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Nutritional Impact of Canola Meal on Performance, Blood Constituents and Immune Response of Broilers

A. A. Ghazalah[1], A. M. El-Kaiaty[1], Hady F. A. Motawe[2] & A. S. Radwan[2]

[1] Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt

[2] Regional Centre for Food and Feed, Agricultural Research Centre, Giza, Egypt

Correspondence: A. S. Radwan, Regional Centre for Food and Feed, Agricultural Research Centre, Giza, Egypt. E-mail: mrahmed_2010sad@yahoo.com

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Abstract

This study was conducted to evaluate the effects of replacing soybean meal (SBM) protein with canola meal (CM) protein on productive performance, nutrient digestibility, immune response, lymphoid organs, blood parameters, carcass fatty acids and cecum microbiota of broilers. A total of 160 one d-old Arbor Acres broiler chicks were randomly allocated to 4 dietary treatments of 5 replicates, where, CM protein replaced SBM protein at 0, 30, 60, and 90% for a 39 days feeding trial. The results showed no significant differences in productive performance parameters among control, 30% and 60% treatment groups, while, at 90% replacement level, all values decreased ($P < 0.0001$) all over the experimental period. The 90% replacement group showed depression of crude protein ($P < 0.001$) and crude fiber ($P < 0.001$) digestibility and spleen relative weight ($P = 0.0386$) with increase of thymus ($P = 0.0555$), bursa ($P = 0.0334$) and thyroid relative weight ($P = 0.0276$) as well as thyroid hormones ($P = 0.0034$, 0339) for T_3, T_4, respectively, while, there were no significant differences among control, 30% and 60% treatment groups for those criteria. However, CM levels had no effect on serum haemagglutination inhibition (HI) titer against Newcastle disease. CM significantly decreased serum cholesterol content ($P = 0.0002$) while increased HDL ($P = 0.0532$), compared to the control. CM levels showed an increase in carcass meat content of unsaturated fatty acids content ($P < 0.0001$) as the replacement level gradually increased. Erucic acid did not detected in carcass. All CM levels decreased cecum content of *E. coli* ($P = 0.0051$) while increased that of *Lactobacillus* ($P = 4094$). Conclusively, CM can be used safely in broiler diet to replace up to 60% of SBM protein without negative effects on growth and immune response of broilers.

Keywords: broilers, canola meal, digestibility, fatty acids, growth, immunity, microbiota

1. Introduction

The poultry feeding costs represent approximately 70% of the overall costs of poultry production. Most countries of the world consider soybean meal (SBM) is the main source of protein in poultry diets (Husak et al., 2008) as in Egypt. Lack of availability of the local soybean and SBM with their highly prices, beside to increasing of globally prices of SBM led to encourage the producers of poultry feeds to use alternatives like cottonseed meal (Aftab, 2009; Yuan et al., 2014), sunflower meal (Aftab, 2009; Moghaddam et al., 2012), cassava leaf meal (Iheukwumere et al., 2008), moringa leaf meal (Gadzirayi et al., 2012; Abbas, 2013) and flaxseed meal (Tarek et al., 2015) otherwise, their combinations. At the same time, the declining profit of poultry companies in light of highly prices of the main raw feedstuffs forced them to search for cheaper alternative protein. If CM used as an alternative protein for SBM in broiler diets may be able to provide liberation to the broiler and soybean industry. According to the USDA (2014), the rapeseed production including canola varieties ranks second among oilseed crops worldwide. On the other hand, Canola Council of Canada (2015) reported that, CM has a widely utilizing as an alternative protein source for soybean meal (SBM) in poultry diets where it has a good balance of essential amino acids. According to Newkirk (2009), the CM contains approximately 40% crude protein, has a good-balanced amino acid profile and high levels of sulfur-containing amino acids compared with SBM.

Although CM glucosinolates have antibacterial, antifungal properties, and cancer-chemoprevention activity, their anti-nutritional effects have limited the use of meals from rapeseed for human food and animal feed (Szydlowska-Czerniak et al., 2011). Besides, canola oil is rich in unsaturated fatty acids like omega 3 fatty acids.

The fatty acid composition of poultry meat is an important quality parameter especially with respect to potentially affecting human health via poultry meat consumption (Mantzioris et al., 2000; Rahimi et al., 2011).

The nutritive value of CM is inferior compared to SBM in chicken diets due to its content of some anti-nutritive factors, like non-starch polysaccharides (NSP) that account for 18-20% and high fiber content which represents 11-12% (Khajali & Slominski, 2012) that led to low energy availability content compared to SBM for poultry. Accordingly, CM could not be used as 100% alternative for SBM in poultry diets (Khajali & Slominski, 2012). Therefore, the objective of the present study was to evaluate the effects of replacing SBM protein with CM protein at 0, 30, 60 and 90% on performance, nutrients digestibility, immune response, lymphoid organs, blood parameters, carcass fatty acids and cecum microbiota of broilers.

2. Materials and Methods

The Institutional Animal Care and Use Committee of Cairo University approved the experimental protocol used in this study, with approval number CU-II-F-22-20 on May 2020.

2.1 Experimental Chicks, Design and Management

This experiment was carried out at Regional Centre for Food and Feed, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. A total of one hundred and sixty day-old-chicks male Arbor Acres broiler chicks were randomly distributed into four treatment groups of five replicates (battery cages) each of 8 chicks using a completely randomized design. Treatment groups were fed diets contain canola meal (CM) at 0, 30, 60 and 90% of SBM protein (Table 1).

Diets were formulated in the Regional Center for Food and Feed to be isonitrogenous, isocaloric and mycotoxins-free, as levels of aflatoxins and other mycotoxins were below the detection limit (detection limit 1 ppb) (Ross et al., 1997) as well as free from any medication or antibiotics. The diets were formulated to meet the nutrient requirements of broiler chicks during starting, growing and finishing periods according to Arbor Acres plus Broiler Nutrition Specifications (2014).

All chicks were fed a starter diet from day one to 14 days of age. From day 15 to 28 days of age, the birds were switched to a grower diet, and from day 29 to 39 days of age, chicks were fed a finisher diet. The composition and determind chemical analyses of the used diets are stated in Table 1. Feed and water were available *ad-libitum* during the experimental period (39 days). Water was provided by drip nipples. At the first week, Temperature was adjusted at 30±0.5 ºC then lowered 2 ºC each successive week, and then maintained at 24±0.5 ºC. Relative humidity was about 60% to 70% at the first week of age then, lowered to 50-60% from the 2nd week of age until the end of experiment. The broiler chicks were exposed to 23 hrs light and 1 hour dark during the day at the first week. From the second week up to the end of the experiment, the light was 20 hrs and 4 hrs dark during the day.

The chicks had an average initial body weight of 46.00±1.00 g. Chicks were vaccinated against IB at 6th day of age, H_5N_1 at 9th day, Gumboro D78 at 13th and 24th days of age. Weekly, body weight (BW) and feed intake (FI) were recorded for each replicate during all periods of growth. The feed conversion ratio (FCR) was calculated by dividing feed intake by body weight gain.

2.2 Digestibility Trial

At 39 day of age, a digestibility trial was conducted for 3 days collection period using 5 birds within each treatment. Birds were individually housed in metabolic cages and the total collection method cited by Abou-Raya and Galal (1971) was executed to determine the apparent digestibility (AD) of nutrients. Nitrogen (N), ether extract (EE), crude fiber (CF), and ash content of dried excreta as well as those of feed were determined according to AOAC (2016) using the N. 928.08, 2003.06, 2011.25 and 920.153 methods, respectively. The total protein content was calculated using Kjeldahl nitrogen and a conversion factor of 6.25. The following equation indicates how to determine % AD:

$$\% \text{ AD} = [(f - e)/f] \times 100 \quad (1)$$

Where, f and e are the intake and excreted nutrient in grams, respectively.

2.3 Immune Response and Lymphoid Organs

To determine the immune response of chicks, whole sheep blood collected in heparinized tube was washed three times in phosphate buffered saline (PBS, pH 7.4) and diluted in PBS to 25 per cent (v v^{-1}). Ten chicks from each treatment group (2 from each replicate) were immunized with 1 mL of 25 per cent sheep red blood cells (SRBCs) (Kundu et al., 1999) in thigh muscles at day 28. Booster dose of SRBCs antigen was given at day 35. Blood samples were collected at day 35 of age for assessing hemagglutination (HA) titer (Abdel-Ati et al., 1984)

against SRBCs by using freshly prepared one per cent SRBCs. At 39 days of age, another blood samples were collected randomly from wing vein of 5 birds, from each treatment group (one bird/replicate). Serum samples were subjected to haemagglutination inhibition (HI) test for determining antibody titers against Newcastle disease (ND) vaccine as described by Swayne et al. (1998). In addition, the differences in relative weight of lymphoid organs including spleen, bursa and thymus, other than the relaive weight of thyroid gland were detected.

Table 1. Composition and calculated analysis of the experimental diets

Ingredients (%)	Starter (1-14 days)				Grower (15-28 days)				Finisher (29-39 days)			
	Replacement levels (%)											
	0	30	60	90	0	30	60	90	0	30	60	90
Yellow corn (7.35%)	59.00	54.71	50.41	46.05	61.21	57.90	54.01	50.01	61.48	57.48	53.13	48.73
Soybean meal (45.32%)	27.29	19.10	10.91	2.73	23.14	16.20	9.25	2.32	25.00	17.50	10.00	2.50
Canola meal (34.11%)	0	10.88	21.75	32.63	0	9.22	18.45	27.67	0	9.97	19.93	29.89
Corn gluten (60.8%)	8.23	8.91	9.30	9.30	9.69	10.00	10.50	11.00	6.00	6.50	7.10	7.70
Vegetable oil	0.97	2.13	3.50	5.00	1.70	2.64	3.86	5.18	3.73	5.00	6.41	7.85
Di calcium phosphate	2.01	1.97	1.94	1.89	1.81	1.76	1.73	1.69	1.63	1.59	1.54	1.50
Lime stone	0.89	0.85	0.79	0.74	0.81	0.77	0.72	0.68	0.66	0.61	0.56	0.52
DL-methionine (98%)	0.31	0.15	0.09	0.05	0.23	0.09	0.05	0.02	0.25	0.10	0.05	0.02
L-lysine-HCL (98%)	0.60	0.61	0.62	0.91	0.52	0.53	0.54	0.55	0.38	0.39	0.40	0.41
Common salt	0.30	0.30	0.30	0.30	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Vit./Min. Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline Chloride	0.10	0.10	0.10	0.10	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26
Total	100	100	100	100	100	100	160	100	100	100	100	100
Calculated analyses												
ME (Kcal kg^{-1})	2988	2982	3004	3006	3121	3102	3100	3104	3208	3202	3205	3210
Methionine %	0.70	0.58	0.56	0.56	0.62	0.52	0.51	0.51	0.60	0.48	0.48	0.48
Lysine %	1.44	1.44	1.44	1.65	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19
Met + Cys %	1.08	1.08	1.18	1.29	0.99	0.99	1.08	1.18	0.94	0.94	1.04	1.15
% Determined chemical analyses												
CP	22.33	22.33	22.24	22.25	21.38	21.25	21.26	21.26	19.85	19.79	19.82	19.85
CF	3.11	3.73	4.33	4.91	2.90	3.39	4.00	4.50	2.88	3.50	4.10	4.65
EE	3.30	5.25	7.50	9.70	4.00	5.70	7.60	9.70	5.85	8.00	10.15	12.40
Ca	0.85	0.86	0.85	0.85	0.76	0.76	0.76	0.77	0.67	0.67	0.67	0.67
Avail. P.	0.48	0.46	0.45	0.43	0.44	0.42	0.41	0.39	0.41	0.39	0.37	0.36

Note. * Vitamins-minerals mixture supplied per kg of diet: vit. (A), 12000 I.U., vit. (D_3), 2000 I.U; vit. (E), 10 mg; vit. (K_3), 2 mg; vit. (B_1), 1 mg; vit. (B_2), 5 mg; vit. (B_6), 1.5 mg; vit. (B_{12}), 10 µg; Biotin, 50 µg; Pantothenic acid, 10mg; Niacin, 30 mg; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Iron, 30 mg; Copper, 10 mg; Iodine, 1 mg; Selenium, 0.1 mg and Cobalt, 0.1 mg.

2.4 Blood Parameters

At the end of experiment, blood samples were taken from wing vein of 5 chickens in each group and directly aliquoted into 2-mL sterile vials and allowed to clot for 4 h. After centrifugation (10 min, 2000 rpm), the serum was collected and stored at -20 °C until the time of analysis. The serum samples were assigned for determination of total protein (TP), liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as kidney function indices including urea and creatinine and cholesterol using commercially available kits (Biosystem S.A., Costa Brava, 30, Barcelona, Spain) according to manufacturer's instructions. Serum thyroid hormones concentration were determined by 125 I labelled RIA kits for T_3 (IM1699, Immunotech, Czech Republic) and T_4 (IM1447 Immunotech, Czech Republic) (Okuliarova et al., 2011).

2.5 Cecal Contents and Fatty Acids Profile of Carcass Meat

Five birds from each treatment group were slaughtered at 39 d of age. Then, cecal contents were taken for bacterial counting (Collin et al., 1995). Fatty acids profile of overall carcass meat was determined by extracting total lipids from about 5 grams of collected samples in a homogenizer with 20 ml of 2:1 chloroform-methanol and then filtered through Whatman No. 1 filter paper (Folch et al., 1957). The fatty acids were determined as methyl esters using Gas Liquid chromatography technique according to AOAC (2016). Saturated and unsaturated fatty acids of meat were expressed as percentage of total fatty acids.

2.6 Statistical Analyses

A polynomial regression analysis was used to predict the effect of the inclusion of various levels of canola meal in the diet on the parameters of growth performance, nutrient digestibility, immune response, lymphoid organs, biochemical parameters, fatty acids profile of carcass meat and cecum microbial content. The polynomial regression models were selected based on the significance of the regression coefficients ($P < 0.05$) and on the value of the coefficient of determination. Data obtained in this study were analyzed by one-way analysis of variance using the SAS software general linear model (SAS, 2004). The main factor was canola meal replacement level. Mean values were compared using the Duncan's New Multiple Range test (Duncan, 1955) when significant differences existed. The fixed effects model used in the analysis was as follow: $Y_{ij} = \mu + T_i + e_{ij}$, Where, Y_{ij}: the observation of the J_{th} chick in the i_{th} treatment; μ: the overall mean; T_i: effect of the i_{th} treatment ($i = 1, 2, 3, 4$) and e_{ij}: the random error effect. The significance level was set at ($\alpha = 0.05$).

3. Results

3.1 Productive Performance

Results presented in Table 2, indicated presence of differences in body weight and body weight gain/period among experimental groups. Body weight and body weight gain of the group that fed 90% CM diet were significantly lower at all ages compared to the other experimental groups and the control. However, both 30% and 60% CM diets groups are nearly similar to the control group without significant difference.

Feed intake of the groups fed 30 and 60% CM substituted from SBM protein were not significantly differ, however, at 90% replacement level, FCR was significantly inferior, compared to the control.

Table 2. Effect of replacing Soybean meal protein by canola meal protein on productive performance

Items	Control	CM (30%)	CM (60%)	CM (90%)	SEM	P-value
0-14 d						
BW, g	408.86^{a}	400.66^{a}	393.00^{a}	322.80^{b}	6.31	< 0.0001
BWG, g	363.46^{a}	355.46^{a}	346.26^{a}	276.73^{b}	6.20	< 0.0001
FI, g	430.86^{a}	417.33^{ab}	410.00^{b}	346.66^{c}	5.24	< 0.0001
FCR	1.18^{a}	1.18^{a}	1.18^{a}	1.25^{b}	0.02	0.0454
15-28 d						
BW, g	1471.13^{a}	1470.33^{a}	1456.27^{a}	1047.73^{b}	12.21	< 0.0001
BWG, g	1062.27^{a}	1069.67^{a}	1063.27^{a}	724.93^{b}	11.82	< 0.0001
FI, g	1390.00^{a}	1386.67^{ab}	1366.87^{b}	1229.33^{c}	7.66	< 0.0001
FCR	1.31^{a}	1.29^{a}	1.28^{a}	1.69^{b}	0.02	< 0.0001
29-39 d						
BW, g	2498.67^{a}	2490.27^{a}	2470.00^{a}	1979.40^{b}	18.30	< 0.0001
BWG, g	1027.53^{a}	1019.93^{a}	1013.73^{a}	931.66^{b}	18.09	0.0011
FI, g	1770.00	1792.33	1795.33	1822.33	31.75	0.7141
FCR	1.72^{a}	1.75^{ab}	1.77^{b}	1.95^{c}	0.01	< 0.0001
0-39 d						
BWG, g	2453.27^{a}	2445.07^{a}	2423.27^{a}	1933.33^{b}	18.19	< 0.0001
FI, g	3590.87^{a}	3596.33^{a}	3572.20^{a}	3398.33^{b}	33.98	< 0.0001
FCR	1.46^{a}	1.47^{a}	1.47^{a}	1.75^{b}	0.01	< 0.0001

Note. CM = Canola meal, BW = body weight, BWG = body weight gain, FI = feed intake, FCR = feed conversion ratio; $^{a-b}$ Means with different superscripts within each row are significantly different, SEM: standard error of the mean, P-value = significance level at $\alpha = 0.05$. Regression equations set for (0-14 d): BW = 421.21 – 0.89x, R^2 = 0.50, P-value < 0.0001, SE = 0.11; BWG = 375.89 – 0.90x, R^2 = 0.53, P-value < 0.0001, SE = 0.11; FI = 440.21 – 0.87x, R^2 = 0.59, P-value < 0.0001, SE = 0.95; FCR = 1.14 + 0.001x, R^2 = 0.56, P-value < 0.0001, SE = 0.0001. For (15-28 d): BW = 1554.007 – 4.281x, R^2 = 0.58, P-value < 0.0001, SE = 0.468; BWG = 1132.79 – 3.39x, R^2 = 0.55, P-value < 0.0001, SE = 0.40; FI = 1428.99 – 1.82x, R^2 = 0.67, P-value < 0.0001, SE = 0.17; FCR = 1.235 + 0.004x, R^2 = 0.46, P-value < 0.0001, SE = 0.0005. For (29-39 d): BW = 2586.69 – 5.33x, R^2 = 0.64, P-value < 0.0001, SE = 0.53; BWG = 1032.69 – 1.05x, R^2 = 0.70, P-value < 0.0001, SE = 0.09; FCR = 1.749 + 0.002x, R^2 = 0.59, P-value < 0.0001, SE = 0.0002. For (0-39 d): BWG = 2541.37 – 5.34x, R^2 = 0.64, P-value < 0.0001, SE = 0.53; FI = 3675.89 – 2.67x, R^2 = 0.31, P-value < 0.0001, SE = 0.52; FCR = 1.434 + 0.003x, R^2 = 0.56, P-value < 0.0001, SE = 0.0003.

3.2 Nutrient Digestibility

No significant differences were observed among CM levels for the average values of organic matter (OM), EE and nitrogen free extract (NFE) digestibility (Table 3). Data showed that the highest CM inclusion level had the lowest crude protein (CP) and CF digestibility, being 82.7 and 22.6%, respectively. The corresponding values of the control were 84.9 and 24.9%, respectively.

Table 3. Effect of replacing of Soybean meal protein by canola meal protein on nutrients digestibility of experimental finisher diets

Canola meal level	Nutrients apparent digestibility (%)				
	OM	CP	EE	CF	NFE
0	86.4	84.9^{a}	73.3	24.9^{a}	85.9
30	86.1	85.1^{a}	73.3	24.7^{a}	86.0
60	86.2	84.7^{a}	73.6	23.8^{ab}	85.0
90	86.1	82.7^{b}	72.5	22.6^{b}	84.8
SEM	0.39	0.39	0.79	0.42	0.23
P-value	0.535	< 0.001	0.166	< 0.001	0.285

Note. $^{a-b}$ Means with different superscripts within each column are significantly different, SEM: standard error of the mean, P-value = significance level at $\alpha = 0.05$; OM = organic matter, CP = crude protein, EE = ether extract, CF = crude fiber, NFE = nitrogen free extract. Regression equations set for: CP = 85.40 – 0.02x, $R^2 = 0.59$, P-value = 0.0033, SE = 0.006; CF = 25.17 – 0.03x, $R^2 = 0.82$, P-value < 0.0001, SE = 0.004.

3.3 Immune Response and Lymphoid Organs

The Haemagglutination Inhibition (HI) titer against ND virus did not differ ($P > 0.05$) at 39 days of age due to the replacement of soybean meal by CM in the broilers diets (Table 4). Also, there were no significant differences ($P > 0.05$) among all groups in response of SRBCs injection.

Increasing level of CM up to 90% of SBM protein significantly lowered percentage of spleen, while, increased those of thymus, bursa and thyroid gland, compared to the control. While, no significant differences were found between groups fed either 30 or 60% CM and the control group fed diet based only on SBM.

Table 4. Effect of replacing of Soybean meal protein by canola meal protein on immune response and lymphoid organs

Items	Control	CM (30%)	CM (60%)	CM (90%)	SEM	P-value
ND titer	6.66	7.66	8.66	8.33	0.66	0.2341
SRBCs titer	6.44	6.44	5.66	5.66	0.42	0.3605
Thyroid (%)	0.08^{b}	0.09^{b}	0.08^{b}	0.16^{a}	0.013	0.0276
Spleen (%)	0.09^{a}	0.10^{a}	0.09^{a}	0.07^{b}	0.014	0.0386
Bursa (%)	0.12^{b}	0.13^{b}	0.13^{b}	0.15^{a}	0.018	0.0334
Thymus (%)	0.31^{b}	0.26^{b}	0.45^{ab}	0.61^{a}	0.093	0.0555

Note. CM = Canola meal, ND = Newcastle disease, SRBC's = sheep red blood cells; $^{a-b}$ Means with different superscripts within each row are significantly different, SEM: standard error of the mean, P-value = significance level at $\alpha = 0.05$. Regression equations set for: Thyroid (%) = 0.0650 + 0.0008x, $R^2 = 0.64$, P-value < 0.0001, SE = 0.0001; Spleen (%) = 0.0985 – 0.0003x, $R^2 = 0.36$, P-value = 0.0012, SE = 0.00007; Bursa (%) = 0.1112 + 0.0008x, $R^2 = 0.47$, P-value = 0.0002, SE = 0.0002; Thymus (%) = 0.245 + 0.004x, $R^2 = 0.77$, P-value < 0.0001, SE = 0.0004.

3.4 Blood Biochemical Parameters

All the levels tend to decrease serum AST ($P = 0.0071$), Creatinine ($P < 0.0001$) and urea ($P = 0.0007$) values compared to the control (Table 5). In addition, no significant differences were recorded among treatment groups in regard to the serum content of total protein, albumin and globulin values. Serum content of thyroid hormones including T_3 ($P = 0.0034$) and T_4 ($P = 0.0339$) were higher in chicks fed CM at 90% of SBM protein compared to control group fed the basal diet (Table 5). Moreover, chicks fed diets contain CM recorded lower values of

cholesterol ($P < 0.05$) and LDL cholesterol but not significantly ($P > 0.05$), compared to the control group fed diet based on SBM only. As the control group recorded the lowest value of HDL cholesterol ($P = 0.0532$), accordingly, the HDL/LDL ratio increased gradually ($P < 0.05$) as the replacement level of CM increased (Table 5).

Table 5. Effect of replacing of Soybean meal protein by canola meal protein on biochemical parameters

Items	Control	CM (30%)	CM (60%)	CM (90%)	±SEM	P-value
Total Protein (g/dL)	3.26	3.30	2.90	3.13	0.15	0.1680
Albumin (g/dL)	2.13	2.16	2.00	2.16	0.05	0.1131
Globulin (g/dL)	1.13	1.14	0.90	0.97	0.17	0.7613
AST (U/L)	119.00^{a}	93.33^{b}	90.33^{b}	85.00^{b}	5.16	0.0071
ALT (U/L)	46.00^{a}	37.00^{b}	33.66^{b}	39.66^{ab}	2.07	0.0165
Creatinine (mg/dL)	2.10^{a}	1.20^{b}	1.20^{b}	1.20^{b}	0.09	<0.0001
Urea (mg/dL)	7.50^{a}	4.00^{b}	2.50^{b}	2.20^{b}	0.58	0.0007
T_3 (ng/dL)	188.33^{c}	208.33^{bc}	230.00^{b}	262.00^{a}	9.55	0.0034
T_4 (µg/dL)	3.5^{b}	3.86^{ab}	4.30^{a}	4.46^{a}	0.19	0.0339
Cholesterol (mg/dL)	150.33^{a}	128.00^{b}	125.00^{b}	76.66^{c}	6.03	0.0002
HDL (mg/dL)	83.33^{b}	97.00^{a}	96.00^{a}	98.66^{a}	2.403	0.0532
LDL (mg/dL)	20.00	19.00	18.66	17.66	2.09	0.8860
HDL/LDL	4.16^{b}	5.10^{a}	5.14^{a}	5.59^{a}	0.57	0.0491

Note. CM = Canola meal; $^{a-c}$ Means with different superscripts within each row are significantly different, SEM: standard error of the mean, P-value = significance level at $\alpha = 0.05$. Regression equations set for: AST = 112.67 – 0.35x, $R^2 = 0.65$, P-value = 0.0015, SE = 0.08; ALT = 42.67 – 0.09x, $R^2 = 0.38$, P-value = 0.0312, SE = 0.03; Creatinine = 1.83 – 0.009x, $R^2 = 0.60$, P-value = 0.0031, SE = 0.002; Urea = 6.66 – 0.06x, $R^2 = 0.84$, P-value < 0.0001, SE = 0.008; $T_3 = 185.76 + 0.81x$, $R^2 = 0.94$, P-value < 0.0001, SE = 0.06; $T_4 = 3.53 + 0.01x$, $R^2 = 0.75$, P-value = 0.0003, SE = 0.002; Cholesterol = 153.60 – 0.75x, $R^2 = 0.77$, P-value < 0.0001, SE = 0.12; HDL = 87.00 + 0.15x, $R^2 = 0.66$, P-value = 0.0013, SE = 0.03; HDL/LDL = 4.35 + 0.01x, $R^2 = 0.86$, P-value < 0.0001, SE = 0.002.

3.5 Fatty Acids Profile of Carcass Meat and Secum Microbial Content

Results indicated highly significant differences ($P < 0.0001$) in fatty acids contents of carcass meat among experimental groups (Table 6).

Chicks fed diets with different levels of CM showed significantly increase in carcass meat content of the unsaturated fatty acids (UFA), particularly palmitolic and oleic as mono UFA with higher values for treatments 30 and 60% CM of SBM protein. Collectively, total saturated fatty acids (SFA) showed lower values by feeding CM at different levels, compared to the group fed basal diet.

Accordingly, the UFA/SFA ratio showed an evident increase in CM treatment groups, compared to the group fed basal diet. Data in Table 6 indicate that the linoleic (n-6) fatty acid is higher in chicks fed 60 or 90% CM instead SBM protein by about 77% over the control fed basal diet and by about 21% over those fed CM at 30% replacement level. Erucic acid did not detected in carcass meat of all treatment groups.

It is worthy to note that the lowest ($P < 0.05$) *Enterococcus* and *E. coli* count had been detected for chicks having CM at 30% replacement of SBM protein (Table 6). Salmonella microbes had not been detected in cecum in all experimental groups.

Table 6. Effect of replacing of Soybean meal protein by canola meal protein on fatty acids profile of carcass meat (% of total fatty acids) and cecum microbial content (log10 CFU/g digesta)

Items	Control	CM (30%)	CM (60%)	CM (90%)	SEM	P-value
Myristic	3.82[a]	0.58[c]	0.60[c]	0.85[b]	0.02	< 0.0001
Palmitic	27.85[a]	23.80[b]	24.06[b]	23.14[c]	0.18	< 0.0001
Palmitolic	2.54[b]	4.00[a]	4.00[a]	1.97[c]	0.03	< 0.0001
Stearic	11.03[a]	7.75[c]	6.77[d]	8.46[b]	0.09	< 0.0001
Oleic	28.50[d]	39.19[a]	35.88[b]	33.66[c]	0.14	< 0.0001
Linoleic (n-6)	13.00[c]	19.23[b]	23.01[a]	23.29[a]	0.11	< 0.0001
Linolenic (n-3)	0.80[c]	1.27[b]	2.07[a]	2.05[a]	0.04	< 0.0001
SFA	42.70[a]	32.13[b]	31.43[b]	32.45[b]	0.29	< 0.0001
UFA	44.84[c]	63.69[a]	64.96[a]	60.97[b]	0.32	< 0.0001
n-6/n-3	16.25[a]	15.14[a]	11.12[b]	11.36[b]	0.27	< 0.0001
UFA/SFA	0.99[b]	1.98[a]	2.07[a]	1.88[a]	0.22	< 0.0001
Erucic	ND	ND	ND	ND	-	-
Lact. Count	7.60	7.77	7.71	7.86	0.10	0.4094
En. Count	6.29[a]	4.94[b]	6.31[a]	6.43[a]	0.22	0.0051
E. coli	5.19[a]	3.26[c]	4.44[b]	4.80[ab]	0.128	< 0.0001
Salmonella	ND	ND	ND	ND	-	-

Note. CM = Canola meal; [a-d] Means with different superscripts within each row are significantly different, SEM: standard error of the mean, P-value = significance level at $\alpha = 0.05$, ND = not detected. Regression equations set for: Myristic = 2.47 – 0.02x, $R^2 = 0.44$, P-value = 0.0261, SE = 0.009; Palmitic = 26.55 – 0.04x, $R^2 = 0.64$, P-value = 0.0033, SE = 0.01; Palmitolic = 2.98 + 0.01x, $R^2 = 0.60$, P-value = 0.0032, SE = 0.005; Stearic = 9.81 – 0.03x, $R^2 = 0.38$, P-value = 0.0334, SE = 0.012; Oleic = 31.7 + 0.1x, $R^2 = 0.60$, P-value = 0.0031, SE = 0.03; Linoleic = 14.8 + 0.1x, $R^2 = 0.85$, P-value < 0.0001, SE = 0.02; Linolenic = 0.88 + 0.01x, $R^2 = 0.86$, P-value < 0.0001, SE = 0.002; SFA = 39.40 – 0.03x, $R^2 = 0.57$, P-value = 0.0045, SE = 0.03; UFA = 51.18 + 0.17x, $R^2 = 0.47$, P-value = 0.0138, SE = 0.06; n-6/n-3 = 16.30-0.06x, $R^2 = 0.76$, P-value = 0.0002, SE = 0.01; UFA/SFA = 1.360 + 0.009x, $R^2 = 0.50$, P-value = 0.0100, SE = 0.003; *En*. Count = 5.89 – 0.01x, $R^2 = 0.62$, P-value = 0.0024, SE = 0.003; *E. coli* = 4.61 – 0.02x, $R^2 = 0.61$, P-value = 0.0028, SE = 0.005.

4. Discussion

4.1 Productive Performance

In the present study, FI of the group that fed 90% CM diet was lowest compared with other experimental groups in starting, growing and allover period. This could be attributed to several reasons. The higher glucosinolates content of 90% CM group compared to other experimental groups comes first (Khajali & Slominski, 2012) which caused palatability reduction and may interfere with the function of thyroid gland causing drastic disturbance of thyroid hormones resulting in negative effects on growth performance particularly on BWG. Therefore, increasing of serum T_4 and T_3 concentrations in our study may also led to reduction of the broilers' BWG. Furthermore, CM at higher level caused an increase of thyroid size and its metabolic activities which led to energy and other nutrients utilization for maintenance rather than growth (Woyengo et al., 2011). The second reason is higher dietary fiber content of 90% CM group compared to other experimental groups (Woyngo et al., 2011), causing low energy as well as low protein digestibility (Khajali & Slominski, 2012; Bovera et al., 2014; Gopinger et al., 2014), that might have reduced the performance of chicks. The third reason is higher content of phenolic compounds such as condensed tannins of 90% CM group compared to other experimental groups (Mansoori & Acamovic, 2007) which can decrease the bioavailability of protein by forming complexes with protein and proteolytic enzymes in the gastrointestinal tract (Khajali & Slominski, 2012). Finally, the higher NSP of 90% CM group compared to other experimental groups accounted one of possible reasons for growth performance's impairing which represent 18-20% (Khajali & Slominski, 2012), that caused an increase in the viscosity of digesta and reduced the digestion as well as the absorption of nitrogen and subsequently resulting in poor growth performance (Mushtaq et al., 2007); all contribute to reduced feed intake in the 90% CM diet which is reflected on BWG and Final BW depression. The results are in agreement with previous studies that found an adverse effect on FI of broiler chickens fed diets contained CM (Payvastegan et al., 2013; Gopinger et al., 2014; Aljuobori et al., 2016; Zhang & Adeola, 2017).

In the same vein, CM contains higher phytic acid (2.9-3.2%) than SBM which contains only 1.4% (Payvastegan et al., 2013) that leading to non-full availability of some nutrients which mainly are amino acids, calcium and phosphorus, for example, reduction of lysine digestibility of CM compared to SBM (Mushtaq et al., 2007). According to our results, the broilers that fed 90% CM as replacement of SBM protein showed an inferior FCR.

4.2 Nutrient Digestibility

In the present study, the digestibility of CP and CF was inversely related to the used level of CM in the diet. This means that both CP and CF digestion coefficient tended to decrease ($P < 0.05$) as the CM replacement level increased more than 60% of SBM protein. Such decrease could be attributed to that CM contains some biologically active chemical compounds such as phytate, NSP and tannins which can reduce the digestibility of nutrients (Khajali & Slominski, 2012). Dietary fiber reduces nutrient digestibility due to its physiochemical properties, leading to a more rapid rate of passage that limits the amount of time available for nutrient breakdown (Thacker & Petri, 2011). Moreover, total dietary fiber values for canola meal are higher than those of SBM due to a much higher content of lignin with associated polyphenols (Khajali & Slominski, 2012). As expected, an increase in the level of inclusion of CM results in an increase in the level of fiber in the diet. Accordingly, this increase decreases the protein digestibility. In this respect, Pustjens et al. (2013) reported that NSP are not degraded by poultry endogenous enzymes, and thus increase gut viscosity and consequently reduce nutrient utilization. Consistent with the present study, Pinheiro et al. (2008) observed significant differences in the nutrient digestibility of dry matter (DM) and CP between the diets of broilers with low CF and the diets of broilers with high CF. In addition, Landero et al. (2012); Gopinger et al. (2014); Toghyani et al. (2017) observed a decrease in digestibility of CP with increased canola meal inclusion in a diet for weaned piglets and broilers, respectively, and attributed this decrease to the high CF content of canola meal.

4.3 Immune Response and Lymphoid Organs

Feeding CM had no significant effect on HI titer against ND virus, that as the same as the results obtained by Mushtaq et al. (2007) and that obtained by An et al. (2016). These results are contrary with results obtained by Ahmad et al. (2007); who reported that HI antibody titer against ND virus was lowest in diets containing 10% CM, whereas, it was highest in those having 15% CM. In another respect, CM at 30% and 60% replacement level did not cause significant effect on the relative weight of lymphoid organs compared to the control which agree with results reported by Ahmed et al. (2015) when they fed broilers diets contained 5, 10 and 20% CM. Also, the present results are compatible with the results of Ashnie et al. (2015) where, they did not found a significant differences of relative spleen weights among broilers fed diets contained 0, 7.5 and 15% CM while, the broilers fed diets contained 22.5 and 30% CM showed a significant increase of relative weights of spleen compared to the control group which was contrary with our results. In this respect, Taraz et al. (2006) reached to 38.2, 28.95 and 23.16% CM as maximum levels in starter, grower and finisher diets, respectively; they reported no significant differences in relative spleen weights between all CM groups and the CM free group; that showed disagreement with the present results of broilers fed 90% CM replacement of SBM compared to control, as the starter, grower and finisher diets contained 32.63, 27.67 and 29.89% CM, respectively. In the same vein, An et al. (2016) reported no significant differences among relative spleen weights of broilers fed wheat-SBM based diets contained 0, 3, 5, 10 and 15% CM. It is well known that spleen, bursa and thymus are considered a part of the secondary and primary lymphoid organs (H. Lillehoj & E. Lillehoj, 2000) responsible for producing cells that protect the birds from the invaded microorganisms.

4.4 Blood Biochemical Parameters

Regard to thyroid hormones, the higher concentration of T_3 in the CM diets may be attributed to effects of glucosinolate hydrolysis products on peripheral T_4 monodeiodination. In line with our results, Newkirk and Classen (2002); Kermanshahi and Abbasipour (2006); Maroufyan and Kermanshahi (2006); Aljuobori et al. (2016) have stated that CM feeding elevated T_3 level in broiler chickens. On the other hand, Woyengo et al. (2011) reported that serum T_4 was increased while serum T_3 not affected significantly by the substitution of CM. In this respect, Aljuobori et al. (2016) suggested that glucosinolates in CM may destroy cellular T_3 receptors and thus increase the thyroid hormones level in blood. In contrary to our results, Taraz et al. (2006); Mikulski et al. (2012); Payvastegan et al. (2017) indicated that an increasing of inclusion levels of CM was followed by plasma T_3 concentrations reduction. However, Taraz et al. (2006); Payvastegan et al. (2017) reported no significant differences in plasma T_4 among all experimental groups. They attributed these discrepancies to differences in glucosinolates content in CM, diet ingredients, composition of gut microorganism besides, the extent of glucosinolates degradation by heat during the oil extraction process (Woyengo et al., 2011).

Obtained results indicated that feeding CM to broiler reduced cholesterol level in blood serum, which is a desirable outcome for consumers. In this connection, Mnisi and Mlambo (2018) with quail and El-Medany and El-Reffaei (2015) with growing rabbits attained the same result. In contrary with our results, Payvastegan et al. (2017) reported no significant differences in plasma concentrations of cholesterol and HDL cholesterol in broilers receiving diets containing different levels of CM up to 30%. In the same line Payvastegan et al. (2013) reported no significant differences in plasma concentrations of cholesterol and HDL in broilers receiving diets containing different levels of solvent extracted CM up to 20%.The higher HDL fraction ($P < 0.05$) and lower LDL cholesterol ($P > 0.05$) of CM fed groups compared to the control indicated the positive effect of CM in lowering serum cholesterol content. CM may prevent the accumulation of LDL cholesterol probably by enriching the monounsaturated as well as the UFA which are considered the heart-friendly fatty acids to the consumer (Dernekbasi & Karayucel, 2010).

4.5 Fatty Acids Profile of Carcass Meat

Chickens as a monogastric is able to incorporate the long chain fatty acids in the adipose tissue directly from the diet. Rahimi et al. (2011) concluded that fatty acids composition of the diet has an effect on fatty acids profile of broiler meat. In the present study, linoleic acid in carcass meat increased as dietary CM increased compared to the control. The results of this experiment are Compatible with the results obtained by Tuunainen et al. (2016); they found a significantly decrease in SFA and significantly increase in UFA of broiler breast meat that fed on rapeseed meal based diet compared to that fed SBM based diet. The increased values of linoleic and other UFA particularly palmitolic and oleic as mono UFA make the carcass meat a rich source of such favorable fatty acids for human consumption that attributed to its protection role of body human from heart diseases like heart attack and cardio vascular disorders (Rahimi et al., 2011).

5. Conclusion

Conclusively, there were no detrimental effects of using CM protein up to 60% replacement from SBM protein on productive performance and immune parameters. While, 90% had adversely effects on broiler chicks, likely because of increased dietary concentration of glucosinolates.

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