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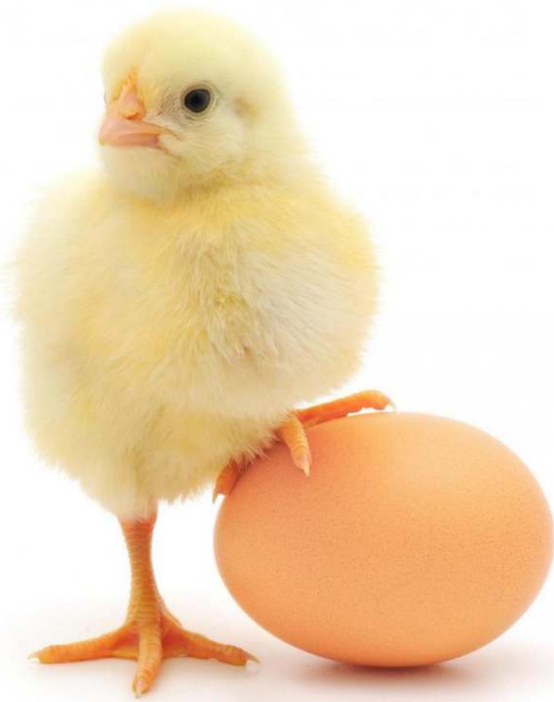

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Replacement Value of Soybean Meal with Rapeseed Meal Supplemented with or Without a Dietary NSP-Dgrading Enzyme on Performance, Carcass Traits and Thyroid Hormones of Broiler Chickens

H. Kermanshahi and A.R. Abbasi Pour
Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, P. O. Box: 91775-1163, Iran

Abstract: In a completely randomized design with 3*3 factorial arrangements and 4 replicates, 432 day-old Cobb male broiler chickens were used to evaluate the replacement of soybean meal with local grown rapeseed meal in the diet of broiler chickens with or without a dietary NSP degrading enzyme. The rapeseed meal was replaced with soybean meal at the levels of 0 (control), 15 and 30 percent for 6 weeks. The enzyme levels added to the diets were 0 (control), 0.025% and 0.050%. Feed intake, body weight gain and feed conversion ratio were adversely affected by added levels of rapeseed meal during the starter (0-21 d), grower (21-42 d) and overall feeding periods (P<0.0001). Adding 0.025% enzyme significantly (0.004) improved body weight gain at starter period. The interactions between rapeseed and enzyme for body weight gain were significant in all periods (P<0.001). Feed conversion ratio was not significantly affected by adding enzyme into the diets. Added levels of rapeseed meal significantly increased the weights of liver, proventriculus, gizzard (P<0.0001) and abdominal fat pad (P<0.028). Adding levels of rapeseed meal were also significantly increased the concentration of serum triiodothyronine (T_3) at 42 days of age (P<0.002) while adding enzyme into the diets had no significant effect among treatments . Therefore under the conditions of this study, use of lower levels of rapeseed meal might be more practical in broiler diets. For higher levels of replacement, further investigations are required.

Key words: Rapeseed meal, thyroid hormones, chickens

Introduction

In comparison to about 44% crude protein in soybean meal (SBM), the protein content of rapeseed meal (RSM) is about 35% and has a physiologically suitable amino acid combination in animal nutrition (Hickling, 2001; Kocher *et al.*, 2000). It has a suitable profile of amino acids but the digestibility of some amino acids is less than that of SBM (Zuprizal *et al.*, 1992). Leeson *et al.* (1987) suggested 100% replacement value of SBM with RSM. However, RSM contains nutritionally unfavorable substances such as glucosinolates, sinapin, tannin and phytate (Ciska and Kozlowska, 1998) and non starch polysaccharides (Kocher *et al.*, 2000). Glucosinolates and their hydrolytic products are commonly referred to as goitrogens. Glucosinolates are hydrolyzed by myrosinase enzyme produced by the plant (Buchwaldt *et al.*, 1986; Ciska and Kozlowska, 1998). Myrosinase activity has been observed in gastro-intestinal bacteria of several animal species and poultry (Marangos and Hill, 1974; Zeb, 1998). Presence of glucosinolates in the diets leads to hypothyroidism in animals and poultry, reducing the level of thyroid hormones and alters the ratio between triiodothyronine (T_3) and thyroxin (T_4) in blood. Enlarged thyroid size, altered thyroid hormones and changed activities of liver enzymes in the blood of poultry fed diets containing RSM has been observed (Pearson *et al.*, 1983; Schone *et al.*, 1990, Nassar and Arscott, 1986; Konicicki *et al.*, 1991). The presence of non starch polysaccharides (NSPs) may adversely affect the performance of broiler chickens fed high levels of RSM (Bedford *et al.*, 1990; Choct and Annison, 1992). There are some reports indicating that use of some exogenous carbohydrases may alleviate the anti-nutritional effect of NSPs (Slominski and Campbell, 1990). Therefore the purpose of this study was to investigate the replacement value of SBM with locally grown RSM on performance, some organ weights and blood thyroid hormones of broiler chickens.

Materials and Methods

Birds and diets: The locally grown rapeseed meal (RSM) was purchased from an oil extraction Co. in Neishabour, Iran (Fazl). For preparing RSM, the oil of the RSM was extracted by hexan. Three levels of 0.0, 15.0 and 30% RSM protein (32.3% crude protein) were replaced with SBM protein (44% crude protein) and three levels of a dietary NSP degrading enzyme (0, 0.025 and 0.050% ; Endofeed DC produced from *Aspergillus niger* (GNC Bioferm Inc., Canada, with minimum activity of 2400 U/g xylanase and 880 U/g Beta- glucanase) were added to the diets during starter (0-21 d of age) and grower (21-42 days of age) periods of broiler chickens (Table 1). All diets met the National Research Council (NRC, 1994) recommendations. Feed and water provided *ad libitum.*

Table 1: Composition of experimental diets

Age (days)	0-21								
Treatments									
Ingredients (%)	1	2	3	4	5	6	7	8	9
Corn	55.70	55.70	55.70	54.18	54.16	54.16	52.30	52.30	52.28
Soybean meal	33.17	33.17	33.17	19.48	19.47	19.38	2.30	2.32	2.33
Rapeseed meal	0.00	0.00	0.00	15.00	15.00	15.00	30.00	30.00	30.00
Fish meal	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Corn gluten meal	-	-	-	1.50	1.50	1.57	5.43	5.44	5.42
Veg. oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Di calcium phosphate	0.70	0.70	0.70	0.74	0.74	0.74	0.94	0.88	0.88
Calcium carbonate	1.00	1.00	1.00	0.94	0.94	0.94	0.88	0.89	0.89
Salt	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vit. Min.premix[1]	0.50	0.50	0.5	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.08	0.08	0.08	0.01	0.01	0.01	-	-	-
L-Lysine	-	-	-	-	-	-	-	-	-
Enzyme[2]	0.00	0.025	0.05	0.00	0.025	0.05	0.00	0.025	0.05
Fine sand	1.20	1.18	1.15	-	-	-	-	-	-
Calculated analysis									
ME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein (%)	20.86	20.86	20.86	20.86	20.86	20.86	20.86	20.86	20.86
Age (days)	21-42								
Treatments									
Ingredients (%)	1	2	3	4	5	6	7	8	9
Corn	62.40	62.40	62.40	60.90	60.86	60.84	59.02	59.00	59.00
Soybean meal	30.48	30.48	30.48	17.44	17.45	17.45	0.25	0.25	0.23
Rapeseed meal	0.00	0.00	0.00	15.00	15.00	15.00	30.00	30.00	30.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Corn gluten meal	-	-	-	1.05	1.05	1.05	5.00	5.00	5.00
Veg. oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Di calcium phosphate	0.71	0.71	0.71	0.81	0.81	0.81	0.95	0.95	0.95
Calcium carbonate	1.20	1.20	1.20	1.15	1.15	1.15	1.10	1.10	1.10
Salt	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vit. Min.premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.01	0.01	0.01	-	-	-	-	-	-
L-Lysine	-	-	-	-	-	-	0.03	0.025	0.02
Enzyme[2]	0.00	0.025	0.05	0.00	0.025	0.05	0.00	0.025	0.05
Fine sand	1.55	1.52	1.50	-	-	-	-	-	-
Calculated analysis									
ME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein (%)	18.12	18.12	18.12	18.12	18.12	18.12	18.12	18.12	18.12

[1]Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3,9790 IU; vitamin E, 121 IU; B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg;
[2]Endofeed DC from GNC Bioferm Inc., Canada.

In a completely randomized design with 3*3 factorial arrangements, 432 day-old Cobb male broiler chickens were randomly assigned in 36 pens of 4 replicates per treatment (1*1.2m). Birds were maintained under continuous light. The environmental temperature in the barn that was initially established on 31°C was gradually reduced to 22°C by week 6.

Sample collection: Feed consumption and body weight gain of chickens were recorded 4 h after the removal of feed, and feed to gain ratio calculated as the unit weight of feed per unit of body weight gain were recorded weekly. At 42 days of age, one chicken from each replicate (pen) was randomly selected, weighed, slaughtered and the liver, gizzard, pancreas, proventriculous and the abdominal fat pad weights were immediately recorded. At 21 and 42 days of age, one bird from each replicate was randomly selected and their blood samples were collected. For measuring T_3 and T_4 concentrations, serum blood samples was separated. The total T_3 and T_4 concentrations in the sera were determined by RIA with using standard commercial

Table 2: Effect of levels of rapeseed meal and NSP-degrading enzyme on performance of broiler chickens

Age (days)	0-21		
Performance treatments	Feed intake (g)	Weight gain (g)	Feed to Gain ratio
Rapeseed level (%)			
A1	935.60^{a}	572.29^{a}	1.635^{c}
A2	902.92^{b}	528.58^{b}	1.708^{b}
A3	699.36^{c}	318.48^{c}	2.194^{a}
P value	0.0001	0.0001	0.0001
Enzyme level (%)			
B1	856.79^{a}	472.08^{b}	1.859
B2	859.62^{a}	485.32^{a}	1.824
B3	821.47^{b}	462.31^{b}	1.855
P value	0.0222	0.0042	0.4771
Rapeseed * Enzyme			
A1B1	931.28^{ab}	550.50^{bc}	1.692
A1B2	953.49^{a}	594.10^{a}	1.604
A1B3	922.05^{ab}	572.28^{ab}	1.611
A2B1	886.63^{b}	522.30^{d}	1.697
A2B2	912.2a^{b}	529.78^{dc}	1.723
A2B3	909.93^{ab}	533.69^{dc}	1.705
A3B1	752.48^{c}	343.45^{e}	2.190
A3B2	713.18^{c}	322.10^{e}	2.145
A3B3	632.43^{d}	280.97^{f}	2.249
±SEM	6.756	2.962	0.015
P value	0.0056	0.0001	0.2524

Age (days)	21-42		
Performance treatments	Feed intake (g)	Weight gain (g)	Feed to Gain ratio
Rapeseed level (%)			
A1	2753.11^{a}	1185.18^{a}	2.326^{c}
A2	2703.25^{a}	1111.57^{a}	2.432^{b}
A3	1728.04^{b}	612.1^{c}	2.827^{a}
P value	0.0001	0.0001	0.0001
Enzyme level (%)			
B1	856.79^{a}	472.08^{b}	2.503
B2	2377.44	949.18^{b}	2.546
B3	2388.32	970.10^{ab}	2.537
P value	0.5402	0.0235	0.5542
Rapeseed * Enzyme			
A1B1	931.28^{ab}	1216.73^{a}	2.293
A1B2	2680.68	1103.63^{b}	2.428
A1B3	2788.88	1235.20^{a}	2.257
A2B1	2703.80	1121.70^{b}	2.412
A2B2	2712.23	1120.95^{b}	2.419
A2B3	2693.73	1092.06^{b}	2.467
A3B1	1762.35	630.30^{c}	2.804
A3B2	1739.43	622.95^{c}	2.790
A3B3	1682.35	583.06^{c}	2.886
±SEM	17.931	6.479	0.019
P value	0.4236	0.0001	0.1204

Table 2: Continued

Age (days)	1-42		
Performance treatments	Feed intake (g)	Weight gain (g)	Feed to Gain ratio
Rapeseed level (%)			
A1	2688.71^{a}	1757.48^{a}	2.099^{c}
A2	2606.17^{a}	1640.16^{b}	2.198^{b}
A3	2427.40^{b}	930.93^{c}	2.610^{a}
P value	0.0001	0.0001	0.0001
Enzyme level (%)			
B1	3275.43	1461.66	2.292
B2	3237.06	1334.49	2.300
B3	3209.78	1432.42	2.316
P value	0.3412	0.1485	0.7474
Rapeseed * Enzyme			
A1B1	3721.05	1767.23^{a}	2.106
A1B2	3634.16	1697.73^{b}	2.140
A1B3	3710.93	1807.48^{a}	2.052
A2B1	3590.43	1644.00^{bc}	2.183
A2B2	3624.43	1650.73^{bc}	2.196
A2B3	3603.65	1625.75^{c}	2.216
A3B1	2514.83	973.75^{d}	2.587
A3B2	2452.60	955.03^{d}	2.566
A3B3	2314.78	864.03^{e}	2.679
±SEM	20.781	7.600	0.015
P value	0.1847	0.0003	0.1568

A1-A3 are 0, 15 and 30% rapeseed meal; B1-B3 are 0, 0.025 and 0.05 % enzyme; $P<0.05$ is statistically significant; [a-f]Means in each column with different superscripts are significantly different.

kits (Kavoshyar kit) according to the procedure of Kloss *et al.* (1994) (Gama manic1, Contron, Italy, with Automatic Gama Counter).

Statistical analysis: Data were analyzed based on a general linear model procedure of SAS (SAS, 1993) and treatment means when significant ($P<0.05$), were compared using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The results of the performance, carcass traits and thyroid hormones of broiler chickens fed rapeseed meal and enzyme are given in Tables 2 and 3. Feed intake, body weight gain and feed to gain ratio were significantly affected by treatments ($P<0.05$). Feed intake of broiler chickens at 0-21, 21-42 and total period was significantly decreased by RSM as its inclusion into the diets increased. The lowest feed intake was seen in 30% replacement. The RSM contains substantial concentrations of phenolic compound that cause a bitter taste and decrease its palatability (Shahidi and Naczk, 1992). Goitrin, isothyocyanates and glucobrassicin are known as bitter taste in Brassica (Drewnowski and Gomez-Carneros, 2000). The evidence indicates that diet palatability can be adversely affected by the glucosinolates of the RSM (Mawson *et al.*, 1993). Also researchers found that the palatability of diets is improved with low glucosinolates RSM, very low glucosinolates RSM (Mawson *et al.*, 1993) and lower dietary inclusion of high glucosinolates RSM (Elwinger and Saterby, 1986). However, a reduction in feed intake at 0-21, 21-42 and 0-42 days of age seen in this study shows that the above mentioned compounds present in RSM are partly responsible for this effect when the inclusion level of RSM was 15 and 30% of soybean meal replacement. Leeson *et al.* (1987) found that even complete replacement of SBM (100%) with canola meal

Table 3: Effect of rapeseed meal and NSP-degrading enzyme on carcass traits and thyroid hormones of broiler chickens

Age (days)	42				21		42	
Carcass traits Treatments	Relative[1] Liver weight	Relative Proventriculus weight	Relative Gizzard weight	Relative Abdominal Fat pad weight	Serum T_3 (η g/ml)	SerumT_4 (μ g/dl)	SerumT_3 (η g/ml)	Serum T_4 (μ g/dl)
Rapeseed level (%)								
A1	2.20[b]	0.47[b]	2.35[b]	1.35[ab]	68.83	0.58	102.33[b]	2.60
A2	2.15[b]	0.46[b]	2.40[b]	1.10[b]	90.00	1.32	110.58[b]	3.13
A3	2.75[a]	0.63[a]	3.20[a]	1.55[a]	83.50	1.38	166.92[a]	2.69
P value	0.0003	0.0001	0.0001	0.0283	0.1606	0.1028	0.0025	0.2020
Enzyme level (%)								
B1	2.40	0.53	2.63	1.30	72.33	0.83	139.67	2.82
B2	2.35	0.52	2.57	1.34	83.00	1.39	113.00	2.82
B3	2.40	0.52	2.73	1.38	87.00	1.06	128.17	2.78
P value	0.7905	0.8539	0.4782	0.8635	0.3965	0.3830	0.3421	0.9899
Rapeseed* Enzyme								
A1B1	2.25	0.47	2.45	1.20	64.75	0.58	100.50	2.32
A1B2	2.15	0.50	2.27	1.33	70.00	0.28	113.25	2.70
A1B3	2.17	0.44	2.37	1.55	71.75	0.90	96.25	2.78
A2B1	2.25	0.50	2.42	1.23	82.75	0.90	118.25	3.32
A2B2	2.10	0.45	2.25	1.00	80.25	1.58	97.25	3.45
A2B3	2.15	0.45	2.55	1.11	107.00	1.50	116.25	2.62
A3B1	2.68	0.63	2.95	1.45	69.50	1.02	200.25	2.80
A3B2	2.68	0.61	3.25	1.68	98.75	2.32	128.50	2.32
A3B3	2.85	0.67	3.40	1.45	82.25	0.78	172.00	2.95
±SEM	0.121	0.055	0.124	0.179	5.165	0.188	8.438	0.146
P value	0.9432	0.4951	0.6383	0.5965	0.5597	0.2448	0.4138	0.3115

[1] Relative to live body weight; A1-A3 are 0, 15 and 30% rapeseed meal; B1-B3 are 0, 0.025 and 0.05 % enzyme; P<0.05 is statistically significant; [a-b] Means in each column with different superscripts are significantly different; T_3, tri-idotyronine; T_4, tyroxine.

(<30 µmoles/g glucosinolate) did not affect the feed intake in broilers and laying hens but Karunajewa *et al.* (1990) found that inclusion 149g/kg (50% replacement), or more, of RSM (42 µmoles/g glucosinolate) in diet reduced feed intake. Replacement of RSM with SBM had an adverse effect on body weight gain and Feed to gain ratio of broiler chickens too, These results can attributed to anti-nutritional factors present in RSM other than its non starch polysaccharides because adding NSP-degrading enzyme did not alleviate the RSM effect (Table 2). Addition of enzyme into the diets itself had no positive effect on performance of the chickens in any periods. The reduction of body weight gain may also be related to the lysine-arginine imbalance in higher levels of RSM in diets (Summers and Leeson, 1978). The use of large amounts of RSM is limited because of the lower energy and higher fiber contents compared with SBM (Mawson *et al.*, 1993). Crude fiber content of RSM negatively affected AME_n value for broiler chicken (Chibowska *et al.*, 2000).

The effect of RSM and enzyme on carcass characteristics, serum T_3 and T_4 is shown in Table 3. RSM significantly increased relative liver, proventriculus, gizzard and abdominal fat pad to live body weight of chickens (P<0.05). Increasing in liver to live body weight of chickens by RSM has been frequently reported (Slominski and Campbell, 1990; Summers *et al.*, 1992). They reported that it is related to the liver damage and its hypertrophy caused by glucosinolate content of RSM fed by the birds. Some changes in liver enzymes such as aspartate transaminase, lactate dehydrogenase and alkaline dehydrogenase in the plasma of layers and broilers fed RSM is reported (Pearson *et al.*, 1983). The more fiber content of the diets containing higher levels of RSM may attribute to more relative weight of proventriculus and gizzard of the birds and the hypertrophy of these organs. The more abdominal fat pad of the birds receiving more RSM may be related to lower AME content of the RSM beyond that is calculated and the imbalance of energy to protein of the diets. In addition to this possibility, the phytate, tannin and other anti-nutritional factors present in RSM may change these balances causes more fat deposition in the body. The reduction in abdominal fat pad content has been observed previously (Karunajewa *et al.,* 1990) and may be related to alteration of T_3 hormone in the blood serum. T_3 not only induces intracellular lipid accumulation but also stimulates adipocyte cell proliferation and fat cell cluster formation (Yen, 2001). Therefore by increasing T_3 in blood serum, abdominal fat pad may increases. RSM only increased the serum T_3 concentration of the chickens at 42 days of age (P<0.05). Newkirk and Classen (2002) reported that the increased level of T_3 caused by RSM may attribute to later heart dysfunction in broilers. According to Clandinin *et al.* (1966) using of goitirin in the diet of chicks caused a physiological equilibrium in thyroid gland of chicks after 3-4 weeks. The hydrolysis products of glucosinolates impair the thyroid uptake of iodide, its oxidation, the iodine binding to thyroglobulin, synthesis and release of hormone (Schone *et al*., 1990). As seen in this study, inclusion of RSM in broiler's diet increased T_3 concentration in blood chicken at 42th day of age (Table 3) that is confirmed by Newkirk and Classen (2002) and Schone *et al*. (1988). Antithyroid compounds of RSM could destroy cellular T_3 receptors (Schone *et al*., 1990) and change outer ring deiodination of T_4 in peripheral tissue (Darras *et al*., 2000).

Conclusion: Researchers classified the RSM to four categories as very low, low, medium and high based on their glucosinolates content (Mawson *et al*., 1993) and based on this classification, the RSM used in this study may had high levels of glucosinolate. Hydrolysis of gluconapin and glucobrassicanapin leads to formation of nitriles, thiocyanides, allyl isothyocyanates and enzymatic hydrolysis of progoitrin and napoleiferin leads to formation of epiniterile, nitrile and goitrin (Ciska and Kozlowska, 1998). Under the condition of this study, higher levels of RSM showed more adverse effect on performance, carcass traits and changed thyroid hormones. Enzyme had no positive effect to alleviate the negative effect of RSM possibly caused by its NSP content. It is possible that the other anti-nutritional factors present in RSM are responsible for poor response of broilers. Further investigations are required to clarify these ideas.

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