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The Effect of Different Levels of Rapeseed Meal with and without Enzyme on the Performance and the Serum Level of Triiodothyronine (T3), Thyroxine (T4) and Thyroid Stimulating Hormone (TSH) in Broiler Chickens

Research Article

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ABSTRACT

In order to investigate the effect of different levels of rapeseed meal on performance and content of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) in broiler chickens' blood, 640 1-d-old Ross male broiler chickens were studied through a 4 × 2 factorial arrangement including 4 levels of rapeseed meal (RSM) (0, 5, 10 and 15 percent) and two levels of enzyme (0 and 0.05%) with a completely randomized design and 4 replicates. Feed intake weight gain and feed conversion ratio were measured at 8, 21 and 42 days of age. In order to investigate the level of T3, T4 and TSH at the end of each period, three chickens from each treatment group were sampled for blood collection. Results indicated that different levels of RSM and enzyme separately had no significant effect on the feed conversion ratio, weight gain and feed intake ($P \geq 0.05$). However, the interaction effect of rapeseed RSM and enzyme significantly improved the feed intake ($P \leq 0.05$). Treatments significantly affected the amount of T3 and TSH in blood serum that was decreased, with adding rapeseed and enzyme into the diet, while the level of T4 was not affected significantly. Therefore, according to obtained results, it seems that rapeseed RSM can be used in the diet of broiler chickens up to 15% without decreasing their performance.

KEY WORDS broiler chicken, enzyme, rapeseed meal, thyroid.

INTRODUCTION

One of the most important factors that should be considered in the feeding and the diet of poultry is protein resource, because it constitutes an important part of the poultry's diet and at the same time it is the most expensive part of it. Oily seeds are among the most important nutrients which play a significant role in human diet due to the oil obtained from them. Further, rapeseed meal (RSM) as a derivative product is used as an important source of protein in poultry and livestock diets. Rapeseed with the scientific name of *Brassica napus* from the order of *Brassicaceae* or *Cruciferea* is an amphidiploids species which is resulted from grafting *Brassica oleracea* and *Brassica rapa*. Three species of rapeseed, *Brassica napobrassica* and Indian mustard are known with two names, colza in French and rapeseed in English in the world market (Ghadami, 2011). Canadian researchers have produced three new types of rapeseed through genetic modifications: 1) number zero (0): a kind of rapeseed who erusic acid has been decreased, 2) two zero (00): a kind of rapeseed that its erusic acid and glucosinolate have been decreased and 3) three zero (000): a kid of rapeseed that besides erusic acid and glucosinolate the amount of its fibers have been reduced (Pusutai, 1989).

Based on previous research, due to nutritionally unfavorable substances in rapeseed such as glucosinolate, erusic acid, tannin, phytic acid and sinapine, it is preferable to use only 5 percent of it in the broiler chickens diet and 0 percent in the egg laying hens' diet (Summers *et al*. 1990; Choct and Annison, 1992). The existence of liquid non-starch polysaccharide in rapeseed' water can increase viscosity of intestine content and decrease digestion and assimilation of nutritious substrates such as protein and fat; in addition, phytate in rapeseed meal can prevent functions of iron, sodium, sulfur, sialic acid, calcium, zinc, copper and other nutrients like nitrogen and amino acids (Cowieson *et al*. 2003). The presence of glucosinolate in poultry's diet together with rapeseed meal decreases the extent of thyroid hormones' secretion, increases the size of thyroid gland and violates the balance between blood hormones namely, triiodothyronine and thyroxin (Nassar and Arscott, 1956; Pearson *et al*. 1983; Schone *et al*. 1993).

Triiodothyronine, also known as T3, is a thyroid hormone. It affects almost every physiological process in the body, including growth and development, metabolism, body temperature and heart rate (Bowen, 2010). Production of T3 and its prohormone thyroxine (T4) is activated by thyroid stimulating hormone (TSH), this is released from the pituitary gland. This pathway is regulated via a closed-loop feedback process; elevated concentrations of T3 and T4 in the blood plasma inhibit the production of TSH in the pituitary gland. As concentrations of these hormones decrease, the pituitary gland increases production of TSH and by these processes, a feedback control system is set up to regulate the amount of thyroid hormones that are in the bloodstream (Bowen, 2010).

In poultry's diet, enzymes are commonly used to increase availability of nutrients and decrease the side effects of nutritionally unfavorable materials existing in nutrients (especially corns); (Bedford and Schulze, 1998).

The researchers already have indicated that β-glocanase enzyme with xylanase in feed ration containing 20 and 30 percent rapeseed meal would not affect the amount of weight gain, feed conversion ratio and feed intake of broiler chickens (Mushtaqe *et al*. 2007; Kermanshahi and abbasipour, 2006). Mikulski *et al.* (2012) showed that final body weight of turkeys was not affected by the graded levels of rapeseed meal. Also, an increase in the inclusion rate of rapeseed meal was followed by a linear increase in feed conversion ratio.

In previous studies different levels of rapeseed meal have increasingly been used instead of soybean meal or it has been mostly used with two enzymes; polysaccharides and phytase. Regarding the use of multi enzymes with rapeseed meal few researches have been done. In these studies researchers tried to use suitable levels of rapeseed meal with multi enzyme (Natuzyme plus). Since the price of rapeseed meal is lower than soybean meal which constitutes a large part of poultry diet and because of its extensively cultivation in Iran, it can be a good substitute for soybean meal. Dehghani (2013), based on her study suggested that, In corn-based diets, 30% rapeseed meal with dietary enzyme for starter and grower and 10% of rapeseed meal with enzyme for finisher are recommended. In wheat based trial, 30% of rapeseed rapeseed meal with enzyme in starter, 20% of rapeseed meal in grower and 20% of rapeseed meal with enzyme in finisher is recommended.

This study was conducted to investigate the effect of different levels of rapeseed meal with and without enzyme on the performance and T3, T4 and TSH serum content in broilers chickens.

MATERIALS AND METHODS

In this experiment a total of 640 one-day-old Ross 308 male broiler chicks were randomly allocated into 4 × 2 factorial design (8 treatment) groups with 4 replicates each and 20 chicks per replicate (initial body weight:45.59±0.27 g). Feed and fresh water were available *ad libitum*. The lighting schedule provided 23 h light per day. In this treatment chickens were reared for 42 days with diets containing 4 levels of rapeseed meal (0, 5, 10 and 15%) and 2 levels of enzyme (0 and 0.05%) instead of soybean meal through completely randomized factorial treatments. At the end of each week feed remained were weighted the data were measured, daily mortality were obtained, and feed adjustment were corrected daily (mortality rate 1.5%). Ingredients of enzyme and experimental diets are presented in Table 1, 2 and 3. Experimental diets were formulated using ration-formulation software (UFFDA, 1992) to be isocaloric and isonitrogenous following national research council recommendations (NRC, 1994). Design's statistical model:

$$Y_{ij}= \mu\ \alpha K + \beta L + (\alpha\beta)KL + e_{ijk}$$

Where:
Y_{ij}: effect of each observation.
μ: sample mean.
αK: effect of rapeseed meal rapeseed level.
βL: effect of enzyme.
(αβ)KL: interaction effect of enzyme and rapeseed meal rapeseed level.
e_{ijk}: effect of error.

Until 7 days of age, chickens were raised based on the basic diet of corn-soybean; after that, they ate nothing for one day; then, those with equal weights were assigned to treatment groups to be exposed with different diets.

Table 1 Composition of natuzyme plus enzyme[1] (BioProtein Company, Australia)

Xylanase	Cellulase	Protease	Phytase	β-gluconase	α-amylase	Pectinase	Lipase
10000000	6000000	3000000	1500000	700000	700000	70000	30000

[1] Numbers are written based on international unit.

Table 2 Experimental diets and composition of nutrient at 8-21 days of age[1]

Feed ingredients (%)	Control 0.0%	Rapeseed meal 5%	Rapeseed meal 10%	Rapeseed meal 15%
Corn	48.77	47.38	46.00	44.61
Soybean meal	33.42	29.39	25.37	21.35
Wheat	10.00	10.00	10.00	10.00
Oil	3.87	4.33	4.80	5.27
Dicalcium phosphate	1.60	1.58	1.56	1.55
Oyster shell	1.03	0.99	0.96	0.93
Salt	0.36	0.35	0.34	0.34
DL-methionine	0.29	0.26	0.24	0.22
L-lysine	0.18	0.20	0.22	0.24
Mineral premix*	0.25	0.25	0.25	0.25
Vitamin premix**	0.25	0.25	0.25	0.25
Rapeseed meal	0	5.00	10.00	15.00
Calculated analysis				
Metabolizable energy (kcal/kg)	3050	3050	3050	3050
Crude protein (%)	20.34	20.34	20.34	20.34
Calcium (%)	0.87	0.87	0.87	0.87
Methionine (%)	0.58	0.57	0.56	0.54
Sodium (%)	0.16	0.16	0.16	0.16
L-lysine (%)	1.20	1.20	1.20	1.20
Methionine-cystine (%)	0.92	0.92	0.92	0.92
Arginine (%)	1.27	1.27	1.21	1.19
Tryptophan (%)	0.28	0.27	0.75	0.26
Phosphorus (%)	0.43	0.43	0.43	0.43

[1] all experimental diets were prepared both with and without enzyme.
* Supplied per kilogram of diet: Copper: 10 mg; Iodine: 99 mg; Manganese oxide: 99 mg; Selenium: 2 mg; Zinc: 84 mg and Iron: 50 mg.
** Supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 9790 IU; vitamin E: 121 IU; B: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg and Thiamin: 4 mg.

During the period of treatment weight gain and feed intake were weekly measured and recorded. For measuring the concentration of thyroid hormones, T3, T4 and TSH at the end of period, from each treatment three chickens were bled and then serum samples were separated. The total T3, T4 and TSH concentrations in the sera were determined by luminescence chmei using standard kits.

Data were analyzed by SAS, (2002) and the means of treatment groups were compared through Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

As the result indicated in Tables 4, 5 and 6 rapeseed meal and enzyme separately did not significantly influence daily weight gain and feed intake, while feed conversion ratio increased with increasing the level of rapeseed meal in the diet at 8-21 days of age. At the age of 22-42 days the effect of rapeseed meal with and without enzyme on daily weight gain, feed intake, and feed conversion ratio was not meaningful except for the interaction effect of enzyme and rapeseed meal that improved feed conversion ratio ($P \leq 0.05$).

According to the data given in Table 7, the experimental diets, at the end of the period, significantly affected the serum content of T3 and TSH, that is, with increasing the amount of rapeseed in the diet the amount of T3 and TSH in chicken's blood decreased. In other words, thyroid function decreased ($P \leq 0.05$). However, diets had no significant effect on the amount of T4 in chickens' blood plasma ($P > 0.05$).

Based on the results of this study, adding rapeseed meal up to 15% into the diet had no negative effect on weight gain, feed intake and feed conversion ratio; also, using enzyme without rapeseed meal rapeseed in diets led to no improvement in productive features. The interactional effect of rapeseed meal with enzyme on weight gain and feed intake was not statistically significant while it significantly affected the feed conversion ratio. In other words, increasing the level of rapeseed meal rapeseed up to 15% in diet and then, adding enzyme into it led to improvement in feed conversion ratio.

This is similar to the study by Dehghani (2013) who found adding enzyme to the diet improved bird weights regardless of level of rapeseed meal.

Table 3 Experimental diets and composition nutrients at 21-42 days of age

Feed ingredients (%)	Control 0.0%	Rapeseed meal 5%	Rapeseed meal 10%	Rapeseed meal 15%
Corn	52.35	50.96	49.58	48.19
Soybean meal	29.10	25.08	21.05	17.03
Wheat	10.00	10.00	10.00	10.00
Oil	4.76	5.23	5.70	6.16
Dicalcium phosphate	1.54	1.52	1.50	1.49
Oyster shell	0.99	0.96	0.93	0.90
Salt	0.36	0.0	0.34	0.34
DL-methionine	0.26	0.23	0.21	0.19
L-lysine	0.15	0.17	0.19	0.21
Mineral premix*	0.25	0.25	0.25	0.25
Vitamin premix**	0.25	0.25	0.25	0.25
Rapeseed meal	0	5.00	10.00	15.00
Calculated analysis				
Metabolizm energy (kcal/kg)	3150	3150	3150	3150
Crude protein (%)	18.70	18.70	18.70	18.70
Calcium (%)	0.83	0.83	0.83	0.83
Methionine (%)	0.54	0.52	0.51	0.49
Sodium (%)	0.16	0.16	0.16	0.16
Lysine (%)	1.07	1.07	1.07	1.07
Methionine-cystine (%)	0.85	0.85	0.85	0.85
Arginine (%)	1.15	1.12	1.09	1.07
Tryptophan (%)	0.25	0.25	0.24	0.23
Phosphorus (%)	0.41	0.41	0.41	0.41

[1] all experimental diets were prepared both with and without enzyme.
* Supplied per kilogram of diet: Copper: 10 mg; Iodine: 99 mg; Manganese oxide: 99 mg; Selenium: 2 mg; Zinc: 84 mg and Iron: 50 mg.
** Supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 9790 IU; vitamin E: 121 IU; B: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg and Thiamin: 4 mg.

Table 4 The effect of rapeseed meal and enzyme levels (treatment) on average daily weight gain broiler chickens (g)

Treatments	Level of rapeseed meal or enzyme (%)	8-21 (days)	22-42 (days)	8-42 (days)
Rapeseed meal level	0	39.63	75.79	61.33
	5	39.65	76.62	62.16
	10	40.05	77.19	62.17
	15	40.51	77.29	62.39
P-value	-	0.706	0.899	0.894
Enzyme level	0	39.87	75.83	61.45
	0.05	40.04	77.61	62.58
P-value	-	0.789	0.265	0.289
Interaction effects (rapeseed meal×enzyme)	0 × 0	39.81	75.18	61.04
	0 × 0.05	39.45	79.19	63.29
	0 × 5	40.33	76.45	62.00
	5 × 0.05	39.76	78.13	62.78
	0 × 10	40.61	76.23	61.98
	10 × 0.05	40.41	77.01	62.37
	0 × 15	38.76	75.46	60.78
	15 × 0.05	40.55	76.12	61.89
P-value	-	0.488	0.862	0.929

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Results showed that, considering the cost effectiveness of rapeseed meal rapeseed compared to soybean meal and the vast cultivation of it in regions with climate conditions like Iran, it can be used up to 15 percent in diets; thus, we can reduce soybean meal imports and exchange rate.

These findings support the findings of other researchers; for instance, Jalali-Hajiabadi *et al.* (2006) indicated that using rapeseed meal rapeseed up to the level of 25% had no negative effect on chickens' weight but higher levels led to weight loss.

Table 5 The effect of rapeseed meal and enzyme levels (treatment) on average daily feed intake of broiler chickens (g)

Treatments	Level of meal or enzyme (%)	8-21 (days)	22-42 (days)	8-42 (days)
Rapeseed meal level	0	86.73	167.68	135.78
	5	86.92	170.36	135.88
	10	88.20	172.32	138.09
	15	90.67	173.48	140.35
P-value	-	0.274	0.213	0.232
Enzyme level	0	88.64	169.49	137.15
	0.05	87.63	171.43	137.91
P-value	-	0.520	0.400	0.669
Interaction effects (rapeseed meal×enzyme)	0 × 0	85.32	161.79^{c}	131.20^{c}
	0 × 0.05	88.52	174.93^{a}	140.37^{a}
	0 × 5	87.77	172.53^{b}	138.63^{ab}
	5 × 0.05	85.70	172.11^{b}	137.54^{ab}
	0 × 10	88.08	166.30^{bc}	135.01^{b}
	10 × 0.05	88.32	169.05^{ab}	136.76^{ab}
	0 × 15	93.37	177.34^{a}	143.75^{a}
	15 × 0.05	87.97	169.62^{ab}	136.95^{ab}
P-value	-	0.271	0.026	0.028

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 The effect of rapeseed meal and enzyme levels (treatment) on feed conversion ratio of broiler chickens (feed intake per gr/weight gain per g)

Treatments	Level of meal or enzyme (%)	8-21 (days)	22-42 (days)	8-42 (days)
Rapeseed meal level	0	2.18^{b}	2.21	2.19
	5	2.19^{b}	2.22	2.20
	10	2.19^{b}	2.23	2.21
	15	2.31^{a}	2.30	2.30
P-value	-	0.043	0.381	0.100
Enzyme level	0	2.24	2.23	2.24
	0.05	2.20	2.22	2.21
P-value	-	0.308	0.762	0.489
Interaction effects (rapeseed meal×enzyme)	0 × 0	2.13^{a}	2.17	2.16
	0 × 0.05	2.26^{ab}	2.20	2.22
	0 × 5	2.19^{a}	2.25	2.23
	5 × 0.05	2.18^{a}	2.21	2.20
	0 × 10	2.19^{a}	2.18	2.18
	10 × 0.05	2.20^{a}	2.21	2.21
	0 × 15	2.45^{b}	2.35	2.39
	15 × 0.05	2.18^{a}	2.25	2.22
P-value	-	0.005	0.749	0.142

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 7 The effect of different diets (treatments) on the density of T3, T4 and TSH in broiler chickens' blood

Treatments	TSH (mg/dL)	T3 (mg/dL)	T4 (mg/dL)
Diet without enzyme and rapeseed (control group)	0.182^{c}	2.007^{ab}	8.102
Diet with 5% rapeseed without enzyme	0.207^{c}	2.428^{a}	8.651
Diet with 10% rapeseed without enzyme	0.242^{c}	2.064^{ab}	8.267
Diet with 15% rapeseed without enzyme	0.365^{bc}	1.904^{ab}	8.429
Diet with 0.05% enzyme without rapeseed	0.609^{c}	1.558^{b}	7.047
Diet with 0.05 % enzyme and 5% rapeseed	0.809^{c}	1.754^{b}	7.565
Diet with 0.05% enzyme and 10% rapeseed	0.169^{c}	1.663^{b}	7.648
Diet with 0.05% enzyme and 15% rapeseed	0.085^{c}	1.543^{b}	6.512
SEM	0.063	0.085	0.355

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
T3: triiodothyronine; T4: thyroxine and TSH: thyroid stimulating hormone.

They identified the presence of higher levels of sulfur especially mineral sulfur in rapeseed meal and also the presence of sulfur in glucosinolates and their hydrolyzed products as the factors that led to weight loss and high calcium defecation at higher levels of replacement. Schone *et al.* (1991) conducted feeding trials with 16 percent ordinary rapeseed meal (high glucosinolate) and new kind of rapeseed meal (low glucosinolate) in the diet of broiler chickens. They indicated that the performance of chickens fed from the control diet without any modification was similar to the performance of those fed by diets containing rapeseed meal. In another study conducted by Clark *et al.* (2001) the effect of rapeseed meal up to 20 percent in the diets of broiler chickens was investigated. Results indicated that weight gain, feed intake, feed conversion ratio, mortality rate and foot rot were not affected significantly. Further, it was shown that there was no significant difference in weight gain and feed intake among different treatment groups as a result of adding colza meal up to 20% into the diets of broiler chickens (Khan *et al*. 1996).

Regarding ordinary effects of rapeseed meal during growth period it should be noted that feed conversion ratio significantly increased as the level of rapeseed meal increased. This is because of the fact that feed intake increased as a result of increasing the level of rapeseed meal in the diet.

During the growth period, the mutual effects of rapeseed meal and enzyme were statistically significant. The highest feed conversion ratio was due to the diet with 15 percent rapeseed meal without enzyme and the lowest ratio was due to control group except for diets containing 15 percent Rapeseed without enzyme, the rest of diets had a conversion ratio near to that of the control group. The reason behind this observation during growth period (8-21 day) can be the lack of physiological evolution in digestive system, so, chickens cannot digest high amounts of fiber in their diet. Although, at this period chickens can cope with high amounts of fiber to some extent, at any rate, this is not a complete adaptation.

As stated earlier, conversion ratio during growth period and during the entire period was not affected by added levels of Rapeseed meal with enzyme or by the mutual effect of them. It seems that this is due to the digestive system maturity and relative capability of chickens to digest fibers.

With reference to obtained results during the whole period, it seemed that compared to the control diet using rapeseed meal to 15 percent would not decrease the productive performance of chickens. These findings were in line with results of other researchers. Mannion (1981) indicated that adding rapeseed meal to levels of 10, 12 and 15 percent in broiler chickens' diet had no negative effect on feed conversion ratio. Zeb *et al.* (1999) stated that negative effects of Rapeseed meal would decrease at old age. They continued that this is due to the complete formation of the digestive system at old age and concluded that replacing Soybean meal with rapeseed meal at old ages would lead to better results.

On the other hand, some researchers have obtained opposing results. Bougon *et al.* (1988) when using Rapeseed meal from two zero variety in the diets of broiler chickens, observed a linear decrease in weight gain, feed intake and feed conversion ratio as a result of increasing the level of Rapeseed meal. Other researchers stated that the increase of conversion ratio related to the presence of glucosinolate in meal. Negative effects of rapeseed meal increase as the period of feeding increases; that is, a decrease in body weight and an upward trend in conversion ratio would be observed (Zeb, 1998).

Considering the results on the effect of rapeseed on the productive potentiality, it seems that this byproduct can be used in the diet of broiler chickens without decreasing their performance. However, the effects of this product on corpus quality should also be investigated to be able to rely on these findings more rigorously. One purpose of this study is investigating the effect of enzyme as a variable on thyroid gland. As can be seen in Table 6, the amount of TSH in serum increased as the level of rapeseed in the diet increased while, T3 and T4 decreased. This observation is due to the presence of nutritionally unfavorable matters in rapeseed meal that prevents iodine absorption which is directly related to the function of thyroid gland. There are considerable reports as to thyroiditis, rise of thyroid stimulating hormone, hypothyroidism, and thyroid hormone deficiency in the blood of poultry fed by rapeseed meal (Chiasson and Sharp, 1979). In a study conducted by Eila *et al.* (2012) the effect of different levels of calcium iodates in poultry's diet was investigated. The result indicated that the amount of thyroxin D in chickens' blood significantly increased but the amount of T3 showed no meaningful changes. Still in another study, the effect of three different levels of Rapeseed meal (5, 7 and 15 percent) on broiler chickens was investigated. The results of this study showed that at 21 days of age, the density of T3 in blood serum increased and at 41 days of age, the density of T4 increased this is while the amount of TSH was not affect by increasing the level of rapeseed (Maroufyan and Kermanshahi, 2006).

According to Woyengo *et al.* (2011), an increase in dietary level of expeller-extracted rapeseed meal from 0 to 40% resulted in a linear increase in serum tetraiodothyronine concentration. There was, however, no effect of dietary treatment on the serum triiodothyronine concentration.

CONCLUSION

To sum up, it can be concluded that using low levels of Rapeseed meal, almost to a maximum of 15 percent would produce no particular problem in poultry's diet. Furthermore, since the amount of fiber in Rapeseed meal is so high, using enzymes in poultry's diet, can be beneficial.

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