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Effect of dietary supplementation with medium chain fatty acids on growth performance, intestinal histomorphology, lipid profile and intestinal microflora of broiler chickens

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Abstract

The aim of the present study was to assess the effects of medium-chain fatty acid (MCFA) diet supplementation on growth performance, intestinal histomorphology, serum biochemistry and intestinal microflora of broiler chickens. The study was performed on 180 one-day-old broilers of the same origin (Cobb 500 hybrid), over a 42-day period. They were fed diets supplemented with three treatments: control group (basal diet without supplementation); group with MCFA supplementation; and group with MCFA and coccidiostat supplementation. Broiler chickens fed diets supplemented with MCFAs had a significantly greater final bodyweight. The weights of carcass cuts (breast, drumsticks with thighs and wings) were greater in broilers receiving MCFAs than in control broilers. The addition of MCFAs to broiler diet significantly increased villus length and crypt depth in the duodenum and caecum, and significantly decreased villus width in the duodenum and ileum. Additionally, serum HDL-cholesterol and triglyceride concentrations were increased significantly in broilers with MCFA dietary supplementation. The results indicated that the MCFA diet supplementation had a beneficial effect on the performance of broiler chickens, their intestinal histomorphology and microflora.

Keywords: Carcass characteristics, coccidiostat supplementation, nutrition, poultry, serum biochemistry
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Introduction

Poultry meat is an important food resource for many people. Consumers are becoming increasingly concerned about the nutritional and health aspects of their food (Baltić *et al*., 2015; Janjić *et al*., 2015). The production of high-quality poultry meat has been a constant objective of the meat industry for many decades. The main goal is to obtain meat with high lean percentage and good meat quality traits at the same time (Baltić *et al*., 2011; Glamočlija *et al*., 2016).

Feed fats are routinely included in broiler diets as a source of essential fatty acids and energy. The use of various fats can influence broiler performance and carcass characteristics owing to differences in fatty acid chain length, degree of saturation and degree of esterification (Wang *et al*., 2015). Fatty acids with a chain length between 6 and 12 carbon atoms are regarded as medium-chain fatty acids (MCFAs). MCFAs are more effectively absorbed and metabolized than saturated long-chain fatty acids, and have antimicrobial properties, as shown in mammals and birds (Zentek *et al*., 2011). Adding MCFAs to a broiler diet to replace part of the soybean oil and animal fat has been shown to improve feed conversion efficiency (Van der Hoeven-Hangoor *et al*., 2013). Additionally, body fat deposition can be reduced by MCFAs (Wang *et al*., 2015) and breast meat yield can be enhanced (Shokrollahi *et al*., 2014).

Many *in vitro* studies have confirmed the antimicrobial properties of MCFAs against enteric pathogens (protozoa and bacteria) (Newell & Wagenaar 2000; Bruggeman 2002; Van Immerseel *et al*., 2004;

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Skrivanova *et al*., 2005; Kabara & Marshal 2005; Hermans *et al*., 2011). To consider such antibacterial properties, MCFAs, such as new non-antibiotic feed additives, are helpful in bolstering healthy gastrointestinal conditions in broilers. Based on research with poultry, MCFAs can be an alternative to antibiotic growth promoters. Aromabiotic is a commercial product, which contains a mixture of MCFAs that demonstrate antimicrobial, physiological and immunological properties (Isaac *et al*., 2013). It was reported that Aromabiotic decreased the invasion of enteric pathogens, decreased mortality and had positive effects on growth performance and intestinal histomorphology in broiler chickens (Khosravinia, 2015; Gutierrez *et al*., 2006) and Japanese quail (Saeidi *et al*., 2016). Numerous natural products, such as sources of fats containing high concentrations of n-3 fatty acids or feedstuffs, have been tested as anticoccidial dietary additives (Tan & Long, 2012). MCFAs have been found to have anticoccidial activity in calves and anti-protozoal activity in dairy cattle, swine, sheep, goat and infant animals (Tan & Long, 2012).

The objective of this study was to evaluate the effect of dietary supplementation with MCFAs on growth performance, intestinal histomorphology and microbiota and serum biochemistry of broiler chickens, for 42 days.

Materials and Methods

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

A total of 180 one-day-old broilers of both sexes and the same origin (Cobb 500) were used in this study in a 42-day period. Birds were randomly assigned to one of three dietary treatments (control and two experimental groups), each having 6 replicates (10 birds in each replicate). Birds were placed in an environmentally controlled room (stocking density 6 birds/m^2) with 5 cm thick sawdust. The temperature in the room was 32 °C from days 1 to 5, then gradually lowered to 22 °C on day 21. This temperature was maintained until the end of the study. Relative humidity was 45% - 50%. The lighting of the rooms was 24 hours. Water and feed were supplied ad libitum throughout the study.

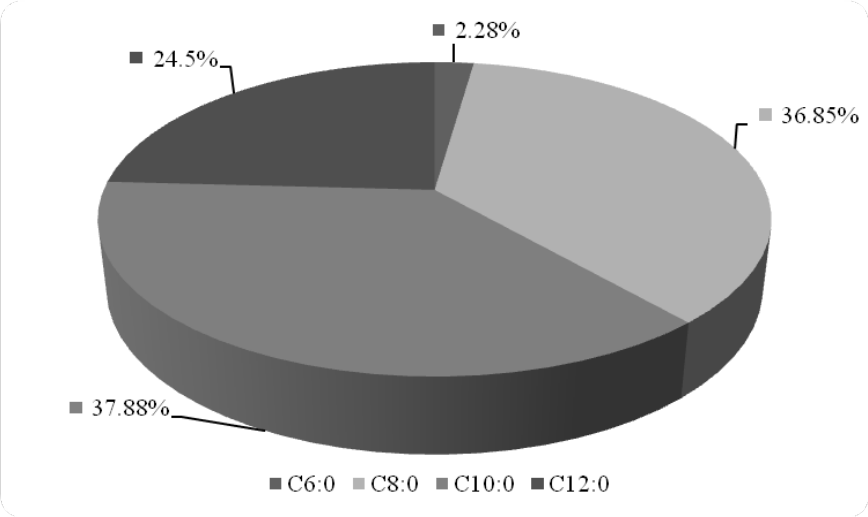

Figure 1 Fatty acid composition* of Aromabiotic^R

*Total lipids for fatty acid determination were extracted from Aromabiotic with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Germany). After evaporation of solvent until dryness under the stream of nitrogen, total lipids were converted to fatty acid methyl esters (FAME) by trimethylsulfonium hydroxide. FAMEs were determined with a Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cianopropyl HP-88 capillary column (100 m x 0.25 mm x 0.20 µm) (Trbović *et al*., 2011).

From the start of the trial, each group of broilers was fed with one of three experimental diets, which comprised the same basal diet, and differed only in additive supplementation (MCFAs or coccidiostat). The basal diet was formulated to meet the maintenance and growth requirements of the animals used in the study. Broilers were fed from day 1 to day 42 in three phases with three nutritionally different concentrated feed mixtures, namely starter (up to 10 days), grower (11 – 21 days) and finisher (22 – 42 days) mixtures. The broilers in the control group were given a diet without MCFAs or coccidiostat (C group). The other two treatment groups were given the same diet as the control group (C group), but were supplemented with

MCFAs (Aromabiotic^R, Nuscience, Belgium) (E-I group) and MCFAs (Aromabiotic) with coccidiostat (salinomycin, 500 mg/t feed, 1 – 35 days) (E-II group). Commercially prepared MCFAs (60%) (Aromabiotic) was added to the feed (starter: 0.16%, grower: 0.12% and finisher: 0.10%) for the experimental groups (E-I and E-II), at the producer-recommended rate in the feed mixture. The fatty acid composition of Aromabiotic is shown in Figure 1.

The ingredients and chemical composition (calculated analyses) of the basal diets are listed in Table 1.

Table 1 Formulation and calculated values of the feed mixtures for broilers

Ingredients (%)	Starter (up to 10 days)		Grower (days 11–21)		Finisher (days 22–42)	
	C* group	E-I and E-II* groups	C group	E-I and E-II groups	C group	E-I and E-II groups
Maize	50.85	50.69	44.15	44.03	44.95	44.85
Wheat	-	-	10.00	10.00	15.00	15.00
Soy grits	15.00	15.00	17.00	17.00	20.00	20.00
Soybean meal	12.40	12.40	1.00	1.00	1.00	1.00
Soybean cake	17.00	17.00	23.30	23.30	14.70	14.70
Monocalcium phosphate	1.20	1.20	1.00	1.00	0.90	0.90
Chalk	1.60	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Premix**	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Adsorbent	0.20	0.20	0.20	0.20	0.20	0.20
Aromabiotic	-	0.16	-	0.12	-	0.10
Parameter			**Calculated values**			
Metabolic energy MJ/kg	12.69	12.71	13.01	13.03	13.11	13.13
Total ash	6.77	6.77	6.66	6.66	6.16	6.15
Total lipids	6.61	6.76	7.39	7.51	7.20	7.29
Crude fibre	3.89	3.89	3.97	3.97	3.44	3.44
Crude protein	22.24	22.22	21.14	21.13	19.62	19.62
Moisture	10.41	10.39	10.20	10.18	10.54	10.53
Lysine	1.50	1.49	1.42	1.42	1.17	1.17
Methionine+ cysteine	0.81	0.81	0.80	0.80	0.76	0.76
Tryptophan	0.31	0.31	0.29	0.29	0.27	0.27
Calcium	1.01	1.01	0.94	0.94	0.90	0.90
Phosphorus	0.59	0.59	0.56	0.56	0.54	0.54
NFE	50.08	49.96	50.63	50.55	53.04	52.97

*Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat

**Mineral-vitamin premix provided per kg of diet: vitamin A, 12 999 IU; vitamin D3, 4 950 IU; vitamin E, 75 mg; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 7.95 mg; vitamin B_6, 4.05 mg; vitamin B_{12}, 0.0195 mg; vitamin C, 19.95 mg; biotin, 0.15 mg; niacin, 60 mg; calcium pantothenate 15 mg; folic acid, 1.95 mg; iodine, 1.0005 mg selenium, 0.3 mg; choline chloride, 399.9 mg; iron, 39.99 mg; copper, 15 mg; manganese, 99.9 mg; zinc, 99.9 mg; methionine, 2100 mg; lysine, 1200 mg

NFE: nitrogen free extract

Growth performance of broilers was evaluated by recording bodyweight gain, feed intake and feed conversion ratio (FCR) during the 42-day experimental period. Feed intakes of birds were recorded on a pen

basis, the uneaten feed was discarded, and fresh feed was placed in feeders at the end of each day. FCR was calculated as the amount of feed consumed per unit of bodyweight gain.

At the end of the study, animals were transported to a slaughterhouse and then weighed individually, stunned electrically and immediately slaughtered by severing the jugular veins. Subsequently, animals were processed following standard industrial techniques and hot carcass weight was recorded. After evisceration, liver weights were measured. During the first 24 hours post mortem, carcasses were stored in a ventilated cold room at 2 °C, after which cold carcass weight was measured. After chilling, the carcasses of broilers from each group were separated into breast, drumsticks with thighs, wings, neck and back with pelvis. The various carcass parts were weighed.

For morphological and histological analyses, tissue samples from the duodenum, ileum and caecum were collected from the slaughtered birds and fixed in 10% buffered formalin saline. Tissues were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene, and embedded in paraffin. A rotary type microtome was used for cutting the paraffin sections. Sections 5 to 8 µm in thickness were cut and stained with Mayer's haematoxylin and eosin (HE) and with a combination of periodic acid Schiff stain and Alcian blue (PAS-AB) (Yamabayashi 1987; Smirnov *et al*., 2005). Analysis was performed using a light microscope (Olympus BX53) with the objective magnifications x 4 and x 10. Morphometric examinations were carried out using Olympus cellSens software (http://www.olympusamerica.com) and included these measurements: ileal villi height and width, ileal crypt depth and caecal crypt depth (Bergamo *et al*., 2016).

The pH of various segments of the digestive tract was determined with a digital pH meter (TESTO 205, Germany).

Blood samples were collected from the slaughtered birds in non-heparinized tubes. The samples were centrifuged at 3000 rpm for 15 minutes, and the serum was stored at -20 °C until analysis. HDL-cholesterol and triglyceride were determined by the auto analyser (CentroLIA LB 961, Berthold Technologies, Germany) using commercial kits from Accurex biomedical company.

Samples for bacteriological examination were taken directly from the intestines with a sterile syringe. Serial dilutions were prepared down to 10^{-8}. Duplicate plates of selective media were inoculated with 0.1 mL of each dilution to determine the bacterial species defined by standard laboratory methods. Total *Enterococcus* species, *Escherichia coli* strains and *Staphylococcus aureus* were counted on UTI agar (urogenital tract infections agar) (HiMedia) after incubation for 24 hours at 37 °C, while lactic acid bacteria (LAB) counts were determined on selective MRS agar (de Man, Rogosa and Sharpe) agar supplemented with 20 mg/mL vancomycin (Sigma Aldrich) and 2 mg/mL cefotaxime (Sigma Aldrich), after incubation for 72 hours at 30 °C. Microaerophilic atmospheres, used for the growth of lactobacilli on agar plates, were obtained using the Anaerocult^{R} C (Merck KgaA, Darmstadt, Germany). Based on the appearance of colonies and cultural characteristics, subcultivation was performed on suitable substrates to obtain pure bacterial cultures. After cultivation, morphological and biochemical characterization of the isolated bacteria were conducted.

Statistical analysis of the results was conducted with GraphPad Prism software version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and standard error of means (SEM). One-way ANOVA with Tukey's post hoc test was performed to assess the significance of differences among control and experimental groups. Values of P <0.05 were considered significant.

Results

Table 2 shows growth performance and carcass characteristics of broilers in the study. At the end of the study, significant (P <0.05) differences in weight gain and final bodyweight were found between control and experimental groups receiving diets containing MCFAs and coccidiostat. Feed intake for the overall period of the study was lower in the control group than in the group with MCFAs (E-I group) added to feed and in the group with MCFAs and coccidiostat added to feed (E-II group) (P >0.05). Broilers fed only with the basal diet showed a higher FCR (from day 0 to day 42) than E-I group and E-II group.

The cold carcass weight was significantly lower in the control group than in the E-I and E-II groups. Dressing percentage was lower in the control group compared with the other groups. Basic cuts of broiler carcasses (breast, drumsticks with thighs, wings, neck and back with pelvis), presented as weight and calculated percentage of carcass, differed significantly among the broiler groups. Supplementation of MCFAs significantly improved the yield of carcass cuts, in which the control group had lower (P <0.05) weight of breast, drumsticks with thighs and wings compared with the other experimental groups.

Table 2 Performance and carcass characteristics of broilers receiving diets containing medium chain fatty acids and coccidiostat

Parameter	Group			SEM	*P*-value
	C	E-I	E-II		
Body weight (g)					
Day 1	41.96	42.06	42.13	0.79	0.963
Day 10	306.0	313.2	308.6	5.34	0.251
Day 21	876.5^{a}	939.8^{b}	937.3^{b}	30.51	0.045
Day 42	2358^{a}	2520^{b}	2510^{b}	53.37	0.0003
Feed intake (g)					
Day 1–10	345.4	343.4	341.0	2.60	0.153
Day 1–21	125 ^{a}	128 ^{b}	128 ^{b}	9.24	0.0012
Day 1–42	4343	4361	4356	29.58	0.740
Weight gain (g)					
Day 1–10	264.0	271.1	266.5	4.59	0.161
Day 1–21	834.5^{a}	897.8^{b}	895.2^{b}	29.74	0.014
Day 1–42	2316^{a}	2478^{b}	2468^{b}	52.65	0.0002
Feed conversion ratio					
Day 1–10	1.31^{a}	1.27^{b}	1.28^{b}	0.01	0.0004
Day 1–21	1.51^{a}	1.43^{b}	1.44^{b}	0.0095	<0.0001
Day 1–42	1.88^{a}	1.76^{b}	1.77^{b}	0.0122	<0.0001
Dressing percentage (%)	73.14^{a}	74.94^{b}	74.05^{a,b}	0.49	<0.0001
CCW (g)	1724^{a}	1889^{b}	1858^{b}	40.37	<0.0001
Liver weight (g)	42.28	46.76	46.56	1.66	0.046
Carcass cuts weight (g)					
Breast	665.0^{a}	734.0^{b}	738.5^{b}	16.78	<0.0001
Drumsticks with thighs	487.9^{a}	545.5^{b}	543.8^{b}	15.03	<0.0001
Wings	163.9^{a}	175.8^{b}	170.7^{b}	2.93	<0.0001
Neck	83.25^{a}	88.98^{b}	80.78^{a}	1.78	<0.0001
Back with pelvis	325.0^{a}	338.6^{a}	307.8^{b}	7.92	<0.0001
Carcass cuts percentage (%)					
Breast	38.55^{a}	39.00 ^{a}	40.05^{b}	0.25	<0.0001
Drumsticks with thighs	28.25^{a}	28.94^{b}	29.44^{b}	0.33	0.0001
Wings	9.56	9.35	9.33	0.16	0.145
Neck	4.83^{a}	4.74^{a}	4.41^{b}	0.07	<0.0001
Back with pelvis	18.80^{a}	17.98^{b}	16.77^{c}	0.20	<0.0001

Data are means and SEM (n = 60 per group). CCW: cold carcass weight
Within a row, means with different lowercase superscript letters (*P* <0.05) are significantly different
Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat

The effects of MCFA and coccidiostat on intestinal morphometric parameters expressed as means of crypt depth, villus width and villus length for all groups are shown in Table 3. Some typical photomicrographs are shown in Figure 2. In this study, the inclusion of MCFAs in the diet increased the duodenal villus length (*P* <0.05). A numerically but not statistically significant increase in crypt depth was observed in the E-II group compared with control and E-I. The dietary treatment affected the villus length/depth ratio (*P* <0.05) of duodenum, as this was greater in both experimental groups (Table 3).

Table 3 Intestinal morphometric parameters of broiler chickens

Parameters	Groups			SEM	*P*-value
	C	E-I	E-II		
Duodenum (µm)					
Crypt depth	177.0	177.4	179.7	4.867	0.704
Villus width	64.47^{a}	59.48^{b}	63.35^{a}	2.047	0.001
Villus length	472.9^{a}	875.4^{b}	705.9^{c}	28.901	<0.0001
Ileum (µm)					
Crypt depth	223.3^{a}	163.9^{b}	164.3^{b}	6.107	<0.0001
Villus width	74.92^{a}	55.42^{b}	65.36^{c}	2.195	< 0.0001
Villus length	503.8^{a}	267.4^{b}	331.2^{c}	19.14	< 0.0001
Caecum (µm)					
Crypt depth	168.3	173.0	173.9	4.870	0.173
Villus width	58.95	58.42	58.15	1.856	0.801
Villus length	201.7^{a}	219.7^{b}	236.0^{c}	6.372	< 0.0001
Villus length / depth ratio					
Duodenum	3.26^{a}	5.01^{b}	3.99^{c}	0.208	< 0.0001
Ileum	2.34^{a}	1.67^{b}	0.13^{c}	0.122	< 0.0001
Caecum	1.22^{a}	1.27^{b}	2.03^{c}	0.050	< 0.0001

Data are means and SEM
Within a row, means with different lower-case superscript letters ($P < 0.05$) are significantly different
Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat

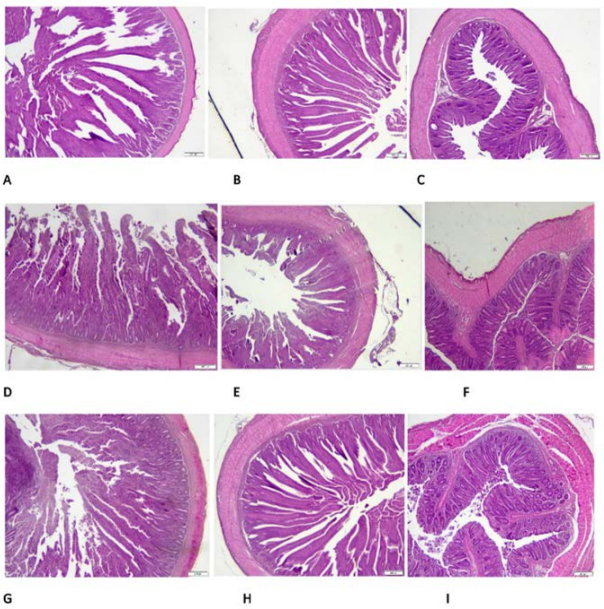

Figure 2 Photomicrographs of intestinal segments in control group and E-I and E-II groups
Histomorphology of the duodenum (A), ileum (B) and caecum (C) of broiler chicken in control group. Histomorphology of the duodenum (D), ileum (E) and caecum (F) of broiler chicken in E-I group (broilers supplemented with medium chain fatty acids). Histomorphology of the duodenum (G), ileum (H) and caecum (I) of broiler chicken in E-II group (broilers supplemented with medium chain fatty acids and coccidiostat. Haematoxylin and eosin staining. Scale bars represent 200 µm

The ileal villus crypt depth was significantly decreased in broilers with MCFA and coccidiostat supplementation compared with the control group (P <0.05). Moreover, MCFA supplementation decreased the villus width and villus length (P <0.05) compared with the other groups (P < 0.05). Furthermore, coccidiostat supplementation decreased the ileal villus length/depth ratio significantly (P <0.05). In the caecum, the crypt depth of E-II group was numerically, but not statistically significantly greater than it was in control and E-I group (Table 3). Supplementation with MCFAs and coccidiostat (E-II group) significantly increased (P <0.05) villus length in caecum. Also, the combination of MCFAs and coccidiostat significantly increased (P <0.05) the villus length/depth ratio.

Feed with MCFAs and coccidiostat resulted in increased pH of the duodenum (P <0.05) (Table 4). The pH of the ileum did not change markedly when MCFAs and coccidiostat were supplemented in the diet, although the pH tended to decrease when diet was supplemented with MCFAs only. In the caecum, the pH was significantly decreased (P <0.05) in E-I group compared to that in the control and E-II group.

Table 4 pH in various segments of broiler gastrointestinal tract

Parameters	Groups			SEM	*P*-value
	C	E-I	E-II		
Duodenum	5.88[a]	5.97[ab]	6.02[b]	0.031	0.225
Ileum	5.65	5.52	5.68	0.061	0.5470
Caecum	6.40[a]	5.77[b]	6.14[a]	0.048	0.0006

Data are means and SEM. Within a row, means with different lower-case superscript (P <0.05) letters are significantly different. Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat

The mean values of serum biochemistry parameters (HDL-cholesterol and triglyceride) of broiler chickens fed MCFA-supplemented diets are shown in Table 5. Supplementation of MCFAs or MCFAs and coccidiostat produced no significant differences (P >0.05) in the mean concentration of HDL-cholesterol in the broilers' sera. There was a significant increase (P <0.05) in the triglyceride levels in the broilers fed diets supplemented with MCFAs compared with the control group.

Table 5 Lipid profile of broiler chickens

Parameters (mmol/L)	Groups			SEM	*P*-value
	C	E-I	E-II		
HDL-cholesterol	2.71	3.01	2.69	0.053	0.05
Triglyceride	0.60[a]	0.78[b]	0.70[ab]	0.017	0.002

Data are means and SEM
Within a row, means with different lowercase (P <0.05) letters are significantly different. Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat

The results of microbial community analyses in the various segments of the intestinal tract are presented in Table 6. The highest counts were measured in the caecum. None of the dietary treatments affected the *E. coli* or *S. aureus* counts in the ileum or caecum. *Enterococcus* spp. and LAB counts, on the other hand, were significantly (P <0.05) influenced by both dietary treatments, except *Enterococcus* spp. counts in caecum, where no significant differences were detected. The lowest LAB and *Enterococcus* spp. counts were observed in the ileum of broilers fed E-I, while in the caecum, the lowest LAB count was in E-II group fed diet with MCFAs and coccidiostat. In duodenum, the lowest numbers of all bacterial groups were observed in control broilers, except *S. aureus* count which was the lowest in E-I broilers.

Table 6 Effect of Aromabiotic and salinomycin on microbiota in various intestinal segments of broilers

	Groups			SEM	*P*-value
	C	E-I	E-II		
Duodenum (log CFU/g)					
LAB	3.65[a]	3.90[a]	4.69[b]	0.24	0.0002
Enterococcus spp.	3.30[a]	3.57[b]	3.54[b]	0.03	<0.0001
E. coli	3.31[a]	3.54[b]	3.48[ab]	0.08	0.0093
Staphylococcus aureus	4.15[a]	3.80[b]	4.19[a]	0.16	0.0181
Ileum (log CFU/g)					
LAB	4.47[a]	3.68[b]	4.14[ab]	0.22	0.0019
Enterococcus spp.	3.80[a]	3.65[a]	4.17[b]	0.08	<0.0001
E. coli	3.78	3.74	4.05	0.18	0.1012
Staphylococcus aureus	4.68	4.34	4.34	0.19	0.0753
Caecum (log CFU/g)					
LAB	7.14[a]	6.33[b]	5.71[b]	0.29	0.0001
Enterococcus spp.	6.33	6.15	6.53	0.43	0.5642
E. coli	6.28	6.00	6.22	0.44	0.7303
Staphylococcus aureus	5.36	5.21	4.80	0.29	0.0786

Within a row, means with different lowercase (P <0.05) letters are significantly different
Control group (no supplementation); E-I: broilers supplemented with medium chain fatty acids; E-II: broilers supplemented with medium chain fatty acids and coccidiostat
LAB: lactic acid bacteria

Discussion

Numerous studies have suggested that the effectiveness of MCFAs on growth stimulation of birds would produce a final result of improved gastrointestinal ecosystem, with ensuing augmented intestinal environment, integrity of the intestinal mucosal barrier, digestive and immune function of intestine and broiler health (Tellez *et al*., 2006; Mountzouris *et al*., 2010; Hayat *et al*., 2014). The results of this study indicate that broiler performance was affected positively by adding MCFAs to the broiler diets. There are relatively few literature data on the effect of MCFAs on broiler production. De Los Santos *et al*. (2008) found that supplementation with 1.4% caprylic acid (an 8-carbon MCFA) in a regular chick starter diet reduced feed consumption. This result is similar to those of the present study. The effect of MCFAs has been studied extensively in other animals. Miller *et al*. (2009) reported that no differences occurred with regard to feed intake, feed efficiency and weight gain in grower and finisher pigs when fed diets with 1%, 3% and 6% MCFA oil compared with diets containing the same amount of tallow, pig fat or corn oil. However, these results are at variance with those of Dove (1993), who showed that dietary inclusion of 5% MCFAs provided the greatest increase in weight gain and a better feed conversion for post-weaning piglets compared with other sources of fat (soybean oil and animal fat). Bodyweight gain and feed intake of White Leghorn chicks decreased with diets containing 10% MCFA oil (Furuse *et al*., 1992).

The beneficial effect of MCFA supplementation in broiler diets in terms of increased final bodyweight and FCR is documented in studies from several research groups (O'Dea *et al*., 2006; Timmerman *et al*., 2006; Onderci *et al*., 2008; Bansal *et al*., 2011). This was also the case in the present study, at 42 days, even though there was no difference in feed consumption between broilers fed diet supplemented with MCFAs and the broilers in our control group. The improvement in live bodyweight at the end of the growth phase due to the supplementation of MCFAs was presumed to be a result of bacterial antagonism, competition for colonization site, competition for nutrients, a reduction in toxic compounds or modulation of immune system (Applegate *et al*., 2010). In the present study, the broilers supplemented with MCFAs and coccidiostat had a significantly higher hot carcass weight compared with broilers from the control group. The MCFAs in diets had a marked positive effect on some carcass cuts (breast, drumsticks with thighs and wings), as these weighed significantly more in broilers fed MCFA-treated diets than in broilers fed only basal diet. Conversely, other studies showed no broiler performance improvement when birds received MCFAs in comparison with control birds and birds fed antibiotics (Gunal *et al*., 2006; Vieira *et al*., 2008). However, at

the moment, numerous studies suggest that MCFAs have growth-promoting properties and can be used as alternatives to antibiotics and anticoccidiostats (Tan & Long, 2012; Khan & Iqbal, 2016).

In the current study, the positive response of broiler growth performance in final bodyweight as a result of inclusion of MCFAs in the diet might partly be explained by intestinal histological changes and villus characteristics.

Good intestinal health in the poultry industry is of great importance in achieving target growth rates and feed efficiency. MCFA supplementation significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks at 14 days old (Garcıá *et al*., 2007; Kum *et al*., 2010; Rodríguez-Lecompte *et al*., 2012; Sultan *et al*., 2015; Abudabos *et al*., 2017). Thus, MCFA supplementation increased both the villus height and crypt depth. Leeson *et al.* (2005) and Panda *et al*. (2009) reported that MCFAs in broilers' diet improved the villus length and crypt depth in the duodenum, so concluded this supplementation could be highly helpful to young birds for intestinal development. In another study, the highest duodenal and ileal villus heights were recorded in birds fed diets supplemented with MCFAs (Adil *et al*., 2010).

In the current study, broilers fed MCFA-supplemented diets had increased duodenum villus length. Increases of villus length could cause greater enzyme production and better digestion by increasing the effective absorptive area and improving the nutrient transport system (Awad *et al*., 2009). The increase of villus length of different segments of the intestine (duodenum and caecum), as the authors measured, may be attributed to the role of the intestinal epithelium, as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. Pathogens and toxic substances cause disturbances in the normal microflora and in the intestinal epithelium that could alter the permeability of this natural barrier, facilitating the invasion of pathogens and modifying the metabolism, so leading to chronic inflammatory processes at the intestinal mucosa (Khan, 2013). Organic acid salts reduced the growth of many pathogenic intestinal bacteria. Consequently, MCFAs reduced intestinal colonization and the infectious process, decreased the inflammatory process at the intestinal mucosa, and this improved villus length and function of secretion, digestion and absorption of nutrients (Khan & Iqbal, 2016; Khan *et al*., 2016).

MCFA supplementation did not affect serum HDL-cholesterol level, while serum triglyceride concentration was significantly increased in treatment groups of broilers. Contrary to these results, Cater *et al*. (1997) demonstrated HDL-cholesterol reductions in human beings on diets rich in MCFAs or oleic acid.

The triglyceride-increasing effect of MCFAs observed in the present study is supported by the results of Hill *et al*. (1990). However, the effects of MCFAs on triglyceride and cholesterol seemed to be contradictory, since triglyceride and cholesterol secretion are regulated in a coordinated manner (Marten *et al*., 2006). One possible explanation could be that higher MCFAs stimulated insulin secretion and promoted anabolic-related processes (Hill *et al*., 1990). Thus, increased *de novo* fatty acid synthesis would lead to an increase in triglyceride production (Hill *et al*., 1990).

The low counts of LAB, *Enterococcus* spp., *S. aureus* and *E. coli* that were determined in the ileum might be explained by MCFAs being incorporated into bacterial cell membranes or entering bacterial cells in undissociated form and so negatively influencing the bacterial metabolism (Desbois & Smith, 2010; Zeitz *et al.*, 2015). Besides reduction of gram-positive and gram-negative pathogens, dietary fatty acids might inhibit beneficial bacteria such as *Lactobacillus* (Van der Hoeven-Hangoor *et al*., 2013), which was shown in the present study. On the other hand, lower counts of these bacteria in the duodenum of the control broilers might be due to the larger influence of dietary MCFAs in those parts of the gastrointestine that are crucial to nutrient absorption (jejunum and ileum) (Zeitz *et al.*, 2015). The lowest LAB count, which was found in the caecum of the E-II broilers, could be because coccidiostat, beside direct suppression of coccidia, was observed to suppress the major groups of gram-positive bacteria such as LAB and *C. perfringens*, thus facilitating the hosts' direct competition for nutrients (Józefiak *et al*., 2013). Coccidiostat also decreased the microbial deconjugation of bile salts, thus improving fat digestibility, growth and feed utilization in birds (Józefiak *et al.*, 2013). The E-I group broilers had lower LAB count than the control broilers, which demonstrates that dietary MCFAs modulated the microbial ecology of the broiler gastrointestinal tract in a manner similar to coccidiostat. Higher LAB count in caecum of broilers fed dietary phytobiotic could also be due to formation of soaps with Ca, which reduce antimicrobial properties (Zeitz *et al*., 2015).

Conclusion

Dietary MCFA supplementation should be considered an alternative non-antibiotic feed additive (growth promoter). The beneficial effect of dietary MCFAs, which are reflected through the better production results and improved microbiological status of broilers, can be attributed to the effects that MCFAs have on the digestive tract, preserving its integrity, improving morphometric parameters, and changing the intestinal microflora.

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Authors' Contributions

BB and RM participated in its design and coordination and participated in drafting of the manuscript. AR carried out histomorphological examination. DŠ performed production performance and VĐ. performed fatty acids analysis. MŽB and JĐ conceived of the study (carcass cuts), and MG conceived of the serum biochemistry analysis. JĆ and BM performed statistical examination. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflicts of interest.

References

Abudabos, A.M., Alyemni, A.H., Dafalla, Y.M. & Khan, R.U., 2017. Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochemical and antioxidant status in broiler exposed to *Salmonella typhimurium* challenge during the starter phase. J. Appl. Anim. Res. 45, 538-542.

Adil, S.B., Banday, T., Bhat, G.A., Mir, M.S. & Rehman, M., 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 1–7. http,//dx.doi.org/10.4061/2010/479485.

Applegate, T.J., Klose, V., Steiner, T., Ganner, A. & Schatzmayr, G., 2010. Probiotics and phytogenics for poultry, Myth or reality? J. Appl. Poult. Res. 19, 194-210. https,//doi.org/10.3382/japr.2010-00168

Awad, W.A., Chareeb, K., Abdel-Raheem, S., Böhm, J., 2009. Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weight and intestinal histomorphology of broiler chickens. Poult. Sci. 88, 49-56. https,//doi.org/10.3382/ps.2008-00244

Baltić, Z.M., Marković, R. & Đorđević, V., 2011. Nutrition and meat quality. Tehnologija Mesa 52,154-159.

Baltic, T., Baltic, Z.M., Misic, D., Ivanovic, J., Janjic, J., Boskovic, M. & Dokmanovic, M., 2015. Influence of marination on *Salmonella* spp. Growth in broiler breast fillets. Acta. Vet. (Beogr). 65, 417-428. doi,10.1515/acve-2015-0034

Bansal, G.R., Singh, V.P. & Sachan, N., 2011. Effect of probiotic supplementation on performance of broilers. Asian J. Anim. Sci. 5, 277-284.

Bergamo, P., Palmieri, G., Cocca, E., Ferrandino, I., Gogliettino, M., Monaco, A., Maurano, F. & Rossi, M., 2016. Adaptive response activated by dietary *cis*9, *trans*11 conjugated linoleic acid prevents distinct signs of gliadin-induced enteropathy in mice. Eur. J. Nutr. 55, 729-740. doi, 10.1007/s00394-015-0893-2

Bruggeman, G., 2002. Evaluation of the effect of alternatives for growth promoters against *Salmonella* infection in pigs. PhD dissertation. Univ. Ghent, Belgium.

Cater, N.B., Heller, H.J. & Denke, M.A., 1997. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. Am. J. Clin. Nutr. 65, 41-45.

de Los Santos, F.S., Donoghue, A.M., Venkitanarayanan, K., Dirain, M.L., Reyes-Herrera, I., Blore, P.J. & Donoghue, D.J., 2008. Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in ten-day-old broiler chickens. Poult. Sci. 87, 800-804. https,//doi.org/10.3382/ps.2007-00280

Desbois, A.P. & Smith, V.J., 2010. Antibacterial free fatty acids, Activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. 85, 1629-1642. doi, 10.1007/s00253-009-2355-3

Dove, C.R, 1993. The effect of adding copper and various fat sources to the diets of weanling swine on growth performance and serum fatty acid profiles. J. Anim. Sci. 71, 2187-2192.

Furuse, M., Mabayo, R.T., Kita, K. & Okumura, J., 1992. Effect of dietary medium chain triglyceride on protein and energy utilization in growing chicks. Br. Poult. Sci. 33, 49-57. https,//doi.org/10.1080/00071669208417443

Garciá, V., Catalá-Gregori, P., Hernandez, F., Megiás, M.D. & Madrid, J., 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J. Appl. Poult. Res. 16, 555-562. https,//doi.org/10.3382/japr.2006-00116

Glamočlija, N., Starčević, M., Ivanović, J. Janjić, J., Bošković, M., Đorđević, J., Marković, R. & Baltić, M.Z., 2016. Comparative analysis of meat chemical composition of different broiler provenances. Meat Technol. 57, 1-5.

Gunal, M, Yayli, G., Kaya, O., Karahan, N. & Sulak, O., 2006. The effects of antibiotic growth promoter, probiotic or organic acids supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci. 5, 149-155.

Gutierrez Del Alamo, A., De Los Mozos, J., Van Dam, T.P. & De Ayala, P.P., 2006. The use of short and medium chain fatty acids as an alternative to antibiotic promoters in broilers infected with malabsorption syndrome. In: Proc. 16th European Sym. Poult. Nutr., Strasbourg, France. pp. 317-320.

Hayat, T., Sultan, A., Khan, R.U., Khan, S., Zahoor ul Hassan, Ullah, R. & Aziz, T., 2014. Impact of organic acid on some liver and kidney function tests in japanese Quails, *Coturnix coturnix japonica*. Pak. J. Zool. 46, 1179-1182.

Hermans, D., Van Deun, K., Messens, W., Martel, A., Van Immerseel, F. Haesebrouck, F., Rasschaert, G, Heyndrickx, M. & Pasmans, F., 2011. *Campylobacter* control in poultry by current intervention measures ineffective, Urgent need for intensified fundamental research. Vet. Microbiol. 52, 219-228. doi, 10.1016/j.vetmic.2011.03.010

Hill, J.O., Peters, J.C., Swift, L.L., Yang, D., Sharp, T., Abumrad, N. & Greene, H.L., 1990. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J. Lipid. Res. 31, 407-416.

https,//doi.org/10.1017/S1466252311000089
Isaac, D., Deschepper, K., Vanmeenen, E. & Maertens, L., 2013. The effect of a balanced mixture of medium chain fatty acids on zootechnical performance in broilers. In: Proc. 24th Ann. Aust. Poult. Sci. Sym., Sydney. pp. 196-199.
Janjić, J., Katić, V., Ivanović, J., Bošković, M., Dokmanović, M., Baltić, T. & Baltić, M., 2016. Temperatures, cleanliness and food storage practices in domestic refrigerators. Int. J. Consum. Stud. 40, 276-282. doi, 10.1111/ijcs.12252
Józefiak, D., Kierończyk, B., Juśkiewicz, J., Zduńczyk, Z., Rawski, M., Długosz, J., Sipp, A. & Højberg, O., 2013. Dietary nisin modulates the gastrointestinal microbial ecology and enhances growth performance of the broiler chickens. PLoS ONE 8 (12), 85347. https,//doi.org/10.1371/journal.pone.0085347
Kabara, J.J. & Marshal, D.L., 2005. Medium-chain fatty acids and esters. In: Antimicrobials in Food. CRC Press, Boca Raton, FL. pp. 327-360.
Khan, R.U., Chand, N. & Ali, A., 2016. Effect of organic acids on the performance of Japanese quails. Pak. J. Zool. 48, 1799-1803.
Khan, S.H., 2013. Probiotic microorganisms-identification, metabolic and physiological impact on poultry. Wrlds Poult. Sci. J. 69, 601-612. https,//doi.org/10.1017/S0043933913000603
Khan, S.H. & Iqbal, J., 2016. Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res. 44, 359-369. https,//doi.org/10.1080/09712119.2015.1079527
Khosravinia, H., 2015. Effect of dietary supplementation of medium-chain fatty acids on growth performance and prevalence of carcass defects in broiler chickens raised in different stocking densities. J. Appl. Poult. Res. 24, 1-9. http,//dx.doi.org/10.3382/japr/pfu001
Kum, S., Eren, U., Onol, A. & Sandikci, M., 2010. Effects of dietary organic acid supplementation on the intestinal mucosa in broilers. Rev. Med. Vet. 161, 463-468.
Leeson, S., Namkung, H., Antongiovanni, M. & Lee, E.H., 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84, 1418-1422. https,//doi.org/10.1093/ps/84.9.1418
Marten, B., Pfeuffer, M. & Schrezenmeir, J., 2006. Medium-chain triglycerides. Int. Dairy. J. 16, 1374-1382. https,//doi.org/10.1016/j.idairyj.2006.06.015
Miller, D., Johnston, Z., Mullan, B., Pluske, J. & Hansen, C., 2009. Nutritional manipulation of the somatotropic axis in grower and finisher pigs. Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry. December. 2009. Australia. pp. 1-12.
Mountzouris, K.C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G. & Fegeros, K., 2010. Effect of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. 89, 58-67. https,//doi.org/10.3382/ps.2009-00308
Newell, D.G. & Wagenaar, J.A., 2000. Poultry infections and their control at farm level. In: I. Nachamkin & M.J. Blaser (ed). Campylobacter. 2nd edition. ASM Press, Washington, DC. pp. 497-509.
O'Dea, E.E., Fasenko, G.M., Allison, G.E., Korver, D.R., Tannock, G.W. & Guan, L.L., 2006. Investigating the effects of commercial probiotics on broiler chick quality and production efficiency. Poult. Sci. 85, 1855-1863. https,//doi.org/10.1093/ps/85.10.1855
Onderci, M., Sahin, N., Cikim, G., Ydyn, A., Ozercan, I., 2008. α-glucanase-producing bacterial culture improves performance and nutrient utilization and alters gut morphology of broilers fed a barley-based diet. Anim. Feed Sci. Technol. 146, 87-97. https,//doi.org/10.1016/j.anifeedsci.2007.11.005
Panda, A.K., Rama, R., Rao, S.V., Raju, M.V.L.N. & Shyam, G.S., 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Austral. J. Anim. Sci. 22, 1026–1031.
Rodríguez-Lecompte, J.C., Yitbarek, A., Brady, J., Sharif, S., Cavanagh, M.D., Crow, G., Guenter, W.J., House, D. & Camelo-Jaimes, G., 2012. The effect of microbial nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration. J. Anim. Sci. 90, 2246-2254. doi,10.2527/jas.2011-4184
Saeidi, E., Shokrollahi, B., Karimi, K. & Amiri-Andi, M., 2016. Effects of medium-chain fatty acids on performance, carcass characteristics, blood biochemical parameters and immune response in Japanese quail. Br. Poult. Sci. 57, 358-363. https,//doi.org/10.1080/00071668.2016.1169508
Shokrollahi, B., Yavari, Z. & Kordestani, A.H., 2014. Effects of dietary medium-chain fatty acids on performance, carcass characteristics, and some serum parameters of broiler chickens. Br. Poult. Sci. 55, 662-667. https,//doi.org/10.1080/00071668.2014.955836
Skrivanova, E., Marounek, M., Dlouha, G. & Kanha, J., 2005. Susceptibility of *Clostridium perfringens* to C2-C18 fatty acids. Lett. Appl. Microbiol. 41, 77-81. doi, 10.1111/j.1472-765X.2005.01709.x
Smirnov, A., Perez, R., Amit-Romach, E., Sklan, D. & Uni, Z., 2005. Mucin dynamics and microbial populations in the chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplements. J. Nutr. 135, 187-192.
Sultan, A., Ullah, T., Khan, S. & Khan, R.U., 2015. Effect of organic acid supplementation on the performance and ileal microflora of broiler during finishing period. Pak. J. Zool. 47(3), 635-639.
Tan, G.H. & Long, K., 2012. Preliminary study of anticoccidial activity of medium chain fatty acids (MCFA) and their corresponding monoglycerides on broiler chicken coccidiosis. Int. J. Biotechnol. Wellness Ind 1, 134-141. doi,http,//dx.doi.org/10.6000/1927-3037/2012.01.02.05
Tellez, G., Higgins, S.E., Donoghue, A.M. & Hargis, B.M., 2006. Digestive physiology and the role of microorganisms. J. Appl. Poult. Res. 15, 136-144. https,//doi.org/10.1093/japr/15.1.136
Timmerman, H.M., Veldman, A., Van den Elsen, E., Rombouts, F.M. & Beynen, A.C., 2006. Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poult. Sci. 85, 1383-1388. https,//doi.org/10.1093/ps/85.8.1383

Trbovic, D., Vranic, D., Djinovic-Stojanovic, J., Petronijevic, R., Milijasevic, M., Matekalo-Sverak, V. & Spiric, A., 2011. Fatty acid profile of carp fish species from two aquaculture systems. Proc. 5th International Conference Aquaculture and Fishery, June. 1-3, Belgrade, Serbia. pp. 80-84.

Van der Hoeven-Hangoor, E., Van der Vossen, J.M.B.M., Schuren, F.H., Verstegen, M.A., De Oliveira, J.E., Montjin, R.C. & Hendriks, W.H., 2013. Ileal microbiota composition of broilers fed various commercial diet compositions. Poult. Sci. 92, 2713-2723. https,//doi.org/10.3382/ps.2013-03017

Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., Sevcik, M., Rychlik, I., Haesebrouck, F. & Ducatelle, R., 2004. Medium-chain fatty acids decrease colonization and invasion through hilA suppression shortly after infection of chickens with *Salmonella enterica serovar* Enteritidis. Appl. Environ. Microbiol. 70, 3582-3587. doi,10.1128/AEM.70.6.3582-3587.2004

Vieira, S.L., Oyarzabal, O.A., Freitas, D.M., Berres, J., Peña, J.E.M., Torres, C.A. & Coneglian, L., 2008. Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. J. Appl. Poult. Res. 17, 128-133. https,//doi.org/10.3382/japr.2007-00054

Wang, J., Wang, X., Li, J., Chen, Y., Yang, W. & Zhang, L., 2015. Effects of dietary coconut oil as a medium-chain fatty acid source on performance, carcass composition and serum lipids in male broilers. Asian-Austral. J. Anim. Sci. 28, 223-230. doi,10.5713/ajas.14.0328

Yamabayashi, S., 1987. Periodic acid-Schiff-Alcian Blue. A method for the differential staining for glycoproteins. Histochem. J. 19, 565-571. https,//doi.org/10.1007/BF01687364

Zeitz, J.O., Fennhoff, J., Kluge, H., Stangl, G. I. & Eder, K., 2015. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poult. Sci. 94, 2404-2413. https,//doi.org/10.3382/ps/pev191

Zentek, J., Buchheit-Renko, S., Ferrara, F., Vahjen, W., Van Kessel, A.G. & Pieper, R., 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim. Health. Res. Rev. 12, 83-93.