Alteration of D-xylose intestinal absorption in broilers with high dietary barley intake

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Abstract:

Low nutritive value and suitability of barley as a feedstuff for broilers is mainly due to its non-starch polysaccharides. An experiment was carried out to evaluate if diets high in barley alter the mechanisms responsible for the absorption of nutrients across the intestinal epithelium of broilers, using D-xylose absorption test. Thirty, 14-d old male broiler chicks (Ross 308) with similar body weights (430g ± 10) were randomly divided into 3 groups of 10 birds, each bird as one replicate. Each group was offered a diet based on maize, high quality wheat or barley as the main source of carbohydrate, for 14 days. On the last day of the experiment, all birds were weighed individually and D-xylose absorption test was performed. Following the test, all birds were sacrificed and the relative size and weight of digestive tract were measured. Those birds fed on barley diet had lower weight gain when compared with maize or wheat fed birds ($p<0.05$). There was no difference in relative weight and size of digestive parts ($p>0.05$). No difference was also observed in absorption capacity of small intestine for D-xylose between the experimental groups ($p>0.05$). It was concluded that the differences observed in weight gain among the experimental groups was not related to the absorption capacity of intestine as this parameter was not changed. It appears that, in short term, barley antinutrients show their negative effect on growth performance of the birds by other means.

Introduction

Barley (*Hordeum vulgare* L.) is an ingredient with low acceptability in broiler feed, because it's low nutritional value. Barley contains phenolic acids and phytates to some extent, however, the major anti-nutritional factor detracting from its nutritional value for broilers is non-starch polysaccharides (NSP), mainly (1,3-1,4)-β-glucan (Jood and Kalra, 2001; Ravindran et al., 2007; Garcia et al., 2008).

The negative influence of NSPs on the digestion and the absorption of dietary nutrients including glucose due to an increase in digesta viscosity is extensively studied (Rainbird et al., 1984; Eastwood and Morris, 1992; Wood et al., 1994; Ellis et al., 1996). The viscosity of the intestinal contents increases exponentially as the concentration of dietary NSP increases (Eastwood and Morris, 1992; Ellis et al., 1996). There is no evidence indicating diets containing high amounts of barley, affect the mechanisms responsible for the absorption and transfer of nutrients across the intestinal epithelium of chicken.

D-xylose absorption test is a useful indicator of intestinal absorptive function in animals (Sorensen et al., 1997; Rutgers, 2005; Semrad, 2005). In healthy animal, D-xylose, similar to D-glucose, passes the intestinal brush border membrane via trans-cellular pathway as well as diffusion and/or solvent drag through para-cellular pathway (Scharrer and Grenacher, 2000; Chediack et al., 2003; Chang and Karasov, 2004; Chang et al., 2004). Small intestine of chicken absorbs D-xylose almost completely, thus any change in plasma concentration of D-xylose over a 3-h period is quite indicative of the absorption capacity of intestinal tract for D-xylose and similar dietary nutrients (Schutte et al., 1991; Doefler et al., 2000).

This study aimed to evaluate the short term effects of diets containing maize, high quality wheat or barley on the weight gain of broiler chickens, relative weights and length of digestive tract and the absorption function of small intestine. The hypothesis for the latter parameter was that the antinutritional factors of barley might interfere with the mechanisms responsible for the intestinal absorption of dietary nutrients. Absorption function of small intestine was measured by D-xylose absorption test.

Materials and Methods

The experimental protocol was in agreement with the standards for animal experiments approved by the Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Tehran.

Day-old Ross-308 male broiler chicks were kept in a controlled environment (temperature decreased gradually from 32 to 26°C) and received a commercial maize-soybean meal based starter diet until they were 14 d old. At this age, 30 chicks, in 3 groups of 10 birds with similar weights (430g ± 10) were randomly assigned to dietary treatments and were transferred to battery cages. The cages (90×60×40cm in dimensions) were located in a room with 23 h light/day and ambient temperature (± 24°C). All groups were provided one of 3 experimental diets varying in carbohydrate source until the end of the experiment (day 28). Experimental diets contained maize as the sole source of carbohydrate or substituted with high quality wheat or barley at the rate of 40% of total diet (Table 1). All diets were formulated to be iso-caloric and iso-nitrogenous and meet or exceed all required nutrients for the bird (NRC, 1994). Birds were given free access to fresh water as well as the diets, which were provided as mash.

On the last day of experiment (day 28), feed and water were removed from each group of the birds and the weight of remained food for each group was recorded. All birds were weighed individually and feed conversion efficiency (FI/WG) was calculated. Twelve hours after feed removal, all birds were given D-xylose solution (50mg/ml of de-ionized water, Fluka BioChemika 95731, Fluka Chemie AG 25 CH-9470 Buchs Switzerland) at the dose of 500 mg/kgBW via an oral gavage. One-blood sample before, and 5 others after the administration of the test material, were collected by wing (ulnar) vein puncture using heparinised micro-haematocrit capillary tubes (Code - No 9100260, Hirschmann Laborgerate Techcolor, Germany), on 30 minute intervals for 150 minutes. The tubes were centrifuged and plasma was collected. The concentration of D-xylose in plasma was measured according to the method of Eberts et al. (1979) and modified by Goodwin et al. (1985), using a spectrophotometer, (Model 6100, Jenway LTD, Felsted, Dunmow, CM6 3LB, Essex, England, UK) set at 554nm.

At the end of blood collection, all birds were sacrificed humanely by cervical dislocation and the gastro-intestinal tract (GIT) of each bird was quickly removed. The weights of empty proventriculus, gizzard, duodenum plus pancreas, jejunum plus ileum, and caecum were measured. The lengths of duodenum, jejunum plus ileum, and caecum were also recorded. Weights of digestive organs (g/100g BW) and length of intestinal parts (cm/100g BW) were expressed relative to the respective live body weight of the birds.

Statistical Analysis: Analysis of data was carried

Table 1: Ingredients and nutritional composition of the experimental diets. *, The vitamin and mineral premix supplied per kg of diet; Vit A 10000IU, Vit D3 2400IU, Vit E 18IU, Vit B1 1.8mg, Vit B2 6.6mg, Vit B3 (Calcium Panthotenate) 10mg, Vit B5 (Niacin) 30mg, Vit B6 3mg, Vit B12 0.015mg, Biotin 0.1mg, Folic Acid 1.0mg, Cholin Chloride 600mg, Vit K 2mg, Manganese 100mg, Iron 50mg, Zinc 84.7mg, Copper 16mg, Iodine 1mg, Selenium 0.2mg.

Diet Ingredients (gkg-1)	Barley Diet	Wheat Diet	Maize Diet
Maize	189	220	554
Wheat	-	400	-
Barley	400	-	-
Soybean Meal	315	294	356
Vegetable Oil	55	25	25
Di-Calcium Phosphate	15.5	17	17
Calcium Carbonate	12.5	9.5	9.5
Salt	3.6	3.6	3.6
Vitamin Premix*	2.5	2.5	2.5
Mineral Premix**	2.5	2.5	2.5
DL- Methionine (99%)	2.2	2.5	2.0
L- Lysine HCl (78%)	2.0	3.25	1.63
Sand	-	20	26
Nutritional Composition (calculated)			
Metabolisable Energy (kcalkg^{-1})	2880	2880	2880
Crude Protein	203	203	203
Total Fiber	48.3	37.4	37.1
Polysacchrides	39.2	31.7	29.4
Lignin and Others	9.1	5.7	7.7
Ether Extract	66.5	42.6	45.9
Methionine	5.3	5.3	5.3
Methionine + Cystine	8.6	8.6	8.6
Lysine	12.2	12.2	12.2
Threonine	7.5	7.5	7.5
Sodium	1.7	1.7	1.7
Calcium	8.8	8.8	8.8
Available Phosphorus	4.5	4.5	4.5

out using one-way analysis of variance (ANOVA) of Minitab system (Minitab 13.2 statistical package, Minitab Inc. State College, 2000). The treatment means of each variable were separated using the Fisher's LSD test.

Polynomial regression analysis was used to investigate the relationship between plasma D-xylose level (mg/dl) and time (min) using the following model (Kaps and Lamberson, 2004):

$Y_i = \beta 0 + \beta_1 x_i + \beta 2x^2 i + \varepsilon_i$

Where:

Y_i = observation i of dependent variable Y (D-xylose level)

x_i = observation i of independent variable x (time)

$\beta_0, \beta_1, \beta_2$ = regression parameters

ε_i = random error

All statements of significance was based on a probability of $p<0.05$.

Results

The influence of barley, wheat or maize as the main sources of dietary carbohydrates on WG, FI/WG of the experimental birds, the relative length of intestinal parts and the relative weight of digestive tract, is presented in Table 2.

Weight gain was lower in birds received barley in comparison to the birds received wheat or maize. However, there was no difference in FI/WG between the experimental groups.

No difference was noted in relative length of intestinal parts (cm/100g BW) among the experimental groups. There was also no difference in relative weight of digestive tract (g/100g BW) among the experimental groups.

Table 3, shows the level of plasma D-Xylose

Table 2: Influence of diets containing barley, wheat or maize as the main source of carbohydrate on the weight gain, feed efficiency, relative weight of digestive tract (g/100g BW) and relative length of intestine (cm/100g BW) in broilers from day 14 to 28. A,b; Means with different superscripts in each row are significantly different (p<0.05). SEM; Standard error of mean.

	Barley	Wheat	Maize	Prob	SEM
Weight Gain (g)	844a	925b	946b	0.015	24.3
Feed Intake/Weight Gain	1.66	1.62	1.59	0.640	0.048
Relative Length of Intestine					
Duodenum	2.09	1.90	2.01	0.132	0.064
Jejunum + Ileum	9.63	9.59	9.87	0.628	0.215
Cecum	1.13	1.12	1.13	0.976	0.037
Duodenum + Jejunum + Ileum	11.7	11.5	11.9	0.548	0.25
Relative Weight of Digestive Organs					
Proventriculus	0.432a	0.435a	0.489b	0.050	0.017
Gizzard	2.29	2.26	2.40	0.473	0.078
Duodenum + Pancreas	1.07	1.08	1.12	0.747	0.044
Jejunum + Ileum	2.76	2.86	2.89	0.536	0.086
Cecum	0.59	0.51	0.56	0.260	0.035

Table 3: plasma D-xylose level (mg/dl) of broilers (28 day old) after administration of D-xylose solution (500 mg/kg BW) on 30min interval, for 150min. Birds received diets containing barley, wheat or maize as the main source of dietary carbohydrate from day 14 to 28. *, Mean ± Standard Error of Mean (n = 10); 1, Regression coefficient for the quadratic fitted line.

	0min	30min	60min	90min	120min	150min
Barley	0	69.5 ± 3.91*	85.0 ± 3.27	73.1 ± 2.27	54.2 ± 2.41	42.6 ± 1.84
Wheat	0	74.4 ± 5.13	79.3 ± 4.92	69.1 ± 3.71	58.3 ± 3.29	43.7 ± 2.46
Maize	0	66.0 ± 4.92	87.8 ± 4.13	63.5 ± 5.20	44.1 ± 3.15	34.2 ± 2.50
Statistical Significance (Polynomial Regression Line Plot)						
	Linear		Quadratic			
	F ratio	P value	F ratio	P value	R2 value1	
Barley	4.9	0.032	187.4	0.001	0.79	
Wheat	5.3	0.025	109.0	0.001	0.69	
Maize	1.1	0.305	112.2	0.001	0.68	

(mg/dl) of experimental birds on 30min interval for 150min post-ingestion of D-xylose. Dietary sources of carbohydrates had no influence on the level of plasma D-xylose of experimental birds at each time of blood sampling. Plasma D-xylose showed similar trends of concentration for all groups. It reached to its peak at 60min post- ingestion of D-xylose solution and then gradually declined. Similar quadratic correlations were also noted between the concentration of plasma D-xylose and time among the experimental groups.

Discussion

The estimated nutritional composition of the experimental diets (Table 1) shows that the diet high in barley contains more NSP than maize or high quality wheat based diet. As mentioned before, the main part of NSP in barley is (1,3-1,4)-β-glucan (Jood and Kalra, 2001; Ravindran et al., 2007; Garcia et al., 2008).

Negative influence of barley on the weight gain of the birds in the study presented here was in agreement with the previous reports (Almirall et al., 1995; Von Wettstein et al., 2000; Jozefiak et al., 2006; Thomas and Ravindran, 2008).

Barley endosperm cell walls is mainly composed of water-soluble (1→3,1→4) -β-D-linked glucopyranosyl-monomers, known as β-Glucan. β-Glucan is responsible for the high viscosity of β-glucan solutions (Wang et al., 1992; Wood et al., 1994; Von Wettstein et al., 2000; Panahi et al., 2007).

It is known that dietary NSPs produce alterations to the intestinal mucosa and influence the morphology, weight and size of gastrointestinal tract (Iji, 1999; Yamauchi, 2002; Ao and Choct, 2004). It is hypothesized that the differences in performance generally observed between different cereal-based diets are partly related to differences in relative gastrointestinal tract size and intestinal morphology. However, the study presented here failed to show any difference in the relative size and/or weight of digestive organs among the experimental groups possibly because the duration of the experiment was not long enough (14 days) to produce such effects. Changes in the morphology of intestinal wall reported by others (Sharma et al., 1997; Yasar and Forbes, 2000; Mathlouthi et al., 2002; Yamauchi, 2002) are related to adaptive changes in the intestine and highly likely the long term effects on digestive and absorptive processes caused by dietary NSPs. Whereas, in short term, the paramount reasons for lower weight gain of the birds fed on barley when compared to maize or wheat, might be that the NSPs of barley in GI lumen slow the diffusion or mobility of enzymes and substrates to the absorptive surfaces due to high viscosity of digesta. The content of ?-glucan and other NSPs in the diet is positively correlated with gut viscosity and inversely related to nutrient utilization (Bedford et al., 1991; Annison, 1992; Li et al., 2004). Besides, Thomas and Ravindran (2008), reported that the performance differences observed between broilers fed diets based on different cereals were not related to differences in gastrointestinal tract weights or intestinal morphology, as these parameters were unaffected by the dietary cereal type.

Similar trends of plasma D-xylose concentration among the experimental groups in this study indicate that barley antinutrients had no effect on mechanisms responsible for the absorption and transfer of nutrients across the intestinal epithelium. In a study on man by Flourie et al. (1984), apple pectin did not alter the intestinal glucose dependent sodium transport. The authors suggested that pectin impaired intestinal absorption by means of an increased unstirred layer resistance.

It is known that dietary NSPs have negative effect on the physical mixing of dietary substrates and enzymes, thereby reducing rates of digestion and movement of nutrients from the lumen to the mucosal epithelium (Tovar et al., 1991; Brennan et al., 1996; Ellis et al., 1996). Viscous digesta produces laminar or 'stream-line' flow, rather than turbulent or disorderly flow, a characteristic of less-viscous fluids. As a result, viscous digesta alters efficient mixing of substrate-enzyme in the GI tract. Laminar-type mixing under viscous conditions would also reduce the rate at which nutrients are presented to the epithelial surface and are then absorbed (Ellis et al., 1996). Thus, β-glucans may impair intestinal absorption of nutrients by means of an enlargement of the unstirred water layer adjacent to epithelial cells (Flourie et al., 1984; Rainbird et al., 1984; Fuse et al., 1989).

In the study presented here, the diet containing 40 percent barley, had no effect on size, weight and the absorption capacity of the gut. Thus it can be concluded that, in short term, the primary mechanisms by which barley exerts its antinutritive properties would be highly likely a reduction in digestion of the dietary ingredients through an alteration in mixing of digestive enzymes with dietary substrates in the intestinal lumen and/or lower diffusion and mobility of nutrients to the absorptive surfaces due to digesta high viscosity.

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