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The effect of mustard seed meal (*Sinapis arvensis*) on thyroid hormones and liver enzymes in Japanese quails (*Coturnix coturnix japonica*)

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ABSTRACT

Objective: To investigate the effect of wild black mustard seed meal on thyroid hormones (thyroxine and thyroid-stimulating hormone) in Japanese quails and also study the ability of $FeSO_4$ to alleviate the possible negative effect of mustard meal on thyroid hormones in these birds for the first time.

Methods: The experimental procedure was undertaken on 28 quails which were randomly assigned to a control and 6 test groups with 4 quails in each group for 28 days, during which the control group received basic diet with no mustard meal whereas the test groups (No. 2, 3 and 4) received mustard meal (5%, 10% and 15%, respectively) and test groups (No. 5, 6 and 7) received $FeSO_4$ (1%)-treated mustard meal (5%, 10% and 15%, respectively) on the basic of basic diet.

Results: The group fed on 15% non-treated mustard seed meal had the least thyroxine level and its level backed to normal in group fed on 15% $FeSO_4$-treated mustard seed meal although this group had the highest alanine transaminase and aspartate transaminase levels.

Conclusions: We concluded that up to 10% $FeSO_4$ mustard seed meal could be incorporated in the quail diet successfully with the least damage to thyroids and livers, but further investigations on these birds are still needed to confirm this hypothesis.

1. Introduction

The demand for quail birds and its products is increasing rapidly due to its medicinal, nutritional and economic benefit in all countries. The more quail birds raise, the more protein sources will be needed for their nutrition. It is well accepted that if a growing animal is provided with insufficient protein, then protein synthesis will be reduced and the efficiency of metabolisable energy utilization will probably be altered[1]. *Brassica* vegetables including wild black mustard (*Sinapis arvensis*) belonging to the family Cruciferae, is highly regarded for its protein value after the extraction of oil[2,3], but feeding it to poultry has been associated with thyroid malfunction because of the highest level of glucosinolates (a family of secondary plant metabolites) in its seeds[4,5].

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Glucosinolates are complex compounds and are composed of glucose and a thio-sulphate radical to which a side chain length is attached. Glucosinolates are usually present in relatively large amounts, as many as six of these compounds have been found in botanical family Cruciferae[6-9]. They can be converted into various bioactive metabolites such as isothiocyanates, oxazolidine-2-thiones, nitriles, thiocyanate ion and indole-3-carbinol by a plant enzyme myrosinase or animal thioglucosidase, which implies a goitrogenic effect[4,5,10-12].

For instance, isothiocyanates and oxazolidine-2-thiones have been shown to implicate in the malfunction of thyroid gland even if iodine was sufficient[12-15]. Attempting to overcome this problem has led to extensive researches into treating and detoxifying mustard meal (MM) by using heat, solid-state fermentation, $FeSO_4$ and ammonium[16-20]. Despite the importance of quails in poultry industry, most of the researches conducted in chicks and the effect of detoxified mustard on endocrine function in Japanese quails is not well studied.

The main objective of the present investigation was to evaluate the effect of different levels of treated and non-treated wild black mustard seed meal on thyroxine (T_4) and thyroid-stimulating hormone (TSH) in Japanese quails. In current experiment, liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST) were also measured in order to check the possibly adverse effect of $FeSO_4$ on livers.

2. Materials and methods

2.1. Birds

This experiment was carried out at the hen house of Agricultural School of Shahid Bahonar University of Kerman, Iran. The temperature of the hen house was around 20 °C and the light was given for 15 h per day from 6:00 am to 9:00 pm. A total of 28 experimental one day-old quail chicks were fed on a regular quail starter diet for 7 days and then they were assigned randomly into 7 groups, namely the control group (No. 1) and test groups (No. 2–7). The feeders were filled with feed twice a day and fresh water was available to the chicks all the time. All experimental procedures involving animals were conducted in accordance to the guidelines for use of animals in research and approved by the hen house of Agricultural School of Shahid Bahonar University of Kerman, Iran.

2.2. Test procedure

The experimental procedure was applied for 28 days during which the control group received basic diet with no MM whereas the test groups (No. 2, 3 and 4) received MM (5%, 10% and 15%, respectively) and the test groups (No. 5, 6 and 7) received $FeSO_4$ (1%)-treated MM (5%, 10% and 15%, respectively) on the basic of basic diet. This MM contained on dry matter basis: 28.6% crude protein, 40.3% ether extract, 5.0% ash, 7.0% moisture and 14.8% crude fiber[21]. Apparent metabolisable energy was 4 630 (Kcal/Kg dry matter). The diets were formulated using National Research Council guideline[22]. Nutrient compositions of quail diets used for 7–35 days of age were shown in Tables 1 and 2.

Table 1
Nutrient compositions of quail diets used for 7–35 days of age.

Ingredients:	MM (%)			
	0	5	10	15
Corn	53.20	50.95	48.70	46.50
Wheat bran	0.50	1.33	2.14	2.85
Soy bean meal	40.00	36.80	33.60	30.50
Mustard meal	0.00	5.00	10.00	15.00
Vegetable oil	2.55	1.70	0.85	0.00
Calcium carbonate	1.32	1.30	1.30	1.28
Dicalcium phosphate	0.80	0.80	0.80	0.78
Dl-methionine	0.16	0.16	0.16	0.15
L-lysine	0.12	0.11	0.10	0.09

Table 2
Calculated chemical composition (%) of quail diets used for 7–35 days of age.

Calculated chemical composition	MM (%)			
	0	5	10	15
Crude protein	22.000	21.990	21.970	21.980
Metabolisable energy (kcal/kg)	2905	2903	2900	2900
Lysin	1.307	1.304	1.302	1.302
Methionin	0.502	0.504	0.506	0.499
Methionin + cystein	0.863	0.877	0.890	0.894
Calcium	0.807	0.804	0.809	0.802
Phosphate, available	0.302	0.304	0.305	0.302
Na	0.151	0.152	0.152	0.152

2.3. Blood samples

Blood samples were collected from the 28 quails on day 35 after birth. Samples allowed clotting for 30 min and sera were separated following centrifugation at 3000r/min for 15 min and stored at -20 °C until assay.

2.4. Serum biochemical analysis

The determination of serum T_4 was carried out by microplate enzyme immunoassay method (Monobind Inc, Lake Forest, USA). TSH was determined using quantitative enzyme immunoassay technique using commercial chicken TSH ELISA Kit (Shanghai Crystal day Biotech Co., China). ALT and AST colorimetric assay was based on Reitman and Frankel Method[23].

2.5. Statistical analysis

All data were statistically analysed by SPSS/PC software (version 16). One-way ANOVA with Tukey HSD post-hoc test and independent sample *t*-test were used for the comparison of the means. The $P < 0.05$ was considered as statistically significant.

3. Results

The results were shown as mean ± SE in standard international units in Figure 1–4 which represented the concentrations of serum T_4, TSH, ALT and AST in the control and test groups at different treated and non-treated mustard meal levels.

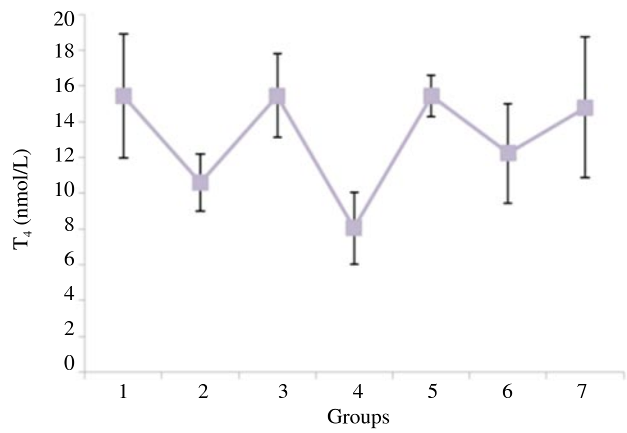

Figure 1. The concentrations of T_4 (nmol/L) in the control group and test groups.

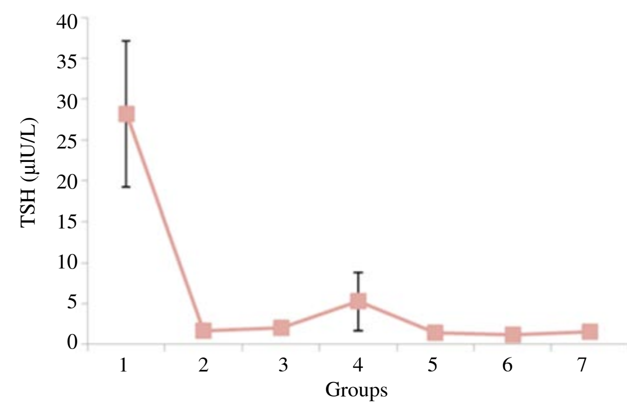

Figure 2. The concentration of TSH (μIU/L) in the control group and test groups.

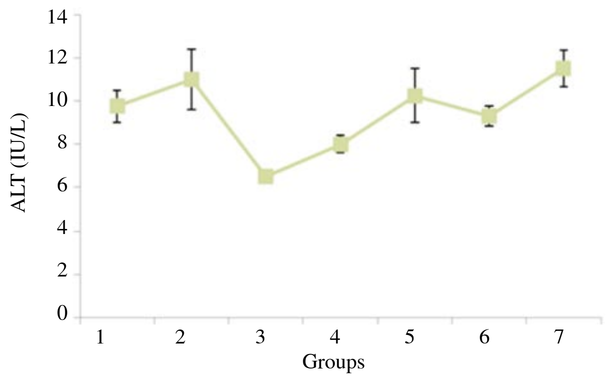

Figure 3. The concentration of ALT (IU/L) in the control group and test groups.

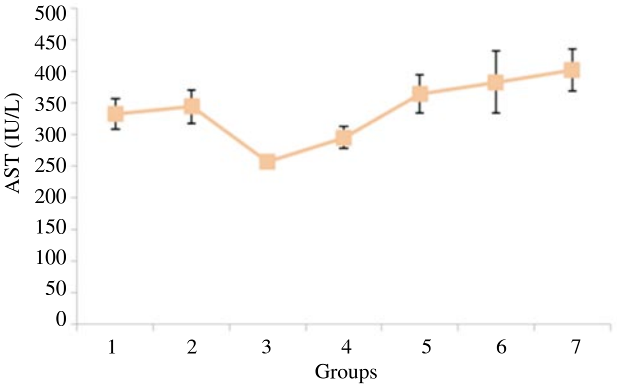

Figure 4. The concentration of AST (IU/L) in the control group and test groups.

According to the results, there were no significant differences in the concentrations of T_4, ALT and AST between the control and test groups. A significant decrease was found in T_4 concentration in group 2 (5% MM) as compared with that of group 5 (5% treated MM). There was a significant difference in TSH concentration between the control and all of test groups. There was no significant difference in TSH concentrations in group 4 (15% MM), group 7 (15% treated MM) and also group 2 (5% MM) in compared to group No. 5 (5% treated MM). We found a significant increase in TSH concentrations in group 3 (10% MM) as compared with group 6 (10% treated MM). The least T_4 concentration was belonged to group 4 (15% non-treated MM). Group 4 had the highest TSH concentration among the other test groups. We found significant increases in ALT and AST concentrations in group 7 (15% treated MM) as compared with group 4 (15% non-treated MM).

4. Discussion

It has been well established that *Brassica* seed meals, rapeseed meals (RSM) and raw soybeans characterized by anti-nutritional factors such as glucosinolates with different mechanisms are proven as growth inhibitors. Their lowering effects on the synthesis and release of thyroxin change the whole metabolism of different tissues and body organs[24-26]. Various detoxification methods are used to reduce glucosinolates contents of mustard seed meal in poultry diet[14,16,20,27]. For instance, in a study that was published by Daghir and Ali Mian[16], the $FeSO_4$ treatment of mustard meal was found to be effective in attenuating oxazolidinethione content of the meal by about 88% and the isothiocyanate content by 74% for starting chick rations. Likewise, treatment of the meal resulted in a significant improvement in body weight and feed efficiency of growing chicks[16]. Another investigation using solid-state fermentation for reducing glucosinolate contents of RSM significantly made progress in broiler performance as compared with birds fed on unfermented rapeseed meal. 20Canibe and Jensen in (2003) had shown improvement in pig feed intake and body weight gain by using fermented RSM in pigs' diet[17]. Surprisingly, the effect of detoxified mustard on thyroid function in Japanese quails is not well cleared. In the current study, treated and non-treated mustard seed meals were incorporated in isocaloric and isonitrogenous diets at the rate of 0%, 5%, 10% and 15%. As shown in Figure 1, thyroxin concentration in the group which recieved 10% MM was close to this value in the control. Similarly, Svetina and his coworkers[18] showed the serum concentrations of triiodothyronine and T_4 in pigs fed on 10% RSM were not significantly increased whereas in groups fed on (10%) RSM, thyroid gland and liver weights were significantly higher than those in the control group. Marked changes in liver histology were not observed and they suggested that 6%–10% RSM could be used as a protein source in the diet of fattening pigs without considerable side effects, which is in agreement with our results[18].

The concentration of T_4 decreased in group 4 as compare to that of the control group. Although it was not significant, but this group had the least T_4 among the other groups therefore these changes in T_4 concentration could be related to glucosinolates content of MM (15%) and probably affect thyroid gland. This result was in contrast with those observed by Elangovan *et al.* which showed the effect of RSM on blood biochemical factors in Japanese quails and indicated that enzymatic and histopathological studies of liver and thyroid gland fed on graded levels of high glucosinolates (92.5 μmol/g) rapeseed meal did not reveal any considerable changes[28]. A recent study conducted by Malik and his colleagues determined the safe dose of mustard seed meal in the diet for growing Japanese quails. They showed that feeding of 5%, 15%, and 25% MM diet for 30 days had no toxic effects on the growth of birds but groups received 15% and 25% MM had higher liver enzyme (alkaline phosphatase) than the control[29]. Findings by Tangtaweewipat *et al.* demonstrated that body weight gain, feed intake and efficiency in broiler chickens (2–7 weeks of age) decreased with the increasing level of MM and the overall performance indicated that MM could be incorporated in broiler diet up to 10% during 2–7 weeks of age without any adverse effects[30]. However, thyroid gland was found to enlarge with the increasing level of MM as a consequence which is in agreement with this study that there was no considerably change in T_4 concentration in the group fed on 10% treated MM. In our experiment, it seems that $FeSO_4$-treated MM attenuated the possible adverse effect of MM on thyroid hormone because T_4 concentration increased in the group fed on 15% treated MM as compare to that of the group fed on 15% MM. Interestingly, TSH concentration showed an increase in birds fed on 15% MM as compare to those fed on 15% treated MM probably to compensating decreased level of T_4. As shown in Figure 1, all of the groups fed on detoxified MM (groups 5, 6 and 7) exhibited almost normal T_4 concentration and we could indicated that $FeSO_4$ (1%) is a good treating agent to be used in the quail farms and even poultry industry to detoxify the goiterogenic *Brassica* species. Malik and Lone reported that those quails raising with 15% high glucosinolate RSM for 30 days had better growth and glucosinolates presented in the meal did not have the drastic effects which envisaged in the literature for mammals and other domestic birds[31], and this is in contrast with the current study that indicated quails like the chicks are sensitive to the toxic substances of MM. An experiment with Blair demonstrated that up to 10% ammoniated MM could be incorporated in the broiler chick diet successfully although the thyroid size was increased[32]. According to Figures 3 and 4, the most ALT and AST concentrations were belonged to quails received 15% treated MM. Although histopathological studies of the liver have been needed to confirm, we could hypothesized that 15% detoxified MM with 1% $FeSO_4$ might link to the accumulation of iron in liver and organ commonly affected by hemochromatosis. Iron overload by $FeSO_4$ causes inflammation in liver cells and diffuse liver enzymes (AST and ALT) in the blood as its consequence. In a pathological study in 2015, an introduction of 5% and 10% rapeseed cake to chicken feed resulted in certain liver and thyroid gland lesions, a mass increase of the liver in treated broilers and degenerative changes to blood vessels accompanied by vasculitis[33]. In another investigation in 2015, dietary canola meal (up to 15%) did not adversely affect the growth performance, while the relative weight of thyroid was significantly increased[34]. In a study in 2013, rapeseed meal at inclusion level of 10% or 15% was found to result in poor egg production and numerically poor feed efficiency values in layer type chickens whereas supplementations with potassium iodide, enzymes or amino acids were helpful in arresting the depressing effect of RSM on egg production and feed efficiency[35].

We concluded that up to 10% $FeSO_4$-treated MM could be incorporated in the quail diet successfully to avoid damage to thyroid and liver and it could be economically cheap protein source for these birds although further researches in this area are needed to support this hypothesis.

Conflict of interest statement

We declare that we have no conflict of interest.

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