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The effects of Wheat with and without enzyme on apparent ileal digestibility coefficient of Ca, P, Cu, Mn, Fe, and Zn in Broilers

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ABSTRACT: The study was conducted on ninety six one-day Ross (308) broiler chicken to investigate the effect of wheat with and without enzyme supplementation on apparent ileal digestibility coefficient of Ca, P, Cu, Mn, Fe, and Zn in (Ross) Broilers. Chicks were allotted into 4 equal groups (24 chicks of mixed sex per group in metabolic cages). Two experimental diets were formulated, basal diet containing 10% and 15% wheat with and without enzyme supplementation from 1 to 21 day and 15% and 20% wheat with and without enzyme supplementation from 21 to 42 day together control diet. The results indicated that the diets contained wheat led to decrease in mineral digestibility. Enzyme supplementation improved the apparent ileal digestibility coefficient of minerals of broilers from 1 to 42 d.

Keywords: Enzyme, NSP, Broilers, apparent ileal digestibility coefficient of minerals.

INTRODUCTION

Corn is a major ingredient (above 50%) in broiler diets, contributing about 65% of broiler ME requirements. However, increasing corn prices by reason of its preferential diversion toward human consumption and corn ethanol industry have compelled the poultry industry to reduce reliance on corn. Despite numerous scientific investigations no alternative grain to corn has yet been identified. The use of wheat and grain in broiler feed is limited by the presence of soluble non-starch polysaccharides, particularly xylans, arabinoxylans and Beta- glucans components (Pourreza *et al.,* 2007). Majority of coarse cereals in poultry diets contains high fiber and low energy (Rama-Rao *et al.,* 2004). It is recognized that poultry diets contain variable levels of poorly digested NSP including arabinoxylans, Beta-glucons and pectin (Campbell and Bedford, 1992) that possess chemical cross-linking between them and minerals by poultry (Adams and Pough, 1993). Poultry produce a number of enzymes, including amylases to digest starch, proteases to digest protein and lipases to digest fats. Birds do not produce enzymes like cellulase, xylanase, required for the digestion of NSPs. However, they do not produce enzymes to digest fibers in feeds. Due to the chemical structure of plant cell wall matrix, NSP degrading enzymes has been recommended to enhance poultries mineral absorption. Soluble high molecular weight fiber polysaccharide complexes are responsible for high digesta viscosity. These complexes are only a fraction of the polysaccharides present in the digesta, and are made up of a number of different components. High digesta viscosity can lead to reduced feed intake, slower digesta passage rate and impaired nutrient digestion (Austin *et al.*, 1999; Naqvi and Nadeem, 2004). The inclusion of appropriate multi-enzyme systems can lower digesta viscosity and improve chick's digestibility. For common poultry diets, the enzymes of the digestive system cause normal hydrolysis of the dietary proteins, carbohydrates and fats. Thus, no benefit may be expected from the use of enzyme preparation as feed additives unless feed composed of higher amounts of wheat grain is fed to chickens (Banerjee, 1992). Enzyme supplementation is well documented as effective in breaking polymeric chains of NSPs and hence improving the nutritive value of feedstuffs (Giraldo *et al,* 2008). The main potential of enzyme addition to feed appears for digestion of substances that an animal is intrinsically incapable of digesting (Cheeke, 1991). These enzymes can open up the complex feed cell walls, allowing the animals own enzymes to digest the enclosed nutrients. Therefore adding NSP-degrading enzymes in poultry diets has increased considerably in recent years.

Supplementation of NSPs degrading enzymes may not only reduce the anti-nutritive effects of NSPs, but also releases some nutrients from these, which could be utilized by the birds (Balamurugan and Chandrasekaran, 2009). However, the effects of exogenous enzymes can be variable and it depends on a large number of factors such as the age of the bird and the quality and type of diet (Bedford, 2000 and Acamovic, 2001).

Therefore, the objective of the present study was to investigate the effects of enzyme on apparent ileal digestibility coefficient of minerals in broilers fed wheat-based diets.

MATERIALS AND METHODS

A. Experimental Design

This study was carried out at Animal Science Research Institute of Iran. A total of 96 one-day-old Ross broiler chicks were randomly allocated to 4 groups with 4 replicates containing 6 birds (3 males + 3 females) in metabolic cages.

The experimental design was CRD. Three experimental diets contain one control diet and two diets were formulated, basal diet containing 10% and 15% wheat (W1) without enzyme supplementation from 1 to 21 day and 15% and 20% wheat with (W1E and W2E) and without (W1 and W2) enzyme supplementation from 21 to 42 days. Formulation and composition of experimental diets are given in Table 1 and 2. Diets were designed in two phases. Combo® multi-enzyme was used contained 1000 unit phytase and 180 unit multi-glycanase activities per each gram.

Table 1: Composition of experimental diets (1-21d).

Ingredients (%)	W_1[3]	W_2[4]	W_2E[5]	Control
Corn	48.48	42.19	42.19	54.12
Wheat	10.00	15.00	15.00	-
Soybean meal	34.29	33.71	33.71	38.37
Sunflower oil	3.66	4.40	4.40	4.08
Dicalcium phosphate	1.85	1.86	1.86	1.83
Oyster Shell	0.66	0.65	0.65	0.66
Salt	0.15	0.14	0.14	0.19
Vitamin mix[1]	0.25	0.25	0.25	0.25
Mineral mix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.26	0.27	0.27	0.23
L-Lysine	0.16	0.18	0.18	0.03
Multi enzyme[6]	-	-	+	-
inert	-	1.10	1.10	-
Calculated composition of				
ANEn (kcal/kg)	3100	3100	3100	3100
Crude Protein	21.00	21.00	21.00	21.00
Calcium	0.95	0.95	0.95	0.95
Available Phosphorus	0.45	0.45	0.45	0.45
Methionine	0.45	0.45	0.45	0.45
Methionine+Cystein	0.95	0.95	0.95	0.95
Lysine	1.30	1.30	1.30	1.30
Sodium	0.16	0.16	0.16	0.16
Clorine	0.16	0.16	0.16	0.16
TNSP	11.75	11.75	11.75	11.75
SNSP	1.23	1.34	1.34	1.09
USNSP	10.52	10.41	10.41	10.66
Crude Fiber	4.79	4.63	4.63	5.05
NDF	15.57	14.98	14.98	15.92
Phytic acid	0.86	0.86	0.86	0.86

[1]Vitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 mg; pyridoxine HCl, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 mg; transretinyl acetate, 1892 mg; all-rac α-tocopheryl acetate, 11 mg; ethoxyquin, 125mg.
[2]Trace mineral mix provided the following (per kg of diet): manganese (MnSO4-H2O), 60 mg; iron (FeSO4-7H2O), 30 mg; Zinc (ZnO), 50 mg; copper (CuSO4-5H2O), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium (NaSeO3), 0.3 mg3 Dietary cation-anion balance.
[3]10%wheat without enzyme, [4] 15% wheat without enzyme, [5] 15% wheat with enzyme [6] Combo® multi-enzyme contained 1000 unit phytase and 180 unit multi-glycanase activities per each gram.

B. Mineral bioavailability

Feed and water offered ad libitum in all period of experiment. The lighting schedule was 23 h light per 1 h darkness at 320C in the first day. This was subsequently reduced 30C each week until week five.

Chromic oxide was included at 0.3 percent in all diets as indigestible marker. At the end of 21 and 42 days, 4 chicks per treatment were randomly selected and killed to obtain samples for measurement of intestinal Cr_2O_3 and mineral concentration. Whole ileal digesta were individually collected, and measured for Cr_2O_3 and mineral concentration. Chromic oxide content of diets and the digesta was measured by spectophtometry method as reported by Fenton and Fenton (1979).

Apparent ileal mineral digestibility in experimental diets was calculated using the following equation (Kadim and Mougham, 1997)

DP=1-[(Dietary Cr_2O_3 Cont./Fecal Cr_2O_3 Cont.) × (Fecal mineral Cont./Dietry mineral Cont.)]

Means of 4 observations were considered to statistical analysis.

Table 2: Composition of experimental diets (22-42d).

Ingredients (%)	W_1[3]	W_2[4]	W_2E[5]	Control
Corn	45.47	42.62	42.62	64.45
Wheat	15.00	20.00	20.00	-
Soybean meal	31.34	29.31	29.31	28.33
Sunflower oil	5.03	4.83	4.83	3.95
Dicalcium phosphate	1.60	1.61	1.61	1.63
Oyster Shell	0.62	0.62	0.62	0.63
Salt	0.25	0.25	0.25	0.16
Vitamin mix[1]	0.25	0.25	0.25	0.25
Mineral mix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.19	0.20	0.20	0.23
L-Lysine	-	0.06	0.06	0.13
Multi enzyme[6]	-	-	+	-
inert	-	-	-	-
Calculated composition of				
ANEn (kcal/kg)	3200	3200	3200	3200
Crude Protein	19.00	19.00	19.00	19.00
Calcium	0.85	0.85	0.85	0.85
Available Phosphorus	0.42	0.42	0.42	0.42
Methionine	0.40	0.40	0.40	0.40
Methionine+Cystein	0.85	0.85	0.85	0.85
Lysine	1.10	1.10	1.10	1.10
Sodium	0.16	0.16	0.16	0.16
Clorine	0.16	0.16	0.16	0.16
TNSP	11.56	11.56	11.56	10.66
SNSP	1.28	1.35	1.35	0.83
USNSP	10.28	10.21	10.21	9.83
Crude Fiber	4.59	4.46	4.46	4.72
NDF	15.22	15.04	15.04	16.27
Phytic acid	0.85	0.85	0.85	0.79

[1]Vitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 mg; pyridoxine HCl, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 mg; transretinyl acetate, 1892 mg; all-rac α-tocopheryl acetate, 11 mg; ethoxyquin, 125mg.
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[3]15%wheat without enzyme, [4] 20% wheat without enzyme, [5] 20% wheat with enzyme [6] Combo® multi-enzyme contained 1000 unit phytase and 180 unit multi-glycanase activities per each gram.

C. Statistical analysis

All data were analyzed for normal distribution using one-sample kromogrov-smirnov procedure of SPSS10 software. A completely randomized design was employed. Duncan's multiple range test were used for comparison of means ($P<0.05$).

RESULTS AND DISCUSSION

The effect of dietary treatments on chicken mineral digestibility response is shown at Table 3 and 4. Table 3 showed the effect of diets on chicken mineral digestibility at 21 days of age and Table 4 showed the effect of diets on chicken mineral digestibility at 42 days of age. The results indicated that the diets contained Wheat led to decrease in mineral digestibility. Soluble NSPs of wheat including xylans and β-glucans, the principal water-soluble non-starch polysaccharides (NSP), limits digestion of nutrients (Yin *et al.*, 2000;Lin *et al.*, 2010; Mirzaie *et al.*, 2012). Several reports indicated that water soluble NSPs have more deleterious impact on physicochemical properties and microbial proliferation of digesta in the intestinal tract. These types of NSPs creates ideal environment for maximum proliferation of bacteria. A slow moving digesta with low oxygen tension could provide a stable media where fermentative microbes such as anaerobic bacteria can establish. These microbial changes result in reduced minerals available for host and produces of detrimental byproducts (Langhout *et al.*, 1999; Jaroni *et al.*, 1999).

Table 3: Duncan's Multiple Range Test for Apparent Ileal Digestibility Coefficient of Ca, P, Cu, Mn, Fe, and Zn (21D).

Tr	Ca	P	Cu	Mn	Fe	Zn
W1	0.53	0.59	0.55^{c}	0.58b^{c}	0.41	0.32^{b}
W2	0.50	0.53	0.50^{d}	0.54^{c}	0.39	0.28^{b}
W2E	0.59	0.63	0.67^{a}	0.69^{a}	0.39	0.43^{a}
Control	0.58	0.66	0.60^{b}	0.64^{a}	0.44	0.36^{ab}
P value	0.449	0.152	0.0001	0.011	0.768	0.037
SE	0.0061	0.0539	0.0582	0.0709	0.0645	0.0508

Means with the same letter are not significantly different.

Table 4: Duncan's Multiple Range Test for Apparent Ileal Digestibility Coefficient of Ca, P, Cu, Mn, Fe, and Zn (42D).

Tr	Ca	P	Cu	Mn	Fe	Zn
W1	0.46	0.53	0.37	0.48	0.27	0.19
W2	0.39	0.51	0.34	0.47	0.19	0.18
W2E	0.47	0.55	0.40	0.49	0.28	0.20
Control	0.48	0.54	0.40	0.49	0.29	0.20
P value	0.114	0.998	0.839	0.994	0.479	0.978
SE	0.0140	0.0549	0.0232	0.0219	0.0243	0.0188

Means with the same letter are not significantly different.

Enzyme supplemented Wheat diets with multi-enzymes resulted in increase in mineral digestibility. The presence of xylans and β-glucans increases the digesta viscosity, motivates the gut microbial propagation resulted to produces high amount of short chain fatty acids which lowers digesta pH (Jaroni *et al.,* 1999; Choct *et al.,* 2006).

It is well documented that supplementing exogenous enzymes to diets for broilers can improve mineral digestibility (Peng, 2003; Wang *et al.,* 2005). The effects of dietary enzyme supplementation on Cu, Mn and Zn Significantly was better ($P<0.05$). The improved mineral digestibility may be due to lowered viscosity and/or disruption of cell wall (Vukic-Vranjes and Wenk, 1995; Choct *et al.,* 1996). However, there was non-significant difference in mineral digestibility of broiler chicks of all groups during finisher phase ($P<0.05$).

During the ileal phase, enzymes remove fermentable substrates, during the cecal phase, degradation products of sugars, such as xylose and xylo-oligomers, are fermented by cecal bacteria, thus stimulating the production of VFA and the growth of specific beneficial bacteria (Bedford, 2000b). According to Bedford and Classen (1992), low level of enzyme could in fact accumulate high-molecular weight soluble NSP in the hind gut section of broilers and reduce mineral absorption.

Graham *et al.* (1993) reported that high digesta viscosity blocked the digestive enzyme efficiency in the gut and reduced the absorption of minerals. Reults reported in Table 3, indicated that wheat diets have more deleterious impacts on bioavailability of Cu, Mn and Zn.

Supplementation of diets with exogenous enzymes releases the encapsulated nutrients and reduces digesta viscosity. These processes are further facilitated by the action of phytases (Ravindran *et al.,* 1999; Slominski, 2011).

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