



EFFECT OF ENZYME ADDITION TO WHEAT, BARLEY BASED DIETS ON PHYSIOLOGICAL AND PRODUCTION PERFORMANCE OF BROILER

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Abstract

This experiment was conducted in the poultry farm of veterinary medicine college, University of Kufa. The purpose of this study was evaluated the effect of Rovabio multienzyme. With five levels (0.125,0.250,0.375,0.500%) on the productivity and physiological characteristics of the 300 bird (Ross-308). With 40 ± 2 g initial body weight at day one. A completely randomized design with 5 treatments and 3 replications were fed experimental diets included, (1) basal diet no enzymes (control), (2) basal diet + 1.25 kg/ton enzymes, (3). Basal diet +2.5 kg/ton enzyme, (4) basal diet + 3.75 kg/ton enzyme, (T5) basal diet+5 kg/ton enzyme. The diets of all groups were formulated in an isonitrogenous and isoenergetic diet based on wheat/barley. Data on feed consumption, weight gain, feed conversion ratio, were collected for 3 periods starter (1-21d), finisher (22-42 d), and total (1-42 d). Carcass characters. Biochemical & hematocrit parameters were collected for end total period (42d). 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 increased the concentration of blood HDL and decreased triglyceride, cholesterol and VLDL at 42 d of age. Results from this experiment suggest that multi enzyme supplementation can improve broiler performance.

Key words : Enzymes, wheat, physiological and broiler.

Introduction

In order to use the high genetic potential of modern broiler strains, nutrient requirements, and nutritional management of poultry have changed (Ravindran, 2013). Feed represents the most significant component of poultry production costs. Poultry diets are primarily based on corn and soybean meal. The continuous rise of corn and soybean prices worldwide generated a growing use of alternative cereals in broiler diets, such as wheat and barley (Alkassar, 2017). However, the cell wall of these cereals are rich in insoluble and soluble polysaccharides other than starch. These complex non-starch polysaccharides (NSP) are a heterogeneous group of compounds that include cellulose, pectins, β -glucans, pentosans, heteroxylans and xyloglucan, which cannot be hydrolyzed by the endogenous digestive enzymes of

humans and monogastric animals (Kumar *et al.*, 2012). Soluble NSP increase digest viscosity due to their high capacity to absorb water and gelatinize the intestinal tract contents of birds (Choct *et al.*, 2010). This phenomenon disturbs digestion with consequences for feed energy, feed efficiency (Choct, 2006) and broiler performance (Chandra Shekhar *et al.*, 2014). Hence, cereal grains high in non-starch polysaccharides (NSP) such as wheat (cellulose and therefore largely insoluble) and barley (largely soluble) exhibit poor nutritional value (low apparent metabolizable energy, AME). Along with this, high dietary levels of NSP lead to high nitrogen and phosphorus excretion, which is a major environmental concern in densely populated regions. Wheat and corn are rich in arabinoxylan, whereas barley has high levels of β -glucan (Knudsen, 2014). The anti-nutritional

Table 1 : Basal diets for all treatments.

Stages	starter					finisher				
Periods	0-3 weeks					4-6 weeks				
Ingredients	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Wheat	30.0	30.0	30.0	30.0	30.0	36.2	38.37	38.25	38.13	38.0
Barley	29.5	29.37	29.25	29.13	29.0	29.5	29.37	29.25	29.13	29.0
Soybean meal	25.0	30.0	30.00	30.00	30.0	17.0	17.0	17.0	17.0	17.0
Protein conc.*	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Corn oil	3.8	3.0	3.00	3.0	3.0	5.6	5.6	5.6	5.6	5.6
Di-Calcium Phosphat	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Limestone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Rovabio Enzyme	—	0.13	0.25	0.37	0.5	—	0.13	0.25	0.37	0.5
Total	100	100	100	100	100	100	100	100	100	100
Chemical analysis%										
ME Kcal /kg ****	2916	2916	2916	2916	2916	3100	3100	3100	3100	3100
Crude protein	23.4	23.4	23.4	23.4	23.4	20.4	20.4	20.4	20.4	20.4
Crude fiber	5.1	5.1	5.1	5.1	5.1	4.8	4.8	4.8	4.8	4.8
Lysine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Meth.+cys	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Total Calcium	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
A. Phosphorous	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.40
C:P ratio	124.6	124.6	124.6	124.6	124.6	152.0	152.0	152.0	152.0	152.0

*WAFI(Holland, Company) Contain per kg: ME 2150Kcal/kg, CP 40%, Fat 5%, CF 5%, Ca 5.6%, A phosphorous 2.65%, Methionine 3.70%, Meth.+cys 4.10%, Lys 3.85%, Tryp 0.4%, Thr 1.29%, Vitamin A 220000 iu, Vitamin D3 60000 iu, Vitamin E 60mg, Vitamin B1 60mg, Vitamin B2 140mg, Vitamin B6 80mg, Vitamin B12 700mg, Vitamin K3 50mg, Niacin 800mg, Biotin 2000mg, Pantothenic acid 320mg, Copper 200mg, Iron 1000mg, Manganese 1600mg, Zinc 1200mg, Iodine 20mg.

**Diets contains according to NRC(1994).

properties expressed by NSPs, such as pentosans (arabinoxylans) and β -glucans can be reduced by including NSP degrading enzymes (xylanase-glucanase and cellulase) in broiler diets. The NSP degrading enzymes (NSPases) are known to increase the digestibility of raw materials for monogastrics and a combination of NSPases there by increasing the use of nutrients and the energy available for growth (Gemma *et al.*, 2016) and production (Lazaro *et al.*, 2003). Therefore, the objective of this study was to evaluate the effect of different levels (0, 0.125, 0.250, 0.375 and 0.05%) of commercial enzyme mixtures (Rovabio®, glucanase, cellulase, xylanase,

Adisseo France S.A.S., Antony, France) on performance, blood constituents and carcass characteristics of broiler chicks fed wheat-barley diets.

Materials and Methods

Housing and management

This experiment was conducted during August of 2017 and January 2018 in the poultry farm of veterinary medicine college-Kufa University. In this study, 300 one day-old unsexed Ross chicks with 40 ± 2 g initial body weight were used. The chicks were divided into 5 groups and 3 replications, 20 chicks in each. Each group was housed separately in pens. The chicks were fed standard starter rations (from 1 to 21 days), and finisher (from 22 to 42 days). The ingredients and nutritional composition of the diets used are presented in table 1. These diets were formulated to be iso energetic and iso nitrogenous according to NRC (1994), nutrient requirements for broiler, in particular the recommendations for Ross 308 strain. The five basal diets (wheat/barley-based). Groups were randomly assigned to following treatment groups (T1) basal diet-no enzymes (control), (T2) basal diet +1.25kg/Ton Rovabio® multi enzymes, included (xylanase 1800,000 mg/kg, β -glucanase 2200,000 mg/kg, cellulase 1500mg/kg); (T3) basal diet + 2.5kg/Ton enzymes; (T4) basal diet +3.75kg/Ton enzymes; (T5) basal diet +5.0kg/Ton enzymes. Birds were housed in an environmentally controlled system and growing conditions were similar in all treatments. Birds were vaccinated against (Newcastle disease Lassota strain +Influenza-Vatro Company, Italia at 3d age. and infectious bronchitis virus (IBH120) Isovac Company, Italia. Newcastle disease and bronchitis virus strain H120 was provided at d 11. Gumboro virus was provided at d 14. Vaccines were prepared per vendor recommendation and were supplied via drinking water after a period of water removal for 3 h. The exogenous enzymes mixtures

used were the commercial multi-enzyme preparations, Rovabio® (Adisseo France S.A.S., Antony, France). The Rovabio® mixture includes endo-1, 3(4)- β -glucanase (2200,000 mg/kg) and endo-1, 4- β -xylanase (1800,000 mg/kg) and cellulase (1500mg/kg) produced by a non-genetically modified strain of *Penicillium funiculosum* Pf 8/403 (International Mycological Institute, under the number IMI 378536).

Experimental procedure

Each experimental group was fed *ad-libitum* with its own diet for 42 d. Feed intake, gain weight and feed

conversion ratio was determined in each period weekly. The study was conducted according to the International Guidelines for research involving animals (Directive 2010/63/EU), specially slaughtering birds according to the Islamic procedures.

Performance traits

Feed intake (FI, g/bird/period) and body weight gain (BWG, g/bird/period) were recorded for the period at the beginning of the experiment (1d) until the end of the starter period 21th d of age, and finisher period 22th-42nd d of age (Alkassar, 2012). Feed conversion ratio-(FCR) was calculated by dividing feed intake by body weight gain (Alkassar, 2010). On the final day of the experiment, at 42d-of-age, two bird from each replicate (six from each treatment) was randomly selected slaughtered and dissected manually, plucked and eviscerated. The viscera were removed as for the usual dressing of poultry carcasses. Heart, liver (minus the gall bladder), empty skinned gizzard were trimmed of extraneous tissue and weighed individually and their sum of weights 'giblets' was taken. The dressed weights obtained were expressed as a percentage of the live weights and yield parts expressed as a percentage of dress carcass weight.

Sampling and blood parameters

At the end of the experiment, 42d-of-age, 2 bird/replicate, totaling 6 birds/treatment, was randomly selected for blood collection. Blood samples (1.5 mL/bird) were collected from the wing vein using a 2 mL syringe and left to stand at 30°C to allow clotting and clot retraction. The serum that remained after clotting was centrifuged at 3000 rpm for 10 min at room temperature. Samples were chilled until analyzes. Blood parameters analyzed in serum were glucose (GLU), total triglycerides (TG), total cholesterol (TC), very low density lipoprotein (VLDL), high density lipoprotein (HDL), total protein (TP), albumin (Alb), uric acid (UAc) and alkaline phosphatase (ALP). The concentrations for these parameters were determined by routine methods using commercial laboratory kits (BIOLABO Co., France), according to the manufacturer's instructions.

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 ($P < 0.05$). by using the following model.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,

Y_{ij} : is the value of observation of traits.

μ : is the overall mean of traits.

T_i : The effect of treatments, control (T_1), (T_2), (T_3), (T_4) and (T_5) in experiment.

e_{ij} : Random error assumed to be mean equal to zero and variance is σ^2e ($N \sim 0, \sigma^2e$).

Results and Discussion

Performance parameters

The effects of enzyme supplementation on broiler performance at 21, 42 and 49 d are shown in table 2. Feed consumption, body weight gain (BWG), feed conversion ratio (FCR), significantly improved ($P < 0.05$) by enzyme supplementation. Among different experimental diets for weight gain, feed intake and feed conversion ratio were significant ($P < 0.05$) as showing in table 2. 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 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 enzyme in corn-soybean based diets, the use of Rovabio enzyme resulted in an increase in body weight 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 micro flora 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). Generally, there are significantly improvement ($P < 0.05$) in weight gain and feed consumption and feed conversion ratio increasing linear with supplemented enzyme in all different ages among birds, in starter, finisher and total period. 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). The mortality

Table 2 : Means of some productive traits of broiler at (0-3), (4-6) and (0-6) weeks of age.

Traits	Treat.				
	T1 Control without enzymes	T2 1.25kg Enzy./Ton	T3 2.5kg Enzy./Ton	T4 3.75kg Enzy./Ton	T5 5 kg Enzy./Ton
Initial BWg/bird (1 d)	40	40	40	40	40
Weight gain (0-3wk)	c**593.0±9.11	c610.0±9.01	b655.0±9.31	a715.0±9.73	a740.0±9.86
Weight gain (4-6wk)	c1767.0±18.0	c1808.0±21.1	b1934.0±23.2	b1956.0±23.7	a2008.0±24.4
Weight gain (0-6wk)	e2320.0±27.1	d2378.0±27.4	c2549.0±29.3	b2631.0±29.7	a2708.0±31.0
Feed Cons.g/bird (0-3wk)	c723.5±6.1	c719.8±5.8	c766.3±6.0	b829.4±6.4	a910.2±6.9
Feed Cons.g/bird (4-6 wk)	b3475.7±28.2	b3513.8±25.3	a3694.4±26.2	a3748.5±25.1	a3693.5±27.0
Feed Cons.g/bird (0-6 wk)	c4199.2±27.8	c4232.8±25.5	b4460.7±25.8	a4577.9±25.7	a4603.7±25.3
Feed Conv.ratio (0-3wk)	a1.22±0.04	b1.18±0.01	b1.17±0.01	b1.16±0.01	a1.23±0.05
Feed Conv.ratio (4-6wk)	a1.96±0.02	a1.94±0.02	a1.91±0.02	a1.91±0.02	b1.83±0.01
Feed Conv.ratio (0-6wk)	a1.81±0.06	a1.78±0.06	ab1.75±0.05	ab1.74±0.04	b1.70±0.04
Mortality%	0%	0%	0%	0%	0%
Significant	*	*	*	*	*

*means significant differences ($P \leq 0.05$) among treatments.

**means the same letters in every row indicated no significant differences and the different letters mean there were significant differences.

Table 3 : Dressing percentage without edibles and edibles weight for all treatments.

Traits	Treatments				
	T1	T2	T3	T4	T5
Final bodyweight(g)/bird	2360.0	2418.0	2589.0	2671.0	2748.0
Dressing percentage without edibles	74.4	74.8	75.1	95.4	75.8
Clean carcass weight(g) without edibles	1755.8	1808.6	1944.3	2013.9	2110.2
Heart weight (g)	9.2	9.3	11.0	11.5	13.0
Liver weight (g)	50.0	52.0	55.0	57.0	60.6
Gizzard weight (g)	40.0	38.0	37.0	36.0	33.0
Significant	NS	NS	NS	NS	NS

NS:means Non Significant differences among treatments.

Table 4 : Carcass cuts percentage of birds slaughtered at 6 weeks of age.

Traits	Percentage of carcass cuts%				
	T1	T2	T3	T4	T5
Breast	31.2	31.4	31.7	31/2	32/2
Thighs	27.6	27.8	27.5	28.3	28.5
Back	19.5	19.1	18.4	17.9	17.0
Neck	9.6	9.8	10.0	9.4	9.8
Wings	12.0	11.8	12.3	12.5	12.5
Significant	NS	NS	NS	NS	NS

NS:means Non Significant differences among treatments.

percent 0% in all treatments, this result due to good management and care, balance diets, optimal temperature in house of chickens.

Carcass characterizes

Table 3 shows the statistical analysis for carcass traits, so there is no significant differences between all treatments in dressing percentage, carcass cuts percentage, edibles weight (heart, liver, gizzard). This result didn't agree with Selle *et al.* (2003), who found that supplementation of wheat based diets with xylanase plus phytase increased breast weight by 5.8%. While our study agree with 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. Also, 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 when compared with the control group. In general, enzyme supplementation decreased the relative size of the digestive organs and increased carcass yield.

Blood parameters

Generally, there is no effect of added enzyme on biochemical and haematocrit pictures among all treatments. Tables 5, 6 in present study showed that adding Rovabio multi enzyme to broilers diet did not significantly increased the concentration of blood total, HDL at 42 d. The concentration of blood VLDL,

Table 5 : Biochemical parameters in blood serum.

Parameters	Treatments				
	T1	T2	T3	T4	T5
Glucose (dL ⁻¹)	151.0	165.0	181.0	151.0	189.0
Cholesterol (dL ⁻¹)	118.0	105.0	98.0	96.0	94.0
Triglyceride (dL ⁻¹)	142.0	135.0	131.0	128.0	126.0
HDL (mg dL ⁻¹)	71.2	75.3	80.6	83.4	86.7
VLDL (mg dL ⁻¹)	29.0	28.0	28.0	27.0	25.0
Uric acid (gdL ⁻¹)	2.5	2.6	2.7	3.0	3.0
Totalprotein (gdL ⁻¹)	3.0	3.1	3.1	3.0	3.0
Albumin (gdL ⁻¹)	0.70	0.68	0.68	0.70	0.70
Globulin (gdL ⁻¹)	2.30	2.42	2.42	2.30	2.30
ALPase (UdL ⁻¹)	277.0	273.0	270.0	263.0	261.0
Significant	NS	NS	NS	NS	NS

NS : Mean No significant differences among treatments.

Table 6 : The hematocrit parameters in blood for all treatments.

Traits	Treatments				
	T1 Control	T2	T3	T4	T5
Hb gdL ⁻¹	9.95	10.13	10.88	9.95	11.20
PCV(%)	32.0	32.33	35.0	33.10	34.40
RBCX (10) ⁶ μL	2.87	3.01	3.20	3.09	3.30
WBCX (10) ³ μL	22.4	22.6	22.9	22.70	23.0
H/L ratio	0.36	0.34	0.31	0.32	0.29
Calcium mgd L ⁻¹	5.26	6.08	5.34	6.76	7.45
Phosphorus mg dL ⁻¹	2.90	3.10	3.50	3.30	3.60
Iron μg dL ⁻¹	143.3	146.1	149.2	147.30	151.1
Significant	NS	NS	NS	NS	NS

NS:Mean No significant differences among treatments

cholesterol and triglyceride was decreased by enzyme addition at 42 d mathematically. Studies with animal models have shown that high level of dietary cholesterol, saturated fatty acids 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 barley 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 viscous nature of intestinal chyme and therefore improves fat digestibility (Van Der Klis *et al.*, 1995).

Conclusion and Recommendation

Results from this experiment suggest that Rovabio enzymes supplementation can improve broiler performance, increasing of available metabolisable energy and improvements digestibility and absorption nutrients which contained high levels of fiber (barley and wheat). Increase in the range of feedstuffs that can be used and increased flexibility in feed formulations by reducing or removing the constraint on the inclusion limit of poorly digested ingredients. Reduced variability in the nutritive value between batches of ingredients. Enzyme supplementation uplifts the value of poor samples and reduces the variation between good and poor quality samples of a given ingredient. This effect, in turn, improves the degree of precision of feed formulation.

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