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Intestinal carbohydrase activity and sodium-glucose transporter expression in layers fed diets containing wheat and rice brans supplemented with phytase

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ABSTRACT. The effect of diets containing wheat and rice brans with or without phytase supplementation on performance, intestinal carbohydrase activities and mRNA expression of sodium-glucose transporter-1 (SGLT-1) in layers was studied. A total of 96 23-wk-old Hy-line W-36 were allocated in a completely randomized experimental design at random with 6 treatments and 4 replicate cages of 4 birds each. A control diet was used in this experiment and then it was formulated to contain 10% wheat bran and 15% rice bran. Then phytase (2 g kg⁻¹; 1000 FTU kg⁻¹) was added to each diet. Feed intake, egg mass, feed conversion ratio, shell weight, shell thickness and Haugh units were not influenced by brans or phytase supplementation ($p > 0.05$). But egg production decreased in rice bran treatments ($p < 0.05$). In the jejunum, adding of phytase to control and diet containing wheat bran increased the concentration of sucrose ($p \leq 0.01$). Also, addition of phytase to control diet increased ($p \leq 0.001$) the mRNA expression of SGLT-1 in the duodenum. It was concluded that feeding layers with diets containing 10% wheat bran were practically feasible without compromising production performance, egg shell quality, and endogenous carbohydrase activity.

Keywords: bran, intestinal enzyme activity, layer hen, performance.

Atividade da carboidrase intestinal e expressão do transportador sódio-glicose em poedeiras alimentadas com dietas contendo farelo de trigo e arroz suplementado com fitase

RESUMO. Este estudo analisou o efeito de dietas para poedeiras contendo farelo de trigo e arroz suplementado ou não com fitase sobre o desempenho, atividade da carboidrase intestinal e expressão de mRNA do transportador sódio-glicose-1 (SGLT-1). Noventa e seis aves da linhagem Hyline W-36 com 23 semanas de idade foram distribuídas num delineamento experimental completamente randomizado com 6 tratamentos e 4 repetições de gaiolas com 4 aves cada. A dieta controle usada neste experimento foi formulada para conter 10% de farelo de trigo e 15% de farelo de arroz. Fitase (2 g kg⁻¹; 1000 FTU kg⁻¹) foi adicionada a cada dieta experimental. O consumo de ração, massa de ovos, razão de conversão alimentar, peso da casca, espessura da casca e unidade de Haugh não foram influenciados pelos farelos ou suplementação com fitase ($p > 0,05$). Mas a produção de ovos diminuiu nos tratamentos com farelo de arroz ($p < 0,05$). No jejuno, a adição de fitase e a dieta contendo farelo de trigo aumentaram a concentração de sacarose ($p \leq 0,01$). Além disso, a adição de fitase à dieta controle aumentou ($p \leq 0,001$) a expressão de mRNA do SGLT-1 no duodeno. Concluiu-se que o fornecimento de dietas para poedeiras contendo 10% de farelo de trigo é viável sem comprometer o desempenho da produção, qualidade da casca do ovo e a atividade da carboidrase endógena.

Palavras-chave: farelo, atividade de enzima intestinal, galinha de camada, desempenho.

Introduction

The effect of dietary phytate and phytase supplementation on the production performance and nutrient digestibility of poultry have received remarkable attention in the scientific studies (Liu, Ru, Li, & Cowieson, 2008a; Attia et al., 2012).

Recent studies have indicated that dietary phytate could cause remarkable negative effects on performance, endogenous secretion and energy utilization in chickens (Cowieson, Acamovic, & Bedford, 2004; Ravindran, Morel, Partridge, Hruby, & Sands, 2006; Cowieson & Ravindran, 2007; Liu

et al., 2008a; Liu, Ru, Cowieson, Li, & Cheng, 2008b). It has been demonstrated that phytate could decrease amylase activity and starch digestion *in-vitro* (Knuckles & Betschart, 1987) and *in-vivo* (Dilworth, Omoruyi, Simon, Morrison, & Asemota, 2004). Kishi, Tanaka, Igawa, Takase, and Goda (1999) have shown that when dietary sugar concentrations were elevated, increased expression of sodium glucose transporter-1 in the rat's jejunum. It is documented that phytate decreased the activities of disaccharidase, Na⁺K⁺-ATPase, and glucose concentrations in chickens intestine, but phytase enhanced the concentrations of amylase, sucrase, maltase, Na⁺K⁺-ATPase, and glucose (Liu et al., 2008b). Also, Kishi et al. (1999) have shown that diets containing increased phytate upregulated the mRNA expression of the sodium/glucose cotransporter gene in duodenum of chickens. The confirmation that stated dietary phytate inhibits sugar digestion and blood glucose in rats (Dilworth et al., 2004) and mice (Lee et al., 2006) resulted in this assumption that anti-nutritional factors capable of altering nutrient utilization might regulate associated gene expression (Liu et al., 2008b). Many studies have shown the effectiveness of phytase supplementation to improve the digestibility of phytate-bound P in poultry diets as well as reducing P excretion (Tangendjaja, Chung, & Broz, 2002). Several experiments have reported the efficacy of microbial phytase in laying hens and broilers diet (Mathlouthi, Larbier, Mohamed, & Lessire, 2002; Panda, Rao, Raju, & Bhanja, 2005; Cowieson & Ravindran, 2007; Liu et al., 2007; Liu et al., 2008a).

As by-products of the wheat and rice milling industry, wheat and rice brans are attractive alternative ingredients to the feed industry due to their moderate nutritional quality and economics. Due to low content of available phosphorous in these ingredients and the low endogenous phytase activity in birds (Rapp, Lantzsich, & Drochner, 2001; Zimmermann et al., 2002), diets must be supplemented with enzyme phytase. Also, with their high phytate P content, wheat and rice brans are excellent candidates for phytase application in diets.

The intent of this study was to evaluate the effect of diets containing wheat or rice brans with or without phytase on production performance, intestinal activity of amylase, sucrase, and maltase and the mRNA expression of SGLT-1 in layers.

Material and methods

All procedures used during this study were approved by Animal Care Committee of Bu-Ali Sina University, Hamedan, Iran.

A total of 96 23-wk-old Single Comb White Leghorn (SCWL) hens, Hy-line W-36, were allocated in a completely randomized experimental design with 6 treatments and 4 replicate cages (24 experimental units) of 4 birds per cage. Six experimental isonitrogenous and isoenergetic diets containing 0 or 10% wheat brans and 0 or 15% rice brans were formulated based on Hy-line nutrition guide (2005). A control diet (without brans), a control diet with Ronozyme NP (2 g kg⁻¹; 1000 FTU kg⁻¹), and four other treatments including 15% rice brans, 15% rice brans+enzyme, 10% wheat brans and 10% wheat brans+ enzyme. The exogenous 6-phytase enzyme used was produced by a genetically modified *Aspergillus oryzae* (Ronozyme NP -Razak Corporation Tehran, Iran). The commercial product had a phytase activity of 5000 FTU g⁻¹. Composition of the experimental diets presented in Table 1. Each experimental diet was offered for 12 weeks. The light was provided for 16 hours daily and the temperature of the barn was maintained at 23°C. All the birds were maintained under similar management conditions throughout the experimental period in 23-35 weeks of age.

Table 1. Ingredients and nutrient composition of the experimental diets (as-fed basis)¹.

	Basal diet	10% Wheat bran	15% Rice bran
Ingredients, %			
Corn	50.85	41.88	38.92
Soybean meal 45%	19.15	13.08	9.00
Wheat screening	15.00	15.00	15.00
Corn gluten	1.46	4.12	7.60
Wheat bran	0.00	10.00	0.00
Rice bran	0.00	0.00	15.00
Soybean oil	2.69	4.98	3.56
Dicalcium phosphate	1.92	1.84	1.89
Oyster shell	7.89	7.95	7.96
Sodium chloride	0.20	0.17	0.22
Mineral-vitamin premix ^{2,3}	0.50	0.50	0.50
DL-Methionine (%)	0.15	0.14	0.07
L-Lysine HCl (%)	0.00	0.13	0.20
Sodium bicarbonate	0.19	0.21	0.08
Total	100.0	100.0	100.0
Calculated composition			
Metabolizable energy, kcal kg ⁻¹	2800	2800	2800
Crude protein, %	16.00	16.00	16.00
Lysine (Digestible), %	0.77	0.77	0.77
Methionine (Digestible), %	0.43	0.43	0.43
Calcium, %	3.50	3.50	3.50
Crude fiber, %	3.50	4.00	4.31
Non-phytate phosphorous, %	0.50	0.50	0.50
Analysed composition			
Phytate, %	0.87	1.20	1.54

¹To evaluate the effect of enzyme, phytase (1000 UFT kg⁻¹) was added to diets. ²Vitamin premix supplied the following per kilogram of diet: thiamine hydrochloride, 3.3 g; riboflavin, 0.72 g; menadione dimethylpyrimidinol bisulfate, 1.6 g; DL- α -tocopherol acetate, 14.4 g; cholecalciferol, 7 g; retinyl acetate 7.7 g; D-calcium-pantothenate, 12 g; pyridoxine hydrochloride, 6.2 mg; vitamin B12, 14.4 g; choline, 440 mg. ³Mineral premix supplied the following per kilogram of diet: MnSO₄·H₂O, 64 g; ZnCO₃, 44 g; FeSO₄·7H₂O, 100 g; CuSO₄·5H₂O, 16 g; KI, 0.64 g.

Phytate contents of the experimental diets was measured based on the method of Thompson and

Erdman Jr. (1982), 2-g samples was placed in Erlenmeyer flask and 100 mL of 1.2% HCl + 10% Na₂SO₄) was added. Then flask was shaken for 2 hours. Then, the extract was vacuum filtered. Deionized water was added, followed by FeCl₃ solution (2g FeCl₃.6H₂O+ 16.3 ml of concentrated HCL). The contents were heated 75 min in boiling water. In the next stage, centrifuged at 1000 × g for 15 min. The supernatant was decanted and discarded, and pellet was thoroughly washed three times with a solution of 0.6 HCL and 2.5% Na₂SO₄. Concentrated HNO₃ and several portions of deionized water were added to the resulting pellet. The contents were approximately heated for 30 min. on hot plate. At about, 4-5 mL of H₂O₂ 30% was added. The residue was dissolved in 15 ml of 3N HCl and heated analyzed for iron.

Egg production and egg weight were recorded daily, and feed intake was recorded weekly. This information was used to calculate average daily feed intake, egg production, egg mass, and feed conversion ratio (FCR) (kg feed kg egg⁻¹). Egg mass was calculated by multiplying percentage egg production by egg weight for each replicate. Egg quality was measured in 6 eggs per replicate produced at two last days bi-weekly, and the average value for each period was used for further analysis. The eggs were individually weighed and the external and internal quality was determined. The shell was separated from the yolk and albumen weighed after drying overnight at 60°C as indicated by Grobas, Mendez, Lazaro, De Blas, and Mateo (2001). Shell thickness was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan). Haugh units were calculated from egg weight and albumen height as indicated by Haugh (1937).

The protein content of the intestinal samples (duodenum and jejunum) were measured according to the method described by Bradford (1976) and the data were used to calculate specific enzyme activities. Protein content of the tissue samples in the reaction mixtures were determined by Coomassie Blue using BSA (Sigma Chemical Co) as a standard. Data on enzyme activities were expressed in units per milligram of protein of intestinal tissue.

The activities of sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) in the intestine were detected based on the method by (Dahlqvist, 1984). Certain amount of duodenum and jejunum tissue weighed and phosphate buffer was added, then homogenized. Briefly, 0.1 mL of serum or mucosal supernatant incubated with 0.1 mL of 56 mM disaccharide substrate (sucrose or maltose) in 25 mM malate buffer at pH 6.4 for 30 min. at 37°C. The reaction

was stopped by heating in a boiling water bath for 10 min. min (Mohamed, Fahmy, & Salah, 2007). The amount of glucose was determined using Glucose Kit (ParsAzmun Co, Tehran, Iran). The amylase activity was measured with quantitative amylase Detection Kit (ParsAzmun Co, Tehran, Iran).

Jejunum and duodenum samples were rapidly discarded and frozen in liquid nitrogen then stored at -20 for total RNA extraction. The mRNA expressions of SGLT-1 in the duodenum and jejunum were analyzed by reverse transcription PCR. The total RNA of jejunum and duodenum scrapings was extracted by using an RNA extraction kit (RNX-plus solution, Cinnagen. Tehran), according to manufacturer's instructions. The optical density of RNA at 260 and 280 nm was measured by spectrophotometer. To evaluate the purity and concentration of RNA, 5 µg of total RNA was reverse transcribed using oligo (dT)₁₈ primers (2-steps RT-PCR Kit, Cinnagen. Tehran) according to manufacturer. A PCR was performed (2-steps RT-PCR Kit, Cinnagen. Tehran) by using a pair of primers (AJ 236903; forward; 5'-TGTTTGTCTTAGGATGGGTG-3', reverse; 5'-CAGTGACAGCATCTCGGAAG-3') according to manufacturer instructions. The PCR reaction used the following program: at 94°C for 2 min.; followed by 35 cycles of 94°C for 30 s, 48°C for 30 s, and 72°C for 40 s; and a final extension step of 72°C for 7 min. Reactions were electrophoresed on a 2.0% agarose gel and stained with ethidium bromide. Expression of SGLT-1 gene identified by the presence of specific band, whilst it lacked the 489-bp band. Reactions was measured by colorimetric method as measured with spectrophotometer (Patterson & Mura, 2013).

Data were analyzed as completely randomized designs using the GLM procedure (Statistical Analysis System [SAS], 2004). Treatment means were compared with Duncan's multiple range tests. All differences were considered significant at $p \leq 0.05$.

Results and discussion

Feed intake, egg mass, egg production, feed conversion ratio, shell weight, shell thickness and Haugh units were not influenced ($p > 0.05$) by treatments (Table 2). The effect of treatments on enzyme activities in the intestinal mucosa of layers is shown in Table 3. Enzyme activity in the duodenum of birds fed experimental treatments indicated no significant effect. In the jejunum, adding of phytase to control and wheat bran diets increased the concentrations of sucrase ($p < 0.05$).

Table 2. Production performance of layers in response to experimental diets.

	Basal	Basal + Enzyme ¹	10% Wheat bran	10% Wheat bran + enzyme	15% Rice bran	15% Rice bran + enzyme	SEMP	-value
Feed intake (g day ⁻¹ hen ⁻¹)	87.67	88.26	88.38	87.67	86.10	87.03	0.23	0.87
Egg mass (g day ⁻¹ hen ⁻¹)	48.01	49.49	48.45	48.69	46.91	47.46	0.26	0.06
Egg production (%)	87.03 ^a	88.92 ^a	86.49 ^a	86.65 ^a	82.06 ^b	82.72 ^b	0.65	0.001
Feed conversion ratio (kg feed kg egg ⁻¹)	1.95	1.90	2.17	1.95	1.99	1.93	0.04	0.48
Shell weight ³	5.52	5.47	5.37	5.40	5.58	5.52	0.16	0.66
Shell thickness (μm)	36.96	37.94	36.65	37.09	36.71	36.95	0.02	0.74
Haugh unit	100.12	100.30	100.40	100.20	100.92	101.97	0.29	0.41

¹Phytase (1000 UFT kg⁻¹) was added to diet. ³% of egg weight.

Table 3. Effect of experimental treatments on specific enzyme activities in the intestinal mucosa of layers (U mg⁻¹ protein).

	Duodenum			Jejunum		
	Amylase	Sucrase	Maltase	Amylase	Sucrase	Maltase
Basal	21.92	1.64	1.88	40.49	6.02 ^{bc}	7.77
Basal + enzyme ¹	22.31	1.65	1.64	42.63	6.52 ^a	7.91
10% wheat bran	21.85	1.58	1.86	39.21	5.79 ^c	7.76
10% wheat bran + enzyme	22.09	1.59	1.91	41.42	6.26 ^{ab}	7.89
15% rice bran	21.45	1.57	1.88	39.93	5.90 ^{bc}	7.68
15% rice bran + enzyme	22.14	1.57	1.90	40.78	6.18 ^{abc}	7.82
SEM	0.12	0.01	0.01	0.37	0.05	0.07
P-value	0.38	0.33	0.89	0.18	0.01	0.94

^{a-c}Means without a common superscript within a column differ ($p \leq 0.05$). ¹Phytase (1000 UFT kg⁻¹) was added to diet.

Effect of experimental treatments on the mRNA expression of sodium/glucose transporter-1 (SGLT-1) in the intestine of layers is shown in Table 4. Also, adding of phytase to control diet increased the mRNA expression of SGLT-1 in the duodenum ($p \leq 0.001$). No significant difference effect of experimental treatments was observed on the mRNA expression of SGLT-1 in the jejunum of layers.

Table 4. Effect of experimental treatments on the mRNA expression of sodium-glucose transporter-1 (SGLT-1) in the intestine of layers (μg μL⁻¹).

	Duodenum	Jejunum
Basal	0.71 ^b	0.96
Basal + enzyme ¹	0.89 ^a	1.02
10% wheat bran	0.68 ^{bc}	0.99
10% wheat bran + enzyme	0.67 ^{bc}	0.98
15% rice bran	0.70 ^b	1.01
15% rice bran + enzyme	0.59 ^c	0.94
SEM	0.01	0.01
P-value	0.00	0.64

^{a-c}Means without a common superscript within a column differ ($p < 0.05$). ¹Phytase (1000 UFT kg⁻¹) was added to diet.

Inclusion of wheat and rice brans into diet had no adverse effect on layers performance, except for egg production in diets containing 15% rice bran, which is in agreement with Tangendjaja, Chung, and Broz (2002) who showed that there were no statistically significant differences in hen-day production, egg weight, egg mass, feed intake, FCR, and number of cracked eggs in response to rice bran with or without phytase enzyme. The results of the present experiment are in accordance with the findings of Samli, Senkoylu, Akyurek, and Agma (2006) who stated rice bran could be included up to 10% without any adverse effect on laying

performance and egg quality, except for egg production which affected negatively by rice bran with or without phytase in current study.

It has been reported that supplementation of enzyme improved egg mass of layers fed wheat bran based diets, but it did not affect the performance of hens (Mathlouthi et al., 2002). Regarding inclusion of wheat bran, result of current study are in agreement with the results of Mathlouthi, Larbier, Mohamed, and Lessire (2002), but application of enzyme showed inconsistency owing to the difference in enzyme used, nutrient concentration, type of diet and birds age. The application of phytase in this study did not improved performance which disagreed with previous reports (Tangendjaja et al., 2002; Selle & Ravindran, 2007; Liu et al., 2008b; Attia et al., 2012). Liu, Ru, Cowieson, Li, and Cheng (2008b) reported that supplementation with phytase improve feed intake, body weight, and gain to feed intake ratio of broilers. It has been reported that phytate is capable of depressing enzymatic digestion *in-vitro* (Knuckles & Betschart, 1987) and *in-vivo* (Dilworth et al., 2004) but in present study this effect of phytate was not observed. Overall, it could summarize that the possible reasons for these contradictions as below: a) source and amount of enzyme, b) type of diets and nutrients density especially the available phosphorous contents of used diets, which had met the strain requirements. c) age and strain of birds.

In the current study, birds were fed a diet containing 0.5% non-phytate phosphorous, which is sufficient for laying hens. Other researchers (Gordon & Roland, 1997) did not observe any deficiency symptoms in hens fed diets containing 0.20 to 0.50%, non-phytate phosphorous and phytase supplementation of these diets gave no further improvements in performance. This observation is in accordance with current results. In this study, birds fed diets containing wheat and rice brans did not reduced activity of endogenous carbohydrase, which it is not in accordance with the results of Liu et al. (2008b) who reported a decrease in intestinal enzyme activity by phytate. They have stated that decreased enzyme activity may result in more nutrients passing through the digestive system

unabsorbed. Based on this study, adding of phytase had no significant effect on amylase, sucrase and maltase activity in the duodenum. The same trend has been demonstrated in jejunum except for sucrase which the addition of phytase to control diet and diet containing wheat bran increased the concentration of sucrase ($p < 0.05$). Regarding enzyme activity, current results are inconsistent with findings of Liu et al. (2008b). The well-known mechanisms reported by other researchers is inhibitory effect of phytate on the activity of digestive enzyme in the gastrointestinal tract of animals which include chelation with co-factors required for optimum enzyme activity, binding the digestion products, as well as forming phytate-protein complexes at pH below the isoelectric point of proteins (Katayama, 1997).

It is affirmed that glucose is the key monosaccharide available for absorption from most practical diets of animals and it is exposed to carrier-mediated transfer through the brush border by SGLT-1 (Wright, 1993). In current study, in diets containing wheat and rice brans, which were efficient in non-phytate phosphorous, the mRNA expression of SGLT-1 did not upregulate. The obtained results are in disagreement with Liu et al. (2008b), stated SGLT-1 of broiler intestine could be regulated by dietary phytate.

Conclusion

It was concluded that feeding layers with diets containing 10% wheat bran was practically feasible without compromising production performance, egg shell quality, intestinal carbohydrase activity and mRNA expression of SGLT-1. In non-phytate phosphorous efficient diets, wheat bran can safely use with or without phytase. However, phytase supplementation improved jejunal sucrase activity and the mRNA expression of SGLT-1 in the duodenum of laying hens. Furthermore, studies are required to confirm the effect of diet containing different levels of wheat or rice brans on performance and nutrient utilization in laying hens.

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