Effects of dietary short- and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens

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ABSTRACT
The objective of this study was to determine the effect of short-chain fatty acids (SCFAs; C4 powder) and medium-chain fatty acids (MCFAs; Bergaprime) on performance, carcass characteristics, and some serum parameters of broiler chickens. A total of 200 one-day-old male broiler chicks (Ross 308) were assigned to five dietary treatments, including control diets (C), C plus Virginiamycin (200 g/ton; ANTI) as positive control, C plus MCFAs supplement (1 kg Bergaprime/ton; M), C plus SCFAs supplement (3 kg C4 powder/ton in starter and 1.5 kg/ton in grower and finisher diets; S), and C plus the combination of SCFAs and MCFAs supplement (SM), as mentioned above according to a completely randomized design. Each treatment consisted of 4 replicates with 10 chicks each. There were no significant differences in body weight, feed intake and feed conversion ratio, and carcass traits among the treatments. SCFAs and MCFAs treatments had higher heterophil and lower lymphocyte percentage compared to the control treatment. Blood glucose and cholesterol concentrations were decreased in MCFAs and SCFAs (p < .05). Lipid percentage of thigh meat of MCFAs and SCFAs was decreased. These results indicated that dietary MCFAs and SCFAs positively decreased broiler chicken meat.

1. Introduction
The modern broiler has been intensely selected for higher growth rates and increased feed conversion (Pakdel et al. 2002). Short-chain fatty acids (SCFAs) are saturated aliphatic organic acids that consist of one to six carbons, of which acetate (C2), propionate (C3), and butyrate (C4) are the most abundant (>95%) (Cook and Sellin 1998). Butyrate, which is a by-product of microbial fermentation of products such as resistant starch, is considered to be important for the normal development of epithelial cells (Przyde et al. 2002). Butyrate appears to play a role in the development of the intestinal epithelium and, therefore, seems to be both bactericidal and a stimulant of villi growth (Leeson et al. 2005). In fact, butyric acid, the major energy source to enterocytes, is essential to the health of intestinal mucosa (Isolauri et al. 2004), and as an organic acid, it did not affect broiler chicken performance challenged with Salmonella enterica subsp. Typhimurium (Abudabos et al. 2017). On the other hand, the addition of 2 ml of Aciflex® as organic acid per litre of Japanese quails drinking water improved the performance and dressing percentage (Khan et al. 2016). SCFAs may also lower the cholesterol synthesis rate by decreasing the enzyme activity of hepatic 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and plasma glucose levels by increasing the gut hormone peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) via activation of receptors Ffar2 and Ffar3 (Rodwell et al. 1976). In addition, gut hormone peptides PYY and GLP-1 play an important role in the communication between tissues, and GLP-1 indirectly regulates blood glucose levels by increasing the secretion of insulin and decreasing the secretion of glucagon by the pancreas (den Besten et al. 2013).

Many potential alternatives for antibiotic growth promoters (AGPs) have been described. Among these, SCFAs and medium-chain fatty acids (MCFAs) affect parameters that are also affected by the banned AGPs (Canibe et al. 2001). SCFAs and MCFAs have been reported to have antibacterial properties due to their ability to cross bacterial membranes in their undissociated form (Dierick et al. 2002). MCFAs have been shown to be good alternatives for nutritional antibiotics in piglets, due to their high antibacterial activity, and they enter the cell undissociated (Dierick et al. 2002). Once in the cell, the MCFA dissociates followed by a drop in pH and results in the inactivation of the bacterial cell. The MCFA inhibits the production of lipases by the bacteria (Dierick et al. 2002). As lipases are needed to allow the bacteria to attach to the intestinal wall, this process will be prohibited and the bacteria will be washed out (Dierick et al. 2002). Furthermore, the antibacterial potency of MCFAs is believed to exceed that of SCFAs (Hermans et al. 2010). During the first week, MCFAs are important players in the build-up and maintenance of the poultry’s health.
(Ding and Lilburn 1997). Therefore, this experiment was carried out to determine the effect of MCFAs (C6–C12) alone or in combination with SCFAs (C2–C6) on broiler chicken performance, carcass characteristics, haematology, and some serum parameters.

2. Materials and methods

2.1. Experimental design

A total of 200 one-day-old male broiler chicks (Ross 308) were purchased from a commercial broiler hatchery located in Gilan Province, Iran. The broilers were housed in identical-sized four-floor battery cages. Environmental temperature was set at 31°C for the first week and 28°C for the second week, which was further decreased to 22°C until the end of the experiment. The relative humidity was allowed to fluctuate, but not to levels below 55% throughout the study. Table 1 lists the basal diet formulated to meet the nutrient requirements of broilers provided by Ross Broiler Manual (Ross 2009). In a completely randomized block design, broilers were allotted to 5 dietary treatments with 4 replicates and 10 birds each. The experimental diets consisted of control diets (C), C plus Virginiamycin (200 g/ton; ANTI) as positive control, C plus MCFAs supplement (1 kg Bergaprime/ton in grower and finisher diets; M), C plus SCFAs supplement (3 kg C4 powder/ton in starter and 1.5 kg/ton in grower and finisher diets; S), and C plus the combination of SCFAs and MCFAs supplement (SM) as mentioned above. The SCFAs (C4 powder, Sana-dam Co. Tehran, Iran) and MCFAs (Bergaprime, Lodestar TM C8-10, Loders Croklaan, Wormerveer, the Netherlands) supplements were added to the C diet as a substitution of soybean oil. The birds were kept under conventional conditions for vaccination, temperature, ventilation, and lighting based on Ross catalogue recommendations and were fed experimental diets from 1 to 42 d of age. The broiler diets were formulated based on standardized ileal digestible amino acids (Hoehler et al. 2005) and other requirements were obtained from Ross catalogue recommendations. Body weight gain (BWG) and feed intake (FI) were recorded for each period and during the whole experiment (1–42 d of age); then feed conversion ratio (FCR) was calculated.

2.2. Determination of blood parameters and internal organ weights

Blood samples were collected from the wing vein of two chicks from each replicate in non-heparinized collection tubes at 42 d of age. Serum was separated by centrifugation of the blood samples for 20 min at 1300 g. Then, the serum samples were stored at −20°C until further analysis. At the same age, two chicks per replicate were randomly selected and slaughtered. The weight of internal organs was measured with a digital scale with an accuracy of 0.001 g. When the head, shanks, and feathers were removed, the carcass was eviscerated by cutting around the vent to remove all of the viscera. Once eviscerated, the carcass without giblets was weighed and expressed as a percentage of its initial live weight and considered as the carcass yield. Breast and thighs were weighed and expressed as percentages of the live weight. The weights of the liver, gizzard, and abdominal fat were also measured and expressed as percentages of the live body weight. Serum glucose, triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and total cholesterol were analysed using diagnostic kits (Pars Azmun, Tehran, Iran) and enzymatic methods. Blood samples were collected from the wing vein of two chicks from each replicate in non-heparinized collection tubes at 28 d of age, then heterophil (H) and lymphocyte (L) percentages were determined, and H:L ratio was calculated. At 42 d of age, two chicks per replicate were randomly selected, slaughtered, and the weight of spleen, bursa, and thymus was measured with a digital scale with an accuracy of 0.001 g.

2.3. Lipid extraction

Four grams of muscle samples was weighed into a test tube with 10 volumes of Folch 1 (chloroform:methanol = 2:1, wt/vol; Folch et al. 1957) and homogenized with a Brinkman polytron (Type PT 10/35) for 10 s at high speed. Twenty-five micrograms of butylated hydroxyanisole (10%) dissolved in 98% ethanol was added to each sample prior to homogenization. The homogenate was filtered through a Whatman #1 filter paper into a 100-mL
graduated cylinder and quarter volume (on the basis of Folch 1) of 0.88% NaCl solution was added. After the cylinder was capped with a glass stopper, the filtrate was mixed well. The inside of the cylinder was washed twice with 10 mL of Folch 2 (3:47:48/CHCl3:CH3OH:H2O), and the contents were stored until the aeous and organic layers clearly separated. The upper layer was siphoned off, and the lower layer was moved to a glass scintillation vial and dried at 50°C under nitrogen (Folch et al. 1957). Then lipid percentage of meat was recorded. Also, breast and thigh meat nitrogen was detected by the Kjeldahl method (Davidson et al. 1970).

### 2.4. Gut morphology

Five birds were selected from each treatment group at 42 d for the collection of small intestine samples (AVMA 2007). Immediately after euthanasia, the intestines were removed. Jejunum samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 h, and 4.0-µm sections were prepared. The sections were stained with standard haematoxylin–eosin solution and observed for villus height (VH), villus width (VW), crypt depth (CD), and lamina propria (LP) thickness at 100× magnification by light microscopy using a calibrated ocular micrometer. Ten microscopic fields per bird were measured.

### 2.5. Statistical analysis

The data were analysed using a completely randomized design by utilizing the General Linear Model procedures of SAS 9.1 (SAS Inst. Inc., Cary, NC, USA) (SAS 2001). Differences among mean treatments were determined using Duncan’s least significant multiple range test (p < .05).

### 3. Results and discussion

#### 3.1. Growth performance

The performance data of different treatments are presented in **Table 2**. No significant differences were observed in body weight and weight gain among the treatments during the starter (1–12 d), grower (13–25 d), and finisher (26–42 d) periods (p > .05). Similarly, various treatments had identical FI and FCR (p > .05) except FCR of 1–12 d of age (p < .05), so that broilers fed diet containing MCFAs had the highest FCR and the control group birds had the lowest FCR (p < .05). These results are in accordance with other studies, which suggested that there were no significant differences among broilers receiving diets containing 0.1%, 0.15%, or 0.2% MCFAs compared to those receiving the control diet (Leeson et al. 2005; Del Alamo et al. 2007; Shokrollahi et al. 2014). Also, none of the MCFAs (10 g/kg level (1% diet)) or SCFAs (acetic, butyric, or caprylic acid) had a significant effect on FI in comparison with maize oil, but 30 g/kg (3% diet) of MCFA containing caprylic (C8:0), capric (C10:0), and lauric (C12:0) acid groups significantly increased FI and weight gain in chicks (Cave 1982). The inclusion of 0.2% or 0.3% butyric acid did not influence broiler chickens’ BWG, FI, or FCR at the whole period of the experiment (Mahdavi and Torki 2009). It has been suggested that in case of well-nourished healthy chicks housing at a moderate stoking density and hygienic condition, dietary inclusion of MCFAs or SCFAs was ineffective on the birds’ performance (Pinchasov and Jensen 1989). Free butyric acid is absorbed very quickly in the upper digestive tract, and will likely be of limited use other than as a feed sanitizer, and, unlike other acids such as propionic acid, did not depress FI (Pinchasov and Jensen 1989). In the current study, adding up to 0.3% SCFAs or 0.1% MCFAs had no detrimental effect on FI. MCFAs and SCFAs have G-protein-coupled receptors (GPR) at the cell surface of some organs such as GPR120 that work in macrophages and adipocytes, mediating potent anti-inflammatory and insulin-sensitizing effects (Lagakos and Oh 2011). SCFA concentrations are also sensed by specific GPRs, which are involved in the regulation of lipid and glucose metabolism. GPR41 and GPR43 were identified as SCFA receptors (den Besten et al. 2013). After the discovery of the SCFA receptors, GPR41 was renamed as free fatty acid receptor (Ffar)3 and GPR43 became Ffar2. The distinct chain length density and hygienic condition, dietary inclusion of MCFAs or SCFAs was ineffective on the birds

### Table 2. Effect of dietary treatments on broiler chicken body weight (g), FI (g), and FCR (feed/gain).

| Item | Treatment* | C | ANTI | M | S* | SM | SEM | p-Value |
|------|------------|---|------|---|----|----|-----|---------|
| Body weight | D 1-12 | 242.67 | 203.18 | 202.29 | 199.43 | 214.61 | 15.15 | 0.35 |
| | D 13-25 | 641.13 | 659.46 | 672.17 | 675.24 | 695.83 | 21.17 | 0.49 |
| | D 26-42 | 1255.36 | 1273.27 | 1281.82 | 1264.19 | 1321.02 | 39.95 | 0.89 |
| | D 1-42 | 2139.16 | 2135.91 | 2156.28 | 2138.86 | 2231.46 | 47.58 | 0.82 |
| FI | D 1-12 | 348.18 | 334.46 | 299.71 | 364.46 | 386.02 | 15.15 | 0.35 |
| | D 13-25 | 1037.16 | 1007.14 | 1068.44 | 1039.3 | 1074.4 | 21.17 | 0.49 |
| | D 26-42 | 2442.81 | 2417.54 | 2477.65 | 2453.52 | 2396.96 | 39.95 | 0.89 |
| | D 1-42 | 3828.15 | 3759.14 | 3845.8 | 3857.28 | 3857.38 | 112.23 | 0.46 |
| FCR | D 1-12 | 1.43 | 1.65 | 1.52 | 1.83 | 1.80 | 0.051 | 0.002 |
| | D 13-25 | 1.62 | 1.53 | 1.59 | 1.54 | 1.54 | 0.033 | 0.38 |
| | D 26-42 | 1.95 | 1.9 | 1.93 | 1.94 | 1.82 | 0.067 | 0.71 |
| | D 1-42 | 1.79 | 1.76 | 1.78 | 1.8 | 1.73 | 0.065 | 0.55 |

Notes: a,b,c values in the same row with no common superscript are significantly different (p < .05). The data were obtained from 4 lots of 10 birds each per group.

*: C means control, ANTI means control plus virginiamycin (200 g/ton), M means control plus MCFAs (1 kg/ton), S means control plus SCFAs, and SM means control plus SCFAs + MCFAs.

**: C4 used 3 kg per ton in the starter and 1.5 kg/ton in the finisher and grower diet.
(Xiong et al. 2004; Zaibi et al. 2010). Although dietary treatments increased the FCR of broiler chickens and it may be due to increasing the energy expenditures during the starter period, it was expected that MCFAs and SCFAs influence the performance of broiler chickens via the above mentioned mechanism, but dietary inclusion of MCFAs or SCFAs were ineffective on the birds’ performance.

### 3.2. Haematology

Birds fed the SM diet had the highest H percentage and H:L ratio and the lowest L percentage compared to the C diet, whereas using MCFAs and SCFAs supplements alone had an intermediate but significant effect on white blood cell population (p < .05; Table 3). Dietary treatments had no effect on bursa, spleen, and lymphoid weight (p > .05; Table 3). These results are not in accordance with those reported previously which suggested there were no significant differences among broilers receiving diets containing 2–3 g/kg butyric acid compared to those receiving control diet (Mahdavi and Torki 2009).

G protein-coupled receptors consist of a family of cell surface receptors that sense various extracellular stimuli, including light, odorants, peptides, nucleotides, neurotransmitters, and hormones. In the past decade, several GPRs have been reported to be activated by free fatty acids (FFAs). GPR84 is a special receptor for MCFAs in macrophages and polymorphonuclear leukocytes (PMNs), and MCFAs elicited chemotaxis of PMNs and macrophages and amplified LPS-stimulated production of the proinflammatory cytokine IL-8 from PMNs and Tumor necrosis factor alpha from macrophages (Suzuki et al. 2013). MCFAs and SCFAs have GPRs at the cell surface of some organs like GPR120 that work in macrophages and adipocytes, mediating potent anti-inflammatory and insulin-sensitizing effects (Lagakos and Oh 2011). Both GPR41 and GPR43 are expressed in PMNs as well, to which SCFAs elicit chemotaxis (Le Poul et al. 2003).

### 3.3. Carcass traits

The effects of dietary treatments on broiler carcass traits are presented in Tables 4 and 5. Carcass, breast, and thigh meat yields were not considerably affected by treatments (p > .05). No significant impact of MCFAs or SCFAs were found on abdominal fat and gall bladder weights (p > .05); however, the mean of them tended to decrease in broilers fed S, M, and SM treatments (p > .05). Dietary treatments had no effect on breast and thigh meat percentage (p > .05; Table 4). On the other hand, breast and thigh meat protein percentage and breast meat lipid were not affected by MCFA or SCFA levels in the experimental diets, while thigh meat lipid percentage significantly decreased by S or M diets and tended to further reduce in broilers fed SM diet (Table 5). These findings were similar to those reported by others who suggested that dietary Medium-chain triglyceride (MCT) decreased the abdominal fat percentage in broiler chickens (Chiang et al. 1990). Experiments in other animal species and human subjects also showed that MCT reduces body fat deposition (Scalfe et al. 1991). Suppression of lipid absorption (Nakai et al. 2005), reduction in calorie intake (Van Gaal et al. 2005), reduction in the biosynthesis of fatty acids, and enhancement of fatty acid oxidation (Murase et al. 2001) are possible mechanisms of the reduction in body fat. Conversely, gall bladder relative weight tended to reduce in MCFA treatments. Therefore, MCFAs may have special effects on lipid digestion and absorption and diminishing of energy consumption (Moharrery 2006); further researches are needed in this regard.

The MCFAs and SCFAs have a different metabolic fate than long chain fatty acids, and they are rapidly absorbed in the small intestine, transported to the liver as FFAs via hepatic

### Table 3. Effect of dietary treatments on carcass trait of broiler chickens (%BW).

| Treatments | ABF | GB | Carcass | BMY | TMY | CW (kg) |
|------------|-----|----|---------|-----|-----|---------|
| C (control)| 27.4 | 0.104 | 69.195 | 19.012 | 20.705 | 1.60 |
| ANTI       | 2.57 | 0.106 | 70.192 | 20.576 | 20.647 | 1.56 |
| M          | 2.35 | 0.082 | 70.501 | 21.766 | 19.258 | 1.69 |
| S          | 2.30 | 0.085 | 70.208 | 19.224 | 20.377 | 1.66 |
| SM         | 2.49 | 0.083 | 68.900 | 20.338 | 19.563 | 1.62 |
| SEM        | 0.051 | 0.015 | 1.99 | 0.88 | 1.16 | 0.07 |
| p-Value    | .94 | .78 | .99 | .26 | .92 | .79 |

- ABF: abdominal fat; GB: gall bladder; BMY: breast meat yield; TMY: thigh meat yield; CW: carcass weight.
- C means control, ANTI means control plus virginamaycin (200 g/ton), M means control plus MCFAs (1 kg/ton), S means control plus SCFAs, and SM means control plus SCFAs + MCFAs.
- C4 used 3 kg per ton in the starter and 1.5 kg/ton in the finisher and grower diet.

### Table 4. Effect of dietary treatments on carcass trait of broiler chickens (%BW).

| Treatments | Protein | Lipid | Protein | Lipid |
|------------|---------|-------|---------|-------|
| C (control)| 23.53 | 4.82 | 20.09 | 10.17 |
| ANTI       | 22.95 | 3.66 | 21.08 | 9.16 |
| M          | 15.78 | 4.33 | 19.65 | 8.68 |
| S          | 23.62 | 3.51 | 21.21 | 8.71 |
| SM         | 22.64 | 2.83 | 19.04 | 7.17 |
| SEM        | 12.45 | 3.61 | 20.04 | 5.17 |
| p-Value    | .38 | .28 | .63 | .03 |

- C means control, ANTI means control plus virginamaycin (200 g/ton), M means control plus MCFAs (1 kg/ton), S means control plus SCFAs, and SM means control plus SCFAs + MCFAs.
- C4 used 3 kg per ton in the starter and 1.5 kg/ton in the finisher and grower diet.
Thigh meat lipid percentage significantly decreased by S or M diets and tended to further reduce in broilers fed SM diet. Thigh meat has more lipid than breast meat, and the reduction in the lipid content of thigh meat by SCFAs may be explained by SCFAs regulating the balance between fatty acid synthesis, fatty acid oxidation, and lipolysis in the body (den Besten et al. 2013). Fatty acid oxidation is activated by SCFAs, while de novo synthesis and lipolysis are inhibited. Besides the receptors Ffar2 and Ffar3 that increase leptin secretion from adipocytes, AMP-activated protein kinase (AMPK) plays an important role in this regulation, and SCFAs have been shown to increase the AMPK activity in liver and muscle tissues (Gao et al. 2009).

The decreasing effects of dietary SCFAs and MCFAs on thigh meat lipid percentage can be attributed to the intensifying leptin secretion and its action on lipid metabolism. In vitro and in vivo experiments showed that SCFAs increase leptin expression via a Ffar2-dependent pathway (Xiong et al. 2004; Zaibi et al. 2010). Leptin, an adipokine that regulates energy expenditure and food intake, stimulates fatty acid oxidation by increasing the AMP/ATP ratio and AMPK activity in liver and muscle tissues (Minokoshi et al. 2002).

### 3.4. Serum parameters

The effects of the treatments on blood parameters of 42-d-old broilers are given in Table 6. The serum glucose and cholesterol concentration of broilers fed M, S, and SM diets significantly decreased ($p < 0.1$). On the other hand, serum triglyceride (TG), LDL-C, and HDL-C were not influenced by the dietary treatments ($p > 0.05$). In agreement with the current results, MCFAs reduced plasma cholesterol in rats (Geelen et al. 1995; Shokrollahi et al. 2014), and on the other hand, the concentration of plasma cholesterol in humans was not affected after treatment with an MCT-containing formula (Hill et al. 1990). Resting oxygen consumption in the MCFAs-fed rats was significantly higher than that of the long chain triglyceride-fed group (Baba et al. 1982). It may be concluded that MCFAs stimulate the release of insulin, then glucose uptake by breast muscle increases, and the resulting energy from the oxidation of glucose can be used for protein synthesis.

SCFA concentrations are also sensed by specific GPRs, which are involved in the regulation of lipid and glucose metabolism. SCFAs may also affect plasma glucose levels by increasing the gut hormones PYY and GLP-1 via the activation of the receptors Ffar2 and Ffar3, which leads to reducing the concentration of blood glucose (den Besten et al. 2013). In addition, the gut hormones peptide YY and GLP-1 play an important role in the communication between tissues so that GLP-1 indirectly regulates blood glucose levels by increasing the secretion of insulin and decreasing the secretion of glucagon by the pancreas (Barrera et al. 2011). In summary, SCFAs seem to beneficially affect glucose metabolism by normalizing plasma glucose levels and increasing glucose handling in blood and immune organs.

The serum TG concentration was affected by treatments. These findings were partly similar to those reported by others who reckoned that dietary MCFAs did not influence the serum concentrations of TG as compared to maize oil in primary hypertriglyceridaemic subjects (Asakura et al. 2000). Also, in vitro studies showed that propionate lowered the cholesterol synthesis rate by decreasing the enzyme activity of HMGCS and HMGCR (Bush and Milligan 1996). However, the observations of the present study did not fully support the earlier reports with respect to the deleterious effects of dietary MCFAs on cholesterol concentration in human subjects, which may be due to differences in species and/or the composition of MCFAs. In this study, the concentrations of serum ketone bodies were not analysed. Moreover, serum triacylglycerol concentration did not differ among the treatments at the end of the experimental period (Table 5); therefore, it is difficult to draw conclusions from our data. Reduction in thigh meat lipid can be explained by the effect of SCFAs and MCFAs on leptin concentration.

### 3.5. Gut morphology

The effects of dietary treatments on broilers’ jejunum morphology are given in Table 7. The VH and VW, CD, and LP thickness were not significantly affected by the treatments ($p > 0.05$). These findings were not in agreement with other studies, which reported that 0.2% dietary sodium butyrate increased the duodenal crypt depth of broiler chickens compared to that in bacitracin-fed birds (Leeson et al. 2005). Also, the addition of 0.2% C4 used 3 kg per ton in the starter and 1.5 kg/ton in the finisher and grower diet.

### Table 6. Effect of dietary treatments on blood metabolites of broiler chickens (%).

| Treatments | Glucose | Cholesterol | TG | HDL-C | LDL-C |
|------------|---------|-------------|----|-------|-------|
| C (control) | 214.80<sup>a</sup> | 128.24<sup>b</sup> | 70.23 | 79.48 | 27.88 |
| ANTI | 220.18<i>+</i> | 140.64<i>+</i> | 95.91 | 73.01 | 34.06 |
| M | 143.39<i>+</i> | 101.04<i>+</i> | 80.23 | 55.43 | 41.26 |
| S<sup>*</sup> | 194.39<i>+</i> | 105.28<i>+</i> | 115.91 | 53.03 | 46.16 |
| SM | 173.77<i>+</i> | 107.20<i>+</i> | 81.36 | 59.85 | 34.68 |
| SEM | 24.2 | 8.83 | 18.73 | 16.46 | 7.28 |
| p-Value | .0228 | .03 | .89 | .74 | .76 |

Notes: a,b,c Values in the same column with no common superscript are significantly different ($p < 0.05$). TG: triglyceride; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol. The data were obtained from 4 lots of 10 birds each per group.

### Table 7. Effect of dietary treatments on jejunum morphology of broiler chickens (μm).

| Treatments | Villus height | Villus width | Crypt depth | Lamina propria thickness |
|------------|--------------|--------------|-------------|--------------------------|
| C (control) | 1085.5 | 181.75 | 187.00 | 16.00 |
| ANTI | 888.8 | 204.50 | 169.75 | 14.50 |
| M | 1266.5 | 224.50 | 237.00 | 15.00 |
| S<sup>*</sup> | 1080.0 | 196.75 | 188.00 | 16.75 |
| SM | 1261.8 | 194.75 | 182.25 | 15.75 |
| SEM | 175.77 | 20.28 | 28.27 | 1.49 |
| p-Value | .55 | .67 | .53 | .84 |

Notes: C means control, ANTI means control plus virgianaminacyn (200 g/ton), M means control plus MCFAs (1 kg/ton), S means control plus SCFAs, and SM means control plus SCFAs + MCFAs.
butyrate to broiler chickens’ diet increased the villus height in jejunum and ileum, while it decreased that of duodenum, and the number of goblet cells decreased in duodenum and jejunum and increased in ileum (Haghighi-Khosro et al. 2010). Infusion of butyrate into fistulated rats increased the proliferation of crypt cells in both the small and large intestines (Sakata 1987), and the effect on crypt cell growth may reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity (Sharma et al. 1995). The levels of SCFAs are quite low in the intestine and caeca of young chicks (van der Wielen et al. 2000) and so the neonate may be the best candidate for diet supplementation. There were indications that unlike antibiotics, butyrate helps in the maintenance of intestinal villi structure, compared to the negative effects of antibiotics.

4. Conclusions

In conclusion, our results suggest SCFAs and MCFAs supplementation in broiler diet to be beneficial, especially by lowering serum cholesterol, abdominal fat, and thigh meat fat percentage, and this might be attributable to the ability of SCFAs and MCFAs to improve meat quality. It is recommended that the supplementation of SCFAs and MCFAs in broiler diet, like in our experiment, may lead to leaner meat, and improvement of growth performance in poultry may be achieved, to some extent, through the modulation of the response to feed additives. This series of studies may be valuable to the poultry industry.

Note

1. CH30, Olympus, Tokyo, Japan.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. 2017. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidative status in broilers exposed to Salmonella typhimurium challenge during the starter phase. J Appl Anim Res. 45:538–542.

Asakura L, Lottenberg AM, Neves MQ, Nunes VS, Rocha JC, Passarello M, Nakandakare ER, Quintão EC. 2000. Dietary medium-chain triacylglycerol prevents the postprandial rise of plasma triacylglycerols but induces hypercholesterolemia in primary hypertriglyceridemic subjects. Am J Clin Nutr. 71:701–705.

AVMA. 2007. AVMA guidelines on Euthanasia (formerly report of the AVMA panel on Euthanasia). Available from: https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx.

Baba N, Bracco E, Hashim S. 1982. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. Am J Clin Nutr. 35:678–682.

Bach AC, Ingenbleek Y, Frey A. 1996. The usefulness of dietary medium-chain triglycerides in body weight control: fact or fancy? J Lipid Res. 37:708–726.

Barrera JG, Sandoval DA, D’alesio DA, Seeley RJ. 2011. GLP-1 and energy balance: an integrated model of short-term and long-term control. Nat Rev Endocrinol. 7:507–516.

Bush R, Milligan L. 1971. Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. Can J Anim Sci. 51:121–127.

Canine N, Engberg RM, Jensen BB. 2001. An overview of the effect of organic acids on gut flora and gut health. Proceedings workshop on alternatives to feed antibiotics and anticoagulants in the Pig and poultry meat production; Oslo, Norway.

Cave N. 1982. Effect of dietary short-and medium-chain fatty acids on feed intake by chicks. Poult Sci. 61:1147–1153.

Chiang S, Huang K, Lee H. 1990. Effects of medium chain triglyceride on energy metabolism, growth and body fat in broilers. J Chinese Soc Anim Sci. 19:11–19.

Cook S, Sellin J. 1998. Review article: short chain fatty acids in health and disease. Aliment Pharm Ther. 12:499–507.

Davidson J, Mathieson J, Boyne A. 1970. The use of automation in determining nitrogen by the kjeldahl method, with final calculations by computer. Analyst. 95:181–193.

Del Alamo AG, De Los Mozos J, Van Dam JTP, De Ayala PP. 2007. The use of short and medium chain fatty acids as an alternative to antibiotic growth promoters in broilers infected with malabsorption syndrome. Proceedings of the 16th European Symposium on Poultry Nutrition; Strasbourg, France. p. 317–320.

den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 54:2325–2340.

Dierick N, Decuyper J, Molly K, Van Beek E, Vanderbeke E. 2002. The combined use of triacylglycerols containing medium-chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition: I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets. Livest Prod Sci. 75:129–142.

Ding S, Lilburn M. 1997. Inclusion of coconut oil in diets for Turkey breeders and its effects on embryonic yolk and liver fatty acids. Poult Sci. 76:1714–1721.

Folch J, Lees M, Sloane-Stanley G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226:497–509.

Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. 2009. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 58:1509–1517.

Geelen MJ, Scoths WJ, Bijleveld C, Beynen AC. 1995. Dietary medium-chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triglyceride synthesis in rats. J Nutr. 125:2449.

Haghighi-Khosro P, Akbari Azad G, Moayer F, Pajouhandeh I. 2010. Effect of dietary butyrate on performance and small intestinal morphology of broilers. J Vet Clin Res. 1:235–242.

Hermans D, Martens D, Verlinden M, Van Immerseel F, Garmyn A, Messens W, Heyndrickx M, Haesebrouck F, Pasmans F. 2010. Intestinal mucus protects campylobacter jejuni in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids. Poult Sci. 89:1144–1155.

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

Hoehler D, Lemme A, Ravindran V, Bryden W, Rostagno H. 2005. Feed formulation in broiler chickens based on standardized ileal amino acid digestibility. Proceedings of the 3rd Mid-Atlantic Nutrition Conference; 15–17 Noviembre, Monterrey, Nuevo León, México: Universidad Autónoma de Nuevo León. p. 78–91.
Isolauri E, Salminen S, Ouwehand AC. 2004. Probiotics. Best Pract Res Clin Ga. 18:299–313.

Khan RU, Chand N, Ali A. 2016. Effect of organic acids on the performance of Japanese quails. Pak J Zool. 48.

Lagakos WS, Oh DY. 2011. The role of G-protein-coupled receptors in mediating the effect of fatty acids on inflammation and insulin sensitivity. Curr Opin Clin Nutr. 14:322–327.

Le Poul E, Loison C, Struyf S, Springael J-Y, Lannoy V, Decobecq M-E, Brezillon S, Dupriez V, Vassart G, Van Damme J, et al. 2003. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem. 278:25481–25489.

Leeson S, Namkung H, Antongiovanni M, Lee E. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult Sci. 84:1418–1422.

Mahdavi R, Torki M. 2009. Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. J Anim Vet Adv. 8:122.

Murase T, Nagasawa A, Hase T, Tokimitsu I, Shimasaki H, Itakura H. 2001. Dietary tea catechins reduce development of obesity accompanied with gene expression of lipid-metabolizing enzymes in mice. J Oleo Sci. 50:711–715.

Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, Mitsunaga T, Hashimoto F, Kiso Y. 2005. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. J Agric Food Chem. 53:4593–4598.

Odle J, Benevenga NJ, Crenshaw TD. 1991. Utilization of medium-chain triglycerides by neonatal piglets: chain length of even-and odd-carbon fatty acids and apparent digestion/absorption and hepatic metabolism. J Nutr. 121:605–614.

Pakdel A, Van Arendonk JA, Vereijken AL, Bovenhuis H. 2002. Direct and maternal genetic effects for ascitesrelated traits in broilers. Poult Sci. 81:1273–1279.

Pinchasov Y, Jensen L. 1989. Effect of short-chain fatty acids on voluntary feed of broiler chicks. Poult Sci. 68:1612–1618.

Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett. 217:133–139.

Rodwell W, Nordstrom JL, Milschen JJ. 1976. Regulation of HMG-CoA reductase. Adv Lipid Res. 14.

ROSS [Manual]. 2009. ROSS broiler management manual. Newbridge, Scotland: Aviagen Ltd.

Sakata T. 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br J Nutr. 58:95–103.

SAS I. 2001. SAS/WATTM user’s guide. Cary, NC: SAS Institute, Inc.

Scalfi L, Coltorti A, Contaldo F. 1991. Postprandial thermogenesis in lean and obese subjects after meals supplemented with medium-chain and long-chain triglycerides. Am J Clin Nutr. 53:1130–1133.

Sharma R, Schumacher U, Ronaasen V, Coates M. 1995. Rat intestinal mucosal responses to a microbial flora and different diets. Gut. 36:209–214.

Shokrollahi B, Yavari Z, Kordestani A. 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.

Suzuki M, Takaishi S, Nagasaki M, Onozawa Y, Iino I, Maeda H, Komai T, Oda T. 2013. Medium-chain fatty acid-sensing receptor, GPR84, is a proinflammatory receptor. J Biol Chem. 288:10684–10691.

Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S, Group R-ES. 2005. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. The Lancet. 365:1389–1397.

Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M. 2004. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. FEBS Lett. 584:2381–2386.