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Research paper



Investigation of Intestinal Enzyme Activity and Effects of Non-starch Polysaccharide on it

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Abstract

This experiment investigates the effects of utilizing Rahnama cultivar with high non-starchy polysaccharide content and supplementation of xylanase enzyme in poultry feed on the productivity features, nutrient digestibility and intestinal enzymes activity of 21-47 week laying hens. The experiment was conducted quite randomly and in factorial design that included eight treatments with 4 wheat levels (zero, 23, 46 and 69% that contained 1.8, 2.0, 2.2, 2.4% of xylose respectively) and two level enzymes (with and without enzyme) and 5 replications (6 hens) in each replication. During the experiment, by xylose level increase in diet, the weight ($p>0.05$) and mass of the egg ($p>0.01$) decreased and feed conversion ratio increased ($p>0.05$) but there was no effect on feed consumption and egg production rate. Diet supplementation with xylanase resulted in egg production increase ($p>0.05$), weight and mass increase of eggs ($p>0.01$) and improved feed conversion ratio ($p>0.01$). Xylose levels increase resulted in decrease in fat and metabolic energy digestibility of the diet ($p>0.05$); and, amylase enzymes activity in duodenum and amino peptidase, and lipase in duodenum and jejunum ($p>0.01$) increased. However, xylanase supplement had no effect on any intestinal enzymes. Diet supplementation with xylanase decreased viscosity of ileum ($p>0.01$). Increased xylanase level in diet resulted in decreased productivity features of laying hens, increased intestinal enzyme activity and decreased fat and metabolic energy digestibility.

Keywords: *Non-Starchy Polysaccharide, Productivity of Laying Hens, Intestinal Enzyme Activity, Nutrient Digestibility.*

1. Introduction

The word non-starchy polysaccharide, except for alpha-glucan (starch), contains a broad range of polysaccharides molecules. These polysaccharides lack alpha-glycosidic bond. Non-starchy polysaccharides along with lignin, named as dietary fiber, are the two main constituents of cell wall (26). Wheat seed contains a large amount of non-nutritive content especially non-starchy polysaccharides inside endosperm that restricts its usage in Poultry feed. A wheat seed contains xylose, arabinoxylan, beta glucan, cellulose, arabinogalactan peptide (11).

According to physical and chemical features, non-starchy polysaccharides are divided into two soluble and insoluble fibers in water. In digestive system soluble part plays an important role in digestion and absorption processes especially at first phases; while, the insoluble part of dietary fiber, actively involved at the terminal end of intestine to increase fecal mass volume and to decrease digestion time for the feed to pass through the body. (6). Many researches showed wheat-based diet supplementation of laying hens with commercial xylanase can eliminate the adverse effects of non-starchy polysaccharides (7). The effect of soluble fiber and diet supplementation with zinc enzyme on laying hens are quite known but the results of investigations differs among different researchers and the reason of these differences are not quite known; nevertheless, it may relate to some features of used wheat and especially its non-starchy polysaccharides features. Moreover, those improved features of wheat by enzyme supplementation are not completely recognized yet. Among investigations Hadorn et al., (1997) and Pan et al., (1998) observed no effect on productivity features of the laying hens by utilizing wheat-based diet supplement with zinc enzyme (12, 21). On the contrary, Lazaro et al., (2003) observed improved egg production and feed conversion ratio, intestinal viscosity decrease, dry matter, fat, non-starchy polysaccharides digestibility increase and increased outer membrane metabolic energy by wheat, barley and rye based diets (19). Non-starchy polysaccharides part of cereals seed perseveres proteins, lipids and starch and restricts the digestive enzyme access to them. In fact, the final stage of nutrient digestion is done by enterocytes brush border on the inner surface of the intestine (13). Quantitative researches were done about the effects of non-starchy polysaccharides levels and xylanase enzyme in wheat-based diets on intestinal enzymes activity in laying hens; since, digestive systems of laying hens are matured this raises the question that whether they are under the negative influence of non-starchy polysaccharides and this same issue is addressed in this research. Therefore the purpose of this study is to investigate the effect of xylose levels and xylanase enzyme supplement on the productivity, nutrient digestibility and intestinal enzymes activity of 21-47 week laying hens.

2. Materials and Methods

In this research, xylose and arabinoxylan content of Pishtaz wheat cultivar, corn, corn gluten, and soybean meal are measured by Megazyme Company's commercial test kit (Megazyme International Ireland Ltd) and the experimental diets got formulated on basis of amounts mentioned earlier. 240 Hy-Line W-36 pullets were taken from a flock of commercial pullets and they were ran-

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domly placed in 120 cages in pairs. The experiment is done during the period between 21st week and 47th week. In 21st week, along with the treatment, cages were split into eight experimental treatments with 5 replications (6 hens in each replication) and received the related experimental dietary. Dietary feed was in form of powder and the poultry received water and feed with no restriction. The experiment is done quite randomly and in factorial design with four wheat levels (zero, 23, 46 and 69%) that contained xylose (1.8, 2.0, 2.2, 2.4%) and two enzymes levels (with and without enzyme). Utilized commercial enzyme was 20xp- that was obtained from Tricodermalangibrachiatum and contained 70100unit/gr xylose. Used enzyme amount was due to the recommendation of producing company and was based on substrate density used in experimental diets. Each unit of enzyme activity is the amount of enzyme that releases 1 µ/mol of reducing sugars in 4.8PH and 50ºC per minute. Feed and nutrient combination of experimental diet represented in Table 1. Productivity of laying hens, egg production rate and egg weight, were daily recorded. The feed consumption was recorded daily and feed conversion ratio was calculated on basis of the data mentioned above.

Table 1: Feed and Nutrient Combination of experimental diets

Foodstuff	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Corn	66.0	44.94	24.75	4.54
Wheat	--	23.0	46.0	69.0
Soybean Meal		21.55	18.60	14.16	9.71
Corn Gluten		--	0.5	1.90	3.30
Soy Oil	1.10	1.50	1.50	1.50
Methionine		0.14	0.16	0.20	0.25
Lysine	0.01	0.1	0.23	0.37
Dicalcium Phosphate		1.68	1.66	1.66	1.66
Oyster Shell Powder		8.54	8.55	8.57	8.58
Salt	0.36	0.35	0.33	0.31
Enzyme1	0.12	0.14	0.20	0.28
Vitamin and Mineral Supplement2		0.5	0.5	0.5	0.5
Calculated					
Metabolic Energy (kcal/kg)		2800	2800	2800	2800
Crude Protein (%)		15.19	15.19	15.19	15.19
Total Lysine (%)		0.76	0.76	0.76	0.76
Total Methionine (%)		0.39	0.39	0.39	0.39
Calcium (%)		3.8	3.8	3.8	3.8
Linoleic Acid (%)		2.2	2.0	1.8	1.4
Xylose (%)		1.8	2.0	2.2	2.4
Arabinoxylan (%)		3.0	3.3	3.6	3.9
Measured (%)					
Dry Matter (%)		93.49	93.42	93.43	93.87
Crude Protein (%)		16.06	16.45	16.0	16.19
Crude fat (%)		4.49	4.44	4.44	4.40

Treatment 1-NSP level one, treatment 2- NSP level two, treatment 3-NSP level three, treatment 4-NSP level four.

1- The above foodstuffs contain enzymes and treatment 5, 6, 7 and 8 were done with the same foodstuff but without enzymes.

2- Xylanase enzyme contains 70100 units xylose/ gr. 2- Vitamin and Mineral Supplement include vitamin A,IU700000; D3330000IU; E,IU6600; k3,550mg; Thiamin,1500mg; Riboflavin,4400mg; Pantothenic acid, 22000mg; Niacin, 5500mg; Pyridoxin, 3000mg; Choline Chloride, 275000mg; Folic acid, 110mg; Biotin, 55mg; B12,8.8mg; Antioxidant, 1000mg, 66000mg; Zn66000mg; Fe, 33000mg; Cu, 8800mg; Iodine, 900mg.

2.1 Apparent Nutrient Digestibility Measurement

To measure nutrient digestibility, chromium oxide, 3gr/kg, as indigestible marker was used in experimental diets during one week. Then, feces samples collected for two days, dried in the oven (60ºC, 72 hours), weighed in, and ground up by laboratory mill.

Crude protein by Leco machine (Model FP-528, Leco Corporation, St. Joseph, MI); crude fat by soxhlet extractor, and crude energy by calorimeter bomb equipment (Model 1356, Parr Instrument Company, Moline, IL) were measured. Nutrients were measured by AOAC International (2000) (1). Chromium oxide was determined by Garcia et al., (2008) design (10). Apparent nitrogen, fat and metabolic energy digestibilities were obtained through feed and feces analysis (10).

2.2 Intestinal Enzymes Activity

At end of the experiment, 1 hen was selected randomly from each of the replications and were kept in isolated cages. Then, they were fed by the experimental diet from 7 to 10 in the morning and then anesthetized by thiopental sodium and 2.5cm of duodenum and jejunum inner part cut vertically out and washed in an ice-cold buffer phosphate (4.7pH). To prevent mucus enzymes from damage, the taken intestinal samples were kept on ice the entire preparation time. For biochemical tests measurement, samples wrapped inside aluminum foils and froze inside liquid nitrogen and preserved inside freezer in -80 ºC till the time of experiment (25). Intestinal inner cell enzyme activity including amylase (EC 3.2.1.1), amino peptidase (EC 3.4.11.2) and lipase (EC 3.1.1.3) are defined as micromole of substrate hydrolyzed per intestinal protein milligram. The above mentioned enzymes were selected on basis of natural substrate (starch, crude protein and lipids) relative density in standard poultry diets. Amylase enzyme activity was measured by Bernfeld (1955) design (2). 1% starch was used as a substrate. Samples with absorbance at 530nm wavelength were determined against control sample. Each amylase enzyme activity unit defined as 1 mgr malt sugar hydrolyzed per minute in 40 ºC. Amino peptidase enzyme activity was done through Gal-Garber and Uni, (2000) method (9). In this method, l-leucine-p-nitroanilide (Sigma L-9125 Chemical Co., St MO) was used as a substrate. P-nitroanilide determined through coloring and its color density was measured by spectrophotometer at 410nm. Each unit of amino peptides activity equals to 1 µ/mol p-nitroaniline from l-leucine-p-nitroanilide hydrolyzed per minute. Lipase enzyme activity was measures by Teng and Xu (2007) method. In this method p-nitrophenyl palmitate (Sigma L-9125 Chemical Co., St Louis, MO) was used as substrate. Samples absorbance occurred at 410nm against control sample. Each unit of lipase activity is the amount of enzyme that releases 1 µ/mol of p-nitrophenyl per minute. Protein density in intestinal cells was measured by Bradford (1976) method (4). Bovine serum albumin (Sigma Chemical Co. St Louis) was used as the standard. Data obtained from measured enzyme activity was subtracted by the amount of protein in intestinal cells and the total enzyme activity was showed per milligram protein.

PH measurement of different intestinal parts: After anesthetizing hens, 1gr sample of crop, gizzard, duodenum, jejunum, ileum, and cecum contents was weighed and mixed with 9 millimeter distilled water in a falcon tube inside a vortex mixer for five minutes and PH of the mentioned solution was measured by PH electrometer (22).

Ileum viscosity measurement: after PH sampling, ileum content was collected, and placed inside 3000g centrifugus immediately for 15 minutes. Then, obtained supernatanat poured into 2cc vial and stored in refrigerator at -20ºC. Then, viscosity was measured using digital viscometer (Model DV-II þ LV, Brookfield, Stroughton, MA, USA) by centipoises at 40 ºC (19).

Table 2: Effects of xylose levels and xylanase enzyme supplementation on productivity of Laying Hens

Main effects	Foodstuff (gr/weight) Egg production rate (%) Egg weight
(gr)	Egg mass
(gr /day)	Feed Conversion Ratio (gr / gr)

Xylose levels						
1.8	95.3	89.5	58.5a	52.4a	1.832ab	
2.0	94.8	89.3	58.8a	52.6a	1.813b	
2.2	94.2	89.8	57.7ab	51.9ab	1.831ab	
2.4	93.6	88.1	56.8b	50.1b	1.882a	
Standard error		0.464	0.544	0.434	0.434	0.014
Enzyme						
-Xylanase	94.4	88.5b	57.7b	50.1b	1.859a	
+Xylanase	94.6	89.8a	58.8a	52.3a	1.819b	
Standard error		0.328	0.385	0.307	0.307	0.01
P-value						
Xylose levels		0.0814	0.1719	0.0114	0.0012	0.0142
Enzyme	0.6356	0.0226	0.0365	0.0064	0.0097	
Enzyme xylose levels		0.2296	0.4709	0.4538	0.529	0.4671

Means with dissimilar letters in each column indicate significant difference at 5% level.

3. Conclusion

Xylose-D amount of Pishtaz wheat, corn, corn gluten and soybean meal were 3.0, 2.3, 1.0 and 1.3 respectively and arabinokesilan were 4.9, 3.7, 1.6 and 2.0 on dry matter basis and experimental diets were formulated on these amounts. Throughout the experimental period, feed consumption and egg production rate had not been influenced by the xylose levels. However, by xylose level increase, egg weight (P>0.05) and egg mass decreased significantly (P>0.01). Furthermore, feed conversion ratio of diet contained 2.4% xylose (P>0.05). External enzyme increased egg production rate (P>0.05), weight and mass of eggs and improved feed conversion rate (P>0.01) but had no significant effect on feed consumption rate. Interactional effect of xylose levels and xylanase enzyme on productivity features of laying hens was not significant (Table 2). Xylose levels increase resulted in decrease in fat and metabolic energy digestibility of diets. Enzyme had no significant effect on nutrient digestibility (Table 3). Moreover, increase in xylose level increased enzyme activity of amylase in duodenum (P>0.001), amino peptidase and lipase in duodenum and jejunum (P>0.001). Diet supplement with xylanase, did not influenced any of intestinal enzyme activity (Table 4). As it is shown in Table 5, xylose levels, enzymes and their interactional effect had no effect on PH of digestion system. Increased xylose level increased intestinal viscosity but deceased xylanase enzyme on viscosity significantly (Table 5).

Table 3: Effects of xylose levels and xylanase enzyme supplementation on apparent nutrient digestibility

Main effects	Protein (%)	Fat (%)	Metabolic energy digestibility (kg/ kcal)	
Xylose (%)				
1.8	67.7	79.9a	2881a	
2.0	67.3	79.1a	2860ab	
2.2	66.8	78.8a	2839ab	
2.4	66.5	76.6	2819b	
Standard error		0.518	0.513	12.67
Enzyme				
-Xylanase	67.07	78.1	2845	
+Xylanase	66.9	79.0	2854	
Standard error		0.366	0.363	8.96
P-value				
Xylose levels		0.3327	0.0002	0.0063
Enzyme	0.8780	0.0639	0.4866	
Enzyme*xylose levels		0.3105	0.4963	0.4110

Means with dissimilar letters in each column indicate significant difference at 5% level.

Table 4: Effects of xylose levels and xylanase enzyme supplementation on intestinal enzyme activity (unit per milligram intestinal protein)

Main effects	Amylase Jejunum	Amino peptidase Duodenum	Duodenum	Lipase Jejunum	Jejunum	Duodenum
Xylose levels (%)						
1.8	24.13b	42.83	10.33b	13.63c	3.68c	5.14c
2.0	25.25b	43.55	11.37a	13.87cb	3.97cb	5.42c
2.2	26.50a	43.74	11.74a	14.60ab	4.13b	6.45b
2.4	27.23a	44.61	11.89a	14.83a	4.61a	7.09a
Standard error	0.15	0.32	1.29	0.17	0.27	0.11
Enzyme						
-Xylanase	26.13	43.20	11.24	14.32	4.15	6.10
+Xylanase	25.42	44.17	11.42	14.03	4.04	5.90
Standard error	0.11	0.22	0.90	0.12	0.19	0.08
P-value						
Xylose levels	<0.0001	<0.0001	0.9381	<0.0001	0.0023	0.0001
Enzyme	0.2950	0.4872	0.2778	0.3560	0.3351	0.2091
Enzyme*xylose levels	0.7645	0.4713	0.5069	0.1040	0.4147	0.1318

Means with dissimilar letters in each column indicate significant difference at 5% level.

Table 5: Effects of xylose levels and xylanase enzyme supplementation on PH of different parts of digestion system and ileum viscosity (centipoises)

Main effects	Cecum	crop Viscosity	gizzard	Duodenum	Jejunum	Ileum
Xylose levels (%)						
1.8	4.70 3.66d	3.36	6.27	6.48	7.25	6.69
2.0	4.62 4.27c	3.38	6.21	6.50	7.18	6.60
2.2	4.61 5.48b	3.32	6.18	6.51	7.00	6.61
2.4	4.67 8.04b	3.37	6.33	6.50	7.07	6.54
Standard error	0.095	0.053 0.078	0.044	0.078	0.065	0.086
Enzyme						
-Xylanase	4.62 5.88a	3.37	6.19	6.51	7.13	6.65
+Xylanase	4.68 4.85b	3.35	6.31	6.48	7.12	6.57
Standard error	0.067	0.037 0.078	0.031	0.055	0.046	0.061
P-value						
Xylose levels	0.6877	0.6084 <0.0001	0.7264	0.5351	0.9741	0.1251
Enzyme	0.2618 <0.0001	0.7459	0.1197	0.5408	0.8299	0.4746
Enzyme*xylose levels	0.7019	0.3595 <0.0001	0.2698	0.6606	0.8967	0.4672

Means with dissimilar letters in each column indicate significant difference at 5% level.

4. Discussion

As it was mentioned, xylose level increase in the diet, decreased egg weight (1.7 gr), egg mass (2.3 gr) and increased feed conversion ratio throughout experimental period. Moreover, by non-starchy polysaccharide level increase in the diet, ileum viscosity increased but fat and metabolic energy digestibility of diet (3.3% and 62 kcal) decreased. In fact, viscosity through intensifying adhesion of digestive content in small intestinal reduces digestion

time, that food passes the digestion system, and inhibits digestive and substrate enzymes contact and results in decreased fat digestibility of the diet and eventually declined productivity features of the laying hens including egg weight and mass. Therefore, it is claimed that increase in ileum viscosity through decreased nutrient digestibility declined productivity of laying hens. Kim et al., (1976) reported leghorn white hens that fed on corn-based diets had higher feed consumption mean and produced larger eggs than hens fed on wheat-based diets (17). In contrast, Lazaro et al., (2003) and Safa et al., (2009) observed no difference in productivity of Hy-Line and lohmann brown strains by replacing 50 % of corn with wheat (19, 24). In this study diet supplement with xylanase enzyme increased egg production rate, egg weight and mass and improved feed conversion ratio. Supplementation of diet that contains polysaccharide with xylanase enzyme decreased ileum viscosity and increased digestion time; this resulted in increased feed consumption and nutrient digestibility (10, 20). There is only limited information about the effects of non-starchy polysaccharide levels increase or diet supplementation with xylanase enzyme on PH of digestion system in laying hens. In experiment mentioned above non-starchy polysaccharide levels and xylanase enzyme had no effect on PH of digestion system and this is consistent with Choct et al. (1999) that observed no effect on volatile fatty acids levels increase by diet supplementation with xylanase (5).

In contrast, Engberg et al., (2004) observed wheat-based diet supplement with xylanase decreased PH in gizzard and cecum of broiler chickens (8). In the present study amylase enzyme activity in jejunum was higher than in duodenum. Johnson et al., (1977) reported that increased amylase enzyme activity of mocos was higher in first part of the intestine than in the terminal end and the least amount of activity observed mainly at the end of intestine (16).

Bird (1971), reported, as well, that amylase enzyme activity in ¼ of the end of duodenum was at maximum this observation is expected since in this part pancreatic juice secreted into duodenum and the maximum activity of the enzyme above is observed in jejunum and in fact jejunum is the main digestion part for corn in the broiler chickens (3). Amino peptides and lipase enzymes in jejunum was higher than in duodenum. Gal-Garber and Uni, (2000) and Kramer et al., (2005) stated that amino peptides enzyme activity and its protein amount increases toward the end of small intestine in broiler chickens and rabbits (9, 18). In this study, by increase in xylose level amylase enzyme activity in duodenum and amino peptides and lipase in duodenum and jejunum increased. Viscosity produced by non-starchy polysaccharide of the diet thickens mucus layers of intestine and acts as an inhibitor between digestive enzymes and substrate contact hence increases intestinal enzyme activity. Ikegami et al., (1990) reported that non-starchy polysaccharide viscosity intensified intestinal viscosity besides, pancreatic juice and amylase, lipase and chymotrypsin enzymes activities increased in the small intestine (14). Petterson and Aman (1989) stated that many intestinal digestive enzymes activity might decrease by enzyme linked to non-starchy polysaccharide or through physical contact limitation of the enzyme with substrate (23). In this study diet supplementation with xylanase enzyme had no significant effect on inner membrane enzymes activities in different parts of intestine; contrary to the results of this study, Inbor et al., (1993) reported that outer membrane enzymes wheat and barley based diets decreased amylase enzyme activity in the small intestine. They suggested that positive effects of xylanase on inner enzyme production might be the result of arabinoxylan analysis along with small intestinal viscosity decrease (15). General findings of this study showed that non-starchy polysaccharide level increase to 2.4% through intestinal viscosity increase causes increased intestinal enzyme activity, decreased nutrient digestibility and as a result decreases productivity features of the laying hens.

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