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ENZYME SUPPLEMENTATION IMPROVES OATS NUTRITIONAL VALUE BUT REDUCES IT'S HYPOCHOLESTEROLEMIC EFFECTS

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ABSTRACT

The effect of different levels of oats and β-glucanase supplementation in a corn-soya based diet was investigated using a 5×2 factorial arrangement. Oats was included in lieu of corn at levels of 0.0, 10, 20, 30 and 40%. β-glucanase was added at levels of 0.0 and 5600 BGU/kg of diet. Body weight (BW), feed intake (FI), feed conversion ratio (FCR), abdominal fat (AF), ileal digestibility of fat and protein, and plasma cholesterol and triglyceride concentrations were determined. Increasing oat content of the diet significantly ($p<0.01$) reduced BW, FI, AF, fat and protein digestibility as well as plasma cholesterol and triglyceride levels. Feed conversion ratio was increased significantly ($P<0.05$). The lowest BW, FI, AF, digestibility of fat and protein, plasma cholesterol and triglyceride levels and highest FCR were observed at 40% oat inclusion. Supplemental β-glucanase improved ($P<0.05$) BW, FI, and digestibility of fat and protein. Enzyme addition increased ($P<0.05$) plasma cholesterol and triglyceride levels, and AF. Males had higher plasma cholesterol levels than females, whereas AF was higher in females ($P<0.05$).

Key words: Enzyme, Oats, Nutrition, Cholesterol.

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مکمل آنزیم، ارزش غذایی یولاف را بهبود می بخشد ولی اثرات ضد کلسترولی آن را کاهش می دهد

جواد پوررضا، علی آقایی، آرش پوررضا، عبدالحسین سمیع و عبدالمجید رضایی

به ترتیب، استاد، دانشجوی پیشین کارشناسی ارشد، دانشجوی پزشکی (دانشگاه اصفهان)، استادیار گروه علوم دامی و استاد گروه زراعت و اصلاح نباتات دانشکده کشاورزی دانشگاه صنعتی اصفهان، اصفهان، جمهوری اسلامی ایران.

چکیده

اثر سطوح مختلف یولاف و مکمل بتا-گلوکاناز در یک جیره پایه ذرت - سویا، در قالب طرح کاملاً تصادفی به روش فاکتوریل ۲×۵ بررسی شد. یولاف به میزان صفر، ۱۰، ۲۰، ۳۰ و ۴۰ درصد جایگزین ذرت، و بتا-گلوکاناز به میزان صفر و ۵۷۰۰ واحد در کیلوگرم به جیره ها افزوده شد. وزن بدن، مصرف خوراک، ضریب تبدیل خوراک، درصد چربی حفره شکمی، گوارش پذیری ایلئومی پروتئین و چربی، و غلظت کلسترول و تری گلیسرید پلاسما اندازه گیری شدند. افزایش میزان یولاف جیره، وزن بدن، مصرف غذا، درصد چربی حفره شکمی، گوارش پذیری پروتئین و چربی و غلظت های کلسترول و

تری گلیسرید پلاسما را کاهش داد (P<۰/۰۱) و برای ۴۰٪ یولاف، کمترین بودند. ضریب تبدیل خوراک، با افزایش یولاف جیره افزایش یافت و در ۴۰ درصد جیره بیشترین بود. مکمل بتاگلوکاناز، وزن بدن، مصرف خوراک، و گوارش پذیری پروتئین و چربی را به طور معنی داری (P<۰/۰۵) بهبود بخشید. افزودن آنزیم به طور معنی داری (P<۰/۰۵)، کلسترول و تری گلیسرید پلاسما و چربی حفره شکمی را افزایش داد. اثر جنس بر چربی حفره شکمی، و غلظت کلسترول و تری گلیسرید پلاسما معنی دار (P<۰/۰۵) بود. نرها، غلظت پلاسمایی کلسترول بیشتری نسبت به ماده ها داشتند در حالی که چربی حفره شکمی ماده ها، بیشتر بود.

INTRODUCTION

The negative effects of oat-containing diets for broiler chickens on performance, digestibility of nutrients and apparent metabolizable energy (AME) content of the diets are well known (24). It is well established that the non-starch polysaccharides (NSPs) of oats and barley have anti-nutritive effects. The NSPs in cereal grains and their by-products reduce the nutrient digestibility and availability; hence, lower their nutritional value.

The β-glucan present in oats and barley increases the digesta viscosity, reducing the feeding value of these grains for poultry (4, 14). Results of many experiments indicate that enzyme supplementation of poultry diets improve the nutritional value of cereal grains. Improvement in AME (2) and starch digestibility (6) due to added enzymes have been reported. Improved performance and normalization of consistency of the feces due to supplemental β-glucanase to barley- and oats- based diets have also been reported (5, 7, 12). Increased overall nutrient digestibility and performance has been attributed to a reduction in intestinal viscosity (9, 10, 23). It has been shown that barley has hypocholesterolemic effects in both humans and male broiler chicks (17, 18). Several investigators have demonstrated a favorable effect of oats and oat bran on blood cholesterol (8, 15). This has been attributed to increased fecal excretion of bile acids, thereby leading to an enhanced conversion of cholesterol to bile acids (15, 23). Also, the absorption of fat and cholesterol may be reduced (29). The objective of the present study was to evaluate the effect of β-glucanase in improving the nutritional value and performance of broiler chicks fed with diets containing different levels of oats. In addition, the effect of oats on plasma triglyceride and cholesterol was investigated.

MATERIALS AND METHODS

Birds, diets and recording

Four hundred and eighty (480) 1-d-old Arian broiler chicks (mixed sex) were divided into 30 groups of sixteen. Ten isoenergetic and isonitrogenous mash form diets were formulated (Table 1) and each diet was given to three replicates from one to 56 days of age. The birds were reared in 1×1.2 m^2 floor pens in an environmentally controlled, windowless house. Food and water were available *ad libitun* and the chicks had access to 24-h light throughout the experimental period.

In a corn-soya-based diet, oat grain was included at levels of 0, 10, 20, 30, and 40% in lieu of corn. SAFIZYM GP500 (Lesaffre, France), was used at levels of 0.0 and 0.1% in order to provide 5600 BGU/kg of diet. β-glucanase was prepared from *Trichoderma longibrachiatum* CNCM MA6-10W. For estimations of glucanase activity of the enzyme preparation, the measurement was based on the reducing sugar release from β-glucan as a substrate. One unit (IU) of enzyme activity is defined as the amount of enzyme producing 1μmol reducing sugar (as glucose equivalent) per minute at 60°C and at pH =6.0. Diets were formulated according to the NRC (16) recommendations. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) of each pen were measured at 21, 42, and 56 days of experiment. On these days, two males and two females from each pen were selected and from wing vein of each bird 7 ml of blood were collected in heparinized tubes; plasma was further separated by centrifugation. Sampling was performed between 9-11 am. Samples from each sex were pooled and placed in refrigerator for further analysis. The chicks were slaughtered and eviscerated and abdominal fat was separated and weighed.

Chemical analysis

The nitrogen content of the diets and excreta was determined by the Kjeldahl procedure (3) using an automatic analyzer. Crude fat was determined with a Soxhlet apparatus (3). Blood samples were centrifuged at 2000 rpm for 5 minutes and plasma samples were analysed for trigyceride and cholesterol using Ziest-Chemi kits (Ziest -Chemi Diagnostics, Tehran, Iran, Cat. No. 10-508, 525). Chromic oxide content of the diets and excreta was measured according to Fenton and Fenton (11).

Table 1. Composition of experimental diets.

Ingredients (%)	0-3 Weeks[a]					3-6 Weeks[b]					6-8 Weeks[c]				
Corn	57.87	48.00	38.00	28.00	18.00	62.78	53.00	43.00	33.00	23.00	65.90	56.00	46.00	36.00	26.00
Soybean meal	29.01	28.26	27.54	26.81	26.10	22.91	22.14	21.41	20.69	19.96	20.12	19.38	18.65	17.93	17.20
Fish meal	6.00	6.00	6.00	6.00	6.00	5.00	5.00	5.00	5.00	5.00	3.50	3.50	3.50	3.50	3.50
Oats	0	10.00	20.00	30.00	40.00	0	10.00	20.00	30.00	40.00	0	10.00	20.00	30.00	40.00
Sunflower oil	0.50	1.53	2.60	3.67	4.73	0.50	1.50	2.50	3.64	4.70	0.50	1.62	2.60	3.67	4.74
Rice hull	4.13	3.71	3.36	3.00	2.64	6.49	6.03	5.67	5.32	4.96	7.67	7.72	6.92	6.57	6.22
Oystershell	1.10	1.09	1.07	1.07	1.04	1.03	1.01	1.00	0.98	0.97	0.99	0.98	0.96	0.95	0.95
Dicalcium phosphate	0.50	0.52	0.53	0.55	0.58	0.47	0.49	0.52	0.53	0.57	0.52	0.54	0.57	0.58	0.61
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Supplements	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
DL-methionine	0.09	0.09	0.10	0.10	0.11	0.02	0.03	0.03	0.04	0.04	—	—	—	—	—

a. All diets were isoenergetic and isonitrogenous and contained 2800 Kcal/ME, and 20.1% crude protein. They contained 0.79, 0.88 and 0.39% total sulfur amino acids, calcium and available phosphorus, respectively. The vitamin and mineral supplement supplied per kg of diet; retinol, 2.175 mg; cholecalciferol, 23 ug; dl-a-tocopheryl, 9.1mg; cynocobalamin, 10 ug; vitamin K, 1.1 mg; riboflavin, 5.5 mg; calcium pantothenate, 11 mg; niacin, 53 mg; choline chloride, 1000 mg; folic acid, 0.7 mg; biotin, 0.25 mg; manganese, 55 mg; zinc, 42 mg; iron, 80 mg; copper, 5 mg; selenium 0.1 mg; iodine, 0.18 mg.
b. All diets were isoenergetic and isonitrogenous and contained 2805 Kcal/ME, and 17.5 % crude protein. They contained 0.63, 0.79 and 0.31 % total sulfur amino acids, calcium and available phosphorus, respectively.
c. All diets were isoenergetic and isonitrogenous and contained 2800 Kcal/ME, and 15.7% crude protein. They contained 0.57, 0.70 and 0.260 % total sulfur amino acids, calcium and available phosphorus, respectively.

Ileal digestibility of protein and fat

Chromic oxide was added at a level of 0.1% to the diet as an indigestible marker from days 49 to 56. On day 56, four birds (two males and two females) from each pen were killed by cervical dislocation, dissected, and the ileum and jejunum were ligated to avoid *post mortem* digesta movement. Ileal contents (from Meckel's diverticulum to 4 cm above ileocaecal junction) were removed and pooled to obtain enough materials; the materials were frozen at -20°C for further analysis.

Statistical analysis

Data on BW, FI, FCR, and protein and fat digestibility coefficients were analyzed in a completely randomized design with a 5×2 factorial arrangement using Proc GLM of SAS (25). Data on plasma triglyceride and cholesterol levels were analyzed using a 5×2×2 factorial arrangement (oats, enzyme, and sex effects). Interaction effects were compared using MSTATC when the F test was significant.

RESULTS

The effect of inclusion of oat and β-glucanase on performance parameters is presented in Table 2. The effect of dietary oats on BW was significant ($P<0.05$). The greatest reduction in live body weight ($P<0.05$) occurred with the diet in which oats were included at a level of 40%. The relationships between dietary oats and 21st-day BW and FCR, 42nd-day BW and 56th-day BW and FCR (Table 2) were linear and significant ($P<0.05$). Added β-glucanase had a significant effect ($P<0.01$) on BW, FI, and FCR. Enzyme supplementation improved the aforementioned criteria except for FI at 6 weeks of age and FI, and FCR at 8 weeks of age. The interaction effects of enzyme and oats on BW, FI, and FCR were not significant.

Plasma cholesterol was affected significantly ($P<0.01$) by dietary oats. Supplemental enzyme had a significant ($P<0.05$) effect on plasma cholesterol and triglyceride (Table 3). Plasma cholesterol was influenced significantly ($P<0.01$) by sex. Males had higher cholesterol than females. Plasma triglyceride was not affected by sex. There was no significant interaction between oats, enzyme, and sex for plasma cholesterol and triglyceride concentrations (Table 3). Included oats showed linear and significant ($P<0.01$) relationship with 21st- and 56th-day plasma cholesterol and AF in males, females, and in both sexes (Table 3).

Abdominal fat (A.F) was influenced significantly (P<0.01) by oats, enzyme, and sex (Table 3). Increasing dietary oats reduced AF in both sexes. Added enzyme increased AF in males and females. The effect of sex on AF was significant and females had higher AF than males. No significant interaction was detected between oats, enzyme, and sex for AF.

The effect of oats and enzyme on digestibility of fat and protein is presented in Table 4. Protein and fat digestibility coefficients were affected significantly (P<0.01) by dietary oats and enzyme supplementation. No significant interaction was detected between oats and enzyme regarding fat digestibility. The digestibility coefficients of fat and protein reduced with increased dietary oats. The greatest reduction in fat and protein digestibility coefficients occurred with the diet in which oats was included at a level of 40%. The relationships between dietary oats and digestibility coefficients of protein and fat were significant (P<0.01) and linear (Table 4).

DISCUSSION

Similar BW and FCR of chicks fed with diets containing 20% oats compared to control chicks was observed by Vetesi *et al.* (30) but higher levels of oats reduced BW and FCR. Results of the present study confirm the findings of these investigators. β-glucan present in oats increases intestinal viscosity, resulting in reduced nutrients availability and, finally, lower BW. Enzyme supplementation reduced the negative effect of β-glucan by increasing nutrient digestibility with the result that there was less substrate available for microbial fermentation in the gut. Results obtained in this study are in agreement with the findings of many experiments regarding the positive effect of β-glucanase on BW, FI, and FCR (4 , 12).

Several investigators (21, 22) have reported the hypocholesterolemic effects of barley and oats. The hypocholesterolemic effect of oats is due to its unsaturated fatty acids and fiber. Presence of fiber and NSPs in the gut impairs fat digestibility and absorption, therefore, bile acid secretion and flow is enhanced, which requires more cholesterol necessary for bile acid production; thus reducing plasma cholesterol, in turn (23 , 29).

Plasma cholesterol and triglyceride levels tended to increase when the diets containing oats were supplemented with enzyme. Enzymes reduced the negative effects of NSPs, therefore, absorption of fat and cholesterol increased. As a result of increased absorption of fat and cholesterol, less bile acid is required and conversion of cholesterol to bile acids is reduced, which leads to higher plasma cholesterol. The results reported herein confirm the findings of

Table 2. Body weight, feed consumption and feed conversion ratios of chickens fed with diets containing different levels of oats with and without supplemental β-glucanase.

Main effects	0-3 weeks			0-6 weeks			0-8 weeks		
	Body weight (g)	Feed consumption (g/h/d)	Feed to gain ratio (f/g)	Body weight (g)	Feed consumption (g/h/d)	Feed to gain ratio (f/g)	Body weight (g)	Feed consumption (g/h/d)	Feed to gain ratio (f/g)
Oats (%)									
0	540	946^{b}	1.748^{b}	1460^{a}	3204	2.188	2090^{ab}	5278	2.527
10	546	951^{b}	1.740^{b}	1462^{a}	3195	2.191	2138^{a}	5326	2.492
20	532	947^{ab}	1.760^{ab}	1436^{ab}	3183	2.216	2104^{ab}	5339	2.540
30	535	943^{ab}	1.769^{ab}	1445^{a}	3186	2.204	2066^{b}	5254	2.543
40	515	940^{a}	1.817^{a}	1392^{b}	3160	2.270	1994^{c}	5232	2.627
Non-enzyme	520^{b}	937^{b}	1.802a	1417b	3148	2.239a	2059b	5264	2.563
Enzyme[1]	548^{a}	954^{a}	1.740b	1469a	3224	2.166b	2099a	5308	2.528
Pooled SEM	11.4	12.66	0.0283	25.70	78.37	0.0403	23.91	76.54	0.0455
	Probabilities								
Source of variation									
Oats in diet (OD)	*	*	*	*	NS	NS	*	NS	NS
Linear (OD)	*	NS	*	*	NS	NS	*	NS	*
Quadratic (OD)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Enzyme (E)	**	*	**	**	NS	*	**	NS	NS
OD X E	NS	NS	NS	NS	NS	NS	NS	NS	NS

a-c. Mean values within a column for oats or enzyme with common superscripts do not differ significantly (P>0.05).

1. Enzyme was added to the diet at a level of 0.1%.

NS. Non-significant.

* P<0.05, ** P<0.01.

Table 3. Plasma concentrations (mg dl^{-1}) of cholesterol and triglyceride and abdominal fat (relative weight) of chickens fed with diets containing different levels of oats with and without supplemental β-glucanase.

Main effects	Cholesterol			Triglycerides			Abdominal fat		
	Male	Female	Both	Male	Female	Both	Male	Female	Both
Oats (%)									
0	120.4^{a}	118.0^{a}	119.4^{a}	78.0	82.6	80.3	1.690^{a}	1.816^{a}	1.753^{a}
10	118.0^{ab}	112.0^{ab}	115.0^{ab}	69.0	80.9	78.4	1.584^{ab}	2.047^{a}	1.732^{a}
20	116.4^{abc}	104.4^{bc}	110.4^{b}	72.7	84.0	74.9	1.305^{bc}	1.843	1.574^{ab}
30	105.6^{bc}	95.8^{c}	100.7^{c}	74.6	72.6	73.6	1.260^{bc}	1.754^{a}	1.557^{ab}
40	103.4^{c}	95.5^{c}	99.5^{c}	71.0	67.0	68.8	1.382^{c}	1.435^{b}	1.408^{b}
Non-enzyme	110.4	102.0	106.2^{b}	62.2^{b}	70.7^{b}	68.5^{b}	1.323^{b}	1.691	1.437^{b}
Enzyme[1]	115.1	108.4	111.7^{a}	79.9^{a}	84.1^{a}	82.0^{a}	1.566^{a}	1.867	1.736^{a}
Sex	112.8^{a}	105.2^{b}	-	73.1	77.3	-	1.444^{b}	1.766^{a}	-
Pooled SEM	6.00	5.00	5.47	8.68	8.46	8.36	0.132	0.136	0.168
				Probabilities					
Source of variation									
Oats in diet (OD)	*	**	**	NS	NS	NS	*	*	*
Linear (OD)	**	**	**	NS	NS	NS	**	**	*
Quadratic (OD)	NS	NS	NS	NS	NS	NS	*	*	NS
Enzyme (E)	NS	NS	*	*	*	**	**	NS	**
Sex (S)	-	-	**	-	-	NS	-	-	**
OD × E	NS	NS	NS	NS	NS	NS	NS	NS	NS
E×S	-	-	NS	-	-	NS	-	-	NS
OD×S	-	-	NS	-	-	NS	-	-	NS
OD×E×S	-	-	NS	-	-	NS	-	-	NS

a-c. Mean values within a column for oats or enzyme (except for sex) with common superscripts do not differ significantly ($P>0.05$).

1. Enzyme add to the diets at a level of 0.1%.

NS. Non-significant.

* $P<0.05$, ** $P<0.01$.

Table 4. Digestibility coefficients of protein and fat in chickens fed with diets containing different levels of oats with and without supplemental β-glucanase.

Main effects	Digestibility coefficients (%) Protein	Fat
Oats (%)		
0	76.5[a]	79.8[a]
10	78.5[a]	76.1[ab]
20	76.3[a]	75.2[ab]
30	72.3[ab]	69.8[bc]
40	68.00[b]	65.4[c]
Non-enzyme	71.9[b]	68.8[b]
Enzyme	76.6[a]	77.6[a]
Pooled SEM	3.38	3.95
	Probabilities	
Source of variation		
Oats in diet (OD)	*	*
Linear (OD)	**	**
Quadratic (OD)	NS	NS
Enzyme (E)	*	**
OD × E	NS	NS

a-c. Mean values within a column for oats or enzyme with common superscripts do not differ significantly (P>0.05).

1. Enzyme was added to the diets at a level of 0.1%.

NS. Non-Significant.

* P<0.05, ** P<0.01.

Schrijver *et al.* (26); and Pettersson and Aman (20), regarding the effect of enzyme supplementation on increasing plasma cholesterol and triglyceride.

Results of the effect of oats and enzyme supplementation on AF in this experiment are consistent with those in Vetesi *et al.* (30). Polyunsaturated fatty acid content of oats might be the cause for reduction in AF (22, 27). Also, reduced fat absorption and plasma cholesterol due to dietary oats may affect AF. The effect of enzyme in increasing AF may be attributed to its effect on fat and cholesterol absorbability.

Oats and barley reduce the activity of digestive enzymes, consequently, the digestibility of nutrients including fat and protein will decrease. Supplementing diets containing these grains with enzymes increases the activity of digestive enzymes; hence, the digestibility of fat and protein increases (1, 13). The results obtained in the present study are in agreement with the observations by other investigators, who indicated lower fat and protein digestibility coefficients under the influence of β-glucanase of barley and oats as well as improvements in their digestibility due to supplemental β-glucanase (19, 26).

Correlation between fat digestibility and plasma cholesterol was significant ($P<0.01$) and positive ($r=0.77$) which indicated that digestibility of fat increased as plasma cholesterol rose. This explains the effect of added enzyme on increasing plasma cholesterol. Correlation between digestibility of fat and plasma triglyceride was significant ($P<0.001$) and positive ($r=0.88$), which justifies the enzyme effect on increasing plasma triglyceride. Also, the correlation between fat digestibility and AF was significant ($P<0.01$) and positive ($r=0.86$), which indicates that as fat digestibility increased by supplemental enzyme, AF increased correspondingly.

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