JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0160147

Evaluation of Different Levels of Canola Meal on Performance, Organ Weights, Hepatic Deiodinase Gene Expression and Thyroid Morphology in Broiler Chickens

Sina Payvastegan, Parviz Farhoomand, Mohsen Daneshyar and Mokhtar Ghaffari

Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran

This study was carried out to determine the effects of dietary inclusion level of canola meal (CM) on performance, organ weights and hepatic type I deiodinase gene expression in broilers. A completely randomized design with 4 levels of CM (0, 10, 20 and 30%) as a substitute for soybean meal (SBM) was utilized with 5 replicates of 9 birds each. The results showed that body weight gain (1 to 42 d) decreased linearly ($P<0.01$) as the inclusion of CM increased. An increase in dietary level of CM also resulted in a linear ($P<0.05$) increase in feed conversion ratio (1 to 42 d). Proportion of thyroids ($P<0.05$) and liver ($P<0.01$) increased linearly with increased levels of CM. A significant linear increase in right ventricular weight: total ventricular weight ratio ($P<0.01$) and heart weight ($P<0.05$) were observed by substituting CM for SBM. The concentration of plasma triiodothyronine and triiodothyronine: tetraiodothyronine ratio decreased linearly ($P<0.01$) with increasing level of CM. Expression of hepatic type I deiodinase gene (D1) decreased linearly ($P<0.01$) as inclusion level of CM in diets increased. Moreover, increasing linear ($P<0.01$) and quadratic responses ($P<0.05$) were observed in follicles number and epithelial thickness in broilers thyroids followed by increased levels of CM. In addition, increases in dietary CM inclusion led to a linear ($P<0.01$) increase in thyroid follicles diameters. In conclusion, the results of this study indicate that feeding increasing CM inclusions from 0 to 30% negatively affect growth performance of broiler chickens. From this study, it can also be concluded that substitution of CM for SBM adversely interferes with thyroid and liver functions and decrease D1 gene expression, likely because of higher dietary concentration of glucosinolates.

Key words: broiler, canola meal, hepatic deiodinase gene expression, organ weights, performance, thyroid morphology

J. Poult. Sci., 54: 282–291, 2017

Introduction

Soybean meal (SBM) is the primary protein source in diets for poultry due to its high protein and energy content, its excellent amino acid quality and composition, as well as the high availability of AA (de Coca-Sinova *et al*., 2008; Chen *et al*., 2013). However, at different locations, some other protein meals other than SBM are available at lower prices and thus preferentially used in least-cost formulations (Aftab, 2009).

Canola is the second most important oil-producing crop after soybean (McNaughton *et al*, 2014). It accounts for 13% of vegetable oil produced in the world (Duan *et al*., 2011). Recently, there has been great interest in canola production in many parts of the world including Iran. In a 10-year strategic plan, Iran's Ministry of Jihad-e-Agriculture is supposed to supply 75% of its oil requirements from domestic production. Accordingly, the country's reliance on self-sufficiency in oil production is based on canola, since nearly 43% of the canola seed contains oil which is more than double the oil content of soybeans (Canola Council of Canada, 2009).

Given that canola meal (CM) must contain less than 30 μmol/g of aliphatic glucosinolates, it could effectively replace SBM in poultry diets. However, excessive levels of CM and thus high dietary glucosinolate content, could lead to abnormalities in thyroid function and liver enzyme activities and subsequently growth depression (Mikulski *et al*., 2012). The goitrogens are potent inhibitors of peripheral tetraiodothyronine (T4) conversion (Tanabe *et al*., 1965). In broiler chickens, it was observed by Davison *et al*. (1981) that plasma triiodothyronine (T3) concentration and T3: T4 ratio decreased after feeding rapeseed meal and they attributed this to inhibition of peripheral T4 monodeiodination caused by glucosinolates degradation products. A linear decrease in

Received: December 5, 2016, Accepted: February 25, 2017
Released Online Advance Publication: May 25, 2017
Correspondence: Sina Payvastegan, PhD, Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran.
(E-mail: sina.payvastegan@yahoo.com)

blood levels of T3 was also observed by Mikulski *et al*. (2012) in a study in which turkeys were fed on diets containing graded levels of CM. Woyengo *et al*. (2011) reported that an increase in dietary CM from 0 to 40% linearly decreased the feed intake (FI), body weight gain (BWG) and plasma T4 concentration and linearly increased the relative weights of thyroids, liver and kidney of broilers. Significant decrease in BWG, FI and feed efficiency were also observed when feeding CM based diet to broiler chickens raised at high altitude (Khajali *et al*., 2011).

Although CM is particularly rich in sulfur containing amino acids (1.58 vs. 1.28%), the lysine (Lys; 1.94 vs. 2.69 %) and arginine (Arg; 2.08 vs. 3.14%) contents of CM are lower than SBM (NRC, 1994). Moreover, essential amino acid digestibility values of CM are lower than those of SBM (Khajali and Slominski, 2012). Lys is one the key amino acid for protein synthesis, muscle deposition and represents approximately 7% of the protein in breast meat (Mahdavi *et al*., 2012). Arg has also been documented to be a precursor of nitric oxide (NO), a potent vasodilator (Khajali and Wideman, 2010). As the chickens are unable to synthesize Arg, feeding high levels of CM to broilers instead of SBM might not probably provide sufficient Arg to fully support the production of NO by the pulmonary vascular endothelium (Izadinia *et al*., 2010). Newkirk and Classen (2002) demonstrated that high levels of CM in the diet increase the incidence of chronic heart failure in broilers, but the high rate of chronic heart failure in chickens fed on CM remained unexplained.

Despite all these, several authors have showed no adverse effect of high dietary inclusion of CM on growth performance of broilers (Kocher *et al*., 2000; Baloch *et al*., 2003). CM can be included effectively in the diet of broilers at a level of up to 30% without affecting growth performance, as long as diets are formulated on the basis of digestible amino acid (Canola Council of Canada, 2009). Kocher *et al*. (2001) indicated no adverse effect of feeding CM when it was added at 35% of the broilers' diet. Payvastegan *et al*. (2013) observed no adverse effect of CM in the inclusion rate of 20% of the broilers diet. Gopinger *et al*. (2014) also found that CM can be added up to 16.7% in diets for broiler without affecting the key variable of growth performance.

Due to the recent challenges in feed costs in poultry industry and the potential benefits of utilizing alternative ingredients in broiler chicken diets, more data on performance, hepatic type I iodothyronine deiodinases (D1) gene expression and thyroid morphology are required. Therefore, the objectives of the current study were to determine the effects of different dietary levels of CM on performance, organ weights, D1 gene expression and thyroid morphology.

Materials and Methods

Animal Ethics

The experiment was performed at the experimental poultry farm of the Animal Science Department, Urmia University, Urmia, Iran. The protocol for this study was approved by animal care and use committee of the Urmia University.

Chickens, Experimental Design and Husbandry

One-hundred-and-eighty one-day-old male broiler chicks (Ross 308) were purchased from a commercial hatchery, weighed on arrival, and randomly assigned to 4 dietary treatments and 5 replicates of 9 birds per floor pen in a completely randomized design. Four levels of CM (0, 10, 20 and 30%) were used to substitute SBM. New wood shavings at a depth of approximately 10 cm were used as bedding material over the concrete floor. The experiment lasted for 42 d and was divided into 3 phases which include starter (1−10 d), grower (11−24 d) and finisher (25−42 d). All diets were fed in mash form. During the first 3 d, room temperature was set at 32℃, and gradually decreased thereafter to a constant value of 22℃ in fourth week and was maintained until the end of the experiment. Fresh water and feed were provided to allow ad libitum access and light was provided for 24 h daily throughout the experiment. All chickens underwent vaccination program scheduled (Newcastle, infectious bronchitis, avian influenza and infectious bursal disease) according to the local practice.

Diets

All diets (Table 1) were formulated to meet the 96.67, 96.77 and 96.87% of Ross 308 nutrient specifications for macro- and micronutrients during the starter, grower and finisher periods of experiment, respectively (with approximately 3.23% dilution). The corn, SBM, CM and gluten used for formulating the experimental diets were analyzed for dry matter, crude protein, and digestible amino acid contents by near-infrared spectroscopy. Metabolizable energy contents of corn, SBM and CM were estimated by using the regression models presented by NRC (1994). Expeller-extracted CM was obtained from a local provider with an amount of 23.5 μmol/g glucosinolate which was obtained by analysis according to the procedure described by Smith and Dacombe (1987) in the Oil Seed Research and Development Company of Tehran, Iran. Chemical composition of experimental diets for crude protein (CP), crude fiber (CF) and crude fat (EE) were determined by Association of Official Analytical Chemists methods 992.93, 962.09 and 920.39, respectively (AOAC, 2000).

Traits Measured

Growth Performance and Organ Weights

Body weight and FI were measured at the end of starter, grower and finisher periods by pen basis after 6 h of feed withdrawal. FI, BWG and feed conversion ratio (FCR) were calculated for 1 to 42 d period. Daily mortality (if any) was recorded and the chickens that were removed or died during the experiment were weighed to adjust FCR and FI. At the end of the experiment, 1 bird per pen (5 chickens per treatment) was selected randomly and weighed individually, then slaughtered by a unilateral neck cut severing the right carotid artery and jugular vein. Eviscerating and rinsing were subsequently done manually. Then, whole carcass, breast, thigh plus drumstick portions, heart, liver, pancreas, abdominal fat, gizzard, small intestine, duodenum, jejunum, ileum and cecal were collected, weighed, and calculated as a percentage of live body weight. The ventricles were also dissected and

Table 1. **Composition of basal diets and calculated and analyzed composition (as-fed basis)**

Item	Starter (1−10 d)				Grower (11−24 d)				Finisher (25−42 d)			
Ingredients (%)	0%	10%	20%	30%	0%	10%	20%	30%	0%	10%	20%	30%
Maize	55.75	53.25	50.75	48.90	58.49	55.00	50.86	48.70	62.87	60.74	58.14	55.87
Soybean meal (45% CP)	38.89	28.95	19.00	8.10	35.51	27.01	19.44	9.00	30.56	20.08	10.29	0.00
Canola meal (34.47% CP)	0.00	10.00	20.00	30.00	0.00	10.00	20.00	30.00	0.00	10.00	20.00	30.00
Soybean oil	0.72	1.79	2.87	3.76	1.82	3.17	4.69	5.67	2.69	3.66	4.76	5.77
Dicalcium phosphate	2.38	2.34	2.29	2.26	2.13	2.07	2.00	1.96	1.93	1.88	1.84	1.79
Calcium carbonate	0.80	0.72	0.64	0.56	0.74	0.66	0.57	0.49	0.68	0.60	0.52	0.44
Gluten (80% CP)	0.00	1.41	2.82	4.67	0.00	0.73	1.03	2.67	0.00	1.66	3.00	4.56
Vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.34	0.34	0.35	0.36	0.34	0.35	0.35	0.36	0.34	0.35	0.36	0.36
L-Lysine HCl	0.19	0.30	0.42	0.56	0.13	0.20	0.26	0.39	0.13	0.25	0.37	0.49
DL-Methionine	0.32	0.27	0.23	0.18	0.27	0.23	0.20	0.15	0.24	0.20	0.15	0.10
L-Thr	0.12	0.13	0.15	0.17	0.08	0.09	0.09	0.11	0.06	0.08	0.10	0.11
Calculated analyses (%)												
ME (Kcal/kg)	2900	2900	2900	2900	3000	3000	3000	3000	3100	3100	3100	3100
CP	22.23	22.23	22.23	22.23	20.81	20.81	20.81	20.81	18.89	18.89	18.89	18.89
Calcium	0.93	0.93	0.93	0.93	0.84	0.84	0.84	0.84	0.77	0.77	0.77	0.77
Available P	0.46	0.46	0.46	0.46	0.42	0.42	0.42	0.42	0.38	0.38	0.38	0.38
Arg	1.36	1.25	1.13	1.00	1.27	1.18	1.11	0.99	1.13	1.01	0.90	0.78
Lys	1.24	1.24	1.24	1.24	1.11	1.11	1.11	1.11	1.00	1.00	1.00	1.00
Met + Cys	0.92	0.92	0.92	0.92	0.84	0.84	0.84	0.84	0.78	0.78	0.78	0.78
Analyzed composition (%)												
CP	22.21	22.20	22.24	22.24	20.81	20.79	20.78	20.79	18.85	18.87	18.87	18.87
CF	3.05	3.86	4.67	5.46	2.97	3.81	4.67	5.47	2.85	3.65	4.47	5.27
EE	3.86	4.92	5.97	6.86	5.00	6.31	7.77	8.74	5.94	6.90	7.99	8.98
Total glucosinolates (μmol/g)	0.00	2.35	4.70	7.05	0.00	2.35	4.70	7.05	0.00	2.35	4.70	7.05

* Provided the following per kilogram of diet: vitamin A (trans retinyl acetate), 3,600 IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-α-tocopheryl acetate), 7.2 mg; vitamin K3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; pyridoxin, 1.2 mg; cobalamine, 0.6 mg; folic acid, 0.5 mg; choline chloride, 200 mg.

** Provided the following per kilogram of diet: Mn (from MnSO4·H2O), 40 mg; Zn (from ZnO), 40 mg; Fe (from FeSO4·7H2O), 20 mg; Cu (from CuSO4·5H2O), 4 mg; I [from Ca(IO3)2·H2O], 0.64 mg; Se (from sodium selenite), 0.08 mg. 3Values in parentheses are amino acid levels obtained by analysis.

weighed to calculate the right ventricular weight: total ventricular weight ratio (RV: TV) as ascites heart index (%) following the separation of hearts. The RV:TV ratio is indicative of pulmonary hypertension and values greater than 0.27 are considered as ascites (Daneshyar *et al*., 2009).

Plasma Parameters

These same 5 chickens from each treatment (1 bird per replicate) were also selected for blood samples collection by severing the carotid artery and jugular vein for biochemical study. A portion of the blood samples was collected into plastic tube containing a solution of dipotassium EDTA (which serves as an anticoagulant) and then centrifuged at 3000 rpm under constant temperature of 4℃ for 10 min to obtain plasma. Blood plasma samples were analyzed for glucose, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), uric acid and hepatic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) by commercial enzymatic kits (Pars Azmon, Tehran, Iran) with a plasma autoanalyzer (Abbott Alycon 300, Autoanalyzear medical system,UK). Extra plasma was employed for the determination of thyroid hormones. The T4 and T3 concentrations in the plasma were determined by radioimmunoassay (RIA) with commercial RIA kits (Pars Azmon, Tehran, Iran). The remaining blood samples were immediately used to determine packed cell volume (PCV), red blood cells (RBC) counts and hemoglobin (Hb) concentration.

D1 Gene Expression:

RNA isolation and cDNA synthesis: After necropsy, liver samples were immediately collected into cryo-tubes and frozen in liquid nitrogen and then stored at −80℃ until further analysis for gene expression. Total RNA was isolated from the hepatocyte monolayers using the Qiazol Lysis Reagent protocol (Qiagen, Hilden, Germany).

The dried RNA pellets were resuspended in 48 μl of diethyl pyrocarbonate-treated water. The optical density of RNA at 260 and 280 nm was measured by spectrophotometric analysis to evaluate the purity and concentration of RNA

(Eppendorf biophotometer Spectrophotometer, Germany). The RNA was shown to have an OD260:OD280 ratio between 1.8 and 2.2. First-strand complementary DNA was synthesized from 1000 $\mu g/\mu l$ of total RNA utilizing Oligo-dT primers and revert aid reverse transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) based on the manufacturer's instructions. Ribonucleic acid samples were subjected to a 5×g DNA Wipeout Buffer and then converted to complementary DNA (cDNA) using a QuantiTec Reverse Transcription kit (Thermo Fisher Scientific). Synthesized cDNA samples were stored at −20℃.

Quantitative real-time PCR: Primer3 software was used to design specific primers for D1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; was used as a housekeeping gene) based on known chicken sequences. Sequences of primers were as follows: D1 forward primer was 5′-CTGAATTTACAGCGTGGCCG-3′ and reverse primer was 5′-ACGCGAGACAAGGAGTGTTT-3′ and GAPDH forward primer was 5′-TCAAATGGGCAGATGCAGGT-3′ and reverse primer was 5′-TGATGGCATGGACAGTGGTC-3′. The sequencing data was analyzed on the National Center for Biotechnology Information website (http://www. ncbi. nlm. nih.gov/) with the Basic Local Alignment Search Tool to determine the degree of similarity between the sequences obtained in this study and those published in Genbank.

Target cDNA (D1) and reference cDNA (GAPDH) were amplified using SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA) by ABI StepOnePlus^{TM} Real-Time PCR system (Applied Biosystems, Grand Island, NY, USA) based on the manufacturer's instructions. An aliquot (1 μl) of cDNA template solution was added to a total volume of 20 μl containing 10 μl of SYBR Green mix, 7 μl of DEPC-water, and 1 μl (10 pm) each of forward and reverse primers. After a pre-denaturation programme (30 s at 95℃), 40 cycles of amplification were performed (95℃ for 15 s, 58℃ for 30 s, 72℃ for 15 s) and the melting curve was recorded from 85℃. The amplification of GAPDH in each sample was used to normalize the expression of selected gene. The relative expression ratio (R) of mRNA was calculated by $R = 2^{-\Delta\Delta Ct(sample\text{-}control)}$, where $-\Delta Ct$ (sample-control)=(Ct D1 −Ct GAPDH)$_{sample}$−(Ct D1−Ct GAPDH)$_{control}$.

Thyroid Morphology

Necropsy was performed and thyroids of 5 chickens from each treatment were weighed and fixed in 10% formalin saline. The thyroid weights were recorded as combined right and left glands. Following fixation, samples were trimmed, cleared, dehydrated, and embedded in paraffin. Serial sections were cut at 5 μm using a microtome and placed on glass slides. Sections were deparaffinized in xylene, rehydrated in graded ethanol solutions, stained with hematoxylin and eosin, and examined under a light microscope. There were three cross sections per sample (15 cross sections for each treatment), and 10 measurements per cross section (150 measurements per treatment).

Statistical Analyses

A polynomial regression (linear, quadratic and cubic) analysis was employed to predict the effect of the inclusion of various levels of CM in the diet on different parameters tested. Data were analyzed using the PROC GLM procedure of SAS 9.1.3 package (SAS Institute Inc., Cary, NC). Pen was considered as the experimental unit for performance criteria and individual chicken for carcass parts, organ weights, plasma parameters and D1 gene expression data. Outlier data were removed after using the UNIVARIATE procedure of SAS to produce a normal probability plot based on residuals and visual inspection of the raw data. Statistical significance was determined at a probability level of 0.05.

Results

Performance

Effects of dietary level of CM on BWG, FI and FCR are summarized in Table 2. The orthogonal polynomial contrasts revealed that the increase in dietary level of CM from 0 to 30% resulted in a linear ($P<0.01$) decrease in BWG. Moreover, there was a linear ($P<0.05$) increase in FCR with increasing levels of CM. No significant ($P>0.05$) effect was found for FI after the addition of CM to broiler diets.

Organ Weights

Effects of inclusion level of CM on organ weights are presented in Table 2. Decreasing linear ($P<0.05$) response was observed to increase CM level from 0 to 30% for breast yield, whereas chicken's relative weights of liver ($P<0.01$), small intestine, duodenum and ileum demonstrated increasing linear ($P<0.05$) responses. However, the carcass yield, leg, gizzard, pancreas, jejunum, cecal, and abdominal fat weights, demonstrated no statistically significant ($P>0.05$) differences.

Heart and Hematological Parameters

Table 3 illustrates the heart relative weight and hematological parameters measured at the end of the experiment. The regression analyses demonstrated that dietary levels of CM linearly ($P<0.01$) increased RV:TV ratio. Proportion of heart weight also demonstrated both linear ($P<0.05$) and cubic ($P<0.05$) responses with increasing level of CM. The level of inclusion of CM demonstrated no statistically significant ($P>0.05$) effects on PCV, RBC count and Hb concentration.

Blood Biochemical Indices

As shown in Table 4, no significant ($P>0.05$) differences were found in plasma concentrations of glucose, cholesterol, triglycerides, HDL, lactate dehydrogenase, creatine kinase and T4 in broilers receiving diets containing different levels of CM up to 30%. Nevertheless, Plasma T3 concentration and T3 to T4 ratio were significantly influenced by dietary inclusion level of CM and value of these indexes decrease linearly ($P<0.01$) with increasing level of CM from 0 to 30%. Moreover, increase in the level of CM in broiler diet linearly increased plasma uric acid ($P<0.01$), ALT ($P<0.01$) and AST ($P<0.05$) concentrations.

D1 Gene Expression and Thyroid Morphology

The effects of CM on D1 gene expression, thyroid weights, follicle numbers, epithelial thickness and follicle diameters of experimental chickens are presented in Table 5. Expression of D1 gene was affected by the addition of CM to broiler

Table 2. **Effects of dietary level of CM on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR; from 1 to 42 d) and organ weights (at 42 d of age) in broilers***

CM level (%) / Item	0	10	20	30	*SEM*	P-value† for contrasts of dietary CM		
						Linear	Quadratic	Cubic
Performance								
FI (g)	4115	4106	4151	4049	111.64	0.764	0.681	0.696
BWG (g)	2419	2337	2292	2198	45.03	0.003	0.893	0.676
FCR (g:g)	1.70	1.76	1.81	1.85	0.047	0.030	0.782	0.917
Organ weights (% of BW)								
Carcass yield	63.43	62.11	62.22	61.39	1.09	0.232	0.824	0.629
Breast yield	25.79	24.67	23.50	23.39	0.816	0.036	0.542	0.769
Leg yield	19.17	19.20	18.86	18.39	0.378	0.132	0.517	0.889
Liver	2.19	2.20	2.31	2.53	0.082	0.007	0.234	0.965
Gizzard	1.54	1.62	1.55	1.42	0.060	0.131	0.087	0.738
Pancreas	0.251	0.221	0.220	0.243	0.014	0.670	0.074	0.906
Small intestine	2.54	2.60	2.72	3.07	0.167	0.035	0.391	0.800
Duodenum	0.493	0.536	0.585	0.653	0.043	0.014	0.784	0.952
Jejunum	1.18	1.17	1.24	1.31	0.061	0.109	0.548	0.823
Ileum	0.870	0.857	0.892	1.09	0.073	0.048	0.165	0.729
Cecal	0.308	0.309	0.293	0.328	0.017	0.592	0.333	0.370
Abdominal fat	1.53	1.61	1.59	1.60	0.048	0.351	0.446	0.484

SEM, standard error of the means
BW, body weight
* Each mean represents values from 5 replicates.
† P: significance level at 5% by the adjusted regression equation.
Linear equation set for BWG=2487.85−70.54x. Linear equation set for FCR=1.67+0.05x. Linear equation set for breast=26.43−0.838x. Linear equation set for liver=2.03+0.114x. Linear equation set for small intestine=2.30+0.172x. Linear equation set for duodenum=0.434 +0.053x. Linear equation set for ileum=0.753+0.071.

Table 3. **Effects of dietary level of CM on hematological indices, relative weight of heart and right ventricle to total ventricle ratio (RV/TV) at 42 d of age in broilers***

CM level (%) / Item	0	10	20	30	*SEM*	P-value† for contrasts of dietary CM		
						Linear	Quadratic	Cubic
Hb (g/d*l*)	10.92	11.20	11.25	11.25	0.589	0.699	0.818	0.943
RBC ($\times 10^6 \mu l$)	3.26	3.04	3.24	3.30	0.225	0.754	0.543	0.586
PCV (%)	30.81	30.85	30.41	31.21	0.881	0.952	0.893	0.248
RV/TV (%)	21.03	21.30	22.10	22.90	0.435	0.005	0.543	0.790
Herat (% of BW)	0.401	0.458	0.413	0.482	0.015	0.021	0.713	0.012

SEM, standard error of the means
BW, body weight
* Each mean represents values from 5 replicates.
† P: significance level at 5% by the adjusted regression equation.
Linear equation set for RV/TV=20.23+0.642x. Linear equation set for heart weight=0.389+0.02x. Cubic equation set for heart weight= $0.026+0.607x-0.268x^2+0.036x^3$.

diets. A regression analysis indicated that D1 mRNA expression decreased linearly ($P<0.01$) with increasing level of CM from 0 to 30%. Chickens thyroid weights showed an increasing linear ($P<0.05$) response. Moreover, increased linear ($P<0.01$) and quadratic ($P<0.05$) responses were observed on follicle numbers and epithelial thickness by substituting CM for SBM. Concomitantly, follicle diameters were also significantly increased in a linear ($P<0.01$) manner following the substitution of CM for SBM.

Discussion

Feeding graded levels of CM in our study did not affect the FI. This could be attributed to the lower CM glucosinolates and sinapine of our dietary CM that was not adequate to affect the FI. The current findings are in line with previous reports on CM usage in broiler chickens without any effect on FI (Payvastegan *et al*., 2013; Gopinger *et al*., 2014; Aljuobori *et al*., 2016; Zhang and Adeola, 2016).

Table 4. **Effects of dietary level of CM on blood biochemical indices at 42 d of age in broilers***

CM level (%) / Item	0	10	20	30	*SEM*	P-value† for contrasts of dietary CM		
						Linear	Quadratic	Cubic
Glucose (mg/d*l*)	231.20	236.60	252.80	246.40	7.49	0.084	0.443	0.334
Cholesterol (mg/d*l*)	137.80	129.80	132.00	116.00	8.90	0.132	0.659	0.486
Triglycerides (mg/d*l*)	79.40	78.60	81.00	66.80	4.07	0.069	0.119	0.292
HDL (mg/d*l*)	51.92	55.44	50.16	46.20	3.72	0.196	0.329	0.552
Uric acid (mg/d*l*)	3.78	3.72	4.52	4.66	0.172	0.0004	0.568	0.065
AST (IU/*l*)	220.60	266.80	247.60	273.20	14.40	0.047	0.485	0.106
ALT (IU/*l*)	6.12	7.66	8.14	8.24	0.409	0.002	0.097	0.715
LDH (IU/*l*)	1192.00	1183.00	1172.00	1163.00	16.52	0.214	0.981	0.949
CK (IU/*l*)	1477.00	1638.00	1456.00	1763.00	118.51	0.220	0.547	0.136
T4 (ng/m*l*)	8.22	8.61	8.21	8.14	0.339	0.679	0.513	0.465
T3(ng/m*l*)	4.39	3.94	2.94	2.96	0.222	<0.0001	0.300	0.135
T3:T4	0.535	0.460	0.363	0.361	0.029	0.0002	0.225	0.381

SEM, standard error of the means
* Each mean represents values from 5 replicates.
† P: significance level at 5% by the adjusted regression equation.
Linear equation set for uric acid=3.31+0.344x. Linear equation set for AST=217.40+13.86x. Linear equation set for ALT=5.83+0.684x. Linear equation set for T3=4.88−0.531x. Linear equation set for T3:T4=0.585−0.062x.

Table 5. **Effects of dietary level of CM on D1 mRNA expression level, thyroids, follicle numbers, epithelium thickness and follicle diameters at 42 d of age in broilers***

CM level (%) / Item	0	10	20	30	*SEM*	P-value† for contrasts of dietary CM		
						Linear	Quadratic	Cubic
D1 mRNA expression level	1.00	0.952	0.904	0.849	0.047	0.001	0.912	0.961
Thyroids (mg/g BW)	0.082	0.103	0.099	0.108	0.006	0.011	0.306	0.190
Follicle numbers	64.41	70.97	78.30	80.99	0.653	<.0001	0.014	0.082
Epithelium thickness (μm)	2.88	2.92	2.98	2.97	0.018	<.0001	0.035	0.105
Follicle diameters (μm)	58.67	60.23	60.65	61.59	0.618	0.001	0.562	0.693

SEM, standard error of the means
BW, body weight
* Each mean represents values from 5 replicates.
† P: significance level at 5% by the adjusted regression equation.
Linear equation set for D1 mRNA expression level=1.052−0.05x. Linear equation set for thyroids=0.079+0.007x. Linear equation set for follicle numbers=58.90+5.84x. Quadratic equation set for follicle numbers=54.34+10.39x−0.911x^2. Linear equation set for epithelium thickness=2.86+0.029x. Quadratic equation set for epithelium thickness=2.80+0.093x−0.013x^2. Linear equation set for follicle diameters =58.07+0.927x.

An increase in dietary level of CM reduced BWG of the chickens. This could be due to presence of antinutritional factors mainly fiber (12.9%) and glucosinolates (23.5 μmol/g) in the CM. Analysis of experimental diets demonstrated increase in crude fiber content from 3.05 to 3.86, 4.67 and 5.46% during starter phase, 2.97 to 3.81, 4.67 and 5.47% during grower phase and 2.85 to 3.65, 4.47 and 5.27% during finisher phase after the addition of 10, 20 and 30% of CM, respectively. Dietary fiber content of CM have been demonstrated to be inversely related to energy digestibility as well as protein digestibility (Khajali and Slominski, 2012; Gopinger *et al*., 2014; Bovera *et al*., 2014).

Generally, it is believed that glucosinolates in poultry diets must be less than 2.5 μmol/g (Mushtaq *et al*., 2007). In the current study, diets containing 10, 20 and 30% CM had 2.35, 4.70 and 7.05 μmol/g glucosinolates, respectively. Thus, the BWG depression of broilers fed on diets containing CM may be due to the higher glucosinolates content of these diets. The negative impacts of glucosinolates on growth performance may interfere with the function of thyroid and drastic disturbance of thyroid hormones. As observed in our study, reduction of plasma T3 concentration may also impaired the BWG in broilers, because T3 can stimulate the transcription of growth hormone mRNA and growth hormone synthesis in the pituitary (Yen, 2001).

In the same vein, antinutritional factors of CM like tannin may form complexes with protein and proteolytic enzymes in the gastrointestinal tract and reduce the bioavailability of protein, thereby impairing the growth performance (Khajali and Slominski, 2012). Another possible reason for the re-

duced BWG in the current study may be attributed to the higher non-starch polysaccharides (NSP) and phytate content of CM in comparison with SBM (Payvastegan *et al*., 2013). CM has approximately 16% NSP of which 1.5% is soluble (Kocher *et al*., 2000). The soluble NSP tends to increase the digesta viscosity and decrease the nitrogen digestion and absorption and subsequently resulting in poor growth performance (Mushtaq *et al*., 2007). CM also contains 2.9 to 3.2% phytic acid, whereas SBM contains only 1.4% (Payvastegan *et al*., 2013). Phytate has the ability to chelate cations such as iron, sodium, sulfur, calcium, zinc, copper as well as proteins (Khajali and Slominski, 2012). In addition, the observed decrease in BWG could be partly attributed to increased liver and thyroid metabolic activities as evidenced by the increase in liver and thyroid size as a result of CM consumption. Increased liver and thyroid size (and hence its metabolic activities) due to CM consumption, increases the utilization of dietary energy and other nutrients for maintenance at the expense of growth and tissue deposition (Woyengo *et al*., 2011).

As shown in the present study, FI was similar among the treatments and BWG was reduced due to increased dietary CM level. The observed increase in FCR, is therefore unsurprising. Several authors have also demonstrated that the dietary Lys needed by the broilers to achieve maximum FCR was higher than that needed for maximum BWG in the order of BWG < breast meat < FCR (Mushtaq *et al*., 2007). Thus, impairment of feed efficiency by inclusion of CM could be related to lower Lys content and lower Lys digestibility of CM based diets relative to SBM based diet.

Heart weight (as proportion of body weight) and RV:TV ratio were linearly increased due to the substitution of CM for SBM. In avian species, Arg is an essential amino acid (Tan *et al*., 2007). For this reason, Arg concentrations in chickens are correlated only with dietary intake (Payvastegan *et al*., 2012). Arg content and Arg digestibility of CM is lower than SBM (Khajali and Slominski, 2012). Thus, substitution of CM instead of SBM in poultry diets may reduce the dietary level of Arg below its requirements (Izadinia *et al*., 2010). Newkirk and Classen (2002) indicated that feeding CM resulted in a linear increase of heart proportional weight. Khajali *et al*. (2011) and Izadinia *et al*. (2010) also showed that substitution of CM for SBM increased heart weight and RV:TV ratio. The values of RV:TV ratio greater than 0.27 is considered as pulmonary hypertension (Daneshyar *et al*., 2009). In the current study, values of RV:TV ratio was lower than 0.27 for all chickens. Therefore, it is expected that no ascites mortalities was observed in our study.

Increasing the CM content was associated with a linear decrease of the breast yield, coinciding with increasing linear response in liver, small intestine, duodenum, ileum and thyroid weights. Considering the lower percentage of breast, it can be suggested that Lys is generally the first limiting amino acid in practical poultry diets, which do not have high SBM contents (Ahmad *et al*., 2007). Because breast yield has higher Lys requirements (Mushtaq *et al*., 2007), the depression in breast yield in low-Lys diets is unsurprising. In addition, it should be noted that Lys deficiency has greater effect on the development of the breast muscle with entirely fast-twitch glycolytic fiber type than of the leg muscle with mixed fiber types (Tesseraud *et al*., 2001). Moreover, the Arg content of CM was approximately two-thirds that of SBM (2.08 vs. 3.14% according to NRC, 1994) as well as having a lower Arg digestibility in comparison to SBM (Izadinia *et al*., 2010). Some evidence also indicates that Arg regulates the partitioning of dietary energy in favor of muscle protein accretion and fat reduction in animals (Ma *et al*., 2010). Furthermore, polyamines and creatine are synthesized biologically via different pathways, starting from Arg (Khajali and Wideman, 2010). Apart from these roles, approximately 7% of the dietary Arg were converted to proline, which are needed for the synthesis of connective tissue (Khajali and Wideman, 2010). Similarly, Mushtaq *et al*. (2007) and Payvastegan *et al*. (2013) also found significant differences in the breast weight of chickens that were fed by different levels of CM.

The observed linear increase in the liver weight due to CM may be attributed to increased activity of antioxidant and detoxification enzymes due to the absorption of gut microbial degradation hydrolytic products of dietary glucosinolates (Woyengo *et al*., 2011). In line with our results, Newkirk and Classen (2002), Maroufyan and Kermnshahi (2006), Kermnshahi and Abasi pour (2006), Woyengo *et al*. (2011) and Payvastegan *et al*. (2013) reported the increased liver size of broilers due to dietary inclusion of CM. Accordingly, the AST and ALT activity determined in the current study also showed the significant rise as a result of increased dietary CM level. Nevertheless, no changes were determined in terms of CK and LDH activities.

In addition, the significant increase in weights of the emptied small intestine and of the duodenum and ileum, relative to live weight, of the broilers fed on diets containing CM could be attributed to increase in intestinal viscosity. As dietary inclusion of soluble NSP is increased, there is also increase in intestinal viscosity (Dänicke *et al*., 2000; Attia *et al*., 2014). The absolute and relative weight of small intestine is also increased in broilers. Hence, it can be stated that intestinal viscosity-related increases in small intestine, duodenum and ileum weights may be attributed to an increase in the synthesis of fractional protein of these tissues (Dänicke *et al*., 2007). Reduced intestinal size in fast-growing chickens may reflect a more efficient absorption and utilization of nutrients (Dibner and Richards, 2005). An increase in intestine size suggests that more energy may have been partitioned towards maintenance rather than growth, which may lead to a decrease in BWG.

In contrast to this study, our previous research revealed that feeding broilers with CM increases the relative weight of pancreases and reduces relative weights of cecal (Payvastegan *et al*., 2103). Therefore, the difference in response of broilers to dietary inclusion of CM between the current study and that of our previous research with regards to relative weights of pancreases and cecal could be due to the differences in

phytate content of CM based diets and fermentable sugars content of SBM based diets, respectively. Phytase was shown to improve phytate, amino acids and energy utilization of ducks (Attia, 2003), laying Japanese quail hens (Attia *et al*., 2008), and broiler chickens (Attia *et al*., 2012).

It was determined that plasma T3 is produced by 5′-deiodination of T4 in non-thyroidal tissues, particularly the liver and kidney (Jianhua *et al*., 2000). It is possible that glucosinolate degradation products impair liver function so that the formation of T3 is depressed (Chiasson *et al*., 1979). The lower concentration of T3 and T3 to T4 ratio in the CM diet in our study may be attributed to effects of glucosinolate hydrolysis products on peripheral T4 monodeiodination. Spiegel *et al*. (1993) reported that feeding of rapeseed meal would lead to the reduction of hepatic deiodinase level in pigs. This is in agreement with our finding with respect to D1 mRNA expression. As found in this study, an increase in CM level in the broiler diets reduced the D1 mRNA expression linearly. It can be speculated that feeding broilers with CM based diets adversely affected the D1 mRNA expression probably associated with glucosinolate degradation products.

Dietary levels of CM also significantly affected the thyroid weights, follicle numbers, epithelial thickness and follicle diameters. Reduction of deiodination of T4 to T3 had a negative feedback that may affect the release of thyroid stimulating hormone (TSH) from the pituitary (Woyengo *et al*., 2011). The increased follicle numbers, epithelial thickness and follicle diameters were most likely due to the trophic effect of TSH. Follicle numbers, epithelial thickness and follicle diameters increased proportionally with increase in CM inclusions from 0 to 30%, indicating hypertrophy and hyperplasia of thyroid cells. Thus, the observed increase in thyroid weights may be partially attributed to hyperplasia and hypertrophy of thyroid cells.

Glucosinolate degradation products can impair the availability of iodine to the thyroid glands, its oxidation and binding to thyroglobulin, leading to reduced production of T4 (Tripathi and Mishra, 2007). On the contrary, Chiasson and Sharp (1979) also demonstrated that thyroids of cockerels attained a physiological equilibrium by using rapeseed meal after 3−5 weeks. These authors reported that the inclusion of rapeseed meal in the diet caused only a slight depression in the concentration of plasma T4, whereas it caused a significant depression in the concentration of T3 in chickens during the ages of 3 and 5 weeks. Therefore, despite the fact that feeding CM increases the size of thyroid glands in the current study, the failure of dietary CM to decrease plasma concentration of T4 may implies that the long times of CM consumption in broiler diets result in a physiological equilibrium in the thyroid glands and subsequently in T4 synthesis. Moreover, T3 is the major circulating thyroid hormone controlling TSH secretion (Condliffe and Weintraub, 1979). Thus, another possible explanation is that glucosinolates of used CM in the present study caused more extra-thyroidal disturbances, which lead to inhibition of the peripheral T4 monodeiodination and reduction of plasma T3 concentration.

Quite different results have been reported in the literature about the effects of feeding low glucosinolate rapeseed meal on broiler thyroid hormones responses. Newkirk and Classen (2002), Kermanshahi and Abbasipour (2006), Maroufyan and Kermanshahi (2006) and Aljuobori *et al*. (2016) have stated that CM feeding elevated the T3 level in broiler chickens. On the other hand, Woyengo *et al*. (2011) reported that serum T4 was increased by the substitution of CM. In line with our results, Taraz *et al*. (2006) and Mikulski *et al*. (2012), however, indicated that an increase in the inclusion level of CM was followed by a reduction in plasma T3 concentrations. The reasons for these discrepancies are not well known, although they could be partly attributed to differences in glucosinolates content in CM, diet ingredients, composition of gut microorganism and the extent of glucosinolates degradation by heat during the oil extraction process (Woyengo *et al*., 2011).

A significant increase in plasma uric acid level was also observed by replacing CM for SBM in the diets. Plasma uric acid and excreta uric acid can serve as an accurate indicator of the amino acid utilization and dietary protein quality in boilers (Miles and featherston, 1976; Donsbough *et al*., 2010). Less nitrogen is incorporated into body protein and more is excreted as uric acid by chicks fed on a poorer quality protein in comparison with that observed when they are fed on a higher quality protein (Miles and featherston, 1976). In our study, based on emerging scientific literature, it is reasonable to hypothesize that the linear increase in plasma uric acid caused by increased level of CM from 0 to 30% is associated with lower protein quality and amino acid digestibility values of CM when compared to SBM.

Based on the results obtained in the current study, it can be concluded that an increase in dietary level of CM did adversely affect the BWG, FCR, thyroid function and linearly reduced the D1 mRNA expression as well. In addition, substitution of CM with SBM may negatively interfere with liver function, likely because of increased dietary concentration of glucosinolates.

Acknowledgments

This study was supported in part by Urmia University, Urmia, Iran. The authors appreciate their thank to Vatankhah A. M (Department of Drug Applied Research, Faculty of Medical Sciences, Tabriz University, Tabriz, Iran) for this helps in determination of blood parameters and Mazaheri Z. (Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran) for technical contribution to deiodinase gene expression assay.

References

Aftab U. Utilization of alternative protein meals with or without multiple-enzyme supplementation in broilers fed low energy diets. Journal of Applied Poultry Research, 18: 292−296. 2009.

Ahmad G, Mushtaq T, Aslam Mirza M and Ahmad Z. Comparative bioefficacy of lysine from L-lysine hydrochloride or L-lysine sulfate in basal diets containing graded levels of canola meal

for female broiler chickens. Poultry Science, 86: 525−530. 2007.

Aljuobori A, Zulkifli I, Soleimani AF, Abdullah N, Liang JB and Mujahid A. Higher inclusion rate of canola meal under high ambient temperature for broiler chickens. Poultry Science, 95: 1326−1331. 2016.

Association of Official Analytical Chemists International. Official Methods of Analysis. 17th ed. Association of Analytical Communities. Gaithersburg, MD. 2000.

Attia YA, El-Din AERET, Zeweil HS, Hussein AS and Arafat MA. The ef fect of supplementat ion of enzyme on laying and reproduct ive per formance in Japanese Quai l hens fed nigel la seed meal. Journal of Poultry Science, 45: 110−115. 2008.

Attia YA, El-Tahawy WS, Abd El-Hamid AE, Hassan SS, Nizza A, El-Kelaway MI. Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Italian Journal of Animal Science, 11: e56. 2012.

Attia YA, El-Tahawy WS, Abd El-Hamid E, Nizza A, Bovera F, Al-Harthi MA and El- Kelway MI. Effect of feed form, pellet diameter and enzymes supplementation on growth performance and nutrient digestibility of broiler during days 21−37 of age. Archiv Tierzucht, 57: 1−11. 2014.

Attia YA. Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. Archiv of Animal Nutrition, 66: 39−48. 2003.

Aviagen. Ross 308: Broiler Nutrition Specifications. Aviagen Inc., Huntsville, Alabama. http://www.aviagen.com. 2014.

Baloch GM, Solangi AA, Wagan MP and Tahira M. Efficiency of canola meal in broiler ration. Journal of Animal and Veterinary Advances, 2: 138−142. 2003.

Bovera F, Nizza S, Attia YA, Di Meo C, Piccolo G and Nizza A. Prediction of digestible energy and gross energy digestibility of feeds and diets in ostriches. British Poultry Science, 55: 518−523. 2014.

Canola Council of Canada. Canola meal: Feed industry guide. Canadian International Grains Institute. 4th ed. Accessed Oct. 2012. http://www.canolacouncil.org. 2009.

Chen X, Parsons CM and Bajjalieh N. Nutritional evaluation of new reduced oligosaccharide soybean meal in poultry. Poultry Science, 92: 1830−1836. 2013.

Chiasson RB and Sharp PJ. Effects of rapeseed meal and methimazole on levels of plasma hormones in growing broiler cockerels. Poultry Science, 58: 1575−1583. 1979.

Chiasson RB, Sharp PJ, Klandorf H, Scanes CG and Harvey S. The Effect of Rapeseed Meal and Methimazole on Levels of Plasma Hormones in Growing Broiler Cockerel. Poultry Science, 58: 1575−1583. 1979.

Condliffe PG and Weintraub BD. Pituitary thyroid-stimulating hormone and other thyroid-stimulating substances. In: Hormones in Blood. 3rd ed. Vol. 1. pp. 499−574. Academic Press. New York. 1979.

Dänicke S, Valenta H and Matthes S. On the Interactions Between Fusarium Toxin-Contaminated Wheat and Nonstarch Polysaccharide Hydrolyzing Enzymes in Diets of Broilers on Performance, Intestinal Viscosity, and Carryover of Deoxynivalenol. Poultry Science, 85: 291−298. 2007.

Daneshyar M, Kermanshahi H and Golian A. Changes of biochemical parameters and enzyme activities in broiler chickens with cold-induced ascites. Poultry Science, 88: 106−110. 2009.

Dänickea S, Jerocha H, BoÈttchera W and Simon O. Interactions between dietary fat type and enzyme supplementation in broiler diets with high pentosane contents: effects on precaecal and total tract digestibility of fatty acids, metabolizability of gross energy, digesta viscosity and weights of small intestine. Animal Feed Science and Technology, 84: 279−294. 2000.

Daun JK, Michael Eskin NA and Hickling D. Canola: Chemistry, Production, Processing, and Utilization. Origin, Distribution, and Production (Daun JK ed). Vol.1. pp. 22−23. The American Oil Chemists Society Press. Urbana. 2011.

Davison TF, Pearson AW, Rea J and Greenwood NM. Thyroid function in relation to trimethylamine oxidation in immature domestic fowl fed on rapeseed meal. British Poultry Science, 22: 437−443. 1981.

De Coca-Sinova A, Valencia DG, Jiménez-Moreno E, Lázaro R and Mateos GG. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poultry Science, 87: 2613−2623. 2008.

Dibner JJ and Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science, 84: 634−643. 2005.

Donsbough AL, Powell S, Waguespack A, Bidner TD and Southern LL. Uric acid, urea, and ammonia concentrations in serum and uric acid concentration in excreta as indicators of amino acid utilization in diets for broilers. Poultry Science, 89: 287−294. 2010.

Gopinger E, Xavier EG, Elias MC, Catalan AAS, Castro MLS, Nunes AP and Roll VFB. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poultry Science, 93: 1130−1136. 2014.

Izadinia M, Nobakht M, Khajali F, Faraji M, Zamani F, Qujeq D and Karimi I. Pulmonary hypertension and ascites as affected by dietary protein source in broiler chickens reared in cool temperature at high altitudes. Animal Feed Science and Technology, 155: 194−200. 2010.

Jianhua H, Ohtsuka A and Hayashi K. Selenium influences growth via thyroid hormone status in broiler chickens. British Journal of Nutrition, 84: 727−732. 2000.

Kermanshahi H and Abbasi Pour AR. Replacement value of soybean meal with rapeseed meal supplemented with or without a dietary NSP-degrading enzyme on performance, carcass traits and thyroid hormones of broiler chickens. International Journal of Poultry Science, 5: 932−937. 2006.

Khajali F and Slominski BA. Factors that affect the nutritive value of canola meal for poultry. Poultry Science, 91: 2564−2575. 2012

Khajali F and Wideman RF. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. World's Poultry Science Journal, 66: 751−766. 2010.

Khajali F, Tahmasebi M, Hassanpour H, Akbari MR, Qujeq D and Wideman RF. Effects of supplementation of canola meal-based diets with arginine on performance, plasma nitric oxide, and carcass characteristics of broiler chickens grown at high altitude. Poultry Science, 90: 2287−2294. 2011.

Kocher A, Choct M, Morrisroe L and Broz J. Effects of enzyme supplementation on the replacement value of canola meal for soybean meal in broiler diets. Australian Journal of Agricultural Research, 52: 447−452. 2001.

Kocher A, Choct M, Porter MD and Broz J. The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. Poultry Science, 79: 1767−1774. 2000.

Ma X, Lin Y, Jiang Z, Zheng C, Zhou G, Yu D, Cao T, Wang J and Chen F. Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs. Amino Acid, 39: 95−102. 2010.

Mahdavi A, Shivazad M, Alemi F, Zaghari M, Moravej H and Darabighane B. Digestible lysine requirement of broilers based on practical diet. Italian Journal of Animal Science, 11: 68−76. 2012.

Maroufyan E and Kermanshahi H. Effect of Different Levels of Rapeseed Meal Supplemented with Calcium Iodate on Performance, Some Carcass Traits, and Thyroid Hormones of Broiler Chickens. International Journal of Poultry Science, 5: 1073−1078. 2006.

McNaughton J, Roberts M, Rice D, Smith B, Hong B, Delaney B and Iiams C. Comparison of broiler performance and carcass yields when fed diets containing genetically modified canola meal from event DP-Ø73496−4, near-isogenic canola meal, or commercial canola meals. Poultry Science, 93: 1713−1723. 2014.

Mikulski D, Jankowski J, Zdunczyk Z, Juskiewicz J and Slominski BA. The effect of different dietary levels of rapeseed meal on growth performance, carcass traits, and meat quality in turkeys. Poultry Science, 91: 215−223. 2012.

Miles RD and Featherston WR. Uric Acid Excretion by the Chick as an Indicator of dietary Protein Quality. Poultry Science, 55: 98−102. 1976.

Mushtaq T, Sarwar M, Ahmad G, Mirza MA, Nawaz H, Haroon Mushtaq MM and Noreen U. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. Poultry Science, 86: 2144−2151. 2007.

National Research Council. Nutrient Requirements of Poultry. 9th revised ed. National Academy Press. Washington. DC. 1994.

Newkirk RW and Classen HL. The effects of toasting canola meal on body weight, feed conversion efficiency, and mortality in broiler chickens. Poultry Science, 81: 815−825. 2002.

Payvastagan S, Farhoomand P, Shahrooze R, Delfani N and Talatapeh A. The effects of different levels of canola meal and copper on performance susceptibility to ascites and plasma enzyme activities in broiler chickens. Annals of Biological Research, 3: 5252−5258. 2012.

Payvastegan S, Farhoomand P and Delfani N. Growth performance, organ weights and, blood parameters of broilers fed diets containing graded levels of dietary canola meal and supplemental copper. Journal of Poultry Science, 50: 354−363. 2013.

SAS Institute. SAS Statistics User's Guide. Version 9.1.3. SAS Inst. Inc., Cary, NC. 2002−2003.

Schöne F, Rudolph B, Kirchheim U and Knapp G. Counteracting the negative effects of rapeseed and rapeseed press cake in pig diets. British Journal of Nutrition, 78: 947−962. 1997.

Smith CA and Dacombe C. Rapid method for determining total glucosinolates in rapeseed by measurement of enzymatically released glucose. Journal of the Science of Food and Agriculture, 38: 141−150. 1987.

Spiegelg C, Besteiti G, Ross G and Blum JW. Feeding of Rapeseed Presscake Meal to Pigs: Effects on Thyroid Morphology and Function and on Thyroid Hormone Blood Levels, on Liver and on Growth Performance. Journal of Veterinary Medicine, 40: 45−57. 1993.

Tan X, Hu SH and Wang XL. Possible role of nitric oxide in the pathogenesis of pulmonary hypertension in broilers: a synopsis. Avian Pathology, 36: 261−267. 2007.

Tanbe Y, Komiyama T, Kubota D and Tamaki Y. Comparison of the effects of thiouracil, propylthiouracil and methimazole on I^{131} metabolism by the chick thyroid and measurements of thyroxine secretion rates. General and Comparative Endocrinology, 5: 60−68. 1965.

Taraz Z, Jalali S and Rafeie F. Effects of replacement of soybean meal with rapeseed meal on organs weight some blood biochemical parameters and performance of broiler chicks. International Journal of Poultry Science, 5: 1110−1115. 2006.

Tesseraud S, Temim S, Bihan-Duval EL and Chagneau AM. Increased responsiveness to dietary lysine deficiency of pectoralis major muscle protein turnover in broilers selected on breast development. Journal of Animal Science, 79: 927−933. 2001.

Tripathi MK and Mishra AS. Glucosinolates in animal nutrition: A review. Animal Feed Science and Technology, 132: 1−27. 2007.

Woyengo TA, Kiarie E and Nyachoti CM. Growth performance, organ weights, and blood parameters of broilers fed diets containing expeller-extracted canola meal. Poultry Science, 90: 2520−2527. 2011.

Yen PM. Physiological and molecular basis of thyroid hormone action. Physiological Reviews, 81: 1097−1142. 2001.

Zhang F and Adeola O. Energy values of canola meal, cottonseed meal, bakery meal, and peanut flour meal for broiler chickens determined using the regression method. Poultry Science, 0: 1−8. 2016.