (P<0.05). Also the highest FCR was observed in control group and best FCR (lowest) were in group that fed multi enzymes (P<0.05).

The need for exogenous enzyme supplements in corn-soybean meal diets is generally ignored. However, some studies reported a positive growth performance response in corn-based diets supplemented with enzymes, either multiple enzymes which contained xylanase, protease and amylase or a single protease enzyme (Ghazi *et al.* 2002; Yu *et al.* 2007).

The results of the present study substantiated the findings of Gracia *et al.* (2003) and Lazaro *et al.* (2003), who reported that fungal enzyme preparation significantly, improved the weight gain of birds fed on corn, rye, wheat and barley based diets. In a review article dealing with the use of Rovabio® Excel in corn-soybean based diets, the use of Rovabio® Excel resulted in an increase in body weight and egg mass of 1.6, 1.6, 3.9 and 3.7% respectively for an unspecified number of broilers, laying hens, turkeys and ducks, with a reduction of 2.5, 2.0 and 1.6% in feed conversion (Dalibard *et al.* 2004). Responses to enzyme supplementation depend on the bird's age, which is apparently related to both the type of gut microflora present and the physiology of the bird. In old birds, due to enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal with the effects of high viscosity (Vukic-Vranjes and Wenk, 1995).

Table 4 Effects of enzyme supplementations on carcass yield in broiler (at 49 day)

Trait	Treatment			
	T1	T2	T3	SEM
Carcass (% LW)	60.18	63.12	62.91	0.48
Breast (% CW)	35.60	38.62	3879	0.64
Legs (% CW)	29.82	31.35	30.07	0.55
Digestive tract (% CW)	8.12	9.24	8.89	0.16
Liver (% CW)	2.10	2.39	2.41	0.04
small intestin length (cm)	188	196.70	198.30	3.61
Cecum length (cm)	19.20	20.30	18.70	0.34

LW: live weight and CW: carcass weight.
The means within the same row with at least one common letter, do not have significant difference (P>0.05) and (P>0.01).
T1: control group; T2: includes Rovabio (xylanase 2200 u/g, ß-glucanase 200 u/g, cellolase 100 u/g and pectinase 100 u/g) and T3: includes Kemin (xylanase 1800 u/g, ß-glucanase 2600 u/g and cellolase 500 u/g).
SEM: standard error of the means.

Enzyme supplementation might improve broiler performance by at least two mechanisms: increasing feed intake and improving nutrient digestibility. Both mechanisms might be induced, at least partially, by a reduction of the viscosity's reduced viscosity decreases retention time of digesta in the gut, allowing more consumption and therefore improving growth and feed conversion ratio (Lazaro *et al.* 2003).

Carcass characterizes

Breast weights were significantly increased by the enzyme addition (P<0.05) Table 3. Selle *et al.* (2003) found that supplementation of wheat based diets with xylanase plus phytase increased breast weight by 5.8%.

Differences were found in the liver weight between enzyme supplementation groups when compared with the control group (P<0.05). Treatment 3 (basal diet + Kemin® multi enzyme) in compared other experimental diets significantly increased for digestive tract weight (P<0.05). Café *et al.* (2002) reported that addition of a commercial multi enzyme to corn-soybean meal-based diets did not improve dressing percentage, yield of breast, thigh and wing component, but resulted in a significant increase in abdominal fat.

Lee *et al.* (2010) reported that no significant differences in the relative weights of the liver, abdominal fat, right leg or right breast muscle among treatment groups. No Significant differences were found between enzyme supplementation treatments for small intestine length and cecum length when compared with the control group. Wu *et al.* (2004) suggested that individual addition of phytase and xylanase led to a significant reduction in the relative length and weight of the small intestine.

In general, enzyme supplementation decreased the relative size of the digestive organs and increased carcass yield. This result is in agreements with findings of Wang *et al.* (2005).

CONCLUSION

Results from this experiment suggest that Rovabio and Kemin enzymes supplementation can improve broiler performance.

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