

The Comparison of Supplemental Cumin Seed and Cumin Seed Meal with Prebiotic Fermacto on Blood Metabolites and Performance of Broiler Chickens

A. Golian, M. Aami Azghadi and M. Sedghi

Department of Animal Science, Excellence Center of Animal Research,
Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: There hundred sixty days old male Ross broiler chicks were randomly divided into 12 feeding regimens with five replicates of six birds each. One corn-soybean meal-based starter diet was first provided and then the levels of 0 (control), 2 g kg⁻¹ Fermacto, 2, 4, 6, 8 and 10 g kg⁻¹ Cumin Seed (CS) and 10, 20, 30, 40 and 50 g kg⁻¹ Cumin Seed Meal (CSM) were replaced with wheat bran to provide 12 starter diets. The grower diets contained half of the same supplementation of the starter diets and an un-supplemented finisher diet was fed to all birds. Body Weight (BW) and Feed Intake (FI) was recorded and Feed Conversion Ratio (FCR) was calculated during all periods. The carcass yields and relative organ weights measured at 28 and 42 day whereas the concentration of blood metabolites and differential leukocyte counting were determined at day 28. The supplementation of diet with CSM increased BW and improved FCR ($p < 0.05$) but birds BW decreased numerically with the increase in CS in grower diets. The FI in all periods was not influenced ($p > 0.05$) by the addition of Fermacto, CS and CSM as compared to control fed birds. The relative organ weights were not influenced by Fermacto, CSM and CS ($p > 0.05$) on day 28 and 42, although abdominal fat pad was decreased in birds fed diet with highest levels of CSM and CS measured at day 28 ($p < 0.05$). The inclusion of Fermacto and high level of CS (10 and 5 g kg⁻¹ in starter and grower diets, respectively) increased Lymphocyte and decreased Heterophile and Monocyte proportion of WBC ($p < 0.05$). This study revealed that the inclusion of CS and Fermacto in broiler diets does not significantly affect performance, although CSM improved final BW of birds. The relative organ weights and blood metabolites were similar in birds fed diet contained Fermacto, CS and/or CSM at day 42 but these supplementations may influence white blood cells differentiation measured at 28 day of age.

Key words: Cumin seed, cumin seed meal, prebiotic fermacto, broilers performance, day body weight, feed intake

INTRODUCTION

Several reports in the past 50 years had shown the bacteriostatic, fungistatic, antifertility, antihelminthic and other medical properties of spices (Shetty *et al.*, 1994; Sagdic *et al.*, 2002; De *et al.*, 2003). The use of most antibiotic growth promoters had been banned recently and so the interest toward the use of medical plants as feed additives in poultry nutrition has grown drastically (Taylor, 2001). *Cuminum cyminum* Linn (Cumin) is an annual plant of the Umbelliferae family. Cumin is an important medical herb in Asia, especially in Iran. The annual production of Cumin Seed (CS) and Cumin Seed Meal (CSM) in Iran exceed 10,000 and 6,000 ton, respectively. Cumin seed contains 62 g moisture, 177-230 g protein, 238 g fat, 91 g fiber, 355 g carbohydrate and 77 g of mineral matter kg⁻¹ (Farrell, 1985). The CS contains 20-40 g kg⁻¹ of essential oil which is mainly

composed of Cuminaldehyde (Gachkara *et al.*, 2007). Cumin seed meal is the major by-product of the cumin seed oil extraction industry (Mansoori *et al.*, 2006a). The crude protein and fat content of cumin seed varied between 96-190 and 100-300 g kg⁻¹, respectively (De *et al.*, 2003; Mansoori *et al.*, 2006a; Milan *et al.*, 2008).

There are extensive number of reports on nutritive value and medical properties of cumin seeds and essential oil used in human diet however there are no reports on the use of CS and CSM in poultry nutrition. The purpose of this study was to compare the various levels of CS and CSM with prebiotic Fermacto in diet on blood metabolites and performance of broiler chickens.

MATERIALS AND METHODS

About 360 days old male Ross broiler chicks were divided into 60 cages of 6 birds each. Each group was

randomly assigned to one of the 12 dietary regimens. One commercial basal starter diet was formulated and divided into 12 sub-diets. The levels of 0 (control), 2 g kg⁻¹ Fermacto; 2, 4, 6, 8 and 10 g kg⁻¹ CS and 10, 20, 30, 40 and 50 g kg⁻¹ CSM were replaced with wheat bran in starter diet to provide 12 starter diets. The twelve grower diets contained half of the same supplementation of the starter diets and an un-supplemented finisher diet was fed to all birds. Corn-soybean meal-based broiler diets were prepared according to the nutrients recommended by Broiler Nutrition Specifications (2007) and ingredients composition from Leeson and Summers (2005). The composition and nutrients of experimental diets is shown in Table 1. Birds were exposed to 24 h light and had free access to feed and water throughout the experiment. The Body Weight (BW) and Feed Intake (FI) in each group of birds were determined 4 h after feed removal and Feed Conversion Ratio (FCR) was calculated during 1-12, 13-28 and 29-42 day of age. Daily mortalities were recorded and used to correct performance criteria.

One chick from each group of birds close to the average replicate weight was selected, weighed and slaughtered after 4 h fasting to determine the carcass, breast, legs, liver, heart, abdominal fat pad, gall bladder and total tract at 28 and 42 day of age. Cumin seed and CSM were analyzed by standard procedure of AOAC (1996) to determine dry matter, crude protein, crude fiber,

crude fat and ash. Total phenol and tannin content of CS and CSM were determined according to Makkar (2000). Glucose, triglyceride, cholesterol, HDL, LDL and VLDL concentration in serum were measured by enzyme method in an auto-analyzer (Selectra E, vital Scientific, Netherland) and Lymphocyte, Monocyte, Heterophile and Eosinophile count were determined based on the procedure of Gross and Siegel (1983) at 28th day of age. This project was reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad.

Statistical analysis: The data were subjected to ANOVA as a completely randomized design using the GLM procedures of SAS software (SAS Institute, 2004). The Tukey's test was applied to compare the treatment means when the treatment effect was significant at $p = 0.05$. Orthogonal contrasts were used to compare the CS and CSM effects on all criteria. All data were tested for normality prior to analysis. Abnormal data were transformed and then analyzed.

RESULTS AND DISCUSSION

Chemical analysis: The chemical analysis of CSM and CS is shown in Table 2. The chemical compositions of CSM were different from those reported by other researchers (De *et al.*, 2003; Mansoori *et al.*, 2006a; Milan *et al.*, 2008). These differences may be related to the stage of maturity, stage of harvesting time, extracted matters from CS (essential oil, water and saline extract or oleoresin), extraction method and varieties.

Performance parameters: The effect of different levels of CSM, CS and Fermacto on performance parameters and their orthogonal contrast on broiler chicks is shown in Table 3. Different levels of CSM and CS did not have any significant effect on BW and FCR in starter and finisher periods ($p > 0.05$), although significant differences were observed in the growing period ($p < 0.05$).

The body weight was significantly increased in birds fed diet contained CSM ($p < 0.05$) as compared to other treatments during the grower period. Feed conversion ratio was significantly improved when birds fed diet with highest level of CSM than those fed diet contained highest level of CS during the growing period ($p < 0.05$). The effect of prebiotic Fermacto on BW and FCR were similar to those fed diet contained CSM and/or CS in all periods with the exception of the last two highest levels of CSM. The FI of birds in all periods was not significantly influenced ($p > 0.05$) by the addition of Fermacto, CS and CSM to control diet. Similar results on FI for starter and finisher periods were reported by

Table 1: Composition (g kg⁻¹) and calculated nutrient (g kg⁻¹) and energy (kcal ME kg⁻¹) content of the starter, grower and finisher diets

Ingredients	Starter (1-12 days) ¹	Grower (13-28 days) ²	Finisher (29-42 days) ³
Corn	495.0	575.5	613.3
Corn gluten meal	55.0	62.0	0.0
Soybean meal	300.5	238.5	289.0
Vegetable oil	44.0	50.0	62.7
Limestone	8.8	8.0	7.2
Bone meal	20.0	18.0	17.5
NaCl	2.8	2.8	3.0
Meth 98	0.8	1.4	1.3
HCL Lys	2.1	2.8	0.0
Vit and Min permix ⁴	5.0	5.0	5.0
Vit E	1.0	1.0	1.0
Wheat bran	65.0	35.0	0.0
Calculated composition (g kg⁻¹)			
AME, kcal kg ⁻¹	3010.0	3175.0	3225.0
Crude protein	220.0	200.0	186.0
Calcium	10.0	9.0	8.5
Available phosphor	5.0	4.5	4.2
Lysine	11.6	10.5	8.9
Methionin	5.2	5.3	4.7
Methionin+Cystine	7.9	7.8	7.0
Linoleic acid	38.7	43.1	42.5

¹The levels of 0 (control), 2 g kg⁻¹ Fermacto, 2, 4, 6, 8 and 10 g kg⁻¹ cumin seed and 10, 20, 30, 40 and 50 g kg⁻¹ cumin seed meal were replaced with wheat bran in starter diet to provide twelve dietary regimens. ²The twelve grower diets contained half of the same supplementation of the starter diets. ³The un-supplemented finisher diet was fed to all birds. ⁴Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3; 9800 IU, vitamin E. 121 IU; B₁₂, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; cholin, 840 mg; biotin, 30 µg, thiamine, 4 mg; zinc sulphate, 60 mg; manganese, 60 mg

Table 2: The comparison chemical analysis of Cumin Seed (CS) and Cumin Seed Meal (CSM) used in the experiments and other reports

Chemical analysis ¹	CS	CSM			
	The experiment	The experiment	Mansoori <i>et al.</i> (2006a, b)	Milan <i>et al.</i> (2008)	De <i>et al.</i> (2003)
Crude protein	210.0	140.0	96.0	190	172
Crude fiber	96.0	170.0	155.0	55	-
Ether extract	195.0	75.0	140.0	100	300
Ash	76.0	119.0	156.0	90	-
Carbohydrate	429.0	581.0	-	230	-
Total phenol	21.6	10.1	18.2	-	-
Total Tannins	13.7	8.0	3.7	1	-

¹gram per kilogram

Table 3: Effect of Fermacto, Cumin Seed (CS) and Cumin Seed Meal (CSM) and the CS and CSM orthogonal contrasts on broiler chickens performance¹

Twelve dietary regimens	Fermacto												Orthogonal contrasts (CSM vs. CS)	
	Control	CS (g kg ⁻¹)		CSM (g kg ⁻¹)								MSE	p-value	
		2	4	6	8	10	10	20	30	40	50			
Starter ²	0	2	2	4	6	8	10	10	20	30	40	50		
Body weight (g)														
Day 1	41.50	41.40	41.00	41.60	40.9	41.40	41.90	41.80	40.6	41.50	41.0	41.9	0.410	0.500
Day 12	224.70	221.30	222.90	224.10	226.3	227.40	223.70	232.30	225.9	227.20	228.6	227.5	3.600	0.140
Day 28	958.50 ^b	970.70 ^b	979.80 ^b	969.90 ^b	968.0 ^b	944.10 ^b	941.80 ^b	1087.50 ^a	1078.6 ^a	1070.10 ^a	1064.3 ^a	1089.7 ^a	17.400	<0.001
Day 42	2403.90	2428.00	2468.30	2413.50	2401.0	2440.00	2406.90	2505.90	2557.3	2554.80	2531.0	2595.3	41.600	0.010
Feed intake (g day⁻¹)														
Day 1-12	25.50	23.90	24.70	24.30	25.60	23.20	24.70	24.90	24.40	23.80	24.2	24.9	0.860	0.270
Day 13-28	82.70	80.60	74.90	80.80	76.60	77.80	77.60	83.30	86.70	84.90	82.7	87.4	4.300	0.230
Day 29-42	203.40	196.10	192.40	196.50	196.70	202.20	195.00	192.30	206.00	203.60	207.3	205.9	8.100	0.580
Day 1-42	109.50	103.10	110.20	102.80	104.80	106.70	104.00	107.50	114.00	107.20	107.4	112.4	4.700	0.350
FCR (g:g)														
Day 1-12	1.67	1.64	1.56	1.59	1.57	1.63	1.60	1.59	1.58	1.58	1.60	1.61	0.024	0.560
Day 13-28	1.82 ^a	1.78 ^{ab}	1.76 ^{abc}	1.73 ^{abc}	1.77 ^{abc}	1.80 ^a	1.81 ^a	1.73 ^{abc}	1.69 ^{bc}	1.71 ^{bc}	1.66 ^{bc}	1.64 ^c	0.029	0.230
Day 29-42	2.01	1.89	1.97	1.90	1.97	1.91	1.95	1.95	1.980	1.92	1.99	1.94	0.076	0.800
Day 1-42	1.91	1.83	1.85	1.82	1.86	1.84	1.87	1.83	1.840	1.81	1.82	1.80	0.042	0.580

^{a,b,c}Means within each row with different superscript are significantly different (p = 0.05). ¹An un-supplemented finisher diet was fed to all birds. ²The Fermacto, CS and CSM content in starter diets (g kg⁻¹) whereas the grower diets contained half of the same supplementation of the starter diets

others (Mansoori *et al.*, 2006b). The body weight decreased numerically when the level of CS increased in the growing period which could be due to the presence of tannins in CS structure. Tannins compounds may bond with dietary macromolecules such as protein, carbohydrate, lipid and minerals and make soluble and/or insoluble complexes.

These compounds influence broiler performance including impairment in absorption of amino acids, fatty acids, soluble carbohydrate and minerals, also dysfunction in secretion of digestive enzyme, lower digestibility and subsequently reduce growth rate of broiler chickens.

Based on the orthogonal contrast, BW of broiler fed diet contained CSM was higher than CS fed birds at day 28 (p<0.001) and 42 (p = 0.01). These results are not in agreement with other report (Mansoori *et al.*, 2006b). They showed that the increase in CSM level from 0, 25-50 g kg⁻¹ in broiler diets as supplemented by polyethylene glycol and enzyme (Giondazyme) did not have any effect on BWG and BW in all periods. Although,

the levels of CSM in starter (1-12 days) was two times more than grower (13-28 days) diet but the feed digestibility probably influenced more in the grower period because the chicks endogenous enzymes system are more developed in the growing period. In general, these results are in contrary with those obtained by others (Mansoori *et al.*, 2006b). This difference may be related to nutrient composition of CSM and CS. Cumin seed increases bile acids and bile salts synthesis and secretion (Platel and Srinivasan, 2000). In addition the use of CS provided higher concentration and secretion of digestive enzyme in pancreatic and small intestine (Milan *et al.*, 2008; Platel and Srinivasan, 2000, 1996; Ramakrishna *et al.*, 2003). Simultaneously with these actions, transit time of feed in gastrointestinal tract could be reduced (Platel and Srinivasan, 2001) and all of these factors probably improved digestibility (Platel and Srinivasan, 2004) and performance.

Organs weights: The effect of Fermacto and various levels of CS and CSM on relative weight of body organs

Table 4: Effect of Fermacto, Cumrin Seed (CS) and cumrin Seed Meal (CSM) on relative organ weights of broiler chickens at 28 day of age²

Twelve dietary regimens													MSE
Starter ²	Fermacto		CS (g kg ⁻¹)				CSM (g kg ⁻¹)						
	Control	(g kg ⁻¹)	2	4	6	8	10	10	20	30	40	50	
BW (g)	903.000	1001.000	990.000	996.00	989.000	910.000	925.000	1035.000	1091.00	989.000	1081.000	1102.000	60.400
(Percentage of live weight)													
Carcass	53.600	51.500	53.600	51.600	52.900	52.100	54.800	51.600	53.3.0	53.300	52.600	53.400	1.510
Abdominal fat	1.360 ^a	1.480 ^a	0.920 ^a	1.050 ^{a*}	1.080 ^{a*}	0.960 ^a	0.890 ^a	1.190 ^{a*}	1.170 ^{a*}	1.000 ^a	0.920 ^a	0.980 ^a	0.062
Legs	18.400	17.900	18.400	18.200	17.900	18.100	18.500	18.400	18.400	17.500	18.200	18.600	0.517
Breast	19.800	17.900	19.500	18.100	18.600	18.200	19.100	19.500	19.100	19.100	19.200	18.500	0.746
Heart	0.760	0.820	0.700	0.750	0.770	0.850	0.790	0.750	0.770	0.800	0.820	0.780	0.058
Liver	2.620	2.490	2.440	2.550	2.580	2.800	2.660	2.400	2.530	2.720	2.670	2.470	0.140
Gall bladder	0.062	0.065	0.051	0.063	0.072	0.088	0.078	0.087	0.087	0.089	0.088	0.087	0.014
Gastrointestinal tract	12.900	14.100	12.800	13.200	13.800	14.500	13.500	12.700	12.800	13.300	13.000	12.800	0.690

^aMeans within each row without a common superscript are significantly different (p = 0.05). ¹An un-supplemented finisher diet was fed to all birds. ²The Fermacto, CS and CSM content in starter diets (g kg⁻¹) whereas the grower diets contained half of the same supplementation of the starter diets

Table 5: Effect of Fermacto, Cumrin Seed (CS) and Cumrin Seed Meal (CSM) on relative organ weights of broiler chickens at 42 day of age²

Twelve dietary regimens													MSE
Starter ²	Fermacto		CS (g kg ⁻¹)				CSM (g kg ⁻¹)						
	Control	(g kg ⁻¹)	2	4	6	8	10	10	20	30	40	50	
BW (g)	2508.000	2405.00	2392.000	2420.000	2537.000	2417.000	2567.000	2637.000	2591.000	2589.000	2662.000	2710.00	104.700
Percentage of live weight													
Carass	59.400	59.200	59.600	59.700	62.000	60.400	60.900	61.600	60.300	60.500	59.700	60.00	1.000
Abdominal fat	1.790	2.200	1.490	1.400	1.500	1.500	1.530	1.550	1.400	1.440	1.470	1.61	0.240
Legs	20.500	19.600	20.800	21.300	21.200	21.300	20.800	21.400	20.800	21.500	19.800	20.50	0.610
Breast	23.000	23.000	22.900	22.700	24.600	24.000	23.800	24.300	23.900	24.000	24.200	23.80	0.728
Heart	0.610	0.630	0.640	0.660	0.630	0.740	0.770	0.640	0.650	0.640	0.620	0.59	0.054
Liver	2.160	2.040	1.850	1.920	2.190	2.160	2.010	1.920	2.200	1.920	1.930	1.92	0.100
Gall bladder	0.102	0.084	0.094	0.099	0.082	0.085	0.082	0.095	0.093	0.092	0.10	0.016	
Gastrointestinal tract	7.030	7.750	7.600	7.810	7.450	7.170	7.640	7.150	7.970	7.070	7.630	7.17	0.350

¹An un-supplemented finisher diet was fed to all birds. ²The Fermacto, CS and CSM content in starter diets (g kg⁻¹) whereas the grower diets contained half of the same supplementation of the starter diets

of broiler at day 28 and 42 are shown in Table 4 and 5, respectively. Relative weight of carcass, legs, breast, liver, heart, gallbladder and gastrointestinal tract were not influenced by Fermacto, CSM and CS (p>0.05) at day 28 and 42.

Birds fed the highest level of CS and CSM had lower abdominal fat pad as compared to these fed diet contained Fermacto at day 28 (p<0.05).

Blood metabolites and differential leukocyte counting:

Effect of Fermacto, CS and CSM on blood parameters of broiler is shown in Table 6. Blood parameters were not significantly affected in birds fed diets contained Fermacto and/or various levels of CSM and/or CS (p>0.05); although the LDL content was numerically increased as compared to control fed birds. Despite there was not any report on the effect of CS or extracted products of CS on blood metabolites and blood cells. Cumin seed inhibit hepatic 3-hydroxy-3-ethylglutaryl coenzyme A (HMG-CoA) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis.

As a result, hypocholesterolemic effect of CS is expected. There are some reports in about the effect of other medical herbs. Investigators reported that the

supplementation of broiler diets with turmeric rhizome powder increased total cholesterol, HDL and hemoglobin and decreased LDL, VLDL and red blood cells at day 42 (Emadi *et al.*, 2007).

The effect of Fermacto, CS and CSM on cell differentiation is shown in Table 7. The inclusion of Fermacto in diet increased Lymphocyte cell and decreased Heterophile and Monocyte cell as compared to control group (p<0.05).

These differences were more pronounced in birds fed diets with the highest level of CS (10 and 5 g kg⁻¹ CS in starter and grower diets, respectively). Although, addition of 1 and 2 g kg⁻¹ CS to starter and grower diets increased Heterophile cell but Lymphocyte and Monocyte counts were reduced as compared to control and other treatments (p<0.05).

Researchers reported that four Chinese herbal ingredients can be applied as immune stimulators for an active vaccine in chickens at both *in vitro* and *in vivo* assays (Kong *et al.*, 2006).

Almost all of the Chinese herbal ingredients used in that study substantially enhanced *in vitro* chick embryo fibroblast proliferation and promoted the humoral immunity in response to Newcastle disease virus infection

Table 6: Effect of Fermacto, Cumin Seed (CS) and Cumin Seed Meal (CSM) on blood parameters of broiler chickens at 28 day of age¹

	Twelve dietary regimens												MSE
	Control	Fermacto (g kg ⁻¹)		CS (g kg ⁻¹)		CSM (g kg ⁻¹)							
		0	2	2	4	6	8	10	10	20	30	40	
Starter ²	----- (mg dL ⁻¹) -----												
Glucose	160.00	165.0	166.50	170.00	152.0	153.0	168.5	150.5	156.3	170.3	146.0	163.2	62.0
Cholesterol	179.00	185.3	164.80	201.70	177.5	189.3	194.8	166.3	173.5	189.2	182.0	198.0	14.8.0
Triglycerides	79.00	94.3	105.70	92.20	106.7	80.5	91.5	80.8	83.3	86.0	95.7	70.0	16.2.0
HDL	90.70	80.2	86.30	89.50	89.5	93.8	81.8	91.0	94.0	91.8	91.5	87.0	7.83
LDL	61.50	81.3	82.20	78.80	66.8	80.0	82.3	63.5	66.5	74.5	71.3	85.8	13.00
VLDL	14.75	19.0	19.25	18.75	19.5	15.8	18.2	15.3	16.5	15.8	19.2	14.3	3.00

¹An un-supplemented finisher diet was fed to all birds. ²The Fermacto, CS and CSM content in starter diets (g kg⁻¹) whereas the grower diets contained half of the same supplementation of the starter diets

Table 7: Effect of Fermacto, Cumin Seed (CS) and Cumin Seed Meal (CSM) on blood cell differentiation of broiler chickens at 28 day of age¹

	Twelve dietary regimens												MSE
	Control	Fermacto (g kg ⁻¹)		CS (g kg ⁻¹)		CSM (g kg ⁻¹)							
		0	2	2	4	6	8	10	10	20	30	40	
Starter ²	----- (portion of WBC) -----												
Heterophile	0.3550 ^b	0.183 ^b	0.503 ^a	0.317 ^{ab}	.2920 ^b	0.265 ^{ab}	0.1730 ^b	0.207 ^{ab}	0.306 ^{ab}	0.230 ^b	0.230 ^{ab}	0.257 ^{ab}	0.05600
Monocyte	0.0125 ^b	0.010 ^b	0.010 ^b	0.017 ^{ab}	0.0120 ^b	0.020 ^b	0.0030 ^b	0.020 ^b	0.046 ^a	0.015 ^{ab}	0.026 ^{ab}	0.020 ^{ab}	0.00670
Lymphocyte	0.6270 ^{ab}	0.806 ^a	0.486 ^b	0.665 ^{ab}	0.6920 ^{ab}	0.715 ^{ab}	0.7830 ^a	0.767 ^{ab}	0.646 ^{ab}	0.755 ^{ab}	0.743 ^{ab}	0.737 ^{ab}	0.05460
Eosinfile	0.0055	0.000	0.000	0.000	0.0025	0.000	0.0067	0.005	0.000	0.000	0.000	0.000	0.00025 ^a

^{a,b}Means within each row without a common superscript are significantly different (p = 0.05). ¹An un-supplemented finisher diet was fed to all birds. ²The Fermacto, CS and CSM content in starter diets (g kg⁻¹) whereas the grower diets contained half of the same supplementation of the starter diets

in vivo. Generally, the increase in Lymphocyte may help to enhance immune system function against diseases and stresses.

CONCLUSION

This study revealed that the supplementation of diets with CSM at the rate of 5% may have some beneficial effects on broiler chickens performance. The CS and Fermacto supplemented may not have a positive effect on performance, carcass cuts, organ weights and serumbiochemical parameters but may cause an increase in Lymphocyte and a decrease in Heterophile and Monocyte proportion of white blood cell.

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