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Full Length Research Paper

Rapeseed meal feeding effects on total proteins and lipids of Japanese Quail

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A detailed study was undertaken, in order to determine the safe dose of mustard seed meal required in the diet of Japanese quails that can be used at the production level without having deleterious effects on the growth of these birds. For this purpose, some 1500 birds were fed different levels of mustard seed meal in isocaloric and isonitrogenous diets. The results of its application, on the diets of these birds for 30 days show that the protein content of the liver remained more or less constant and capable of catering for the needs of new cell production. While lipids where low in the early stages of the birds growth, they provided extra energy for growth. When lipid deposition was started the lipid levels increased in tissues. Biochemical studies performed on the liver show that the glucosinolates present in the mustard did not have drastic effects on the liver as reported in the literature for mammals and other domestic birds. In this study, it was discovered that, in the case of protein and lipid contents of liver, there was no significant difference between the controls and the experimental groups.

Key words: Japanese, lipids, proteins, quail, rapeseed meal.

INTRODUCTION

Rapeseed is known to contain high quality protein due to its amino acid profiles. Most commonly, soybean meal is used as protein supplement for poultry and generally high quality product. Unfortunately, the use of soybean meal is cost effective and many poultry producers, looking for alternative sources of supplementary protein which may be available at a lower cost. In China, rapeseed meal is used as an alternative to soybean meal (Britzman, 2006).

In rapeseed meal there are three compounds that account for almost all the glucosinolate present. These are progoitrin, gluconapin and glucobrassicanapin. It is confirmed that low glucosinolates rape seed meal are improved by heat treatment. Heat effect does not appear to be related to the low levels of the glucosinolates present in these meals, to destruction of trypsin inhibitors in the meals or to an increase in the digest ability of their protein (Newkirk et al., 1997).

In Thailand, mustard meal (MM) is an important by-product from oil ex-traction process of the seed because of its high nutrient content and the availability of around 4,000 tons of fresh matter a year. Since mustard (*Brassica juncea*) is in the same genus as rapeseed (*B. napus or B. compestris*), it contains similar toxic substances i.e. glucosinolate, erucic acid, sinapine, tannin and some anti-nutrient factors (Rozan et al., 1996). The substances induce unpalatability, growth retardation, thyroid gland enlargement, low feed efficiency and reproductive problem, particularly when the seed is incorporated in the diet at a high level (Hyankova et al., 2008).

Rats fed polished rapeseed oil consumed less food and gained less weight than animals fed the other oils. Liver and adrenal weights did not vary significantly. The difference in weight gains between the two groups of rats receiving approximately the same amount of erucic acid (C-22:1) from rapeseed oil was significant at $P<0.05$, but

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an analysis of co-variance indicated that the variations in weight gain could be accounted for by food consumption (Chiang et al., 2010). It was evident that the diet containing Swedish rapeseed oil and palm oil was more acceptable to the rats than the ones containing polish rapeseed oil. When rapeseed oil was present in the diets the level of arachidonic acid (C-20:4) in the liver was less than when the diet contained no erucic acid. It appeared that in the presence of erucic acid the conversion of linoleic acid to arachiodonic acid was decreased (Tangtaweewipat et al., 2004).

The pectoral muscle contained soluble proteins with molecular masses corresponding to 181, 128, 93, 76, 72, 62, 56, 43, 41, 28 and 20 with low amounts of kiloDalton but those of 60, 50 and 37 had higher amounts of kDa in them. The heart contained soluble proteins with a molecular mass of 181 and kDa were present in lower amounts while in the brain those of 43 kDa were present in lower amounts but those of 221 kDa were present in higher amounts. The specific activity of glyceraldehyde-3-phosphate dehydrogenase decreased markedly both in the liver and pectoral muscle of niacin deficient quail whereas that of 6-phosphogluconate dehydrogenase and malic enzyme decreased markedly in the liver or pectoral muscle, respectively (Koha et al., 1998).

It has been established by feeding rapeseed oil, high in C-22:1 (erusic acid) concentration, that severe fat accumulation in heart, adrenal and skeletal muscles occur within the first week of feeding. This fatty infiltration gradually disappears after rats are kept for several months on these diets (Abdellatif and Vles, 1970). The early cardiac fat accumulation is due mainly to an increase in the concentration of triglycerides and free fatty acids while the phospholipids and cholesterol concentrations remains constant (Iwasaki et al., 2000).

The comparative effects of rapeseed oils containing high and low levels of erucic acid (C-22:1) were studied. Rats fed the experimental diets showed a slightly reduced body weights compared to other groups. Liver weight, which increased in both experimental groups, was significantly higher in rats tested for cholesterol regimen. These animals had a progressive rise of cholesterolemia, which was approximately doubled at the end of 8 weeks of dietary treatment (Sirtori et al., 1984).

Rapeseed meal is used in Canada as a protein supplement. Diet which contained diet Rapeseed oil (RSO) caused a 13 fold rise in triacylglycerols and a 3 fold rise in total fatty acids in hearts compared to the diet containing the same percent of peanut oil. The concentration of heart phospholipids was not influenced by the diet. The increase in the triacylglycerol fraction caused by the RSO diet could almost completely account for the increase in the total fatty acids. The concentration and composition of fatty acids in heart lipids are apparently reflections of the oxidative capacity of the heart mitochondria and the rate of absorption of fatty acids from the blood directly. Blood triacylglycerol can be taken up after previous hydrolysis by heart lipoprotein lipase (Nagata et al., 1996).

MATERIALS AND METHODS

Animal husbandry, formulation of feeds and sampling

For rearing of Japanese quails, feeding and sample collection, Malik and Lone (2011) method was used. The analyses were always done within a week.

Sampling

The birds used for this experiment, were weighed at regular intervals, till the age of 30 days. During this period, samples were taken from each of the four groups consisting of at least 5 male quails for biochemical analyses. In carrying out the samples, care was taken in selecting those birds which did not deviate from the mean of the sample with more than 10% in weight.

At the time of taking the samples, the quails were slightly anaesthetized and then slaughtered. After all the blood was drained off , the liver was immediately dissected out, cleaned and blotted with tissue paper and weighed to the nearest milligram and then frozen immediately for analysis.

Data analyses

Tissue-body index

Tissue-Body Index was calculated using the formula:

$$\text{Tissue-Body Index} = \frac{\text{Weight of organ (grams)}}{\text{Weight of animal (grams)}} \times 100$$

Statistical procedures

All weights and other values were analyzed for statistical difference from respective control values by applying Single-Factor Analysis of Variance according to Sokal and Rohlf (1969). The detailed analyses were made according to Campbell (1974).

Preparation of tissue homogenates

For this preparation, a suitable amount of tissue (about 200 mg) was taken and homogenized in 1 ml ice-cold water using a motor driven homogenizer (Ultra Turrax). Finally this homogenate was separated to extract and estimate total proteins and lipids.

Determination of total proteins

Proteins were determined by using the method of Lowry et al.(1951) as modified by Schacterle and Pollack (1973). Furthermore, 1 ml of NaOH hydrolyzate was added 24 ml of distilled water to dilute it 25 times. Then Commercial Folin-ciocalteau solution (Merck) was diluted 16 times before use then Folin mix was prepared by dissolving 5 grams of NaOH and 25 grams of sodium carbonate in about 100 ml of distilled water and by separately dissolving 250 mg of sodium tartrate and 125 mg copper sulphate

Table 1. Effect of feeding different percentages of Rapeseed meal on the Protein (mg/100 mg) content of Liver of Japanese Quail. Values given are Mean ± S.E. of 5 animals each.

Age (day)	Control	Percentage rapeseed meal in diet		
		5.0	15.0	25.0
Zero day	10.89±0.85			
6	18.92±2.07	18.01±1.2	15.09±2.04	-
8	-	-	-	-
10	16.39±2.00	16.04±1.08	16.16±0.57	12.50±0.54
14	16.50±1.37	18.70±2.08	17.41±1.82	17.51±1.13
18	19.61±1.67	19.57±1.08	17.46±0.92	16.28±1.18
22	13.63±1.00	16.16±0.99	15.89±0.49	16.20±0.26
30	18.25±0.52	18.09±1.08	19.95±1.00	18.32±0.99

in another 100 ml of distilled water. This solution was then poured into the NaOH-sodium carbonate solution and volume made up to 250 ml with distilled water. Thereafter, Protein was determined by taking 1 ml of diluted hydrolyzate and 1 ml of Folin mix and after 10 minutes, 4 ml of diluted Folin-ciocalteau and Folin-phenol solution was added to the above solution, Tubes were shaken and incubated in a water bath at 55±1℃ for 5 min after wh ich the tubes were then cooled and the blue color was read at 650 nm in Beckman-Spinco spectrophotometer. After that Standard curve was prepared using Bovine Serum Albumin (BSA) and Proteins were calculated using this standard curve and expressed as mg/100 mg of tissue.

Determination of total lipids

In this experiment, total lipids were determined by using the method of Woodman and Price (1971). Vanillin solution was prepared by dissolving 6 grams of anhydrous KH_2PO_4 and 0.3 grams of vanillin in 100 ml of distilled water. For test 0.1 ml of homogenate was taken in a test tube and 1 ml of concentrated sulphuric acid was added. The tubes were placed in a boiling water bath for 20 min. The contents of the tubes were then cooled in cold water and 2 ml of vanillin solution was added and the tubes again cooled. After this, a Violet colour obtained was read after exactly 10 minutes at 530 nm against a reagent blank, the values where then expressed as mg/100 mg of tissue.

Statistical procedures

All values were analyzed for statistical difference from respective control values by applying Single-Factor Analysis of Variance according to Sokal and Rohlf (1969). The detailed analyses were made according to Campbell (1974).

RESULTS

Proteins

In their early stages of hatching the liver proteins were 10.89±0.85 mg. The proteins then increased at 6 days, slightly decreased at 8 days and then remained more or less constant till 14 days of age. At this time there was no difference between the controls and the experimental groups (ANOVA: Not Significant). The values then remained around 16mg/100mg of the liver, and then at the end of experiment values increased but at 30 days, there was no significantly difference between the controls and the experimental groups (Tables 1, 2 and 3).

Lipids

At zero-day of age liver lipids, 23.25±2.12 mg/100 mg was contained in their tissue. Thereafter, the liver lipids decreased for another 10 days of life. Measurements after 10 days of the controls showed the highest values while there was a dose response relationship between the experimental groups. This was an inverse relationship between the concentrations of the rapeseed meal. Therefore, the highest concentration of rapeseed induced the minimum fat values while minimum rapeseed in diet caused the highest hepatic lipid values. After 14 days, dose response relationships were present. During this period time controls had the minimum fat stores. After 14 days, there was a sudden increase in the liver lipids. This increase caused a peak in the lipid values. After this peak, liver lipid decreased until the end of the experiment. There was no change in the lipids both at 14 and after 30 days (Tables 4, 5 and 6).

DISCUSSION

The present study was undertaken to observe the effects of the consumption of *Brassica* seed meal on the lipids and proteins of Japanese quail *Coturnix coturnix japonica.* As reported earlier seeds belonging to the family Cruciferae contain certain toxic substances, which upon inclusion in the diet cause growth retardation, thyroid hypertrophy, liver damage and other biochemical and metabolic derailment. The Brassica seeds were purchased from the local grain market and were expelled with the aid of an oil expeller to extract oil from them. The meal obtained after removing the oil from the seeds was

Table 2. Detailed statistical analyses based on results of Table 1. This statistics is according to single-factor ANOVA of Proteins (14 days).

Item	Sum of squares	Df (n-1)	Mean squares	*F*-value
Rations	12.2346	3	4.0782	0.2400747(not significant
Error	271.7954	16	16.987213	
Total	284.03	19		

Table 3. Detailed statistical analyses based on on results of Table 1. This statistics is according to single-factor ANOVA of Proteins (30 days).

Item	Sum of squares	Df (n-1)	Mean squares	*F*-value
Ratios	11.664	3	3.888	0.3455269 (not significant)
Error	87.368	16	4.5983158	
Total	99.032	19		

Table 4. Effect of feeding different percentages of rapeseed meal (mg/100mg) on the lipid content of Liver of Japanese quail. Values given are Mean ± S.E. of 5 animals each.

Age (day)	Control	Percentage rapeseed meal in diet		
		5.0	15.0	25.0
Zero day	23.25±2.12	-	-	-
6	18.94±4.09	17.76±2.18	19.59±2.74	-
8	6.24±0.18	8.01±0.42	14.23±1.53	-
10	10.30±0.51	8.67±0.54	7.94±0.69	6.65±0.65
14	5.92±0.87	8.78±0.43	9.50±0.09	11.46±0.75
18	21.28±5.46	27.28±1.80	24.66±2.35	19.54±1.60
22	11.50±0.58	10.25±1.36	10.76±1.08	9.81±0.77
30	6.48±0.56	7.02±0.95	6.66±0.62	6.56±0.69

Table 5. Detailed statistical analyses based on results of Table 4. This statistics is according to single-factor ANOVA of lipids (14 days).

Item	Sum of squares	Df (n-1)	Mean squares	*F*-value
Ratios	69.06756	3	23.02252	1.318727 (not significant)
Error	29.45814	12	17.45814	
Total	98.5257	15		

Table 6. Detailed statistical analyses based on results of Table 4. This statistics is according to single-factor ANOVA of lipids (30 days).

Item	Sum of squares	Df (n-1)	Mean squares	*F*-value
Rations	0.90456	3	0.30152	0.948764 (not significant)
Error	44.49237	14	3.1780264	
Total	45.39693	17		

incorporated in isocaloric and iso nitrogenous diets at the rate of zero, 5, 10, 15, 20 and 25% of the diet. The diets were prepared from the local ingredients and were designed to be low cost diets. The feeding of these diets was started from the day of hatching and continued up to 4-weeks of age (Malik and Lone, 2011).

Hepatic proteins were quite low at the time of hatching. At the age of 6 days, they nearly doubled and after a slight depression they remained more or less constant with occasional increase or decrease here and there. As reported by Malik and Lone (2011), the growth of the liver was more due to hyperplasia, so the protein content of the liver remained more or less constant to cater need for new cell production. It's worth mentioning here that when expressed here as protein/liver, the protein concentration does reflect the weight of the liver also.

Liver lipid decreased with age from zero days to at least 10 days of age, then there was a trend towards increase and the lipids reached their peak at the 18th day of their life span. Thereafter the peak subsided and values came back to the pre-peak values. Lipids, as recorded previously, are generally quite high at the time of hatching in tissues of animals having megalecithal eggs, e.g. birds. This is probably due to the fact that yolk is the major source of energy and other materials for development. During the early growth period, when growth is quite fast and the food is not rich in fats, these lipids provide extra energy for increased developmental demands thereby causing depletion in cellular lipids. Later on, the lipids start depositing again after which the animal generally slows down a bit, a condition quite conducive for lipid deposition.

After studying in details the effects of consuming mustard meal, it is concluded that the consumption of mustard seed meal did not impair growth of Japanese quails. The changes observed in their liver points to the fact that at the highest dose of the mustard seed meal there were some indications of the toxic effects of glucosinolates in the meal. Nevertheless, these effects were restricted to the weight of liver. The changes seen in the biochemistry of their liver were more due to aging than due to the application of glucosinolates in their diet.

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