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EFFECT OF DIFFERENT MEDIUM CHAIN FATTY ACIDS, CALCIUM BUTYRATE, AND SALINOMYCIN ON PERFORMANCE, NUTRIENT UTILIZATION, AND FERMENTATION PRODUCTS IN GASTROINTESTINAL TRACTS OF BROILER CHICKENS

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ABSTRACT

The objective of this study was to study efficacy of medium chain fatty acids (MCFAs), salinomycin, and butyric acid on growth performance, energy and nutrient availability, development of internal organs, and content of short chain fatty acids (SCFAs) in the gastrointestinal tract of broiler chickens. Nine hundred and sixty Ross 308 male broilers were used in this study. Birds were randomly assigned to 10 dietary treatments (12 replications each/8 birds in one replication). We used two types of diet [provocative (PD) and corn diet (MD)]; each diet was then split into five batches that were supplemented with either salinomycin (70.0 mg/kg); triglyceride form of capric and caprylic acid (3.0 g/kg; MCT 1.38:1); calcium butyrate (10.0 g/kg; CB); mixture of caproic, caprylic, and capric acids (8.3 g/kg; MCFA; 1:1:1); or without any supplement [control (C)]. The examined supplements exerted a positive effect on growth performance in MD-fed chickens only. Chickens fed with MCFA-supplemented MD diet were characterized with 10% gain in body weight and 5% lower feed conversion ratio (FCR) than that of birds in the control group. CB positively influenced the value of nitrogen corrected apparent metabolizable energy (AME_N) by about average 6% (PD and MD) in comparison to the control birds. Nitrogen retention was found to be changed only in PD. The highest positive change was found in birds of MCT group (25%) than that of birds in the control group. MCFA, MCT and CB showed a favorable influence on the weight of gastrointestinal tract, in particular, the weight of the ileum, which was about 8% heavier than that of the control birds. The diet type and tested supplements significantly enhanced the content of SCFAs in broiler crop, ileum, and cecum digesta. In conclusion, the effect of tested supplements on the parameters determined depended on the type of diet, as confirmed by significant interactions. MCFA was found to be the best supplement in this study.

Keywords: Broilers, Salinomycin, Medium chain organic acids, Calcium butyrate.

INTRODUCTION

Feed components and dietary constituents commonly used in feed mixtures for chicks exert a substantial influence on the histological structure of intestinal walls and digestive functions of the alimentary tract (Uni *et al.,* 1998; Jamroz *et al.,* 2000, 2009; Kaczmarek *et al.,* 2016c). The decisive role in the correct formation of mucosa, villi, the depth of crypts, and the release of mucus in the intestinal walls is played by the cellulose complex and nonstarch polysaccharides (NSPs) in the diet. These components of the feed are responsible for the length of the intestinal segments and viscosity of the contents of the gastrointestinal tract (GIT)(Bach Knudsen *et al.,* 1997; Santos *et al.,* 2007; Kaczmarek *et al.,* 2016b). Microbiological fermentation of constituents of NSPs and the formed short chain fatty acids (SCFAs), influence the pH of the intestinal digesta. NSPs present in plant feeds, especially in cereals, to a large extent cause morphological changes of the intestinal walls: the height of villi and the thickness of muscular layers (Hermans *et al.,* 2010, 2012). Furthermore, some feed additives' different dietary fats and medium chain fatty acids (MCFAs), for example, caproic, caprylic, capric, or lauric acids can control the colonization of enteric *Campylobacter jejuni, Salmonella* spp., and *Clostridium* spp. and protect the intestinal mucus (Kollanoor-Johny *et al.,* 2012; Wang *et al.,* 2012). Hermans *et al.,* (2010, 2012) also reported the dose-dependent action of MCFAs against *Campylobacter*; however, capric acid had the highest microbicidal activity. The aforementioned acids penetrate the bacterial cell wall in a nondissociated form (Sun *et al.,* 1998). Lower intracellular pH favors inactivation of bacterial enzymes (Viegas *et al.,* 1991) resulting in the microbial cell death. Butyrates are an important source of energy for epithelial cells in the intestinal tract and participate in the maintenance of colonic homeostasis (Guilloteau *et al.,* 2010).

Despite a few publications confirming the positive effect of MCFA in chickens, their impact on growth performance, nitrogen retention (NR), weight of organs and segments of GIT, and fermentation processes in GIT is still unknown. Moreover, it is unknown that the type of diets, especially diets stimulating increased counts of *Clostridium perfringens* which predisposes chickens to necrotic intestinal inflammation (Santos *et al.,* 2007) will be decided about MCFA efficiently. Therefore, the objective of this study was to determine the impact of MCFA, salinomycin, and butyrate on the performance [body weight gain (BWG), feed intake, and feed conversion ratio (FCR)]; crude fat digestibility and NR; weight of liver, pancreas, small intestine, and cecum; and SCFA in the contents of GIT (crop, ileum, and cecum) of broiler chickens fed with corn diet (MD) and provocative diet (PD).

MATERIALS AND METHODS

All animal procedures were conducted in accordance with the guidelines of the Polish Council of Animal Care. The protocol for this study was approved by the Local Animal Care Committee of Poznan University of Life Sciences (permission number: 33/2013).

Animals and diets: Male Ross 308 broiler chickens were obtained from a commercial hatchery. On arrival, the chicks were individually weighed and the heaviest and lightest birds discarded. Finally, and a total of 960 birds were used for the experiments (average body weight 44 g, and there were no significant differences between treatment groups (P>0.05). The birds were allocated to 120 pens, 8 birds in each pen. Birds were reared on a wood-shaving litter in 1.2 × 0.8 m pens and equipped with individual feeder and four drinkers. Feed and water were offered *ad libitum* to birds throughout the experiment. Each diet was offered to birds in 12 pens in a randomized block design (10 positional blocks). Information on growth and feed intake was obtained from 14 and 35 days of age. Room temperature and lighting regime met commercial recommendations.

The chickens were fed isonitrogenous and isoenergetic diets: on days 1–14 with starter; on days 15–35 with grower mixtures based on wheat, rye, barley, rapeseed meal, and with fish meal, that is, PD; or a diet based on corn, wheat, and soya bean meal, that is, MD (Table 1). This procedure was followed by transferring the screened and premixed portions to a stainless steel horizontal feed mixer (100 or 300 MPW, Zuptor sp. z o.o., Gostyń, Poland; mixing time was 4 min, mixing band: 27.4 rev/min) for mixing of the completed diet. All ingredients except minerals, vitamins, amino acids, and fat were ground in a Skiold Disk mill (SK2500, Skiold A/S, Sæby, Denmark) with disk distance set at 1.8 mm. Each kind of diet comprised five treatments.

The basic diets were supplemented as per the following: control—without additives; salinomycin (S)—70 mg/kg; triglyceride form of capric and caprylic acid (MCT; 1.38:1)—3g/kg; calcium butyrate (CB)—10 g/kg; or mixture of caproic, caprylic, and capric acids (MCFA; 1:1:1)—8.3 g/kg. CB as well as MCT and MCFA were provided by Sigma Aldrich (Poznan, Poland).

The composition of diet was calculated using linear optimization. The diets were offered to the chickens *ad libitum* in mash form. Because the used additives indicated anticoccidial properties (Czerwiński *et al.,* 2012), no coccidiostats were introduced into the diets.

Data collection: The aim of calculated of fat digestibility coefficient, NR, and nitrogen corrected apparent metabolizable energy (AME_N) value in excreta, 3 g/kg titanium dioxide was included as a nonabsorbable marker as to diets fed during days 33 and 34 of growth experiment. The floor of each cage was covered with thick plastic foil and excreta were collected twice a day (Rutkowski *et al.,* 2016). The samples were immediately homogenized and frozen for chemical analysis (n=10).

At the end of the experiment (at day 35), 36 birds (three birds per replication) from each treatment were randomly selected and sacrificed by cervical dislocation and their organs (liver, pancreas, ileum, and caeca) and digestive tracts were immediately removed. The organs were weighted and the fresh contents of crop, ileum, and cecum from 21 chickens per treatment were stored at −20°C for the determination of SCFAs and succinic acid.

Analytical procedures: The following parameters were determined by using AOAC standard methods (2007), in grower-type mixtures as well as in excreta samples following lyophilization (Christ 1825 Medizinische Apparatebau 326 Osterode/Harz, Germany) and grinding (coffee grinder): nitrogen concentration (method 976.05) using a Kjel Foss Automatic instrument, model 16210 (A/S N. Foss Electric, Denmark) and crude fat (method 920.39) using a Soxtec System HT 1043 Extraction Unit (Foss Tecator, Denmark). Calcium (Ca) and phosphorus (P) in diets were analyzed according to the procedure of the AOAC (2007). Phytate in diet was determined according to the method of Haug and Lantzsch (1983). Nonphytate-P was calculated as total P minus phytate. The amino acid content in diets was determined via Amino Acid Analyzer AAA-400, (INGOS s.r.o., Praha, Czech Republic) using ninhydrin for 10 postcolumn derivatization. Before analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110°C (procedure 994.12; AOAC 2005). Methionine and cystine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation before hydrolysis (procedure 994.12, alternative 3; 15 AOAC 2005). Titanium dioxide levels were determined in both grower diets and in excreta according to the method proposed by Short *et al.,* (1996), considering the sample preparation method described by Myers *et al.,* (2004). Using a bomb calorimeter (KL 12Mn, Precyzja-Bit PPHU, Poland) standardized with benzoic acid, gross energy was evaluated in the analyzed samples (diets and excreta). Crude fiber concentrations in the diets were analyzed using PN-EN ISO 6865. NSP concentrations were calculated on a the data presented by Bach Knudsen (1997) (Table 1).

The level of fatty acids in crop, ileum, and cecum was analyzed via gas chromatography with a Hewlett Packard apparatus (Model 6890, Agilent Technologies, Naerum, Denmark) equipped with a flame-ionization detector and a 30-m B-5 column with an internal diameter of 0.32 mm and coated with 5%-phenyl 95% dimethylpolysiloxane with a film thickness of 0.25 µm.

Calculations and statistical analysis: Digestibility coefficients for the crude fat (CF), NR, and AME_N value were determined using the following formula:

$$TTD=\{1-[(TiO_{2\,[g/kg\ diet]}/TiO_{2\,[g/kg\ EX]})\ (CF_{[g/kg\ EX]}/CF_{[g/kg\ diet]})]\}\ 100\%$$

where TTD = total tract digestibility, EX = excreta, and CF = crude fat.

$$AMN_N= [GE_{[kcal/kg\ of\ EX]} \cdot (TiO_{2[g/kg\ diet]}/TiO_{2\,[g/kg\ EX]})] - 34.4\ [(N_{[g/kg\ EX]} - (TiO_{2\,[g/kg\ diet]}/TiO_{2\,[g/kg\ EX]}]$$

where GE = gross energy, N = nitrogen, and 34.4 = the energy equivalent of uric acid nitrogen (Hill and Anderson, 1958).

Statistical analysis was performed using the SAS statistical software package (SAS, 1990 Iowa, USA). A randomized complete block analysis of variance was performed and a 5 × 2 factorial structure was used to study the primary treatment factors (five dietary supplements and the presence of two basal diets) and their interaction. Means from experiments were compared using the Duncan's test. Differences were reported as significant at $P\leq0.05$ and trends were noted when the P value was near to 0.1.

RESULTS

Growth Performance: No health problems were associated with use of the dietary supplements in chickens throughout the experiment, and there were no obvious health problems. Mortality was low (<1%) and not associated with treatment. The average weights of the MD-fed chickens at days 14 and 35 were 436 g and 2046 g, respectively. This observation was in agreement with breeders recommendations.

A statistically significant ($P\leq0.05$) diet-dependent influence on broiler chicken performance was determined (Table 2). MD-fed chickens from days 1–14 were characterized by greater BWG and higher (feed intake) FI than that of PD-fed birds. No statistically significant influence of the applied additives on BWG and FI of broiler chickens was observed during the starter period (days 0–14) ($P>0.05$). The effect of organic acids on final BWG, FI, and FCR depended on the kind of diet, which showed an interaction. The most positive effect on BWG and FCR was obtained for the birds fed with MCFA-supplemented MD ($P\leq0.05$).

Digestibility, NR, and AME_N: The kind of diet significantly affected the utilization of individual nutrients by the experimental birds (Table 3). PD-fed chickens were characterized with lower CF digestibility as well as lower AME_N dietary values than that of MD-fed chickens ($P\leq0.05$). The effect of additives on NR depended on the kind of diet, which showed a significant interaction ($P\leq0.05$). The positive effect of MCT and CB on NR was found only in PD. The highest level of crude fat digestibility occurred in the group supplemented with S. The effect of remaining additives, did not differ from the control. The AME_N values estimated in group of birds fed with diets supplemented with S, MCT, and MCFA were similar as in control birds, whereas the highest AME_N value was determined for birds fed with CB-supplemented diet ($P\leq0.05$). Our experiment did not confirm interactions between the kind of diet and supplements for crude fat digestibility as well as AME_N value.

GIT measurements: Anatomical analysis of the examined organs and segments of the GIT demonstrated a statistically significant impact of the kind of diet on the relative percentages of BWF (Table 4). PD-fed chickens were characterized by a greater mass of the liver and pancreas as well as the ileum than that of MD-fed chickens ($P\leq0.05$).

Our experiment failed to demonstrate any influence of the examined additives on the weight of the pancreas of broiler chickens (Table 4). Significantly lowest liver weight was determined in birds fed with diets supplemented with S and MCFA, whereas in the remaining treatments, the liver weight did not differ from the control group ($P\leq0.05$). Mean ileum weights of birds fed with diets containing organic acids were higher than that of control and from S-supplemented diets ($P\leq0.05$). In case of cecum weights, we found a significant interaction between the kind of diet and additives ($P\leq0.05$). The supplementation of MCT and CB in PD caused an increase in the weights of cecum of broiler chickens.

Concentration of SCFAs in intestine: Overall, we observed a great diversification of fatty acid content in GIT of chickens fed with PD and MD. In crop contents of 35-day-old broiler chickens, visibly greater concentrations of acetic acid, lactic acid, and succinic acid than that of other acids (formic acid, propionic acid, *n*-butyric acid, and *n*-capronic acid) were found (Table 5). The kind of diet significantly modified the level of succinic acid in the crop. The concentration of succinic acid was significantly higher in digesta obtained from MD-fed chickens than that of PD-fed chickens (P≤0.05). Highly insignificant and diversified concentration of formic acid, propionic acid, butyric acid, and capronic acid in crop digesta of broiler chickens from many treatments and the significant interactions of propionic and capronic acid in digesta make it impossible to formulate precise conclusions with respect to the action of the feed supplements used.

Concentration of fermentation products in the ileum was lower than that of the crop, except for lactic acid (Table 6). Significantly more acetic acid was found to be in the ileum digesta obtained from PD-fed chickens, and more butyric and lactic acids were noted in MD-fed chickens (P≤0.05). Numerous significant differences between treatments in terms of the levels of SCFAs in ileum digesta were caused by the additives used. Supplementation with CB led to an increase in acetic acid concentrations, whereas MCT supplementation increased acetic acid, lactic acid, and succinic acid concentrations. The concentration of other acids were inconsistent and differed greatly. In addition, the significance of interactions was only clear for acetic acid concentration.

Great concentrations of fermentation products were found in chicken cecum (Table 7). Lower levels of (P≤0.05) SCFAs were present in cecum contents in PD-fed chickens than that of MD-fed chicken. The highest total fatty acid concentration was obtained in the birds fed with a diet supplemented with MCFAs, salinomycin, and MCT (P≤0.05). Significant interactions between diet and supplement were observed for formic acid, propionic acid, isobutyric acid, valeric acid, and lactic acid concentrations.

Table 1. Basic diet composition, provocative (PD) and maize diets(MD), g/kg DM.

Ingredient[g/kg DM]	PD		MD	
	Starter	Grower	Starter	Grower
Maize	–	–	396.4	442.1
Wheat	283.2	325.2	100.0	130.0
Rye	50.0	49.0	–	–
Barley	200.0	270.0	–	–
Soybean meal [44%]	255.0	173.0	384.0	299.0
Rapeseed meal	60.0	50.0	–	–
Fish meal	30.0	30.0	–	–
Soybean oil	50.0	30.0	81.3	86.4
Lard	40.0	40.0	–	–
Monocalcium phosphate	9.0	9.0	11.1	13.5
Limestone	4.2	4.0	5.7	4.9
$NaHCO_3$	1.0	1.0	1.0	1.8
NaCl	2.6	2.0	2.9	2.4
DL-methionine	2.1	1.5	3.7	2.9
L-lysine HCl 98	2.6	2.0	2.8	3.0
L-threonine	0.3	0.3	1.1	1.0
Vitamin-mineral premix	10.0*	10.0**	10.0*	10.0**
TiO_2	–	3.0	–	3.0
Calculated nutrient composition				
ME [MJ/kg]	13.00	13.20	13.00	13.20
NSP#	147.7	145.5	133.1	122.7
Analyzed nutrient composition				
Gross energy [MJ/kg]	17.96	18.31	17.02	17.37
Crude protein	222.00	187.0	213.00	189.0
Crude fat	66.7	81.9	67.7	82.5
Crude fiber	44.1	41.9	33.2	29.7
Non phytate P	5.2	4.6	4.9	4.5
Calcium	10.2	9.3	10.6	9.1
Lysine (total)	14.3	12.6	14.7	12.5
Methionine + Cystine (total)	10.6	9.4	10.4	9.6
Threonine (total)	9.6	8.5	9.8	8.5
Valine (total)	11.0	9.4	11.1	9.5
Tryptophan (total)	2.3	2.1	2.3	2.0

Notes *provides per kg diet: vit. A 12000 IU; vit. $D_3$3000 IU; vit. E 35 mg; vit. K 2.5 mg; vit. B_1 3 mg; vit. B_2 6 mg; vit. B_6 8 mg; vit. B_{12} 0.03 mg; niacin 30 mg; d-panthothenic acid 15 mg; folic acid 2 mg; d-biotin1 mg; choline 200 mg; betaine 125 mg .
** provides per kg diet: vit. A 10000 IU. vit.$D_3$2400 IU; vit. E 30 mg; vit .K 2 mg; vit. B_1 2 mg; vit. B_2 5 mg; vit. B_6 5 mg; vit.B_{12} 0.03 mg; niacin 24 mg; d-panthothenic acid 17.4 mg; folic acid 0.8 mg; d-biotin 0.8 mg; choline 200 mg; betaine100 mg
# –The NSP concentration was calculated in accordance with the data presented by Bach Knudsen (1997)

Table 2. Performance results of broiler chickens.[#]

Supplements	Diet	FI[g]		BWG[g]		FCR[g]	
		0–14 day	0–14 day	0–14 day	0–35 day	0–14 day	0–35 day
Control	PD	491	491	340	1793de	1.44^{b}	1.75^{a}
S*	PD	491	491	342	1852^{d}	1.39^{b}	1.73^{a}
MCT	PD	475	475	318	1792de	1.54^{a}	1.66^{b}
CB	PD	483	483	328	1751^{e}	1.45^{b}	1.77^{a}
MCFA	PD	476	476	336	1734^{e}	1.43^{b}	1.76^{a}
Control	MD	520	520	441	1942^{c}	1.19^{c}	1.50^{c}
S	MD	510	510	434	2072^{ab}	1.17^{c}	1.44^{de}
MCT	MD	509	509	422	2022^{bc}	1.19^{c}	1.48^{cd}
CB	MD	530	530	451	2066^{ab}	1.16^{c}	1.45^{cde}
MCFA	MD	508	508	432	2141^{a}	1.17^{c}	1.43^{e}
SEM^		3.29	3.29	5.40	16.0	0.01	0.02
p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	PD	483^{b}	483^{b}	333^{b}	1785	1.45	1.74
	MD	516^{a}	516^{a}	436^{a}	2046	1.18	1.46
	SEM^	2.68	2.68	3.85	11.3	0.01	0.01
Control		506	506	389	1871	1.32	1.63
S		501	501	388	1962	1.28	1.59
MCT		493	493	370	1902	1.36	1.57
CB		508	508	390	1901	1.31	1.61
MCFA		492	492	383	1928	1.30	1.59
SEM^		2.97	2.97	4.12	14.8	0.01	0.02
Diet		≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Supplements		0.311	0.311	0.082	0.023	0.001	0.008
Diet ×Supplements		0.685	0.685	0.404	≤0.001	0.013	0.001

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; BWG - body weight gains; FI - feed intake; FCR - Feed conversion ratio

[a-b] Means in a column with no common superscripts differ significantly ($p \leq 0.05$).

[#] Means represent 12 pens of 8 chicks each.

^ Pooled standard error of mean.

Table 3. Nitrogen retention (NR), fat digestibility (CF) and AME$_N$ (MJ/kg) of broiler chickens.[#]

Supplements	Diet	NR [%]	CF[%]	AME$_N$[MJ/kg]
Control	PD	48.3^{c}	51.6	9.73
S*	PD	48.8^{c}	60.8	10.02
MCT	PD	60.4^{a}	47.4	9.97
CB	PD	54.9^{b}	47.2	10.70
MCFA	PD	51.3^{c}	52.6	9.92
Control	MD	61.0^{a}	87.7	13.79
S	MD	62.8^{a}	89.9	13.90
MCT	MD	62.5^{a}	88.4	13.63
CB	MD	63.0^{a}	88.8	14.26
MCFA	MD	61.1^{a}	88.9	14.03
SEM^		0.60	1.90	0.184
p		≤0.001	≤0.001	≤0.001
	PD	52.7	51.9^{b}	10.07^{b}
	MD	62.1	88.8^{a}	13.02^{a}
	SEM^	0.42	1.57	0.144
Control		54.9	70.5^{b}	11.76^{b}
S		56.1	76.0^{a}	12.04^{b}
MCT		61.4	68.8^{b}	11.80^{b}

CB	59.1	68.0[b]	12.48[a]
MCFA	56.1	71.6[ab]	11.98[b]
SEM^	0.51	1.66	0.159
Diet	≤0.001	≤0.001	≤0.001
Supplements	≤0.001	0.019	≤0.001
Diet ×Supplements	≤0.001	0.102	0.474

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; [a-b]Means in a column with no common superscripts differ significantly ($p \leq 0.05$).
[#] Means represent 12 pens of 8 chicks each.
^ Pooled standard error of mean.

Table 4. Percentage of organs in terms of the body weight of broiler chickens.[#]

Supplements	Diet	Liver	Pancreas	Ileum	Cecum
Control	PD	2.67	0.31	3.84	0.33[cd]
S*	PD	2.39	0.30	3.68	0.32[cd]
MCT	PD	2.55	0.32	4.37	0.41[a]
CB	PD	2.56	0.31	4.18	0.40[ab]
MCFA	PD	2.51	0.31	4.09	0.38[abc]
Control	MD	2.21	0.19	2.41	0.34[bc]
S	MD	2.50	0.18	2.38	0.33[cd]
MCT	MD	2.17	0.17	2.43	0.28[d]
CB	MD	2.13	0.18	2.44	0.33[cd]
MCFA	MD	2.03	0.17	2.52	0.35[abc]
SEM^		0.20	0.01	0.07	0.01
p		≤0.001	≤0.001	≤0.001	≤0.001
	PD	2.54[a]	0.31[a]	4.03[a]	0.37
	MD	2.12[b]	0.18[b]	2.44[b]	0.33
	SEM^	0.16	0.01	0.05	0.02
Control		2.48[a]	0.27	3.19[b]	0.34
S		2.25[c]	0.25	3.12[b]	0.33
MCT		2.39[ab]	0.26	3.46[a]	0.35
CB		2.38[ab]	0.26	3.44[a]	0.37
MCFA		2.31[bc]	0.25	3.43[a]	0.37
SEM^		0.18	0.01	0.05	0.01
Diet		≤0.001	≤0.001	≤0.001	≤0.001
Supplements		0.004	0.742	0.006	0.041
Diet ×Supplements		0.788	0.491	0.056	0.002

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; [a-b]Means in a column with no common superscripts differ significantly ($p \leq 0.05$).
[#] Means represent 15 chickens per treatment.
^ Pooled standard error of mean.

Table 5. Organic acid content in the crop of broiler chickens (µMol/g of digesta).[#]

Supplements	Diets	Formic acid	Acetic acid	Propionic acid	n-Butyric acid	n-Capronic acid	DL-Lactic acid	Succinic acid
Control	PD	nd	13.69	nd	Nd	nd	50.97	3.58
Salinomycin	PD	0.50	11.92	nd	Nd	nd	57.61	3.32
MCT	PD	nd	16.16	nd	Nd	0.72[a]	61.64	6.71
CB	PD	nd	14.01	nd	14.70	nd	58.41	4.09
MCFA	PD	0.21	19.36	nd	Nd	1.57[b]	90.98	5.20
Control	MD	nd	12.57	nd	Nd	nd	65.19	5.51
Salinomycin	MD	nd	14.37	0.29[a]	Nd	nd	78.80	6.14
MCT	MD	nd	17.60	nd	Nd	1.68[b]	72.90	6.71

CB	MD	nd	19.62	nd	11.59	nd	85.75	6.54
MCFA	MD	nd	18.33	nd	Nd	0.92[a]	78.09	6.07
SEM^		0.05	0.84	0.16	0.88	0.10	3.49	0.36
p		0.143	0.323	≤0.001	≤0.001	≤0.001	0.120	0.200
	PD	0.14	14.99	nd	3.06	0.44	64.00	4.51[a]
	MD	nd	16.50	0.05	2.79	0.52	76.14	6.19[b]
	SEM^	0.05	0.73	0.16	0.78	0.07	2.74	0.26
Control		nd	13.18	nd	Nd	nd	57.43	4.46
Salinomycin		0.27	13.03	0.13	Nd	nd	67.24	4.60
MCT		nd	16.88	nd	0.76[a]	1.20	67.27	6.71
CB		nd	16.56	nd	13.29[b]	nd	70.84	5.20
MCFA		0.11	18.89	nd	0.45[a]	1.27	85.12	5.60
SEM^		0.05	0.78	0.16	0.84	0.9	3.13	0.30
Diets		0.090	0.380	0.010	0.861	0.653	0.072	0.023
Supplements		0.196	0.125	0.001	≤0.001	≤0.001	0.133	0.280
Diet ×Supplements		0.277	0.682	≤0.001	0.732	0.001	0.373	0.703

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; nd–not detected - concentration is equal to 0.

[a-b] Means in a column with no common superscripts differ significantly ($p \leq 0.05$).

[#]Means represent 21 chickens in 7 pooled replicates per treatment.

^ Pooled standard error of mean.

Table 6. Organic acids in the ileum of broiler chickens (μMol/g of digesta).[#]

Supplements	Diet	Formic acid	Acetic acid	Propionic acid	n-Butyric acid	DL-Lactic acid	Succinic acid
Control	PD	nd	4.04[cd]	nd	nd	54.15	1.04
Salinomycin	PD	0.37	3.94[cd]	nd	nd	35.06	0.17
MCT	PD	nd	8.65[b]	0.16	nd	83.70	2.06
CB	PD	nd	13.55[a]	nd	0.11[a]	70.23	0.88
MCFA	PD	nd	3.92[cd]	nd	nd	55.51	1.00
Control	MD	nd	1.35[d]	nd	nd	56.79	0.87
Salinomycin	MD	nd	1.33[d]	nd	nd	51.24	0.39
MCT	MD	nd	2.52[cd]	0.16	nd	81.94	0.99
CB	MD	nd	4.00[cd]	0.09	0.46[b]	85.86	1.09
MCFA	MD	0.60	5.24[c]	nd	nd	83.76	1.43
SEM^		0.06	0.58	0.03	0.02	3.73	0.12
p		0.154	≤0.001	0.611	≤0.001	0.007	0.031
	PD	0.07	6.82	0.03	0.02	59.72	1.03
	MD	0.12	2.89	0.05	0.09	71.91	0.95
	SEM^	0.03	0.41	0.02	0.02	3.04	0.07
Control		nd	2.82	nd	nd	55.35[bc]	0.96[ab]
Salinomycin		0.20	2.75	nd	nd	42.41[c]	0.27[b]
MCT		nd	5.87	0.16	nd	82.90[a]	1.57[a]
CB		nd	9.21	0.04	0.29	77.33[a]	0.97[ab]
MCFA		0.27	4.52	nd	nd	68.35[ab]	1.20[a]
SEM^		0.05	0.47	0.03	0.02	3.39	0.10
Diets		0.659	≤0.001	0.701	0.006	0.067	0.733
Supplements		0.292	≤0.001	0.181	≤0.001	0.002	0.011
Diet ×Supplements		0.084	≤0.001	0.965	≤0.001	0.610	0.231

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; nd–not detected - concentration is equal to 0.

[a-b] Means in a column with no common superscripts differ significantly ($p \leq 0.05$).

[#]Means represent 21 chickens in 7 pooled replicates per treatment.

^ Pooled standard error of mean.

Table 7. Organic acid content in cecum of broiler chickens(µMol/g of digesta).[#]

Supplements	Diet	Formic acid	Acetic acid	Propionic acid	Isobutyric acid	n-Butyric acid	Iso-valeric acid	n-Valeric acid	DL-Lactic acid	Succinic acid
Control	PD	nd	65.98	6.41^{cd}	0.61^{cd}	13.17^{cd}	0.23	1.00^{ef}	23.47^{a}	1.55
Salinomycin	PD	nd	69.01	8.62^{cd}	1.32^{a}	13.34^{cd}	0.60	1.47^{de}	nd	1.70
MCT	PD	nd	70.89	10.62^{c}	1.10^{ab}	14.58^{cd}	0.48	1.8^{cd}	nd	1.57
CB	PD	nd	56.30	5.44^{d}	0.38^{d}	10.01^{d}	0.07	0.83^{f}	38.96^{b}	1.82
MCFA	PD	nd	81.40	9.61^{cd}	0.97^{abc}	19.60^{bc}	0.45	1.76^{cd}	nd	2.38
Control	MD	nd	72.91	16.38^{b}	0.97^{abc}	15.67^{cd}	0.44	2.20^{c}	nd	1.85
Salinomycin	MD	nd	89.02	26.16^{a}	0.91^{abc}	26.54^{a}	0.38	3.85^{a}	nd	0.88
MCT	MD	0.97^{a}	89.87	18.10^{b}	1.26^{a}	18.59^{bc}	0.57	2.82^{b}	nd	0.42
CB	MD	nd	85.65	17.89^{b}	0.76^{bcd}	24.17^{ab}	0.28	2.92^{b}	nd	2.24
MCFA	MD	nd	92.87	20.61^{b}	1.11^{ab}	28.14^{a}	0.55	3.32^{ab}	nd	1.21
SEM^		0.05	2.22	0.96	0.05	0.98	0.03	0.13	2.65	0.18
p		≤0.001	≤0.001	≤0.001	≤0.0001	≤0.0001	0.001	≤0.0001	≤0.001	0.475
	PD	nd	68.72^{A}	8.14	0.88	14.14	0.37	1.37	nd	1.80
	MD	0.19	86.06^{B}	19.83	1.00	22.62	0.44	3.02	12.57	1.32
	SEM^	0.05	1.76	0.69	0.02	0.68	0.01	0.09	2.56	0.15
Control		nd	69.13^{b}	10.94	0.77	14.31	0.33^{bc}	1.55	12.80	1.69
S		nd	78.10^{ab}	16.60	1.13	16.40	0.50^{ab}	2.55	nd	1.33
MCT		0.44	79.51^{ab}	14.02	1.17	19.34	0.52^{a}	2.26	0.22	1.05
CB		nd	69.64^{b}	11.10	0.55	16.45	0.17^{c}	1.78	21.25	2.01
MCFA		nd	86.62^{a}	14.61	1.03	23.48	0.49^{ab}	2.47	nd	1.85
SEM^		0.05	1.94	0.81	0.03	0.84	0.02	0.12	2.56	0.16
Diets		0.016	≤0.001	≤0.001	0.120	≤0.001	0.145	≤0.001	0.005	0.204
Supplements		0.002	0.013	0.001	≤0.001	0.001	≤0.001	≤0.001	0.005	0.476
Diet ×Supplements		≤0.001	0.316	0.017	0.025	0.021	0.092	0.003	0.014	0.484

Notes: *S – salinomycin, 0.07 g/kg; MCT –triglyceride form of capric acid and caprylic acid (1.38:1), 3 g/kg; CB – calcium butyrate, 10g/kg; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1), 8.3 g/kg; PD – provocative diet; MD – standard diet; nd–not detected - concentration is equal to 0.

[a-b] Means in a column with no common superscripts differ significantly ($p \leq 0.05$).

[#]Means represent 21 chickens in 7 pooled replicates per treatment.

^ Pooled standard error of mean.

DISCUSSION

Performance: The application of PD, which consisted mainly of cereals rich in NSP, animal fats, and fish meal, significantly decreased performance indices of broiler chickens. This can be attributed to the considerable impact of the aforementioned components on *C. perfringens* counts in the small intestine of chickens (Dahiya 2005). PD was characterized by a higher concentration of crude fiber than that of MD, but the concentration of NSPs were relatively small differ. Authors did not determine soluble NSP but only calculated total NSP content.

Crude fiber as well as NSP (soluble polysaccharides) reduce digestibility and absorption and cause disturbances in water management in the intestine (Zhao *et al.,* 1995; Jorgensen *et al.,* 1996; Bach Kundsen 1997; Jamroz *et al.,*1998a). In contrast, the use of MD (corn and soybean meal) radically (P ≤0.05) improves production indices. Higher body weights were obtained, although not in all experimental treatments with diets containing feed additives.

The most favorable FCR in the group of birds fed with MCT- and MCFA-supplemented MD could be attributed to lower crude fiber concentration and the effect of capric acid on the decrease in feed intake and a simultaneous absence of any influence on growth reduction (Cave, 1982; Furuse *et al.,* 1992). Since MCT is made up, primarily, of capric acid, it can be assumed that this acid, due to its properties to reduce feed intake, caused worse FCR in first period of chickens life.

There is majority of the data in the literature with respect to the effect of SCFAs and MCFAs on the morphology of the ileum (e.g., villus height, crypt depth, and surface area) (Leeson *et al.,* 2005; Adil *et al.,* 2010; Khan and Iqbal 2015) but the information about mass of internal organs is not too much (Furuse 1991; Khatibjoo *et al.,* 2017). In this study, broiler chickens fed with diets supplemented with the examined organic acids were found to have higher mass of the ileum. Nevertheless, in the few available studies, organic acids have been confirmed to lead to an increase in the mass of the small intestine (Furuse *et al.,* 1991) or an elongation of the intestinal villi (Adil *et al.,* 2010), which had a positive effect on broiler growth performance. Similar results have been found in this study.

Digestibility, NR, and AME$_N$: In our own experiment on broiler chickens, the positive impact of CB in terms of the increase in the values of dietary AME$_N$ was determined, which was probably due to the bactericidal and bacteriostatic properties of butyric acid (Lawhon *et al.,* 2002) and their impact on energy utilization in birds (Kirchgessner *et al.,* 1999). But, in literature, it is difficult to find reports confirming the influence of SCFAs on the increase in secretion of pancreatic enzymes, particularly amylase (Katoh 1994). This enhancement (caused as the effect of organic acid supplementation) increased simultaneously with the increase in the length of carbon chains. This higher secretion as seen only for use of organic acids with not more than five carbon atoms in the chain. This theory is confirmed by the study of Greenberger *et al.,* (1966), in which MCFA decreased amylase secretion. From this information, it can be presumed that the factor responsible for the increase in the value of AME$_N$ of CB-supplemented diets was the presence of butyric acid. This also contributed to the improvement of NR in birds. The impact of fatty acids on extended digesta-retention in the stomach could exert some influence on protein digestibility and, consequently, act indirectly on NR in those groups of birds fed with MCT- and CB-supplemented diets. The absence of a positive MCFA impact on NR is surprising, but this could have been caused by a different MCFA metabolism. A more advantageous effect of MCT and CB on the performance of broiler chickens was also confirmed by Hejdysz *et al.,* (2012a, b; Kaczmarek *et al.,* 2016a).

Concentration of SCFAs in intestine: The concentrations of SCFAs synthesized in the GIT of chickens fed with diets varied in terms of the kind of grains and carbohydrate source, and they have also been shown in previous studies to be influenced to a small degree by the use of carbohydrates (Jamroz *et al.,* 1998a, b). In this study, the use of PD or MD led to numerous differences between treatments in terms of SCFA concentrations, in particular, segments of GIT. In two-way ANOVA, an insignificant dietary influence was noted for many fatty acids (formic acid, acetic acid, propionic acid, *n*-butyric acid, capronic acid, and DL-lactic acid), except succinic acid in the crop. More acetic acid was found ($P \leq 0.05$) in the ileum of PD-fed chickens (6.82 μMol/g of digesta) than that of MD-fed chickens (2.89 μMol/g of digesta). In total, the greatest concentration of SCFA ($P \leq 0.05$) was determined in cecum digesta of MD-fed chickens (52% more than that of PD-fed chickens).

Variation in the concentration of dietary NSP—carbohydrate fermentation products in the intestine may, to a limited degree, influence their energetic utilization in chickens. Based on NSP, digestibility and SCFA share the calculated energetic value of fermented NSP derived from triticale-rich diets amounted to 7.8–8.6 KJ/g NSP, but from barley-based diets, this was only 2.7–3.2 KJ/g NSP (Kirchgessner *et al.,* 1999; Jamroz *et al.,* 2000). Despite significant numerical differences between the treatments, greater SCFA concentrations were found in the chickens fed with MCFA-, MCT-, and CB-supplemented diet than that of control chickens. The supplementation of diet with MCT, CB, or MCFA enhanced the content of lactic acid and acetic acid in the ileum. In cecum, the highest concentrations of acetic acid, butyric acid, and isobutyric acid than that of control chickens (25, 34, and 64% more, respectively) were determined when feed was supplemented with MCFA. A decrease in the concentration of SCFA in the ileum obtained via S supplementation (Czerwiński *et al.,* 2012) was not observed in this study.

Conclusion: Results obtained in this study suggest that MCT, CB, and MCFA can increase growth performance of MD-fed chickens , whereas there was a lack of growth performance in case of PD-fed chickens. A positive effect of supplemented additives on AME$_N$ value was found only for CB. The type of diet used affected mass of internal organs (except cecum). Supplemented additives (MCT, CB, and MCFA) decreased the mass of the liver and increased the mass of the ileum. The use of CB and MCFA in broiler diets increased lactic acid content in ileum, which had an impact on their performance.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication and dissemination of the information provided herein.

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