The effects of non-starch polysaccharides content of wheat and xylanase supplementation on the intestinal amylase, aminopeptidase and lipase activities, ileal viscosity and fat digestibility in layer diet

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Abstract
The effects of non-starch polysaccharide (NSP) content of wheat and xylanase supplementation (XS) of the diet, on intestinal enzyme activity (amylase, aminopeptidase and lipase), fat digestibility and ileal viscosity of laying hens has been studied. Two hundred forty Hy-Line W-36 layer from 20 to 25 week of age were studied under a factorial experiment (4×2) in completely randomized design with 8 treatments including four levels of wheat (0, 23, 46 and 69%) corresponding to a dietary xylose content of 1.9, 2.1, 2.3 and 2.5% and two levels of xylanase (none or added at the dosage recommended by the supplier). Each treatment was replicated five times each with six hens. Wheat inclusion in the diet increased amylase and lipase activity in the duodenum and jejunum, respectively (P<0.001). Wheat inclusion, increased ileal viscosity while adding xylanase to diet, reduced it (P<0.001). Fat digestibility was decreased by wheat increment levels (P<0.001). The pH of the digesta content in different segments of gastro-intestinal tract (GIT) was not affected by wheat levels or XS, except for cecum that decreased with increasing the level of wheat. Relative weight and length of duodenum, jejunum and ileum were not affected by dietary treatment. Results suggested that wheat inclusion at high levels increased endogenous enzyme activity but could not alleviate the adverse effect of NSP content of diet on fat digestibility.

Keywords: Fat digestibility; Intestinal enzymes; Laying hen; Wheat; Xylanase

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INTRODUCTION

Starch is the predominant carbohydrate in cereals. Some of the constituent carbohydrates are water-soluble non starch polysaccharides (NSP) which are considered the major antinutritive factors in cereals (Mathlouthi *et al*., 2003). The NSP content and the nutritive value of wheat are very variable depending on factors such as cultivar, genotype, environmental factors, and storage conditions after harvesting (Gutierrez-Alamo *et al*., 2008). Xylans are the principle NSP of wheat, and high levels of wheat in poultry diets can increase the viscosity of the gut contents, which impedes the circulation and absorption of nutrients (Annison and Choct, 1991). The NSP fraction of the cereal protects lipids, starch, and protein, thereby compromising the access of digestive enzymes to dietary components (Garcia *et al*., 2008). Intestinal enzymes are responsible for the terminal digestion of most dietary macromolecules and play a vital role in regulating the amount nutrients available for absorption (Iji *et al*., 2001 b). In broiler chickens, enzyme activities are dependent on several factors, variation between lines (Uni *et al*., 1995) and dietary carbohy-

drate concentrations (Biviano *et al*., 1993). The dietary regulation of enzyme activity can be fully appreciated if the natural pattern of development is understood. Further studies are required to enable closer assessment of the impact of dietary factors, especially the stress imposed by anti-nutritional factors present in the diet in laying hens. Furthermore, amount and type of fiber play an important role on organ size and pH of the GIT of birds (Jimenez-Moreno *et al*., 2009). The poultry feed industry has greatly increased its use of exogenous enzymes over the past 15 years (Silversides *et al*., 2006). Xylanase has been used solely or in combination with other enzyme in layer diets containing wheat. However, the results obtained with different experimental conditions and diets have been inconclusive. Adding enzymes to wheat based diets may reduce the anti-nutritional effect of NSP and improve performance of laying hens (Jaroni *et al*., 1999). The available information on the effects of xylanase supplementation on endogenous enzymes activity in wheat-based diets for laying hens is very limited. So, the main objective of the present study was to investigate the effect of diet xylan content on endogenous enzyme activity and its effect on dietary fat utilization.

Table 1. D-xylose, and arabinoxylan content of Iranian wheat cultivars (%, Dry matter).

Cultivar of wheat	Dry matter	D-Xylose	Arabinoxylan
Arta	91.9	2.5	4.0
Atrak	91.7	2.6	4.2
Akbari	91.4	2.6	4.2
Alamut	92.4	2.9	4.7
Alvand	91.3	2.9	4.7
Bahar	91.2	2.5	4.0
Bam	90.7	2.9	4.7
Chamran	91.0	2.9	4.7
Darya	91.5	2.6	4.2
Dez	91.9	2.7	4.3
Kavir	92.0	2.6	4.2
Moghan	92.0	2.9	4.7
Niknejad	90.9	2.6	4.2
Pishgam	91.9	2.4	3.8
Pishtaz	91.7	3.0	4.9
Roshan	91.8	2.5	4.0
Shiraz	91.2	2.4	3.9
Shahriar	92.6	2.6	4.2
Tajan	91.8	2.9	4.7
Means± SD	91.63±0.5	2.68±0.2	4.33±0.34

MATERIALS AND METHODS

Husbandry, diets and experimental design a total of 240 pullets (Hy-Line W-36) allocated at random in groups of two in 120 cages. At 20 week of age, 3 adjacent cages were assigned at random to eight experimental treatments (5 replicates of six pullets each). Feed in mash form and water were provided for *ad libitum* consumption. The experiment was conducted as a completely randomized design with four levels of wheat (0, 23, 46, and 69%) that corresponded to a dietary xylans content of 1.9, 2.1, 2.3, and 2.5%, respectively, with or without xylanase supplementation (XS). The Pishtaz wheat cultivar (3% D-xylose on DM basis) was the source of wheat used in these diets in substitution of corn (Table 1). The exogenous enzyme used was a xylanase produced by *Tricoderma langibrachiatum* (Batch number 4278, Safizyme XP-20 (Lsaffre®, Marquette-lez-lille, France). The commercial product had by analysis a xylanase activity of 70100 Units/g and the unit of activity was defined as the amount of enzyme that releases one mmol of reducing sugars per minute at a pH of 4.8 and a temperature of 50ºC. The ingredient composition, calculated and determined nutritive value of the diets are presented in Table 2.

D-xylose and arabinoxylan measurements content of wheat cultivars analyzed using the Megazyme Kit (Catalog number, K-XYLOSE, Megazyme International, Wicklow, Ireland Ltd.). From all these samples, the Pishtaz cultivar was chosen for the in-vivo laying hen study because of its high D-xylose content. Intestinal enzyme activities determinations at the end of 25 week of age, the hens fed their diet treatments for 3 h (7:00 to 10:00 AM).The hens were weighed and euthanized by intravenous injection with thiopental sodium (Sandoz GmbH, Kundl, Austria). Each segment of the GIT was excised and the contents were removed by gentle flushing with a buffered phosphate saline at pH 7.4. To prevent mucosa damage and endogenous enzyme degradation, samples (2.5 cm) from the mid region of the duodenum, jejunum, and ileum were kept on ice during the preparation process. The samples were wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80ºC as indicated by Shirazi-Beeche *et al*. (1991) until performing biochemical assays were performed. The enzyme activities analyzed were chosen on the basis of the relative concentration of the natural substrates (starch, crude protein, and lipid) in standard poultry diets. The specific activities of amylase (EC 3.2.1.1), aminopep-

Table 2. Composition ingredient used in experimental diets (as fed basis; g/100g).

Ingredient	20 to 25 weeks			
Corn	72.47	52.38	32.27	12.13
Wheat	--	23.0	46.0	69.0
Soybean meal, 44% CP	13.91	9.27	4.63	--
Corn gluten meal, 62%	4.01	5.63	7.24	8.86
Soybean oil	0.8	0.8	0.8	0.8
DL-Methionine, 99%	0.08	0.07	0.05	0.04
L-Lysine-HCl, 78%	0.17	0.27	0.37	0.46
Dicalcium phosphate	1.99	1.97	1.95	1.93
Sodium chloride	0.36	0.35	0.34	0.32
Oyster shell	5.59	5.62	5.65	5.68
Enzyme[1]	0.12	0.14	0.2	0.28
Vitamin and mineral permix[2]	0.5	0.5	0.5	0.5
Calculated				
AMEn (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	15.30	15.30	15.30	15.30
Total Lys (%)	0.76	0.76	0.76	0.76
Total Met (%)	0.37	0.37	0.37	0.37
Calcium (%)	2.6	2.6	2.6	2.6
Xylose (%)	1.9	2.1	2.3	2.5
Arabinoxylan (%)	3.1	3.4	3.7	4.0
Linoleic acid (%)	1.3	1.1	1.0	0.8
Determined				
AMEn (kcal/kg)	2883	2859	2847	2824
Dry matter (%)	93.7	93.4	93.5	93.7
Crude protein (%)	15.5	15. 5	15.4	15.3
Ether extract (%)	4.8	4.7	4.8	4.7
Xylose (%)	1.88	2.13	2.33	2.41
Arabinoxylan (%)	3.02	3.42	3.75	3.90

[1]The product contained xylanase by analysis 70100 Units. xyl/g (Lesaffre, Marquette-lez-lille, France). [2]Provided per kg of diet: retinol, 2310 µg; cholecalciferol, 82.5 µg; DL-alpha-tocopherol acetate, 6.6 mg; menadione , 0.55 mg; thiamine, 1.5 mg; riboflavin, 4.4 mg; pantothenic acid, 22 mg; niacin, 20 mg; pyridoxine, 3 mg; choline chloride, 275 mg; folic acid 1.1 mg ; biotin 0.055 mg; vitamin B_{12} (cyanocobalamin), 0.088 mg; antioxidant (BHT, BHA and ethoxyquin), 1 mg; Manganese, 66 mg; zinc, 66 mg; iron, 33 mg; copper, 8.8 mg; iodine, 0.9 mg; selenium, 0.3 mg.

tidase (EC 3.4.11.2), and lipase (EC 3.1.1.3) in the duodenal, jejunal, and ileal region were assayed in homogenized tissue (Silent Crusher M, Heidolph, Schwabach, Germany). Amylase activity was measured using soluble starch as a substrate, as described by Bernfeld (1955). One unit of α-amylase activity was defined as the amount of enzyme that produced one mg maltose per min at 40ºC. Aminopeptidase activity was determined as described by Gal-Garber and Uni (2000) using L- leucine-p-nitroanilide (Sigma L-9125 Chemical Co., St Louis, MO) as a substrate. One unit of aminopeptidase activity was defined as the production of one mMol of p-nitroaniline per min from the L-leucin-p-nitroanilide substrate. Lipase activity was measured as described by Teng and Xu (2007) using para-nitrophenyl palmitate (Sigma L-9125 Chemical Co., St Louis, MO) as a substrate. The unit of enzyme activity was defined as the amount of enzyme liberating one mmol of p-nitro phenol per min. The protein content of the intestinal samples was measured according to the method Bradford (1976). The result of enzyme activity was expressed in units per mg of intestinal tissue protein. For determination of fat digestibility of the diets, one bird randomly selected per replicate, and placed at individual cages and fed with respective diet treatments together with 0.3% chromium oxide as an indigestible marker for one week. Representative samples of excreta were collected during the last two days, were thawed overnight, dried (60ºC, 72 h), and grounded (1 mm). Chromium oxide content of feeds and excreta was analyzed by atomic absorption spectrophotometry (Smith-Hieftje 22, Thermo Jarrell Ash Corporation, Franklin, MA), using method (Garcia *et al*., 1999). The apparent digestibility of fat was determined as indicated by Garcia *et al*. 2008. Ileal viscosity at the end of the metabolism study, the hens were euthanized, and the ileal digesta were collected and homogenized. Two eppendorf tubes were filled (1.5 g) with ileal digesta and centrifuged (3000 g for 15 min) and the viscosity of the supernatant (1 ml) was measured (cP) at 25ºC, using a digital viscometer (DV-II þ LV, Brookfield, Stroughton, MA, USA) as indicated by (Silva and Smithard, 2002). pH of the gastrointestinal tract the digesta of the crop, gizzard, duodenum, jejunum, ileum and cecum was gently flushed with distilled water, and placed into clean beakers. Nine-fold of distilled water of the digesta weight (wt/vol) was added to the beaker and stirred for five min, and then the pH of the solution was measured using a pH meter (Corning Glass Works, Medfield, MA) as indicated by (Pang and Applegate, 2007). Relative weight of gastrointestinal tract the digestive tract with contents was removed aseptically, and the proventriculus, gizzard, duodenum, jejunum, ileum and ceca were excised, and weighed. The weight of the empty organs was expressed relative to live body weight (%) as indicated by Jimenez-Moreno *et al*. (2009).

Statistical analysis the experiment was conducted as a completely randomized design with eight treatments in a 4×2 factorial arrangement and the main effects of the main effects (level of wheat inclusion and XS) were analyzed by ANOVA using the GLM procedure of SAS institute (1990). When the main

effect was significant, the Tukey 's test was used to make pairwise comparisons between sample means. All differences were considered significant at $P \leq 0.05$.

RESULTS

D-xylose and arabinoxylan measurement content of the 19 wheat cultivars studied were 2.68 ± 0.2 for xylose and 4.33 ± 0.34 for arabinoxylan, respectively (Table 1). Intestinal enzyme activities determinations amylase activity (21.34 vs 18.55 Unit/mg of protein; P<0.001) increased by NSP content of wheat in the duodenum. However, levels of wheat did not have an effect on amylase activity in the jejunum and ileum of birds. Dietary treatment did not influence aminopepti-

Table 3. Influence of dietary wheat inclusion and enzyme supplementation on specific activity (Unit/mg of intestinal protein) of amylase, aminopeptidase and lipase of small intestine of laying hens.

Item	Amylase			Aminopeptidase			Lipase		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Wheat									
0	18.55^{c}	36.83	28.31	8.41	12.21	14.86	3.20	3.66^{b}	6.15
23	19.57^{b}	37.77	29.76	8.45	12.56	14.83	3.51	3.63^{b}	6.32
46	21.11^{a}	37.90	30.12	8.51	13.02	14.81	3.63	5.30^{a}	6.35
69	21.34^{a}	38.71	30.74	8.84	13.31	15.13	3.43	5.41^{a}	6.59
SEM[2]	0.16	2.60	1.71	0.39	0.45	0.40	0.18	0.10	0.23
XS[1]									
- xylanase	20.20	37.36	29.79	8.20	12.95	14.56	3.45	4.51	6.24
+ xylanase	20.09	38.25	29.67	8.91	12.59	15.25	3.44	4.36	6.47
SEM[2]	0.11	1.84	1.21	0.27	0.32	0.28	0.12	0.07	0.17
Probability[3]									
Wheat	***	NS	NS	NS	NS	NS	NS	***	NS
XS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]The product contained xylanase by analysis 70100 U. xyl/g. [2]SEM: standard error of mean (n = 5). [3]The interactions among main effects were not significant (P>0.05).

Table 4. Influence of dietary wheat and enzyme supplementation on fat digestibility and viscosity

Item	Fat digestibility (%)	Viscosity (cP)
Wheat		
0	79.92^{a}	3.39^{d}
23	78.97^{ab}	4.67^{c}
46	78.27^{ab}	5.84^{b}
69	76.22^{b}	6.76^{a}
SEM[2]	0.847	0.028
XS[1]		
- xylanase	78.27	5.75^{a}
+ xylanase	78.42	4.58^{b}
SEM[2]	0.599	0.019
Probability		
Wheat	*	***
XS	NS	***

[1]The product contained xylanase by analysis 70100 U. xyl/g. [2]SEM: standard error of mean (n = 5).

Table 5. Influence of dietary wheat inclusion and enzyme supplementation on GIT pH.

Item	Crop	Gizzard	Duodenum	Jejunum	Ileum	Caecum
Wheat						
0	4.69	3.44	6.29	6.65	7.42	6.61^{a}
23	4.59	3.43	6.27	6.59	7.31	6.09^{ab}
46	4.62	3.55	6.32	6.66	7.38	6.04^{ab}
69	4.55	3.51	6.19	6.67	7.33	5.65^{b}
SEM[2]	0.09	0.07	0.16	0.11	0.07	0.18
XS[1]						
-xylanase	4.59	3.50	6.25	6.69	7.41	6.24
+xylanase	4.63	3.46	6.29	6.59	7.30	5.96
SEM[2]	0.06	0.05	0.11	0.07	0.05	0.13
Probability[3]						
Wheat	NS	NS	NS	NS	NS	**
XS	NS	NS	NS	NS	NS	NS

[1]The product contained xylanase by analysis 70100 U. xyl/g. [2]SEM: standard error of mean (n = 5). [3]The interactions among main effects were not significant (P>0.05).

Table 6. Influence of dietary wheat inclusion and enzyme supplementation on the relative weight (% of BW) of gastrointestinal tract.

Item	Proventriculus	Gizzard	Duodenum		Jejunum		Ileum		Caecum	
	(% of BW)	(% of BW)	(% of BW)	Long (cm)	(% of BW)	Long (cm)	(% of BW)	Long (cm)	(% of BW)	Long (cm)
Wheat										
0	0.38	1.59	0.6	23.95	1.04	44.45	0.82	40.4	0.58	14.1
23	0.37	1.62	0.64	25.50	1.01	45.5	0.81	43.2	0.58	14.1
46	0.34	1.57	0.66	26.05	1.0	42.50	0.81	39.5	0.56	13.3
69	0.35	1.50	0.60	24.45	1.0	46.35	0.80	41.1	0.52	13.2
SEM[2]	0.023	0.06	0.029	0.955	0.041	1.25	0.05	1.81	0.024	0.43
XS[1]										
-xylanase	0.35	1.56	0.63	25.05	1.03	44.35	0.83	41.02	0.56	13.45
+xylanase	0.37	1.58	0.62	24.92	1.0	45.05	0.79	41.10	0.57	13.92
SEM[2]	0.016	0.04	0.02	0.67	0.03	0.88	0.035	1.28	0.02	0.30
Probability										
Wheat	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
XS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]The product contained xylanase by analysis 70100 U. xyl/g. [2]SEM: standard error of mean (n = 5). [3]The interactions among main effects were not significant (P>0.05).

dase activity in duodenum, jejunum or ileum. Increasing the level of wheat enhanced jejenal lipase activity (5.41 vs 3.66 Unit/mg of protein; P<0.001). Enzyme activity was not affected on any of the segments of the small intestine by xylanase supplementation (P>0.05) (Table 3). The apparent digestibility of fat reduced with wheat inclusion in diet (79.92 vs. 76.22%; P<0.001), however XS had no positive effect on this (Table 4). Ileal digesta viscosity was increased with NSP content of wheat (6.76 vs 3.39 cP; P<0.001). Exogenous enzyme also was raised due to reduction of ileal viscosity (4.58 vs. 5.75 cP; P<0.001) (Table 4). The pH of the different segments of the GIT was not affected by dietary wheat except caecum that was decreased by increasing the wheat inclusion in the diet pH of the different segments of the GIT was not influenced by XS (Table 5). Relative weight of organs and length of the duodenum, jejunum and ileum were not affected by NSP content of wheat and exogenous enzyme supplementation (Table 6).

DISCUSSION

In the current study, Pishtaz cultivar was chosen because of its high xylose content. The arabinoxylans content was below the range of values (5.7 to 8.0% DM) that has been reported by Gutierrez-Alamo *et al*. (2008). While similar values of 2.8 for xylose and 5.2% for arabinoxylan have been reported by Mathlouthi *et al*. (2003). The data for wheat obtained in the current research for xylose and arabinoxylan content varied from 2.4 to 3% and 3.8 to 4.9% dry matter, respectively. The reasons for the variability in the nutritional value of wheat are difference in origin (variety, site of growth), physical measurements (storage time, inclusion form), or gut structure and function (Gutierrez-Alamo *et al*., 2008). The information available on the effects of increasing levels of NSP in the diet on endogenous enzyme activity in laying hens is scarce. The results of the current study indicated that amylase and lipase activity increased in the duodenum and jejunum, respectively with increasing level of wheat. Ikegami *et al*. (1990) reported that soluble NSP increased activities of lipase, amylase, and chymotrypsin in gut. Secretion of pancreatic and biliary juices was stimulated by viscosity-producing fibers. Enzyme activity of the digesta in the small intestine depends on actual secretion of the enzyme by the pancreas and of its eventual break down in the GIT. Therefore the enhanced enzyme activity observed in the duodenum contents after feeding viscosity-producing fibers. Also, these results are consistent with the findings of Johnson *et al*. (1977) who reported that mucosal contribution to amylase activity was high in the proximal segments of the intestine of rats with a more reduced activity observed generally in the caudal part. There are no reports on the direct inhibition of intestinal enzyme synthesis by NSP but the activities of most enzymes may be reduced through coupling to NSP or physical restriction of enzyme access to substrates (Iji *et al*. 2001 a). Exogenous enzyme did not have effect on any enzyme activity in three segments of intestine. Almirall *et al*. (1995) observed that sup-

plementation β-glucanase in diet based on high viscosity barley compared with corn increased trypsin, amylase, and lipase activity in the chyme of 21d old broilers. In contrast, Inborr *et al*. (1993) found that exogenous enzymes in the diet based on barley or wheat decreased endogenous enzymes activity. They reported that, exogenous enzymes might promote beneficial effect of endogenous enzyme. But this may result from the fact that exogenous enzymes degrade β-glucan and arabinoxylan in endosperm cell wall and decrease the viscosity of digesta in small intestine. Viscosity may act as a barrier to prevent contact of digestive enzyme with their substrates, thickening of the unstirred layer of mucosa and prevent micelle formation required for absorption of lipids and lead to reduction of fat digestibility of the diet. A reduction in fat digestibility is frequently correlated with digesta viscosity suggesting that viscosity could hinder enzyme digest contacts and hamper the rate of nutrient absorption. The information available on the effects of increasing levels of wheat or XS of the diet on pH values of the intestinal digesta in poultry is limited. In the current experiment, pH of the different segments of the GIT was not affected by wheat inclusion or exogenous enzyme, but pH of the caecum was decreased by increasing levels of NSP content. Bedford (2000) suggested that sugars such as xylose and xylo-oligomers escape enzymatic digestion in the proximal GIT and may enter the caecum where they will be fermented by the cecal microflora and produce short chain fatty acid, lead to decreasing the pH. Engberg *et al*. (2004) reported that gizzard and caecum pH was reduced with xylanase supplementation in 42d old broilers. In contrast, to the results of Choct *et al*. (1999), no significant increase of volatile fatty acid concentration was found in caecal contents of xylanase supplemented birds. The pH value of the digesta of the different segments of the GIT, across dietary treatments were within the range reported for young broilers (Jimenez-Moreno *et al*., 2009; Rynsburger and Classen, 2007) and adult chickens (Herpol, 1966).

CONCLUSIONS

The results of this study confirmed that viscosity induced by wheat NSP content make a reduction in fat digestibility. Therefore, treatment that included 69% of wheat (2.5% xylose) increased lipase activity in the jejunum. Exogenous xylanase supplementation had no beneficial effect on intestinal enzyme activity, fat digestibility and pH of GIT. Results suggested that an increased viscosity in the intestinal content decreases the diffusion processes of substrates and enzyme and hinder their effective interaction at the mucosal surface. Our observations confirmed that in the present of high digestive enzyme secretion, fat digestibility decreased in hen fed viscous fibers.

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