

Using different levels of coated calcium sodium butyrate in the diet of quail on growth performance, duodenal histomorphology, and some biochemical parameters

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Abstract: The aim of this study was to determine growth performance, duodenal histomorphology, and some biochemical parameters in quails' diets containing coated calcium sodium butyrate (CSB). One hundred sixty 1-day-old Japanese quails were arranged into four trial groups consisting of 5 replicates with eight chicks per replicate. One group was formed as a control group without CSB, and the other experimental groups were formed by adding CSB at the level of 0.06% (CSB - 1), 0.08% (CSB - 2), and 0.1% (CSB - 3). All diets were prepared isonitrogenically and isocalorically, with 24% CP and 3000 kcal/kg ME content. The trial was completed at 35 days. At the end of the study, the highest body weight (BW) and body weight gain increase in the CSB - 3 group, which was supplemented with 0.1% CSB. The feed conversion ratio was to determine that ad a linear and quadratic improvement in the CSB - 3 group. The highest villi height was found in the CSB - 2 group, the highest villi: crypt ratio was found in the CSB - 3 group, and the lowest crypt depth was found in the CSB - 3 group. Serum aspartate aminotransferase, alanine aminotransferase, low-density lipoprotein, high-density lipoprotein, and triglyceride values decreased in the experimental groups compared to the control group. However, Ca and P values increased in the control group. No differences were noticed in carcass yield and visceral weights of the experimental groups.

Key words: Blood parameters, butyric acid, crypt, growth performance, quail, villi

1. Introduction

In poultry, growth, and productivity depend on intestinal digestion and absorption of the nutrients taken. Since the deterioration of intestinal health will adversely affect nutrient absorption, growth performance can be significantly damaged [1]. At the same time, it negatively affects digestion and absorption, impairing the essential elements of the whole organism, such as immunity and metabolism. It is crucial to protect intestinal health for sustainable production [2,3]. Research has been focused on probiotics, prebiotics, phytobiotics, enzymes, and organic acids (OA) [4, 5].

Among these alternatives, OA's and acidifiers are feed additives which are natural and were widely used in the poultry industry [6, 7]. Organic acids increase the acidity of the feed, support flavor and consumption, and increase pancreatic secretion. In addition, it regulates the electrolyte balance in the intestines by providing the digestion and

absorption of Ca, P, and Mg [8–10]. In the intestine, short-chain fatty acids (SCFA) produced by colonic flora are also among organic acids. SCFA has been used for sanitation in the poultry industry due to bacteriostatic and bactericidal impact. It has been stated that when added to feeds in the following periods, it supports growth [11], increases nutrient absorption, improves feed efficiency, and protects epithelial cells by reducing the production of pathogens and toxins in the intestines [12, 13]. The most abundant SCFAs in the intestine are propionic acid, acetic acid, and butyric acid. It is estimated that these SCFAs, produced from indigestible carbohydrates, can meet 5%–10% of humans' total daily energy needs [14].

Butyric acid can also be produced synthetically that stands out with its potent on growth promoter in animal production [15]. It has been determined that butyric acid is effective in intestinal villi development and the inhibition of pathogens [16]. It has been revealed that

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butyric acid is a substance that regulates the differentiation and proliferation of epithelial cells of the digestive system, provides apoptosis of genetically defective cells, and is used as an energy substance in colonic epithelial cells [17, 18]. Since butyric acid is corrosive and volatile in free form, making it difficult to use as a feed additive, it is converted into salts with calcium and sodium, providing ease of use and high stability. In addition, it has been determined that coating with particular oil forms delays the release and contributes to more stimulation of absorption and longer activity [15, 19]. In addition, butyric acid is used in the poultry diet in sodium and calcium salts. To the best of our knowledge, till day no literature is available on the supplementation of calcium and sodium butyrate in poultry diets. Therefore, the aim of this study was to estimate the effect of different levels of calcium and sodium butyrate in the quails' diets on the performance, duodenal histomorphology, and some biochemical parameters.

2. Materials and methods

Ethical approval was confirmed by the Local Ethics Committee of Kafkas University (Decision no: 2021/140). One hundred and sixty 1-day-old Japanese quail (*Coturnix coturnix japonica*) were used in the trial. The study consisted of four groups (40 chicks/group) and each group was arranged five replicates consisting of eight chicks. While the control group was supplied basal diet, calcium butyrate and sodium butyrate (CSB) was added to the diets of the treatment groups at 0.06%, 0.08%, and 0.1%, respectively. CSB, which was added to the diets of the experimental groups, was procured from a private commercial company (Intest-Plus Quattro/Arkor Hayvancılık A.Ş.-İzmir). According to the information received from the manufacturer, Intest-Plus Quattro product; contains 58% butyric acid, 2% sodium, 12% calcium, and 23% refined/hydrogenated palm oil. All animals in the study were fed a diet containing 24% CP and 3000 kcal ME/kg. The diet formulation was prepared according to NRC [20], and nutrient analyses were performed according to AOAC [21] (Table 1). Animals were free to access ad libitum to feed and water. For the care and feeding of the chicks, 60 × 20 × 100 cm plastic quail cages were used. The temperature was retained at 32–33 °C for the first three days, then it was lowered by 1–2 °C every week and fixed at 25 °C. The cages are provided with 24 h/day lighting. The trial was completed in 35 days.

2.1 Growth performance

Body weight (BW) and feed consumption (FC) were recorded weekly. The quails were fasted for 4 h before being weighed¹. Body weight gain (BWG), FC, and feed conversion ratio (FCR) were used to determine growth performance.

Table 1. Composition of the experimental diet of quail.

Ingredients	%
Corn	55.65
Soybean meal (%48 CP)	39.90
Vegetable oil	1.85
Limestone	1.35
Dicalcium phosphate	0.65
Salt	0.35
Vitamin-Mineral mix ^a	0.25
Total	100
Nutrient analysis	
Crude protein (%)	24
Metabolized energy (kcal/kg)	3000
Calcium (%)	0.79
Total phosphorus	0.64
Available phosphorus	0.40
Lysine (%)	1.41
Methionine (%)	0.90
Threonine (%)	0.91
Tryptophan (%)	0.33

^aVitamin and mineral premix provided the following per kg of diet: α -tocopherol acetate, 20 mg; thiamine, 2.2 mg; cholecalciferol, 0.025 mg; riboflavin, 10 mg; 400 mg; nicotinamide, choline chloride, 50 mg; pyridoxine HCl, 4 mg; 0.04 mg; folic acid, biotin, 1 mg; vitamin B12 (cobalamin), 1.013 mg; calcium pantothenate, 10 mg; Fe, 60 mg; Zn, 60 mg; Cu, 10 mg; Mn, 100 mg; Co, 0.2 mg; I, 1 mg; and Se, 0.15 mg.

2.2. Carcass and visceral organs

At the end of the experiment, 2 quails from each subgroup were randomly selected and slaughtered by cervical dislocation. After the bled, the feathers were plucked. After the internal organs (heart, liver and gizzard) were removed, carcass and visceral organ were weighed. Carcass yield was calculated.

2.3. Duodenal histomorphology

Duodenum tissue (2 samples from each subgroup) were fixed in 10% formaldehyde solution for 24 h. Following routine histological processing for light microscopy, tissues were embedded in paraffin. Serial sections at 5 μ m were cut and stained with Mallory's modified triple staining. Stained sections were examined and photographed using a light microscope (Olympus BX43, Japan).

Villi lengths and crypt depths were measured at 10 × magnification using Image J (v1.50i) software from quail

¹ <https://en.engormix.com/poultry-industry/articles/feed-withdrawal-effects-contamination-t34272.htm>

duodenum tissue samples. Villi length and crypt depth measurements were made on 50 villi and crypts, 5 villi and crypts from different regions of 10 serial sections from each group.

2.4. Serum biochemical parameters

Serum (2 samples from each subgroup) was obtained from the blood taken from the under-wing (V. brachialis) before slaughter by centrifugation (3000 rpm/10 min.) and stored at -20 °C. Glucose, total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG) were detected in samples that were thawed on the day of analysis, using commercial kits by spectrophotometric method. Calcium (Ca), and phosphorus (P) levels were measured [22].

2.5. Statistical analysis

SPSS 20.0 (IBM - USA) statistical program was used to determine treatment effects using one way analysis of variance. Polynomial contrasts (linear, quadratic, and cubic) were determined to effect of CSB levels. Duncan multiple comparison test was used for pairwise comparisons between groups. Significance was determined at a $p < 0.05$ grade.

3. Results

Performance results of quails are presented in Table 2. At the end of the trial, BW and BWG were found to be statistically the highest in the CSB - 3 group ($p < 0.05$). BW and BWG were influenced linearly by supplementation of CSB ($p = 0.004$). It was determined that the lowest FC and the best FCR were in CSB - 3. Increasing levels of CSB addition showed linear ($p = 0.001$), quadratic ($p = 0.001$), and cubic ($p = 0.001$) effects on FC. Besides, FCR was affected linear ($p = 0.008$) and quadratic ($p = 0.015$) by CSB supplementation.

At the end of the experiment, the carcass parameters of quails during the growth period of adding CSB to the ration are given in Table 3. Carcass yield, visceral weights, and digestive system weights were not statistically significantly affected by the addition of CSB to the ratio between the experimental groups ($p > 0.05$). Addition of CSB was linearly effective on the liver ($p = 0.046$) and gizzard ($p = 0.044$).

It was seen that the structure of the quail duodenum tissues in all groups was similar to each other (Figure 1). Statistical evaluation of the villi length and crypt depth averages of intergroup are given in Table 4. When the duodenum samples taken from the animals at the end of

Table 2. Growth performance of quails fed diets containing CSB^{*}.

Parameters ^{**}	Control	CSB - 1	CSB - 2	CSB - 3	P ^{***}	L	Q	C
Initial BW, g	9.83 ± 0.19	9.92 ± 0.22	9.85 ± 0.21	9.73 ± 0.24	0.943	0.719	0.632	0.891
Final BW, g	161.08 ± 3.55 ^b	161.75 ± 4.89 ^b	167.92 ± 4.82 ^{ab}	177.42 ± 2.82 ^a	0.026	0.004	0.289	0.907
BWG, g	5.40 ± 0.12 ^b	5.42 ± 0.17 ^b	5.65 ± 0.17 ^{ab}	5.99 ± 0.10 ^a	0.025	0.004	0.276	0.904
FC, g	17.89 ± 0.00 ^c	18.26 ± 0.01 ^b	18.85 ± 0.07 ^a	17.98 ± 0.00 ^c	0.001	0.001	0.001	0.001
FCR	3.33 ± 0.08 ^b	3.40 ± 0.11 ^b	3.37 ± 0.10 ^b	3.01 ± 0.05 ^a	0.008	0.013	0.015	0.571

*CSB - 1: 0.06% Calcium-sodium butyrate; CSB - 2: 0.08% Calcium-sodium butyrate; CSB - 3: 0.1% Calcium-sodium butyrate.

** BW: Body weight; BWG: Body weight gain; FC: Average feed consumption; FCR: Feed conversion ratio.

*** Superscripts in a row remarked significant differences ($p < 0.05$); L: linear; Q: Quadratic; C: Cubic.

Table 3. Carcass yields and some organ weights of quails fed diets containing CSB^{*}.

Parameters	Control	CSB - 1	CSB - 2	CSB - 3	P ^{**}	L	Q	C
Carcass yield, %	65.44 ± 1.62	68.00 ± 2.68	68.74 ± 2.63	68.15 ± 1.30	0.711	0.369	0.475	0.959
Heart, g	1.45 ± 0.14	1.60 ± 0.07	1.44 ± 0.08	1.61 ± 0.07	0.379	0.435	0.923	0.123
Liver, g	3.23 ± 0.14	3.91 ± 0.39	3.72 ± 0.25	4.17 ± 0.24	0.136	0.046	0.686	0.234
Gizzard, g	2.72 ± 0.18	2.81 ± 0.08	2.96 ± 0.17	3.21 ± 0.20	0.214	0.044	0.648	0.972
Gastrointestinal tract, g	5.38 ± 0.25	6.48 ± 0.46	6.68 ± 0.40	5.96 ± 0.46	0.139	0.302	0.037	0.966

*CSB - 1: 0.06% Calcium-sodium butyrate; CSB - 2: 0.08% Calcium-sodium butyrate; CSB - 3: 0.1% Calcium-sodium butyrate.

**Superscripts in a row remarked significant differences ($p < 0.05$); L: linear; Q: Quadratic; C: Cubic.

the experiment were examined, it was determined that the villi length and villi: crypt ratio increased compared to the control group. The highest villi length was detected in the CSB - 2 group ($p = 0.09$), while the lowest villi: crypt depth was detected in the CSB - 3 experimental group ($p = 0.003$). The lowest crypt depth among the trial groups was seen in the CSB - 3 group ($p = 0.001$). The villi length was affected cubically by supplementation of CSB. When the addition of CSB showed linear and cubic effect on crypt depth, it had linear effect on V/C ($p < 0.001$).

Serum biochemical parameters are shown in Table 5. There were no differences between the control and treatment groups regarding glucose and total protein values ($p > 0.05$). Lower AST and ALT values were statistically ($p < 0.05$) recorded for the group with the supplementation of CSB at different rates to the diet compared to the control group. Likewise, serum TC, LDL, and TG levels decreased

significantly ($p < 0.05$) with the supplementation of CSB to the diet, while HDL levels increased significantly ($p < 0.05$). CSB supplementation also increased serum calcium and phosphorus levels in treatments groups compared to the control group ($p < 0.05$).

4. Discussion

The performance of poultry is greatly affected by the digestion and absorption of nutrients, resistance to disease, and intestinal health [23, 24]. Many digestive system disorders affect the intestinal health of poultry [25]. Added to poultry diets, CSB stands out as an essential additive used to combat digestive system disorders and ultimately improve the intestinal health performance of poultry. CSB and its derivatives may be a practical approach to evolve immune health and functions. It has been demonstrated that adding sodium butyrate or calcium butyrate to

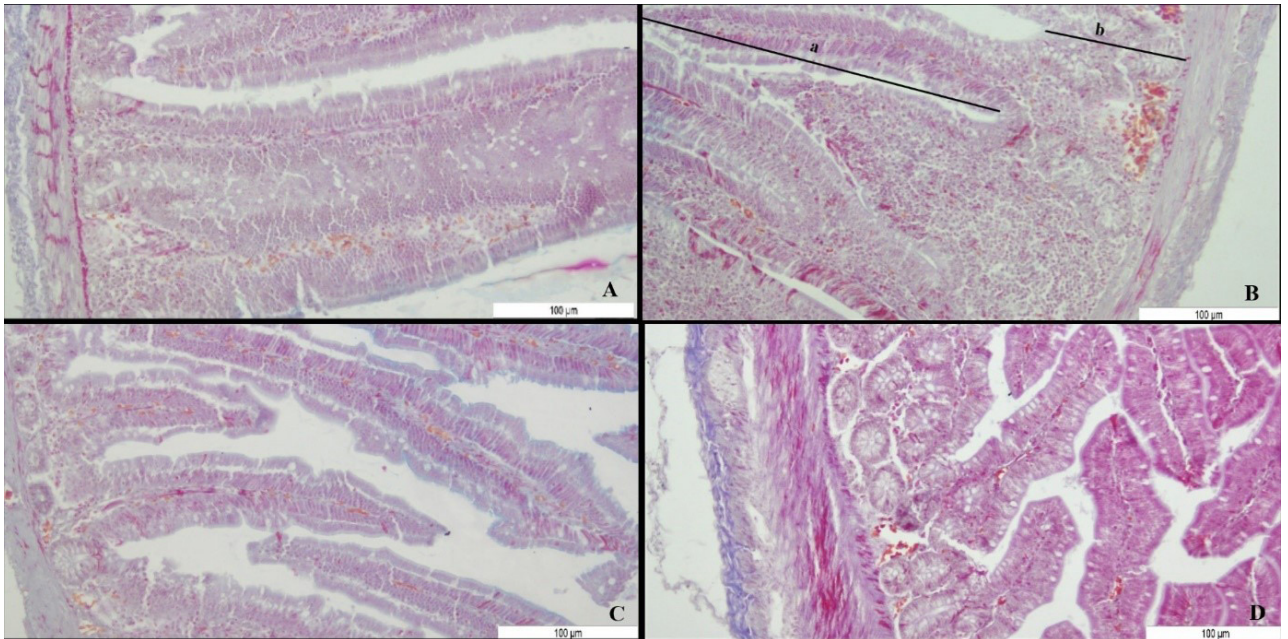


Figure 1. Quail duodenum tissue. A: Control group, B: CSB - 1; A: CSB - 2; D: CSB - 3. a: Villi length; b: Crypt depth. Triple staining.

Table 4. Duodenum histomorphology of quails fed diets containing CSB*.

	Control	CSB - 1	CSB - 2	CSB - 3	P***	L	Q	C
Villi length	389.69 ± 11.37 ^b	381.97 ± 11.46 ^b	422.94 ± 7.75 ^a	392.48 ± 7.94 ^b	0.019	0.262	0.243	0.007
Crypt depth	54.43 ± 2.95 ^a	44.92 ± 2.04 ^{bc}	49.97 ± 1.54 ^{ab}	40.97 ± 1.10 ^c	0.001	0.001	0.900	0.002
V/C**	7.86 ± 0.43 ^b	8.96 ± 0.39 ^a	8.79 ± 0.33 ^{ab}	9.82 ± 0.31 ^a	0.003	0.001	0.925	0.134

*CSB - 1: 0.06% Calcium-sodium butyrate; CSB - 2: 0.08% Calcium-sodium butyrate; CSB - 3: 0.1% Calcium-sodium butyrate.

** V/C: Villi height: crypt depth.

*** Superscripts in a row remarked significant differences ($p < 0.05$); L: linear; Q: Quadratic; C: Cubic.

Table 5. Serum biochemical profile of quails fed diets containing CSB^{*}.

	Control	CSB - 1	CSB - 2	CSB - 3	P	L	Q	C
Glucose, mg/dL	93.38 ± 1.04	94.62 ± 1.43	95.70 ± 1.12	94.98 ± 0.90	0.545	0.255	0.398	0.748
Total protein, g/dL	2.97 ± 0.01	2.99 ± 0.02	2.98 ± 0.01	2.97 ± 0.02	0.906	0.942	0.483	0.828
AST, IU/L	449.17 ± 6.00 ^a	426.17 ± 2.21 ^b	433.33 ± 4.47 ^b	435.50 ± 6.25 ^{ab}	0.018	0.137	0.016	0.123
ALT, IU/L	12.78 ± 0.20 ^a	10.83 ± 0.49 ^b	9.97 ± 0.08 ^c	10.13 ± 0.14 ^{bc}	0.001	0.001	0.000	0.968
COL, mg/dL	286.38 ± 0.09 ^a	285.20 ± 0.15 ^b	285.32 ± 0.14 ^b	285.38 ± 0.15 ^b	0.001	0.001	0.001	0.027
HDL, mg/dL	55.47 ± 0.93 ^b	58.75 ± 0.36 ^a	57.58 ± 0.42 ^a	59.27 ± 0.54 ^a	0.001	0.000	0.195	0.010
LDL, mg/dL	180.76 ± 0.96 ^a	177.95 ± 0.84 ^b	180.27 ± 0.66 ^{ab}	178.25 ± 0.70 ^b	0.035	0.152	0.623	0.011
TG, mg/dL	250.80 ± 0.81 ^a	242.50 ± 3.12 ^b	237.33 ± 1.84 ^b	239.33 ± 1.21 ^b	0.001	0.001	0.011	0.646
Ca, mg/dL	9.46 ± 0.18 ^b	10.20 ± 0.15 ^a	10.46 ± 0.12 ^a	10.66 ± 0.19 ^a	0.001	0.001	0.107	0.561
P, mg/dL	4.43 ± 0.09 ^b	4.80 ± 0.07 ^a	4.93 ± 0.06 ^a	5.03 ± 0.09 ^a	0.001	0.001	0.104	0.567

*CSB - 1: 0.06% Calcium-sodium butyrate; CSB - 2: 0.08% Calcium-sodium butyrate; CSB - 3: 0.1% Calcium-sodium butyrate.

** TP: Total protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; Ca: Calcium; P: Phosphorus.

*** Superscripts in a row remarked significant differences ($p < 0.05$); L: linear; Q: Quadratic; C: Cubic.

poultry feeds has beneficial effects on performance and animal health [26, 27]. In the current study, with the addition of CSB, a significant improvement was observed in the BW, BWG, FC, and FCR compared to the control group. Similarly, researchers reported that increasing microencapsulated butyric acid supplementation in the broiler diet improved growth performance in broilers without affecting FC [27, 28]. The current study Tomar et al. [29] showed a close similarity with the result of the study, which reported that the FCR increased and FC decreased. Likewise, Adil et al. [30] found that chicks fed a diet supplemented with organic acids significantly improved the FCR. On the contrary, Mohamed et al. [31] determined that different levels of the organic mixture did not affect the FCR of broiler chickens. Panda et al. [32] showed that encapsulated butyrate significantly affected BWG, FC, and FCR of broilers. Chamba et al. [19] reported that with the addition of coated sodium butyrate, BW, FC, and FCR increased in broilers. In contrast, Miao [15] reported that different levels of coated sodium butyrate increased egg production and improved FCR. This production performance can be attributed to the duodenal morphology resulting from microencapsulation of butyric acid with palm oil.

Sikandar et al. [33] reported that sodium butyrate supplementation increased the BWG. While it did not affect feed consumption statistically, there was a numerical decrease. In another study, Hu and Guo [34] reported that this application increases BWG, which is achieved by adding sodium butyrate to increase protein, DNA, and RNA concentrations in the duodenum. The results of our study are similar to studies showing that

adding sodium butyrate to broiler diets improved growth performance [35], increased BWG, and decreased FCR [36]. However, some studies have shown that butyric acid additions do not positively affect performance [37, 38]. These differences among the studies may be related to health, age, race, diet, and different butyrate levels. The improvement in the production parameters of the studied groups supplemented CSB can be attributed to the highest stability and absorption of calcium butyrate. This increased absorption and bioavailability of CSB helps the production of energy. This increase in the available energy is utilized by the birds for higher performance.

In the present study, no statistically significant difference was found between the groups regarding carcass yield and visceral and digestive system weight parameters during the trial period. However, Tomar et al. [29] reported that adding butyric acid at increasing doses significantly increased carcass yield. Contrary to the current study, there are also studies reporting that the weight of the digestive organs increases with the addition of butyrate in broilers [35, 39]. Moreover, the results of the previous studies reporting that the heart and liver weights increased significantly, and the gizzard weight decreased with the addition of butyric acid at different rates also differ [29]. Furthermore, Aghazadeh and TahaYazdi [40] reported that the addition of butyrate increased the relative weight of the liver and intestine but had no effect on the relative weight of the gizzard. However, our study agrees with studies reporting that butyrate has no beneficial effect on the relative weight of the liver and gizzard [32, 41].

Intestinal histomorphological features indicate increased absorption surface area and higher nutrient absorption capacity. The villi and crypts' morphology in the duodenum is the leading intestinal health indicator [42]. The V/C can picture the intestinal functions in detail. The enhanced V/C rate indicates the appropriate intestinal mucosa and improved digestion and absorption capacity [43]. Many studies have reported that the supplementation of organic acids to broiler diets has positive effects on intestinal histomorphometry by increasing villi height in different parts of the small intestine, decreasing crypt depth, and increasing villi: crypt ratio [33, 44]. Another study revealed that dietary coated sodium butyrate increased the villi height and the ratio of V/C of the jejunum and ileum and improved intestinal functions [15]. Possibly, the addition of butyric acid increases the proliferation of intestinal epithelial cells and causes the formation of longer villi [45]. Butyrate, the active component of CSB, is absorbed by enterocytes as the primary energy source to support intestinal development and function. The increase in the height of the villi indicates that it allows for higher absorption capacity and a larger surface area, and the development of intestinal health [37, 46]. In our study, with the addition of CSB to quail diets, the highest villi height was reached in the CSB - 2 group compared to the control group, while the lowest crypt depth was found in the CSB - 3 group. However, when the V/C ratio was examined, it was observed that the CSB - 1 and CSB - 2 groups gave statistically better results than the control group. These findings are consistent with studies showing that butyric acid tends to increase duodenal villi height [35–48]. Kaczmarek et al. [27] and Panda et al. [32] reported that butyric acid increased villi height independent of the concentrations in the feed. This may be attributed to butyric acid, which is a ready source of energy for intestinal villi and stimulates their differentiation and proliferation [49].

These results were contrary to the study that reported that butyrate addition did not affect duodenum villi height, crypt depth, and villi - crypt ratio [15–50]. Differences in duodenal morphology results may be explained due to different animal species and doses.

The effect of adding CSB to the diet on some serum parameters in quails is given in Table 5. In the presented study, while serum glucose and TP levels were not affected by the addition of CSB, it was determined that the serum calcium level increased, TC and TG levels decreased significantly with the addition of CSB. These results are consistent with Miao et al.'s [15] findings, which are similar to the finding that the calcium level increases and the TC and TG levels decrease with the addition of coated sodium butyrate in laying hens. Likewise, Deepa et al. [51] and

Elnesr et al. [5] show similar results with the results of the studies reporting that the addition of butyrate to the ration did not affect the serum TP level in broiler chickens, and the TC level decreased. Salah et al. [52] disagree with the result that the addition of 1 g/kg sodium butyrate to the ratio does not affect the serum TC level. The concentrations of body fat metabolism may reflect its status. These results show that CSB can reduce TC and TG levels by inhibiting hepatic lipogenesis and may be beneficial for improving mineral substance levels and lipid metabolism. Contrary to the present study, the serum TC, HDL, calcium, and phosphorus levels in laying hens differ from the literature, reporting that they are not affected by the addition of sodium butyrate to the diet. Likewise, researchers stated that adding butyrate at different levels significantly increased the amount of serum total protein compared to the control group [9, 10]. It has been reported that the use of organic acid in the diet of poultry reduces the pH in minor intestine conditions and improves the absorption of Ca, P, and other minerals [8, 53]. The increase in serum calcium and phosphorus observed in this study can be attributed to this result. The current study was similar to the literature reports that serum calcium and phosphorus were elevated [54, 55].

Liver ALT and AST enzymes manage transamination reactions and are used for liver function. It has been stated that a diagnosis of the hepatocellular disease can be made with high serum AST and ALT values [56]. Serum AST and ALT levels significantly decreased with the addition of CSB in present study. While some studies reported that the addition of various forms of butyric acid did not change ALT and AST levels [51, 55], some researchers reported that in Japanese quail [5] and broilers [35] said that AST and ALT levels decreased significantly.

Conclusion

The present study results revealed that the supplementation of CSB to the diet could improve growth performance and lipid metabolism, increase intestinal digestive capacity, increase serum mineral availability, and protect liver function in quail. In addition, it was concluded that the addition of at least 0.06% calcium-sodium butyrate could provide positive contributions.

Conflict of interest

The authors declare that there is no conflict of interest for this study.

Ethical approval

Ethical approval was confirmed by the Local Ethics Committee of Kafkas University (Decision no: 2021/140).

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