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Inclusion a Multi-Enzyme (Natuzyme Plus®) in Broiler Chicken Diets Containing High Canola Meal

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Abstract: The response of broiler chickens was investigated to Natuzyme Plus® with canola meal. A total of 380 unsexed 1-d-old broiler chickens (Ross 308) assigned to a 2 × 3 factorial arrangement as completely randomized design with 6 treatments of 4 replicate for each treatment. Treatments were inclusion of canola meals (0, 6, or 12 %) and enzyme (with 0.35 gkg^{-1} Natuzyme Plus® or without enzyme) in broiler chicken diets. Body weight gain of broiler chickens decreased by the inclusion of 6 and 12% of canola meal in diets, while feed conversion ratio improved by the inclusion of 12% of canola meal in diets from 1 to 21 d of age ($P<0.05$). Dietary inclusion of enzyme increased body weight gain of broiler chickens ($P<0.05$). Except control, the inclusion of enzyme in diets containing canola meal increased body weight gain rather diets without enzyme ($P<0.05$). The inclusion of enzyme in broiler chicken diets increased ileal digestibility of dry matter, organic matter and crude protein ($P<0.05$). Natuzyme-supplemented diets containing canola meal improve growth performance slightly compared with un-supplemented diets. The use of canola meal at level of 6% and canola meal with enzyme at level of 12% was suitable.

Key words: Natuzyme Plus® · Broiler performance · Canola meal · Digestibility

INTRODUCTION

Canola is an offspring of rapeseed that was bred to have low levels erucic acid (<2%) in the oil portion and low levels of glucosinolates (<30 ìmol/g) in the meal portion [1]. The production of new cultivars of canola with low erucic acid and low glucosinolate lead to increase the use of canola as a canola meal in poultry diets [2, 3]. Canola meal is a protein source with a good balance of amino acids and a lower amino acid digestibility than soybean meal [1]. Their nutritive values are limited by the presence of several anti-nutritive factors (e.g. non starch polysaccharides (NSP), tannins, glucosinolates and phytic acid [1,3-6]. The major NSP components of canola meal are pectic polysaccharides, including rhamnogalacturonan with associated side chains consisting of arabinose, galactose and xyloseresidues [7]. Other polysaccharides of canola meal are cellulose, xylans, arabinoxylans and xyloglucans. Higher fiber content of the canola meal [8] resulted in lower energy content [2-4]. The inclusion rate of canola meal in poultry diets is limited [9], because of their anti-nutritional components which interference with metabolism and growth, induced liver hemorrhages, lower digestibility of nutrients [1, 3, 10-14] increased leg problems, litter problems [15] and growth performance [1, 10-14, 16-18].

Many studies have clearly demonstrated that the use of exogenous enzymes in diets propose as effective way to increase the digestibility of complex molecules especially in young animals, which do not have a well-developed intestinal enzyme profile [9, 11, 19]. It is reported that the negative effects of non-starch polysaccharides (NSP), oligosaccharides and phytic acid can overcome by the supplementation of diets with suitable exogenous enzyme [20]. It is very beneficial to hydrolyze NSP, reduce digesta viscosity, improve nutrient absorption and growth performance [10, 20, 21]. However, limited information is available on the ability of commercial enzyme products to improve canola meal quality. Because of differences in composition and structure of anti-nutritional factors in local canola meal, it seems that using multiple enzymes would be the most beneficial for improving the performance of broiler chickens [22]. Therefore, the present study was conducted to investigate the effects of a commercial multi-enzyme [23]

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on performance, morphological parameters, nutrients digestibility and carcass traits of broilers fed diets containing canola meal substituted with soybean meal.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Ethics Committee of the Islamic Azad University. In addition, the broiler chickens used in the experiment were cared based on guidelines of Animal Care (Chalous Branch, Iran).

Experimental Design and Birds: A 2 × 3 factorial arrangement of treatments with total of 380 unsexed 1-d-old broiler chickens (Ross 308) was conducted as completely randomized design. Broilers were randomly divided to 6 treatments with 4 replicate for each treatment. Treatments were including of the inclusion of different levels of canola meals (0, 6, or 12 %) and enzyme (with 0.35 gkg^{-1}Natuzyme Plus ®or without enzyme) in broiler chicken diets. Diets were designed from1 to 21 d of age based on National Research Council [4] recommendations to meet their nutrient requirements (Table 1). Feed (as mash) and water were offered *ad libitum*. The lighting schedule was 23 h light / 1 h darkness at 32°C at the first day. This was subsequently reduced 3°C each week. Body weight gain (BWG) and feed intake (FI) was measured. Feed conversion ratio (FCR), mortality and production index (PI) were measured accordingly.

Carcass Characteristic, Organ and Morphology Assay: On d 21, final body weights of broiler chicks were measured, 2 birds from each replicate were randomly selected and tagged and they were fasted for 8h (no limitation of water access). Birds were weighted and slaughtered by exsanguination. Carcass weights were measured after removal of feather, head, legs and abdominal contents. Proventriculus, gizzard, pancreas, liver and abdominal fat were dissected and recorded. The breast and thighs weights were calculated as the percentage of fasted live body weights. Immediately after dressing, the gastrointestinal tract (GIT) of slaughtered broilers was removed. The digestive tracts between the gizzard and bile duct and from bile duct to Meckel's diverticulum were removed and considered as duodenum and jejunum, respectively. The ileum was isolated as the section between Meckel's diverticulum and the ileocecal junction. The ceca also removed. Length and weight of all mention segments were recorded.

Table 1: The formulation and composition of diets (gkg^{-1} as feed/or as stated) from 1 to 21 d

Items	T1	T2	T3
Ingredients			
Corn grain	633.6	619.6	605.7
Soybean meal (46% crude protein)	300.0	240.0	180.0
Canola meal	00.0	60.0	12.0
Soybean oil	12.8	19.8	26.8
Calcium carbonate	19.0	18.6	18.2
Dicalcium phosphate	5.5	5.1	4.6
Common salt	3.0	3.0	3.0
Min-VitPremix^{z}	5.00	5.00	5.00
DL-methionine (98%)	1.9	1.8	1.7
L-Lysine HCl	1.9	2.7	3.5
Total	1,000	1,000	1,000
Calculated			
Metabolisable energy (kcalkg^{-1} DM)	3,000	3,000	3,000
Crude protein	216	216	216
Calcium	9.4	9.4	9.4
Available phosphorus	4.6	4.7	4.6
Methionine	6.1	6.1	6.1
Lysine	10.8	10.6	11.2
Methionine + Cysteine	8.4	8.4	8.4

T1, T2 and T3 are diets containing 0, 6 and 12% canola meal, respectively. For make of other diets Natuzyme Plus® was added as 0.35 g kg^{-1} in corresponding diets.

Natuzyme Plus® provided per kilogram of complete feed: phytase, 105,000 U; β-glucanase, 350,000 U; α-amylase, 262,500 U; cellulose, 1,470,000 U; pectinase, 24,500 U; xylanase, 1,750,000 U; and protease,1,050,000U.

^{z}Mineral premix (mg kg^{-1}of diet): Mn, 80 mg; Zn, 84.5 mg; Fe, 80 mg; Cu, 5 mg; I, 1.0 mg; Co, 0.48 mg; Se, 0.30 and vitamin premix: vitamin A, 11,000 IU (retinol); vitamin D3, 3,000 IU; vitamin E, 50 mg (*DL*-α-tocopheryl acetate); vitamin K3, 5 mg; tiamin, 2 mg; riboflavin, 8 mg; calcium pantotenat, 12.40 mg; niacin, 50 mg; pyridoxine, 7 mg; pholic acid, 2 mg; vitamin B12, 1.60 mg; biotin, 5 mg; choline chloride, 1,100 mg; antioxidant, 100 mg kg^{-1} of diet.

Light Microscopy: On d 21, the middle sections of jejunum (3-4 cm) of two birds from each replicate were cut and histological indices were measured according to Iji *et al*. [10] method. Formalin-fixed jejunal tissue samples were dehydrated, cleared, impregnation with paraffin. The processed tissue was then embedded in paraffin wax. Section were cut (6 μm) from the waxed tissue on LEICA RM 2145 microtome, cleared of wrinkles by floating on warm water (55- 60°C) prior to mounting on 10% poly -L-lysine coated slides. The slides were stained by haematoxylin and eosin. Histological indices were determined by use of a computer-aided light microscopic image analyzer (Motic Images, 2000 1.2, Scion Image). Villous height (from top of villous to the crypt opening), crypt depth (from the base of the crypt to the level of

crypt opening) were measured and calculation was made for villous height: crypt depth rate (villous index). Used values for analysis were means from 5 adjacent, vertically oriented villous-crypt units per section.

Nutrients Digestibility: To determine nutrients digestibility, a balance trial (18 to 21 d of age) was conducted using titanium oxide (TiO_2; 3 g kg^{-1}) [25]. On d 21, after 3 d adaptation, 2 more birds from each replicate were sacrificed and the ileal digesta between the yolk sac and the terminal ileum (2 cm above the ileucecal junction) removed and stored at -20°C until further processing. Both diets and obtained freeze-dried digesta were ground through a 0.5-mm screen and stored at 4°C until analysed for the nutrient contents. Titanium was determined according to the method described by Short *et al.* [25]. In brief, samples were ashed before digestion in 60% sulfuric acid (v/v). The mixture was incubated in 30% H_2O_2 and absorbance was read at 405 nm by an atomic absorption spectrometer (AA 670; Shimadzu, Kyoto, Japan). The coefficients of apparent ileal digestibility were calculated according to the standard digestibility equation.

Statistical Analysis: All data were analysed as a 3×2 factorial arrangement of treatments with the pen being considered as the experimental unit using the GLM procedure [26]. The model included the effects of different levels of canola meal, enzyme addition and their interaction. The P-value of = 0.05 was considered as significant.

RESULTS

The effects of the dietary treatments on broiler chicken performance from 1 to 21 d of age are presented in Table 2. No significant difference was observed in feed intake (FI) and mortality. In addition, BWG and PI of broiler chickens decreased by inclusion of 6 and 12% of canola meal in broiler chicken diets and FCR by the inclusion of 12% of canola meal in broiler chicken diets (P<0.05). The inclusion of enzyme in broiler chicken diets increased BWG and PI of broiler chickens (P<0.05). Except control, the inclusion of enzyme in diets containing canola meal increased BWG and PI rather diets containing canola meal without enzyme (P<0.05). There was no pronounced effect of dietary treatments in FCR (P<0.05). There were no significant differences in the length of duodenum, jejunum, ileum and ceca by the inclusion of canola meal and enzyme in broiler chicken diets at d 21 (Table 3).

Table 2: The feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), mortality and production index (PI) of broiler chickens in response to diets from 1 to 21 d of age

Item	FI (gd^{-1})	BWG (gd^{-1})	FCR	Mortality (%)	PI
Canola meal (%)					
0	59.62	39.15^{a}	1.53^{a}	1.25	181.63^{a}
6	53.95	35.83^{b}	1.51^{a}	2.50	162.76^{b}
12	49.41	35.49^{b}	1.40^{b}	2.50	160.14^{b}
SEM	0.266	0.262	0.013	0.489	1.61
P-value	<0.0001	0.004	0.044	0.76	0.007
Enzyme					
-	52.98	35.54^{b}	1.50	2.50	160.78^{b}
+	55.67	38.11^{a}	1.46	1.67	175.57^{a}
SEM	0.177	0.175	0.009	0.326	1.080
P-value	0.006	0.008	0.45	0.61	0.012
Interaction					
0-	60.66	39.98^{a}	1.47^{ab}	2.5	180.72^{a}
0+	60.58	38.33^{a}	1.58^{a}	0.00	182.54^{a}
6-	52.84	33.87^{b}	1.56^{a}	5.00	145.57^{b}
6+	55.05	37.79^{a}	1.46^{ab}	0.00	179.96^{a}
12-	47.46	32.77^{b}	1.45^{ab}	0.00	156.05^{b}
12+	51.37	38.20^{a}	1.34^{b}	5.00	164.23^{ab}
SEM	0.532	0.525	0.026	0.977	3.239
P-value	0.61	0.008	0.003	0.05	0.049

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of themeans.

Table 3: Effect of dietary treatments on the length (cm) of intestinal segments of broiler chickens at d 21

Item	Duodenum	Jejunum	Ileum	Ceca
Canola meal (%)				
0	3.68	9.16	9.45	1.92
6	3.81	9.46	9.55	1.97
12	3.97	9.17	9.38	1.95
SEM	0.073	0.121	0.149	0.027
P-value	0.61	0.78	0.96	0.89
Enzyme				
-	4.03	9.31	9.49	2.04
+	4.61	9.22	9.42	1.85
SEM	0.049	0.081	0.099	0.018
P-value	0.09	0.82	0.89	0.05
Interaction				
0-	3.95^{ab}	9.19	9.57	2.01
0+	3.41^{b}	9.12	9.32	1.83
6-	3.88^{ab}	9.41	9.39	1.13
6+	3.75^{ab}	9.51	9.71	1.81
12-	4.28^{a}	9.32	9.52	1.97
12+	3.66^{ab}	9.03	9.24	1.92
SEM	0.146	0.243	0.287	0.055
P-value	0.67	0.92	0.85	0.46

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of themeans.

Table 4: Effect of dietary treatments on the weight (g) of intestinal segments of broiler chickens at d 21

Item	Duodenum	Jejunum	Ileum	Ceca
Canola meal (%)				
0	1.40	4.58	3.25	0.37
6	1.41	4.70	3.37	0.32
12	1.43	4.56	3.16	0.42
SEM	0.029	0.075	0.052	0.013
P-value	0.97	0.87	0.60	0.17
Enzyme				
-	1.49	4.62	3.27	0.39
+	1.34	4.61	3.25	0.36
SEM	0.019	0.050	0.034	0.009
P-value	0.12	0.98	0.89	0.48
Interaction				
0-	1.41	4.66	3.28	0.36
0+	1.39	4.50	3.19	0.38
6-	1.50	4.68	3.34	0.35
6+	1.32	4.73	3.40	0.30
12-	1.56	4.51	3.18	0.45
12+	1.30	4.60	3.14	0.40
SEM	0.058	0.149	0.104	0.026
P-value	0.59	0.90	0.92	0.70

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of themeans.

Table 5: Effect of dietary treatments on the weight (g) of organs and abdominal fat of broiler chickens at d 21

Item	Liver	Pancreas	Abdominal fat	Proventriculus	Gizzard	Crop
Canola meal (%)						
0	3.24^{b}	0.41^{a}	1.30	0.71	2.42	0.58
6	3.26^{b}	0.51^{a}	1.41	0.72	2.44	0.59
12	3.83^{a}	0.49^{b}	1.52	0.73	2.40	0.60
SEM	0.055	0.010	0.054	0.011	0.032	0.017
P-value	0.025	0.048	0.59	0.88	0.96	0.95
Enzyme						
-	3.47	0.48	1.47	0.72	2.42	0.59
+	3.42	0.46	1.34	0.72	2.42	0.58
SEM	0.037	0.007	0.036	0.007	0.021	0.011
P-value	0.78	0.47	0.48	0.81	0.95	0.83
Interaction						
0-	3.25	0.44	1.27	0.71	2.41	0.59
0+	3.23	0.38	1.32	0.70	2.43	0.56
6-	3.31	0.52	1.41	0.71	2.44	0.58
6+	2.21	0.49	1.40	0.73	2.44	0.59
12-	3.84	0.48	1.73	0.75	2.41	0.61
12+	3.81	0.50	1.31	0.71	2.40	0.59
SEM	0.110	0.020	0.108	0.022	0.064	0.034
P-value	0.98	0.64	0.51	0.85	0.99	0.94

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of the means.

Table 6: Effect of dietary treatments on the weight (g) of carcass of broiler chickens at d 21

Item	Carcass	Breast	Thighs
Canola meal (%)			
0	71.67	13.97	20.34
6	70.95	13.36	18.21
12	69.87	12.53	18.35
SEM	0.183	0.35	0.238
P-value	0.07	0.59	0.67
Enzyme			
-	70.59	14.32	18.40
+	71.06	12.24	19.53
SEM	0.122	0.233	0.158
P-value	0.43	0.08	0.17
Interaction			
0-	71.86	14.91	19.48^{ab}
0+	71.48	13.03	21.20^{a}
6-	69.46	15.22	17.57^{b}
6+	70.27	11.49	18.84^{ab}
12-	70.45	12.84	18.15^{b}
12+	71.44	12.21	18.52^{ab}
SEM	0.366	0.699	0.476
P-value	0.60	0.55	0.77

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of the means.

Table 7: Effect of dietary treatments on morphology characteristic of jejunum of broiler chickens at d 21

Item	Villi height (im)	Crypt depth (im)	Villi index
Canola meal (%)			
0	1160.06	150.30	7.82
6	1107.54	136.16	8.32
12	1012.63	134.86	7.58
SEM	17.445	2.405	0.185
P-value	0.13	0.23	0.60
Enzyme			
-	1060.42	138.81	7.63
+	1126.41	142.07	8.18
SEM	11.630	1.603	0.123
P-value	0.26	0.68	0.38
Interaction			
0-	1099.24	138.35	7.94
0+	1220.88	162.26	7.67
6-	1116.70	139.54	8.01
6+	1098.38	132.77	8.62
12-	965.30	138.53	6.94
12+	1059.97	131.18	8.21
SEM	34.890	4.093	0.370
P-value	0.58	0.20	0.59

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of themeans.

Table 8: The coefficient ileal digestibility of nutrients of broiler chickens in response to diets at d 21

Item	Dry matter	Organic matter	Ether extract	Crude Protein
Canola meal (%)				
0	73.10	65.88	62.00	70.30
6	70.69	66.80	63.87	66.93
12	70.79	63.90	62.21	66.17
SEM	0.455	0.388	0.686	0.581
P-value	0.35	0.19	0.76	0.07
Enzyme				
-	69.56[b]	63.33[b]	62.02	65.76[b]
+	73.49[a]	67.73[a]	63.37	69.83[a]
SEM	0.303	0.259	0.457	0.388
P-value	0.016	0.003	0.56	0.046
Interaction				
0-	72.55	64.89	63.06	67.56
0+	73.65	66.88	60.94	73.03
6-	67.07	64.36	62.16	68.06
6+	74.30	69.24	65.58	65.79
12-	69.05	60.74	60.84	61.67
12+	72.52	67.06	63.67	70.67
SEM	0.910	0.777	1.372	1.163
P-value	0.26	0.38	0.56	0.071

Means with different superscripts in same column are different ($P < 0.05$).
SEM: standard error of the means.

However, the inclusion of enzyme in diets containing canola meal decreased duodenum length rather diets containing canola meal without enzyme (P<0.05). No significant interactions were observed in other parameters. Moreover, no significant effects were observed between treatments on the weights of intestinal segments (Table 4). Liver weight increased by the inclusion of 12% canola meal in broiler chicken diets (P<0.05). In contrast, the pancreas weight decreased by the inclusion of 12% canola meal in broiler chicken diets (Table 5; P<0.05). No significant effects were observed in other parameters and between diets. Table 6 showed that the inclusion of different levels of canola meal and enzyme in broiler chicken diets had no significant effects on carcass, breast and thighs weights. In addition, there were no significant interactions on carcass and breast weights. However, the inclusion of enzyme in broiler chicken diets containing canola meal increased thighs weights rather diets containing canola meal without enzyme (P<0.05). No significant effects were observed between diets on morphology characteristic of jejunum (Table 7). Table 8 showed that the inclusion of enzyme in broiler chicken diets increased ileal digestibility of dry matter, organic matter and crude protein on d 21 (P<0.05).

DISCUSSION

Canola meal has several anti-nutritional factors [1, 3-6], so could induce significant reduction in broiler performance. A finding that could help to explain lower BWG and PI of broiler chickens fed the diets containing canola meal (6 and 12%). High levels of NSP and phytate content are important factors (2, 7, 27, 28]. In this regards, it was reported that fiber may interfere with protein and mineral digestion [10], elevated digesta viscosity [29, 30, 31] and phytate binds to divalent cations as well as to amino acids and reduces protein and mineral availability lead to reduced nutrient digestion, absorption and performance. Reduction in performance with high levels of canola meal in young broilers was also reported by other researchers [3, 32, 33]. Probably lack of effect of canola meal on FI may attribute the cause of the low glucosinolate of tested canola meal. Lesson *et al.* [34] found that even complete replacement of soybean meal (100%) with canola meal didn't affect FI of broilers laying hens. The inclusion of enzyme in diets reduce digesta viscosity [10] and in turn improve broiler chicken performance [35], a finding that may explain better BWG obtained by enzyme addition in diets in the current study. In addition, the inclusion of Natuzyme in diets containing canola meal increased BWG and PI rather diets containing canola meal without Natuzyme supplementation. Inclusion of enzyme in piglet diets containing canola meal lead to increases in FI [36] which could lead to increases in BWG. It seems that Natuzyme with several enzymes degrading phytate and other anti-nutritional factors of canola meal increased BWG and PI. These results are in agreement with the observation of Cowan [37] but are in contrast to the result of Kocher *et al.* [9]. No significant difference of the diets on mortality cold implies that the tested materials were safe, which is in agreement with other researchers [38, 39].

Except the length of duodenum, the diets (inclusion of different levels of canola meal, enzyme addition and their interaction) had no effects on the weights and lengths of intestinal segments. It is reported that [40, 41] diets containing fiber material (wheat and barley) increased duodenum length in broiler chickens. It appears that increased duodenum length is effort to increasing expose of nutrition to digestive enzymes. Actually, broilers adapt to presence of high level of insoluble fiber consumption and anti-nutritional material by gastrointestinal tract enlargement and naturally their increased weights and length. Positive effects of Natuzyme inclusion in the diets was observed by reduce

the length of duodenum in the present study. The short time of experiment may be the reason of uninfluenced of the length of other segments by tested diets.

The liver weight increased and the pancreas weight decreased by the inclusion of 12% canola meal in broiler chicken. The liver is responsible of refining toxins body and biliary secretions. It seems that anti-nutritional materials in canola meal at levels of 12% could have detrimental effects on the absorption of nutrients. The use of diets containing anti-nutritional materials led to enlargement of liver [42]. On the other hand, increase in the pancreas weights may be an attempt to enhance ability of birds to cope with effects of anti-nutritional materials of canola meal, especially NSP. Increase in pancreas weights was also reported because of viscose material [43].

There is limited information regarding carcass traits of broiler chickens when fed by diets containing canola meal and enzyme. The various levels of canola meal and enzyme had no significant effects on carcass, breast and thighs weights. This observation could imply that carcass traits unimpressed by inclusion of various levels of canola meal and enzyme. However, their interaction showed that the inclusion of enzyme in broiler chicken diets increased thighs weights. Saki [41] demonstrated that higher intestinal viscosity resulted in the loss of economical parts of carcass. Positive effects of enzyme inclusion in broiler chicken diets were also reported [10], which is in agreement with the present study.

The structure of the intestinal mucosa can reveal some information on gut health. The small intestine changed absorption surface area with diet changes. On the other hand, crypt length and villous height are important parameters in mucus size and nutrients surface area [44]. Enzymes improve morphological status in gut of broiler chickens [45], a finding that is opposite with the results of current study, where no significant effects were observed between diets on morphology characteristic of jejunum. The exact mechanism is not clear but the presence of enzyme can induce changes in basal diets and intestinal ecosystem which maintain intestinal morphology.

Little study also is available on effect of the inclusion canola meal in broiler chickens on nutrients digestibility. The results showed that the use of various levels of canola meal had no effects on nutrient digestibility. Type of tested canola meal, because of differences in composition, bird's age, basal diets and the levels of tested canola meal could explain at least partly of obtained results. On the other hand, the use of enzyme increase digestibility of dry matter, organic matter and crude protein. Anti-nutritional factors of canola meal have important role on apparent digestibility [3]. The use of enzyme in diets containing canola meal degraded anti-nutritional factors and lead to increases in digestibility of protein and amino acids [46]. Similar results were observed by other composition containing anti-nutritional factors [47, 48].

The results of this study indicated that feeding broiler chickens with diets containing canola meal at levels of 6 and 12% had negative effects on performance. The use of Natuzyme Plus in diet to some extent modulates broiler chicken performance. The use of canola meal at levels of 6% and canola meal with enzyme at levels of 12% was suggested.

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