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Effects of Replacing Canola Meal with Soybean Meal in Broiler Chicken Diet on Growth Performance, Carcass Traits, and Liver Enzymes during Different Rearing Periods

Research Article

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ABSTRACT

Soybean meal is more beneficial than canola meal (CM) due to the better balance of amino acids in the nutrition of broilers. Three experiments were conducted to assess the impact of replacing soybean meal with CM at different levels during the rearing stages of Ross 308 male broilers on performance, carcass traits, and liver enzyme concentrations. In the first experiment, 420 one-day-old chicks received starter diets with six graded levels of CM from 0 to 15% for 10 days. In the second experiment, 360 eleven-day-old chicks were fed six experimental grower diets varying in CM from 0 to 25% over 14 days. In the third experiment, three hundred 25-day-old chicks were subjected to six finisher diets ranging in CM from 0 to 40% over 18 days. The findings revealed that the inclusion of the CM at the examined levels had no adverse effects on broilers' performance, carcass traits, and liver enzymes in the first and second experiments ($P>0.05$). Dietary CM inclusion of more than 32% in the third experiment had significant adverse effects on the performance, European broiler index, and liver enzymes of broilers ($P<0.05$). In conclusion, the CM can be included in broiler diets at levels up to 15% during the starter and 20% during the grower periods without adversely affecting growth performance and carcass traits. Nonetheless, an inclusion level of CM exceeding 32% of the diet during the finisher period resulted in decreased weight gain and harmful effects on liver function.

KEY WORDS alternative protein source, feed ingredient, growth, nutrition, production phase.

INTRODUCTION

Soybean meal (SBM), a highly desirable potential feed ingredient, has been commonly used in poultry diets for many years. Due to the widespread use of soy in animal and human food, soy-based feed ingredients continue to increase in price (Manyeula *et al.* 2020). However, high market prices of SBM have negatively affected poultry businesses, with farmers struggling to cope with increased feed costs. As demand for sustainable feed ingredients has increased, attention has been redirected toward alternative protein sources in poultry diets (Watts *et al.* 2020), including canola meal, sunflower meal, or dried distillers' grains with solubles (DDGS) (Liebl *et al.* 2022). The possibility of replacing SBM with canola meal (CM) in poultry diets has been highlighted as one of the opportunities for improving profitability for avian enterprises. As the second worldwide feed protein ingredient, canola stands out as a promising alternative to soybean due to its high crude protein and offering high content of sulfur amino acids and a well-balanced and comparable amino acid profile with soybeans (Khajali and Slominski, 2012; Liebl *et al.* 2022). Even though CM can be used to replace SBM in poultry diets, it may not fully substitute SBM because of its high content of anti-nutritional factors, such as non-starch polysaccharids, phytic acid, glucosinolates, and sinapine electrolyte imbalance (Khajali and Slominski, 2012), lower energy due to its higher fiber content, lower nutrient utilization, and a problem for Maillard reaction products associated with processing (Teodorowicz *et al.* 2018).

The inclusion of CM in broiler diets has been studied for decades. Researchers paid attention to the effects of dietary CM on broiler growth performance during different rearing ages. There are diverse reports for the maximum levels of dietary CM in broiler starter diet as 38% (Leeson *et al.* 1987), 28% (Newkirk and Classen, 2002), 30% (Ramesh *et al.* 2006; Mushtaq *et al.* 2007), 25% (Min *et al.* 2011), 20% (Payvastegan *et al.* 2013), 17% (Gopinger *et al.* 2014a), 15% (An *et al.* 2016), 10% (Ahmed *et al.* 2015; Gorski *et al.* 2017) and 6% (Michalik-Rutkowska *et al.* 2017). For the grower-rearing periods, Gorski *et al.* (2017) reported that up to 24% of CM in the diet had no adverse effects on broiler performance which indicated more tolerance than in the starter period. Other studies did not recommend adding more CM during the grower phase compared to the starter phase (Min *et al.* 2011; Gopinger *et al.* 2014b; Ahmed *et al.* 2015; An *et al.* 2016). Some researchers suggested similar levels of CM for the finisher-rearing period as those for the grower and starter (Ramesh *et al.* 2006; Payvastegan *et al.* 2013; Ahmed *et al.* 2015). Michalik-Rutkowsla *et al.* (2017) found the optimal level of CM for the grower-finisher phase as 8-10% (more than the value suggested for the starter as 4-6%) provided that its glucosinolate level was lower than 9.5 µM/g. On the other hand, Newkirk and Classen (2002) recommended a dietary CM level of up to 15% of a wheat-based feed for broiler chickens' finisher period, which was about half of that they suggested for the starter feed.

While these studies have contributed valuable insights, it’s worth noting that, in each case, the broiler chickens were consistently exposed to dietary CM throughout all the investigated production phases. In pursuit of determining the optimal CM levels for the grower phase, Gorski *et al.* (2017) took an approach by conducting a separate experiment in which the birds received starter feed containing lower CM levels than those previously determined in the same study. The potential impacts and carryover effects of dietary CM consumption on the bird’s performance in subsequent production phases deserve to be questioned. Some researchers have implemented specific strategies to avoid potential carryover effects of dietary interventions from earlier phases (Dozier *et al.* 2008; Amos *et al.* 2021). As the impacts of feeding CM on broilers may persist and interfere with the following production phase, it is appropriate to determine the optimal level of dietary CM for each rearing phase by conducting independent experiments. In the present study, three independent experiments were innovatively designed to determine the optimal levels of dietary CM for the starter, grower, and finisher-rearing periods. In all three experiments, birds received corn-SBM feed before the beginning of the experiment, and the effects of different levels of CM on growth performance, carcass traits, and serum liver enzymes were examined.

MATERIALS AND METHODS

This study was performed in a commercial broiler production farm located in the north part of Iran (Babol, Iran), and the study procedure was reviewed and approved by the Animal Ethics Committee of the Animal Science Department, Gorgan University of Agriculture and Natural Resources (Gorgan, Iran).

The birds and experiments

Three separate experiments were carried out concerning the three-rearing period of Ross 308 broiler chickens. In the first experiment, 420 one-day-old Ross 308 male broiler chicks with a mean body weight of 46.0 ± 0.18 g were purchased from a private hatchery and reared for 10 days. In the second experiment, 360 Ross 308 male broiler chicks with a mean body weight of 192.4 ± 2.4 g were provided from a flock at the age of 10 days and reared for 14 days. The chicks were fed a commercial corn-SBM diet from hatching up to 10 days. For the third experiment, 300 Ross 308 male chicks with a mean body weight of 872.6 ± 16.8 g were supplied from the flock at the age of 24 days and reared for 18 days.

The chicks were previously fed a commercial corn-SBM diet before starting the experiment. In all three experiments, birds were randomly distributed to 30 pens with nearly a similar body weight between the pens. Each of the experiments had six dietary treatments with five replicates of 14 (experiment 1), 12 (experiment 2), or 10 (experiment 3) birds per each. Birds were reared on deep litter floor pens and had free access to mash feed forms and tape water. The lighting program consisted of 24 continuous lights, and the temperature was set up according to the Ross manual guide (Aviagen, 2017).

Experimental diets

Two isoenergetic and isonitrogenous basal diets were formulated to meet Ross broiler chickens' minimum nutritional requirements for all experiments. The SBM basal diets had no CM, while the CM basal diets contained CM at 15%, 25%, and 40% for the first, second, and third experiments (Table 1).

Table 1 Ingredients and chemical composition of the soybean meal (SBM) and canola meal (CM) basal diets for each experiment (Exp.)

Ingredients, %	**Starter (Exp. 1)**		**Grower (Exp. 2)**		**Finisher (Exp. 3)**	
	SBM	CM	SBM	CM	SBM	CM
Corn (CP=7.2%)	53.7	48.66	58.62	50.2	64.46	51.03
Soybean meal (CP=44.5%)	39.66	28.51	35.29	16.69	29.76	0
Canola meal (CP=35.4%)	0	15	0	25	0	40
Oil	1.86	3.45	1.86	4.52	1.86	6.11
Calcium carbonate	0.86	0.84	0.78	0.75	0.72	0.67
Dicalcium phosphate	2.13	1.76	1.86	1.25	1.66	0.68
Salt	0.25	0.24	0.24	0.23	0.24	0.23
Sodium bicarbonate	0.16	0.16	0.16	0.16	0.16	0.15
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.29	0.35	0.22	0.32	0.21	0.37
DL-Methionine	0.4	0.33	0.33	0.22	0.3	0.11
L-Threonine	0.14	0.15	0.09	0.11	0.08	0.1
Coxistac	0.05	0.05	0.05	0.05	0.05	0.05
Chemical composition (%), unless mentioned						
Metabolizable energy (kcal/kg)	2800		2930		3000	
Crude protein	22.1		20.36		18.28	
Calcium	0.922		0.824		0.74	
Available phosphorous	0.461		0.412		0.371	
Sodium	0.154		0.151		0.15	
Lysine	1.384	1.384	1.222	1.222	1.087	1.087
Methionine	0.705	0.664	0.625	0.559	0.565	0.455
Methionine + cystine	1.038	1.038	0.938	0.938	0.853	0.853
Threonine	0.957	0.983	0.849	0.892	0.752	0.821
Arginine	1.451	1.389	1.328	1.224	1.17	1.004
Digestible lysine	1.259	1.241	1.109	1.079	0.989	0.941
Digestible methionine	0.678	0.628	0.599	0.515	0.543	0.408
Digestible methionine + cystine	0.948	0.927	0.855	0.819	0.779	0.723
Digestible threonine	0.826	0.826	0.729	0.729	0.647	0.647
Digestible arginine	1.33	1.257	1.216	1.095	1.07	0.878

[1] Provides per kg of diet: vitamin A: 10000 IU; vitamin E: 40 IU; vitamin B_{12}: 0.015 mg; Cholecalciferol: 4000 IU; Menadione: 3.5 mg; Riboflavin: 6 mg; Niacin: 45 mg; Pantothenic acid: 15 mg; Folic acid: 1.6 mg; Thiamin: 2.5 mg; Pyridoxine HCl: 3.5 mg and Biotin: 0.2 mg.

[2] Provides per kg of diet: Manganese: 110 mg; Zinc: 100 mg; Iron: 20 mg; Copper: 16 mg; Selenium: 0.30 mg and Iodine: 1.25 mg.

The basal diets were blended to prepare six levels of CM using ratios of SBM diet and CM diet at 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. Therefore, the percentages of CM in six dietary treatments were respectively as 0, 3, 6, 9, 12, and 15 within the 1st experiment, 0, 5, 10, 15, 20, and 25 within the 2nd experiment, and 0, 8, 16, 24, 32, and 40 within the 3rd experiment. CM had 9.29% moisture, 35. 4% crude protein, 5.1% ether extract, 12.4% crude fiber, 7.6% ash, and 4236 kcal/kg gross energy.

Data collection

Birds were weighed by pen group at the beginning and ending period of the experiment after four hours of fasting, and then weight gain was calculated. Feed intake was measured for each pen by subtracting feed residual from the feed offering during the experiment. Feed conversion ratio (FCR) was calculated by dividing feed intake to weight gain and European broiler index (EBI) according to Biesek *et al.* (2020) formula as, (average daily weight gain × % liveability) / (feed conversion rat).

At the end of each experiment, two chickens per pen with a body weight close to the average pen body weight were randomly selected to determine liver enzyme concentrations and carcass characteristics. The birds were weighed individually first and then 5 mL of blood was collected from the wing vein and poured into sterile glass tubes, and after that, they were slaughtered. Blood serum samples were prepared and immediately transferred to the laboratory for further analysis. Liver enzymes concentration including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using traditional kits manufactured by Pars Azmoun Company (Pars Azmoun, Iran), with a photometric spectrometer (CLima-617).

After slaughtering, de-feathering, and removing offal, carcass, breast, thigh, and liver were weighed. The values were expressed as absolute weight and percentage of live body weight.

Statistical analysis

Data from all three experiments were checked for normal distribution through the UNIVARIATE procedure and Shapiro–Wilk test, then analyzed in a completely randomized design using the GLM procedure of SAS software (SAS, 2013).

The statistical model was:

$Y_{ij}=\mu+T_{ij}+\varepsilon_{ij}$

Where:

Y_{ij}: observed response variables.

μ: overall mean.

T_{ij}: effect of diet.

ε_{ij}: random error.

Tukey test was used to determine significant differences between treatments. Differences with P-values less than 0.05 were considered to be statistically significant. Orthogonal polynomial contrasts were also used to examine the linear and quadratic trends in response to increasing levels of CM.

RESULTS AND DISCUSSION

The effects of replacing different levels of CM with SBM on growth performance, carcass traits, and liver enzymes in experiment 1 are shown in Table 2. Results indicated that the inclusion of CM up to 15% of the diet from hatching to 10 days of age had no significant effect on body weight, weight gain, feed intake, FCR, EBI, carcass traits, liver weight, and serum concentrations of liver enzymes of broiler chickens ($P>0.05$).

Results from experiment 2, in which the CM was replaced with SBM from zero to 25% of the diet, are shown in Table 3. The results indicated that CM can be used up to 25% of broiler diets at 11 to 24 days of age without significantly impacting growth performance, carcass traits, and liver enzymes ($P>0.05$). However, relative liver weight significantly decreased when the CM was replaced with soybean meal at above 15% of the diet ($P<0.05$).

Results from the 3rd experiment in Table 4 show that including CM at levels more than 32% of diet during the 25 to 42 days of the finisher period leads to a significant decrease in body weight and weight gain ($P<0.05$). Although feed intake was not affected by CM level, FCR and EBI significantly worsened when CM was replaced with SBM at more than 24% of the diet ($P<0.05$).

Replacing SBM with CM significantly affected carcass characteristics, liver weight, and liver enzyme concentrations ($P<0.05$). Absolute carcass weight decreased significantly when CM was replaced with SBM at 40% of the diet ($P<0.05$). At the same time, no significant difference was observed between CM treatments with the SBM diet for relative carcass weight. Replacing SBM with CM led to a significant decrease in the absolute and relative weight of the thigh but an increase in absolute liver weight ($P<0.05$). ALT and AST concentrations increased significantly when the CM level was 40% of the diet ($P<0.05$).

The results from the first experiment confirm that CM can be used at 15% of the diet for the 10 days post-hatching in broilers without any adverse effects on growth performance, carcass traits, and liver enzyme concentrations. Inconsistent with these results, Min *et al.* (2011) reported no adverse effects of incorporating CM at 25% of the diet on the growth performance of broiler chickens from hatching to 14 days of age. On the other hand, Olukosi *et al.* (2017) found negative impacts of CM on the weight gain of broilers in the first 21 days of the rearing period when its levels of inclusion reached 15% and on FCR when the value was 20%. Ahmed *et al.* (2015) reported that the inclusion of CM in diets of broilers without multi-enzyme supplements, even at 5% of the diet, led to a decrease in weight gain and feed efficiency for the first seven days of age while supplementing the multi-enzyme caused to use of CM at 5% of the diet. They concluded according to weight gain criteria, CM could be used at 5% of the diet without enzyme supplementation and 10% of the diet with enzyme addition for 8 to 14 days of age, while the inclusion at the high level of 20% of diet had no significant effect on feed efficiency. They emphasized adding carbohydrase enzyme complexes to broiler diets containing CM. A quadratic effect was observed by Gopinger *et al.* (2014b) for weight gain at 7 to 14 days of age to the inclusion of 16.4% CM and decreased after that.

In the 2nd experiment, including CM up to 25% of the diet from 11 to 24 days had no adverse effect on growth performance, carcass traits, and liver enzyme concentration. Similarly, Min *et al.* (2011) indicated that CM can be incorporated into broiler diets at 25% from 15 to 28 days. Ahmed *et al.* (2015) reported that during 15 to 28 days of production, the inclusion of CM at 5%, 10%, and 20% without multi-enzyme had no significant effect on growth performance. Supplementing the multi-enzyme to CM diets caused an increase in feed intake but could not improve weight gain. Gopinger *et al.* (2014b) observed no significant effect on body weight gain by replacing CM with SBM at 10%, 20%, 30%, and 40% of broiler chickens' diet. Still, they found a quadratic response for feed intake with a maximum corresponding to the inclusion of 22.9% of CM in the 14 to 21 days.

Table 2 Effect of different levels of canola meal (CM) on the growth performance, carcass traits, and liver enzymes of broiler chickens in the 1st experiment (age of 0 to 10 days)

Variable	Treatments (CM %)						SEM	P-value		
	0	3	6	9	12	15		Anova	Linear	Quadratic
Body weight (g)	179.4	178.7	179.0	181.2	182.4	171.0	4.22	0.505	0.856	0.287
Weight gain (g)	133.4	132.7	133.0	135.1	136.3	125.0	4.23	0.511	0.854	0.284
Feed intake (g)	218.0	222.5	228.6	217.5	212.9	209.8	4.99	0.150	0.183	0.881
FCR	1.64	1.69	1.72	1.65	1.56	1.71	0.05	0.351	0.254	0.392
EBI	81.72	78.27	77.66	80.78	87.43	72.77	4.38	0.321	0.445	0.292
Carcass (g)	117.6	116.5	117.1	118.1	121.0	111.5	3.06	0.433	0.864	0.324
Breast (g)	48.8	48.6	48.7	49.3	50.9	46.6	1.25	0.300	0.835	0.383
Thigh (g)	49.9	49.7	49.8	50.42	51.1	47.8	1.28	0.628	0.967	0.393
Carcass (% of LBW)	66.1	64.2	64.4	64.9	65.2	64.3	0.54	0.159	0.085	0.029
Breast (% of LBW	27.4	26.8	26.8	27.1	27.5	26.8	0.26	0.212	0.113	0.106
Thigh ((% of LBW)	28.0	27.4	27.4	27.7	27.6	27.6	0.26	0.521	0.330	0.116
Liver (g)	4.616	4.6	4.606	4.634	4.752	4.346	0.12	0.299	0.924	0.312
Liver (% of LBW)	2.593	2.535	2.533	2.551	2.564	2.506	0.02	0.191	0.171	0.042
ALT (IU/L)	6.256	6.362	6.278	6.37	6.118	6.576	0.16	0.477	0.532	0.488
AST (IU/L)	253.0	257.4	253.8	257.8	247.4	263.6	6.50	0.625	0.503	0.605
ALP (IU/L)	1607	1620	1593	1618	1617	1667	19.3	0.178	0.690	0.189

FCR: feed conversion ratio; EBI: European broiler index; LBW: live body weight; ALT: alanine aminotransferase; AST: aspartate aminotransferase and ALP: alkaline phosphatase.

SEM: standard error of the means.

Table 3 Effect of different levels of canola meal (CM) on the growth performance, carcass traits, and liver enzymes of broiler chickens in the 2nd experiments (age of 11 to 24)

Variable	Treatments (CM %)						SEM	P-value		
	0	5	10	15	20	25		Anova	Linear	Quadratic
Body weight (g)	849.1	865.0	842.9	850.7	847.9	821.6	13.4	0.379	0.911	0.375
Weight gain (g)	658.0	671.9	651.4	658.9	653.9	628.7	13.0	0.345	0.975	0.332
Feed intake (g)	1013	1077	1029	1053	1115	1111	33.4	0.201	0.034	0.254
FCR	1.54	1.606	1.588	1.601	1.711	1.772	0.06	0.115	0.066	0.131
EBI	299.9	300.8	296.2	295.0	271.6	255.9	14.0	0.164	0.246	0.163
Carcass (g)	543.2	562.8	548.4	553.5	558.1	534.5	12.8	0.671	0.577	0.997
Breast (g)	227.1	235.2	229.2	231.4	233.3	223.4	5.37	0.670	0.577	0.997
Thigh (g)	237.0	245.5	239.2	241.4	243.4	233.1	5.60	0.667	0.583	0.983
Carcass (% of LBW)	64.8	65.2	65.2	65.1	65.2	65.0	0.16	0.349	0.201	0.318
Breast (% of LBW)	27.0	27.3	27.2	27.2	27.2	27.2	0.07	0.348	0.201	0.318
Thigh (% of LBW)	28.3	28.4	28.4	28.4	28.4	28.3	0.07	0.377	0.232	0.396
Liver (g)	21.51	22.28	21.71	21.91	22.09	21.16	0.51	0.672	0.578	0.996
Liver (% of LBW)	0.447^{a}	0.445^{a}	0.443^{ab}	0.439^{b}	0.434^{c}	0.427^{d}	0.001	< 0.001	< 0.001	< 0.001
ALT (IU/L)	6.33	6.17	6.25	6.35	6.42	6.46	0.10	0.424	0.894	0.196
AST (IU/L)	234	227	236	233	240	244	5.00	0.289	0.970	0.166
ALP (IU/L)	1542	1515	1529	1524	1551	1546	21.9	0.848	0.768	0.652

FCR: feed conversion ratio; EBI: European broiler index; LBW: live body weight; ALT: alanine aminotransferase; AST: aspartate aminotransferase and ALP: alkaline phosphatase.

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

SEM: standard error of the means.

They expressed that the increase in crude fiber content of diets after adding the CM may be the reason for decreasing feed intake. For 21 to 28 days of age, they observed an improvement in weight gain up to 30% level of CM inclusion and up to 20% inclusion for feed conversion ratio.

The data of the 3rd experiment revealed that including the CM in the broiler diet, more than 32% decreased body weight and weight gain. Meanwhile, FCR and EBI get worse when diets contain more than 24% of CM, which may be related to the high content of crude fiber and anti-nutritional factors in CM (Khajali and Slominski, 2012). Additionally, this could be attributed to the elevated starch content relative to protein in CM, altering digestive dynamics by increasing the dietary starch:protein ratio.

Table 4 Effect of different levels of canola (CM) on the growth performance, carcass traits, and liver enzymes of broiler chickens in the 3rd experiments (age of 25 to 42)

Variable	**Treatments (CM %)**						**SEM**	**P-value**		
	0	8	16	24	32	40		Anova	Linear	Quadratic
Body weight (g)	2269^{a}	2263^{a}	2278^{a}	2279^{a}	2216^{ab}	2157^{b}	23.9	0.008	0.034	0.278
Weight gain (g)	1416^{a}	1388^{a}	1396^{a}	1406^{a}	1340^{ab}	1281^{b}	22.1	0.002	0.007	0.088
Feed intake (g)	2720	2726	2791	2771	2770	2780	25.7	0.288	0.986	0.041
FCR	1.92^{c}	1.97^{bc}	2.0^{bc}	1.97^{bc}	2.07^{ab}	2.17^{a}	0.02	< 0.001	< 0.001	0.001
EBI	409^{a}	393^{ab}	388^{ab}	396^{ab}	360^{bc}	328^{c}	10.0	< 0.001	0.002	0.007
Carcass (g)	1439^{ab}	1486^{a}	1387^{bc}	1451^{ab}	1428^{ab}	1341^{c}	15.4	< 0.001	0.565	0.012
Breast (g)	527^{abc}	559^{a}	495^{c}	555^{a}	543^{ab}	508^{bc}	10.2	0.001	0.040	0.695
Thigh (g)	693^{a}	678^{ab}	602^{c}	646^{b}	656^{b}	605^{c}	7.67	< 0.001	0.393	< 0.001
Carcass (% of LBW)	64.1^{ab}	65.4^{a}	60.9^{c}	63.8^{abc}	64.5^{a}	61.5^{bc}	0.69	0.001	0.135	0.083
Breast (% of LBW)	23.5^{ab}	24.6^{a}	21.7^{b}	24.4^{a}	24.5^{a}	23.3^{ab}	0.46	0.001	0.013	0.921
Thigh (% of LBW)	30.8^{a}	29.8^{ab}	26.4^{d}	28.4^{bc}	29.6^{abc}	27.7^{cd}	0.45	< 0.001	0.958	0.000
Liver (g)	41.6^{d}	43.8^{cd}	46.1^{c}	46.4^{c}	50.9^{b}	55.8^{a}	0.64	< 0.001	< 0.001	< 0.001
Liver (% of LBW)	0.275	0.268	0.285	0.281	0.294	0.284	0.01	0.307	0.749	0.041
ALT (IU/L)	5.81^{b}	5.95^{b}	5.90^{b}	5.86^{b}	6.15^{ab}	6.44^{a}	0.09	0.001	0.005	0.095
AST (IU/L)	233^{b}	238^{b}	236^{b}	234^{b}	246^{b}	256^{a}	4.01	0.003	0.008	0.126
ALP (IU/L)	1598^{ab}	1575^{b}	1641^{a}	1608^{ab}	1630^{ab}	1602^{ab}	13.7	0.033	0.084	0.014

FCR: feed conversion ratio; EBI: European broiler index; LBW: live body weight; ALT: alanine aminotransferase; AST: aspartate aminotransferase and ALP: alkaline phosphatase.

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

SEM: standard error of the means.

This change leads to poor digestibility, negatively impacting intestinal structure and function (Ajao *et al.* 2022). Moreover, McNeill *et al.* (2004) associated the diminished performance of birds fed CM with the presence of a trypsin inhibitor, impacting both feed intake and nutrient utilization.

Ahmed *et al.* (2015) showed that including CM in broiler diets at 20% of diet from 29 to 35 days of age does not harm growth performance but leads to a decrease in feed efficiency. They observed similar growth performance between broilers fed a 20% CM diet, and birds fed a control SBM diet at the older age of 36 to 42 days. Gopinger *et al.* (2014a) reported that CM can be added even up to 40% of the diet from 28 to 35 days of age without affecting the growth performance of broilers. Part of the discrepancy in the literature about the tolerance of broilers to the dietary level of CM could be attributed to the variability in CM quality. For variability in genotype, weather and agronomic condition, oil extraction procedure, and processing (Watts *et al.* 2021), there is great variation in CM quality, considering energy and amino acid digestibility and sources of anti-nutritional factors, such as glucosinolate and complex dietary fiber (Khajali and Slominski, 2012). The results from the 3rd experiment indicate that the birds could bare higher levels of dietary CM compared to most of the reports in the literature. Conducting separate experiments, along with each rearing period, might also account for the higher tolerance of broiler chickens to CM in this research. Younger chicks are more sensitive to the adverse impacts of CM fiber (Gorski *et al.* 2017).

The birds in the 3rd experiment fed diets lacking CM and were protected from potential damages of anti-nutritional factors in dietary CM during the starter and grower phases of production, and this may be reduced the accumulation of detrimental effects of CM inclusion.

The inclusion of CM up to 15% in the 1st experiment and 25% in the 2nd experiment had no adverse effect on carcass characteristics, liver weight, and liver enzymes. Effects of CM inclusion on carcass characteristics and liver enzymes of broilers in all research were assessed at the slaughtering age of 42 days, and we could not find any report at 10 or 24 days of age. Therefore, it seems that anti-nutritional factors in CM were not at the level to damage the liver or other tissues of broilers at 10 and 24 days. Carcass analysis in the 3rd experiment, in which the birds were slaughtered on day 42, showed that in contrast to some studies (An *et al.* 2016; Toghyani *et al.* 2017; Ajao *et al.* 2022), the carcass component was affected by the CM level and the most reduction was related to high percentage. Khajali *et al.* (2011) explained that this alteration in carcass yield is related to the lower content of arginine in CM because arginine is the precursor of several growth factors and amino acid required for synthesizing connective tissues. Some researchers demonstrated that dietary CM levels below 20% had no significant effect on carcass components in broiler chickens (Ahmed *et al.* 2015; Manyeula *et al.* 2020).

An increase in liver weight and liver enzyme concentration in the 3rd experiment was expected as reported in previous studies (Woyengo *et al.* 2011; Payvastegan *et al.* 2017).

Liver functions include detoxifying and metabolizing chemicals. The observed increase in liver size could be due to increased absorption of toxic products from glucosinolates degradation by gut microbes leading to increased activity of detoxification enzymes and then hepatic hyperplasia and hypertrophy (Woyengo *et al.* 2011). AST and ALT levels as indicators of tissue damage in birds are valuable ways to determine the safe inclusion levels for a non-conventional feedstuff (An *et al.* 2016). The AST and ALT levels of broiler chicken in this study highly increased when dietary CM exceeded 24%, as well as with a decrease in growth performance. Higher hepatic weight and metabolic activities imply increased use of dietary energy for maintenance instead of growth and also increase the utilization of amino acids, minerals, and B-complex vitamins for maintenance instead of tissue deposition (Woyengo *et al.* 2011).

CONCLUSION

This study investigated the impact of replacing CM with SBM at different dietary levels during distinct rearing periods of Ross 308 broiler chickens. The findings revealed that substituting CM with SBM up to 15% during the starter phase and 25% during the grower phase had no adverse effects on growth performance, carcass traits, and liver enzymes. However, caution is warranted, as surpassing 32% CM in the diet during the finisher phase detrimentally affected broiler performance, carcass characteristics, and liver function. These results emphasize the importance of carefully adjusting CM inclusion levels based on the specific rearing period to optimize broiler health and productivity.

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