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Lipidemic Responses of Male Broiler Chickens to Enzyme-supplemented Wheat-soybean Meal-based Diets with Various Levels of Metabolizable Energy

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Abstract: Effects of 2 various levels of AME (according to the manual recommendation and 100 kcal kg^{-1} less than it), 2 levels of endo-β-D-mannanase enzyme (0, 1 g kg^{-1}) and 2 levels of xylanase enzyme (0 and 1 g kg^{-1}) on serum lipid parameters as a 2^3 factorial arrangement were tested in 120 male broiler chicks fed wheat-soybean meal-based diet. These birds were randomly assigned to 8 experimental groups with 3 pen per group and 5 birds per pen. The serum HDL-cholesterol (HDL), LDL-cholesterol (LDL), Total-cholesterol (TC) and Triglycerides (TG) concentrations were measured at 31 and 41 day of age. The concentrations of serum TG, TC and LDL of 41-day-old birds demonstrated to be lower than those of 31-d-old ($p<0.001$). Some hypolipidemic responses were observed in the broiler chicks fed on (1) Diet supplemented with only β-mannanase, (2) Normal-AME diets supplemented with β-mannanase, (3) Normal-AME diets supplemented with Xylanse and (4) Normal-AME diets supplemented with both β-mannanase and Xylanase ($p<0.01$). In the other hand, some hyperlipidemic responses were detected in the broiler chicks fed on low-AME diets supplemented with xylanse or β-mannanase enzymes, alone or in combination ($p<0.01$). Regardless of AME, adding both xylanse and β-mannanase to the wheat-soybean meal-based diets have both hyperlipidemic and hypolipidemic effects together ($p<0.01$).

Key words: β-mannanase, xylanase, hypolipidemia, hyperlipidemia, broiler chickens

INTRODUCTION

Corn and soy bean meal are two common components of poultry feeds. Partial substitution of corn with wheat grain is sometimes a good economical strategy. Soybean meal contained 1.61% β-mannan (Hsiao *et al*., 2006). Betta-mannan is a polysaccharide with mannose, galactose and glucose repeating units (McCleary, 1988). On the other hands, wheat grain contained at least 5-8% pentosan (mostly arabinoxylan), resulting in 10-15% reduction in Apparent Metabolizable Energy (AME) of wheat-based diets. This part of dietary fibers is known as Non-starch Polysaccharides (NSP_s) and may be water soluble or insoluble (Asp, 1987). Because chickens have no digesting enzymes for xylans and β-mannans (Silversides and Bedford, 1999), their diets must be supplemented with appropriate exogenous NSP-degrading enzymes. These enzymes degrade NSP_s to xylose, manose, glucose and galactose units which would be more available for monogastrics, resulting in more dietary AME (Odatellah, 2000). Addition of multi-carbohydrase enzymes to corn and soybean diets improved AME_n in broilers due to an increase in cell wall degrading activity (Meng and Slominski, 2005).

On the other hands, high plasma lipids and cholesterol concentrations (hyperlipidemia and hypercholesterolemia) are important risk factors for coronary heart diseases. Researchers are usually trying to diminish the effects of these risk factors in animal models by new methods (especially by natural and organic materials) for two following purposes: firstly to produce the low-cholesterol meat, egg and other animal products and secondly to extend the results of these animal models to humans diseases associated with blood lipids.

There are several factors, such as sex, age and nutritional factors, that can affect plasma cholesterol concentration in the broiler chickens (Ozdogan and Aksit, 2003; Buyse *et al*., 2002; Swennen *et al*., 2005). The main dietary factor affecting the plasma cholesterol concentration was most likely the dietary concentration of fat and carbohydrates. For example, high fat and normal-carbohydrate diets inhibited fatty acid synthesis and may have elevated levels of plasma triglycerides (Mekki *et al*., 2006). Dietary cholesterol, fat and fibers are

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other nutritional factors that can affect blood lipid parameters in the broiler chicks (Aman and Graham, 1987; Trowell, 1972). Water-soluble fibers, such as xylans and β-mannans, can significantly diminish plasma cholesterol concentration; whereas insoluble forms, such as cellulose and lignin, cannot (Jenkins *et al*., 1975). Habibian Dehkordi *et al.* (2010) reported that reduction in serum total cholesterol and triglyceride may be influenced by feed additives (such as some herbal medicines) due to the activity reduction of cholesterol-synthetase.

Therefore, the main goal of the present study was to understand the lipidemic response of broiler chickens to wheat-soybean meal-based diets containing various levels of AME, Xylanase and endo-β-D-mannanase enzymes (alone or in-combination). These broiler chickens were evaluated at two different ages (31 and 41 day-old) and moreover, an attempt has been made to assess the effects of age on plasma lipids contents. In this study, increase in serum HDL and decrease in serum TG, TC and LDL concentrations were considered as a positive physiological response and was mentioned as hypolipidemia (in contrast with hyperlipidemia).

MATERIALS AND METHODS

Birds and husbandary: The experiment was carried out in Tehran province, Iran. The Animal Welfare Committee of Islamic Azad University, Varamin-Pishva branch (Varamin, Iran) approved all animal procedures for this experiment.

A total of 120 one-day-old sexed mail broiler chicks (Ross308) were ran-domly assigned to 8 dietary treatments, each consisting of 3 pens of 5 birds per pen. The chicks were raised in the floor pens and were provided with 23L:1D per day. Room temperature was maintained at 33°C for the first 3 day and was gradually decreased according to normal management practices until a temperature of 22°C was achieved. The birds were given free access to mash diets. All diets were fed throughout the 3 experimental periods of 2 week each (i.e., 0 to 2, 2 to 4 and 4 to 6 week).

Enzymes: Hemicel®, a rich source of endo-β-D-Mannanase, was obtained from CemGen Company. This enzyme originated from *Bacillus lentus* and the dried soluble of *Bacillus. l* has 158 million units/kg minimum enzyme activity. Recommendation daily dose of Hemicell is 0.05% of feed.

Rovabio™1500 (Addiseo Co.), as a rich source of Xylanase enzyme, was obtained from their exclusive representative in Iran (Vetaque Co.). The origin of this multienzyme was *Penicillium Funiclosom* fungus and each gram of it contained 2000 IU xylanase. The other enzymes, such as β-glucanase, pectinase, protease and cellulose were also provided by Rovabio™1500. Manufacturer's recommendation for this multienzyme was 500 g per ton but with regard to the exceed amounts of dietary wheat in the current study and for more effectiveness, twice of this recommendation were considered.

Experimental design and diets: Treatments were arranged as a 2^3 factorial with 0 or 1 g kg^{-1} xylanase , 0 or 1 g kg^{-1} β-mannanase and two AME treatments: (1) Normal-AME diets include 3010, 3175 and 3225 Kcal kg^{-1} AME at starter, grower and finisher phases, respectively and (02) Low-AME diets include 2910, 3075 and 3125 Kcal kg^{-1} AME at starter, grower and finisher phases, respectively. Thus, the following eight dietary groups were tested: (1) L-MX: low-AME diet without enzymes, (2) L+M-X: low-AME diet with β-mannanase, (3) L-M+X: low-AME diet with xylanase, (4) L+MX: low-AME diet with β-mannanase and xylanase, (5) N-MX: normal-AME diet without enzymes, (6) N+M-X: normal-AME diet with β-mannanase, (7) N-M+X: normal-AME diet with xylanase and (8) N+MX: normal-AME diet with β-mannanase and β-xylanase.

The diets were formulated to meet or exceed manual nutrient allowance for Ross 308 broiler chickens. Diets were offered in mash form. The composition of the basal and experimental diets is shown in Table 1. Similar nutrients were used for starter (0 to 2 week), grower (2 to 4 week) and finisher (4 to 6 week) diets.

Sampling and biochemical determinations: In day 31 and 41 of rearing period and after one night starving time, from each pen, two birds were selected and 2 mL of blood samples were collected from the wing vein using sterilized syringes and needles (No. 21). Blood samples were placed in test tubes without anticoagulant and then serum was isolated 6 to 8 h after blood collection. Serum samples were maintained at -20°C until biochemical analysis.

Serum total cholesterol and HDL-Cholesterol concentration were measured by the enzymatic-calorimetric methods (CHOD-PAP), triglycerides were measured by the enzymatic-calorimetric methods (GPO-PAP) followed by spectrophotometer. Wavelength of 546 nm was used for spectrophotometer according to instructions of the company (Pars Azmoon Co, Iran). Because the serum TG concentrations in the current experiment were below 400 mg dL^{-1}, the Friedewald *et al.* (1972) formula was used to calculate the LDL-cholesterol concentrations.

Statistical analyzing: The experiment was a 2^3 factorial arrangement of treatments with 3 pens per treatment by 5 birds in each. Mean of each variable from each pen as an experimental unit was used as a data for analyzing. The data were analyzed as a one-way ANOVA using general

Table 1: Composition of the basal diets (g kg^{-1}) for broiler chicks

Items	Diets		
	Starter	Grower	Finisher
Ingredients			
Wheat	450	450	450
Soybean meal (440 g kg^{-1} CP)	390. 5	369. 6	300
Corn	42.5	42. 9	127.8
Corn gluten(600 g kg^{-1} CP)	20	0	0
Limestone	21.5	13	12.7
Di-Ca-Phosphate	4.1	16	15
Sodium Chloride	2.4	6.8	3.2
Vitamin and mineral premix a	6	6	6
HCl-Lysine	2.4	1.8	1.4
DL-methionine	2.2	1.9	1
Soy oil (in low or normal AME Diets)	44.4/56.4[b]	78/90	68.9/80.9
Xylanase	+/-[c]	+/-	+/-
β-Mannanase	+/-	+/-	+/-
Filler	+/-	+/-	+/-
Calculated analysis[f]			
AME (kcal kg^{-1})[d]	2910/3010[e]	3075/3175	3125/3225
Crnde protein	240	222	199
Calcium	10	9	8.5
Available phosphorus	5	4.5	4.2
Sodium	1.6	1.6	1.6
Lysine	11.6	10.5	8.8
Methionine	4.4	4.2	3.7
Methionine+cystine	8.1	7.8	6.9

[a]Provided the following per kg diet: retinyl acetate (vitamin A), 108 mg; cholecalciferol (vitamin D3), 2 mg; DL-α-tocopheryl acetate (vitamin E), 360000 mg; menadione (vitamin K3), 800 mg; thiamin (vitamin B1), 2640 mg; riboflavin (vitamin B2), 4000 mg; niacin (vitamin B3), 12000 mg; Ca-pantothenate (vitamin B5), 12000 mg; pyridoxine (vitamin B6), 1182 mg; folic acid (vitamin B9), 400 mg; Colin Chloride, 100000 mg; Antioxidant, 150 mg; Mn, 40000 mg; Zn, 37000 mg; Fe, 20000 mg; Cu, 4000 mg; Colin chloride, I2, 100000 mg and Se, 80 mg. [b]4.44 g kg^{-1} in Low ME diets and or 5.64 g kg^{-1} in high ME diets(MJ kg^{-1}) and so in other column, [C]For enzymes+are presence 1 g kg^{-1} xylanase, β-mannanase but - are presence of 1 g kg^{-1} indigestible ingredients instead of the enzymes absence. For indigestible ingredient +/- is presence or absence 12 g kg^{-1} sand, [d]Calculated as NRC (1994), [e]2910 kcal kg^{-1} in low AME diets or 3010 Kcal kg^{-1} in normal AME diets and so in other column

linear model of SPSS® statistics 17.0 software package (SPSS Science, Chicago, IL, USA) by the following model:

$$y_{ijk} = \mu + E_i + X_j + M_k + EX_{ij} + EM_{ik} + XM_{jk} + EXM_{ijk} + R_l + e_{ijkl}$$

where, y_{ijkl} is l-th observation of kth β-mannanase level in the j-th dietary xylanase level in the i-th AME level, μ is the overall mean, E_i is the i-th AME level (I = 1, 2), X_j is the j-th dietary xylanase level (j = 1,2), M_k is the k-th β-mannanase level (k = 1, 2) and EX_{ij} is the ij-th interaction between dietary AME and xylanase levels, Em_{ik} is the ik-th interaction between dietary AME and β-mannanase levels, XM_{jk} is the jk-th interaction between dietary xylanase and β-mannanase levels, EXM_{ijk} the ijk-th interaction between dietary AME, xylanase and β-mannanase levels, R_l is L-th block and e_{ijkl} is experimental error effects.

If the F-test for treatment effect was significant in the ANOVA, differences among treatment means were determined using Duncan's multiple range tests. Differences were considered significant at $p<0.05$ (Snedecor and Cochran, 1989).

RESULTS

Age: Effect of sampling age is presented in Table 2. When compared with 31-day-old chicks, serum TG, TC and LDL concentrations in 41-day-old chicks were lower by 55, 9 and 44%, respectively ($p<0.001$). But there was no significant difference in HDL concentration among the ages.

Table 2: Effect of sampling days on serum TG, TC, LDL and HDL concentration of male broiler chicks at 31 and 42 day of age

	Day 31	Day 41	SEM	p-value
TG	80.85	44.93	4.42	0.001
TC	139.23	127.53	2.97	0.048
LDL	67.55	37.76	3.02	<0.001
HDL	77.79	81.39	2.03	0.383

Each value represented mean of twelve pens. TG: Triglyceride, TC: Total cholesterol HDL: High density lipoprotein, LDL: Low density protein, SEM: Standard error of the means

The means for main effects, first and second order interactions are reported in Table 3.

AME: The reduction of dietary AME resulted in a higher serum LDL (at 31 and 41 day) and TG (at 31 day) concentration and lower serum concentration of HDL (at 31 and 41 day), TG at 41 day and TC at 31 day than normal-AME diets in the present study. Therefore, it is still unclear that whether or not the reduction of dietary AME has hyper- or hypo-lipidemic effects.

Xylanase: Compared with no xylanase enzyme diets (+X diets), xylanase supplementation (+X diets) resulted in higher serum TG and LDL concentrations at 41 and 31 day, respectively.

Table 3: Main effects and interactions of enzyme supplements in the different levels of ME on the plasma TC, TG, LDL and HDL of male broiler chicks, day 31 and 42

		TG		TC		LDL		HDL	
Main effects or interactions	n	Day 31	Day 41	Day 31	Day 41	Day 31	Day 41	Day 31	Day 41
AME									
Normal	24.000	74.390^{b}	52.620^{a}	144.920^{a}	125.560	65.800^{b}	29.770^{b}	87.850^{a}	89.27^{a}
Low	24.000	87.320^{a}	40.900^{b}	130.220^{b}	128.330	69.300^{a}	45.750^{a}	67.740^{b}	80.92^{b}
SEM	11.200	5.390	9.270	5.890	5.170	5.830	5.800	5.600	
P-Value	0.001	0.001	0.018	0.269	0.025	<0.001	<0.001	0.003	
X (Xylanase)									
-X	24.000	79.400	53.400^{a}	141.200	127.68	63.810^{b}	39.380	79.230	82.84
+X	24.000	82.600	40.110^{b}	133.940	126.20	71.280^{a}	36.080	76.350	87.35
SEM	11.200	5.390	9.270	5.890	5.17	5.830	5.800	5.600	
P-Value	0.354	<0.001	0.206	0.548	<0.001	0.224	0.081	0.068	
M (β-mannanase)									
-M	24.000	87.340^{a}	50.430^{a}	137.000	126.50	65.830^{b}	35.400	75.420^{b}	88.36^{a}
+M	24.000	75.500^{b}	43.080^{b}	138.140	127.39	69.270^{a}	40.050	80.170^{a}	81.82^{b}
SEM	11.200	5.39	9.270	5.890	5.17	5.830	5.800	5.600	
p-value	0.014	0.011	0.838	0.717	0.028	0.096	0.007	0.013	
Interactions									
AME×M									
N-M	12.000	90.960^{a}	55.870	145.750	127.140^{ab}	70.540^{b}	35.950^{b}	81.620^{b}	85.48^{a}
N+M	12.000	60.580^{b}	49.360	144.080	125.410^{b}	61.050^{c}	23.580^{c}	94.080^{a}	86.03^{a}
L-M	12.000	83.610^{a}	44.990	128.250	127.290^{ab}	61.120^{c}	38.150^{b}	69.210^{c}	84.21^{a}
L+M	12.000	90.410^{a}	36.800	132.200	130.740^{a}	77.470^{a}	53.350^{a}	66.260^{c}	67.51^{b}
SEM	7.930	3.810	6.550	4.160	3.660	4.150	4.100	3.960	
p-value	0.009	0.736	0.616	0.028	<0.001	<0.001	<0.001	0.008	
AME×X									
N-X	12.000	78.000^{b}	53.860^{a}	158.470^{a}	134.320^{a}	64.480^{b}	40.360^{a}	86.290^{a}	81.63
N+X	12.000	70.050^{b}	51.380^{a}	131.360^{ab}	116.450^{b}	67.110^{b}	19.170^{b}	89.400^{a}	89.88
L-X	12.000	80.800^{b}	51.950^{a}	123.930^{b}	121.050^{b}	63.150^{b}	41.080^{a}	72.170^{b}	75.22
L+X	12.000	95.150^{a}	28.850^{b}	136.520^{ab}	138.220^{a}	75.440^{a}	50.420^{a}	63.300^{b}	77.79
SEM	7.930	3.810	6.550	4.160	3.660	4.150	4.100	3.960	
p-value	0.004	0.001	0.003	<0.001	0.004	<0.001	0.001	0.165	
M×X									
-MX	12.000	87.070	60.58^{a}	154.730^{a}	119.380^{b}	70.640^{b}	38.190	69.73^{c}	73.98^{c}
+M-X	12.000	71.730	46.57^{b}	127.660^{bc}	135.990^{a}	56.990^{c}	43.250	88.73^{a}	84.39^{b}
-M+X	12.000	87.610	38.64^{c}	119.260^{c}	136.630^{a}	61.020^{bc}	35.910	81.10^{b}	95.71^{a}
+MX	12.000	79.260	37.08^{c}	148.610^{ab}	118.040^{b}	81.540^{a}	33.680	71.61^{c}	71.96^{c}
SEM	7.930	3.810	6.55	4.160	3.660	4.150	4.100	3.96	
p-value	0.420	0.014	<0.001	<0.001	<0.001	0.859	<0.001	<0.001	
AME ×M×X									
N-MX	6.000	114.00^{a}	63.23	178.420	132.87^{b}	80.13^{b}	43.69	73.94	78.20^{cd}
N+M-X	6.000	41.94^{e}	44.49	138.520	135.78^{b}	48.83^{e}	35.3	98.64	85.07^{bc}
N-M+X	6.000	56.32^{e}	48.52	113.080	118.56^{c}	60.96^{d}	24.35	89.30	92.77^{ab}
N+MX	6.000	79.21^{b}	54.23	149.640	115.04^{cd}	73.28^{c}	15.72	89.51	86.99^{bc}
L-MX	6.000	60.08^{e}	57.93	131.050	105.89^{d}	61.15^{d}	29.95	65.53	69.77^{d}
L+M-X	6.000	101.50^{a}	47.96	116.810	136.21^{b}	65.14^{d}	48.50	78.81	83.38^{bc}
L-M+X	6.000	118.90^{a}	32.0	125.450	148.69^{a}	61.08^{d}	43.63	72.90	98.65^{a}
L+MX	6.000	79.31^{b}	25.65	147.590	122.53^{c}	89.81^{a}	60.62	53.71	56.94^{e}
SEM	5.850	3.03	4.84	3.070	2.58	3.21	2.90	2.86	
p-value	<0.001	0.054	0.089	<0.001	0.004	0.906	0.215	<0.001	

Means of each item in a column with different superscripts are significantly different (p = 0.05). Each value represented mean of twelve pens for main effect, six pens for first order interaction and three pens for second order interaction. ME: Metabolizable energy, L: Low ME, N: Normal ME, -M: Absence of β-manannase, ,+M: Presence of β-manannase, -X: Absence of xylanase ,+X: Presence of xylanase, -MX: Absence of both enzymes,+MX: Presence of both enzymes, TG: Triglyceride, TC: Total cholesterol, LDL: Low density lipoprotein cholesterol, HDL: High density lipoprotein Cholesterol, SEM: Standard error of the mean, AME: Apparent metabolizable energy

β-mannanase: β-mannanase supplementation reduced serum TG (at 31 and 41 day) and LDL (at 31 day) concentration and resulted in an increase in serum HDL concentration at 31 day despite 41 day. Subsequently, inclusion of β-mannanase in the poultry feeds seemed to have some beneficial changes on serum lipids of broiler chicks.

AME×ß-mannanase interaction: Some interactions were detected for serum LDL and HDL concentration in 31 and 41 day-old broiler chicks, so that, by adding β-mannanase to normal-AME diet(N+M), serum TG (at 31 day) and LDL (at 31 and 41 day) concentrations were reduced compared with no β-mannanase supplemented diet (N-M) but when adding β-mannanase to low-AME diet (L+M), serum LDL

concentration at 31 and 41 day of age was increased and serum HDL concentrations at 31 day was reduced compared with no β-mannanase supplemented diet (L-M). Thus we didn't observe a proper interaction for blood parameters by adding β-mannanase to low-AME diet but we observed an appropriate interaction when β-mannanase enzyme was added to diet with normal-AME.

AME×xylanase interaction: This interaction was significant for all parameters, with the exception of serum HDL in 41 day-old broiler chicks. In normal- AME diet, serum levels of TC and LDL at 41 d was reduced by adding xylanase (N+X diets) when compared with no xylanase supplemented diet (N-X), but in low-AME diet, serum TG (at 31 day despite of 41 dau), TC (at 41 dau) and LDL (at 31 day) levels were increased by xylanase addition (L+X) when compared with no xylanase supplemented diets (L-X).

β-mannanase × xylanase interaction: serum TG at 41 day of age was decreased by adding both β-mannanase and xylanase (+MX) to wheat-soybean meal-based diets rather than no enzyme diet (-MX), regardless of the AME content of the diets. This reduction in serum TG levels at 41 day was similar in+MX, -M+X and +M-X treatments. Adding both β-mannanase and xylanase also resulted in higher serum LDL level in 31 day-old chicks rather than no enzyme diets (despite each enzyme separately). Therefore, we did not detect an appropriate interaction for blood lipids after adding both mannanase and xylanase to the diets.

AME×ß-mannanase × xylanase interaction: Serum TG, TC and LDL concentrations, at 31, 41 and 31 day of age respectively, was reduced in normal-AME diet supplemented with both β-mannanase and xylanase (N+MX) when compared with no enzymes (N-MX) diets but in low-AME diets, adding both β-mannanase and xylanase, in combination (L+MX), increased serum TG (at 31 day), TC (at 41 day) and LDL (at 31 day) levels and reduced the serum HDL concentration at 41 day ($p<0.001$) rather than no enzymes diets (L-MX).

DISCUSSION

Age: In the present study, serum TG, TC and LDL-cholesterol were reduced at 41 day of age compared with those of broilers at 31 day. These results are consistent with those of Kalavathy *et al.* (2003) in which, found that serum triglycerides, total cholesterol and LDL-cholesterol of broiler chicks decreased significantly with increasing age from d 21 to day 28, 35 and 42. The reason for the lower serum TG, TC and LDL-cholesterol levels in the older chicks compared to younger ones appears to reflect the better utilization of dietary lipids in younger chicks.

AME: In the present study, feeding low-AME diets (100 kcal kg^{-1}) had negative effects on some serum lipid parameters, especially on LDL and HDL. Some studies have shown that AME is positively correlated with fat digestion (Friesen *et al.*, 1992; Steenfeldt and Heindal, 2000). Qujeq and Gharejeh (2001) reported that increasing the HDL:TC ratio caused by enhance in the reverse cholesterol transportation which, in turn, is due to intestinal losses of dietary fat. The hypercholesterolemic and hyperlipidemic properties of low-AME diets in the current study were probably a reflection of higher lipids digestion and absorption which, in turn, reduced depletion of lipids reserves. All of these resulted in an increase in serum LDL and a reduction in serum HDL concentration.

Xylanase: In the present study, concentrations of serum TG (at 41 day), LDL (at 31 day) were increased by adding xylanase to wheat-soybean meal-based diets. The higher concentration of serum TG and LDL in the Xylanase supplemented diets compared to no xylanase diets appears to reflect hyperlipidemic properties of xylanase addition. Some researchers reported that adding xylanase to wheat-soybean meal-based diets resulted in an increase in serum cholesterol. These authors concluded that adding NSP-degrading enzymes to the broiler diets may play a role in the emulsification of fat by bile salts in the intestine which, in turn, result in higher fat absorption (Hajati *et al.*, 2009; Mancini and Parillo, 1991; Pettersson and Aman, 1992; Sutton *et al.*, 1985). Although present results for xylanase addition weren't similar in broilers with two different ages but adding xylanase appear to have some emulsifying properties which, in turn, resulted in higher lipid digestion and absorption.

β-mannanase: Serum TG (at 31 and 41 day) and LDL concentrations (at 31 day) were reduced by adding β-mannanase to the wheat-soybean meal-based diets but serum HDL concentrations at 31 day of age was increased. These hypolipidemic properties for β-mannanase were probably a reflection of a reduction in lipid absorption in β-mannanase supplemented diets compared to no β-mannanase diets. These results were inconsistent with results of Zangeneh and Torki (2011), who found that serum parameters were unaffected by adding xylanase to hens diet. Sarikhan *et al.* (2009) and

Daggy *et al*. (1997) reported that serum TG concentration in broiler chickens reduced by increasing dietary fiber. But in this study dietary fiber levels didn't change. Therefore, the positive effects of β-mannanase on serum lipids may be due to reduction of lipid absorption. In the other words, intestinal lipid absorptions maybe depressed or became inefficient by adding β-mannanase to broilers diets. Garcia-Diez *et al*. (1996), Moundas *et al*. (1997) and Adrizal and Ohtani (2002) reported a tendency for dietary fiber and Non-starch Polysaccharides (NSPs) to bound to bile acids and thereafter, serum cholesterol will reduce due to more fecal losses of bile acids but in the present study, NSPs would be expected to decreased by adding β-mannanase and therefore, the described mechanism cannot be used for this study.

AME×ß-mananase: However, no comparable published data is available for dietary AME×mananase interaction on serum parameters of broiler chickens but in the present study we found an undesirable hyperlipidemic interaction, so that when β-mannanase was added to the low AME diets, serum LDL increased and HDL decreased. In the present study, AME reduction had hyperlipidemic properties and adding β-mannanase had hypolipidemic effects, but their interaction had hyperlipidemic properties which showed that hyperlipidemic properties of AME reduction were more dominant than hypolipidemic properties of β-mannanase addition.

According to the findings of Annison and Choct (1991) and Auclair and Larbier (2000), AME increased by dietary enzyme supplementation and According to the Qujeq and Gharejeh (2001) research, serum LDL and cholesterol concentration increased by high dietary AME. But despite mentioned researches in the present study, it appears that adding β-mannanase to low-AME diets couldn't increase serum LDL due to an increase in AME. When dietary AME was normal, our results were similar to those of concluded by Annison and Choct (1991) and Auclair and Larbier (2000).

Thus we concluded that perhaps this hyperlipidemic interaction was a reason for higher lipid absorption from small intestine of broilers chicks. This higher lipid absorption suppressed by normalizing of dietary AME by unknown mechanism.

AME×xylananase: We didn't detect optimal interaction (or hypolipidemic properties) by adding xylanase to low-AME diets for blood parameters but optimal interaction observed when xylanase added to normal-AME diets. Adding xylanase to low-AME diets may block intestinal fat digestion and absorption in the broiler chickens. AME can increase by dietary enzyme supplementation (Annison and Choct, 1991; Auclair and Larbier, 2000). Smulikowska and Mieczkowska (2000) reported that increasing AME_n values (by 62%) was due to better fat digestibility when broiler fed on wheat-based diet supplemented with enzyme containing xylanase and β-glucanase activities. Addition of xylanase to broiler diets resulted in a 12.6 to 18.7% increase in AME of wheat. These enzymes were also improved amino acid digestibility in wheat (Hew *et al*., 1998). According to the above findings if AME could increased by exogenous enzyme addition and if AME increasing had hypercholesterolemic and HDL-reduction effects (Qujeq and Gharejeh, 2001), therefore, from our results, hypolipidemic properties couldn't have detected by adding xylanase to normal-AME diets, but inversely we concluded that this interaction had some hypolipidemic effects (reduction of serum TC and LDL levels in 41 day-old birds).

But because some hyperlipidemic properties (include increase in serum TG and LDL at 31 day and TC at 41 day) observed when xylanase was added to low-AME diets, we can interpret that xylanase was able to increase dietary AME in low-AME diets despite normal-AME diets. In the other words, adding xylanase to broiler diets, in order to lowering serum lipids, is an appropriate solution but only in normal-AME diets not also in low-AME diets.

AME×ß-mannanase×xylanase interaction: To the authors' knowledge, however, no comparable published data on influence of xylanase and β-mananase interaction on blood characteristic in wheat-based diets is available.

In this study only when dietary AME was normal (despite low-AME diets), adding both xylanase and β-mannanase resulted in reductions in concentration of serum TG and LDL at 31 day and serum TC at 41 day of age. So, it can be interpreted that supplementation of normal-AME diets with both xylanase and β-mannanase resulted in some hypolipidemic and hypocholesterolemic interactions. Although the reason for this is unknown, it likely relates to this fact that adding both xylanase and β-mannanase to wheat-soybean meal-based diets was unable to normalize dietary AME in broilers given low-AME (100 Kcal kg^{-1} lower than normal) diets. These findings are inconsistent with Scott (2002) who reported that variation in AME among wheat or wheat-based diets was significantly reduced by enzyme supplementation.

CONCLUSION

In conclusion, regardless of dietary AME, β-mannanase supplementation at 0.1% of diet DM to broiler chicks consuming wheat-soybean meal-based

diets, despite xylanase in this study, resulted in some hypolipidemic effects and it can be used as hypolipiedemic agent in practical broilers diets based on wheat-soybean meal.

When normal-AME diets supplemented with 0.1% xylanase or 0.1% β-mannanase (alone or in combination) were fed to broiler chicks, the hypolipidemia were considered as a positive physiological response.

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