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Polish Journal of Veterinary Sciences Vol. 26, No. 3 (2023), 377–383

DOI 10.24425/pjvs.2023.145043

Original article

Effects of dietary betaine and protected calcium butyrate supplementation on growth performance, blood biochemical status, and meat quality in growing Japanese quail (*Coturnix coturnix Japonica*)

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Abstract

This study aimed to evaluate the impact of betaine (Bet) and protected calcium butyrate (PCB) supplementation individually and together on the performance, carcass traits, blood biochemistry, and meat quality of growing Japanese quails (*Coturnix coturnix Japonica*) from 1 to 42 days old. 144 one-day-old unsexed Japanese quails were randomly assigned to four dietary treatments with six replicates each. All birds were fed a maize-soybean meal diet for 42 days. The control group received no feed additives, while the treatment groups received 1.2 g/kg Bet, 1.0 g/kg PCB, or a combination of both in their diets. The results indicated that Bet and PCB supplementation individually and together did not differ performance, relative weights of heart, gizzard, proventriculus, bursa of Fabricius and pancreas, water holding capacity (WHC), cooking loss (CL), blood biochemical values except for glucose and triglyceride. Bet supplementation significantly increased relative liver weights, while PCB supplementation decreased glucose levels in serum. Moreover, carcass yield was increased and triglyceride value in blood serum, malondialdehyde (MDA), and the pH levels of breast meats both on the 1st and 30th day of post-mortem were decreased in all treatment groups. Therefore, based on these results, the combination of betaine and butyrate improves both carcass yield and meat quality in growing Japanese quails. More research is needed to determine the impact of betaine and butyrate on the structure of amino acids in meat, antioxidant enzyme activity, and the immune system in poultry.

Keywords: betaine, calcium butyrate, meat quality, quail, performance

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Introduction

Japanese quails (*Coturnix coturnix Japonica*) have important potential advantages in poultry production due to their early reaching of sexual maturity, rapid growth rate, and low floor space and feed requirements (El-Bahr et al. 2021). Sub-therapeutic levels of antibiotics used to be administered as growth promoters in the poultry industry and were selected for gut health-improving and infectious agent-reducing ability (Chamba et al. 2014). However, healthier feed additives have been preferred to improve performance and meat-egg quality in poultry feeding instead of antibiotics due to increasing public concern over potential antibiotic side effects and the emergence of drug-resistant microorganisms in recent years (Abd El-Wahab et al. 2019, Mátis et al. 2019). As a result, there is much interest in the establishment of healthier and more effective growth promoters as alternatives to antibiotics.

Short-chain organic acids are promising alternatives for antibiotic growth promoters (Abd El-Wahab et al. 2019). Butyric acid, one of the short-chain fatty acids, could promote the lower gastrointestinal tract by utilizing intestinal epithelial cell proliferation and adjusting the composition of the intestinal microbiota to compete with pathogen bacteria, thus enhancing the performance of poultry (Chamba et al. 2014, Elnesr et al. 2019). It has been also demonstrated that butyric acid, a short-chain fatty acid, can contribute to the protection of the metabolism through its antioxidant, immunostimulant, and anti-inflammatory effects (Zhang et al. 2011a, Jiang et al. 2015, Gümüş et al. 2021). Dietary supplementation of butyrate has been found to improve meat quality and muscle growth by regulating cell growth and differentiation programming in poultry (Pascual et al. 2020). Non-protected butyrate added to poultry feed is readily metabolized in the upper gastrointestinal tract and, microencapsulation techniques can prevent the dissociation of SCFA in the upper parts of the gastrointestinal tract and direct their bioactivity towards the intestines, resulting in a more targeted and effective use of these compounds (Czerwiński et al. 2012).

Betaine (Bet), a trimethyl derivative of the amino acid glycine, has an important role in the protein and energy metabolism of the poultry by donating its labile methyl group (CH_3) (Uzunoğlu and Yalçın 2019, Abudabos et al. 2021). Bet could utilize digestibility in the gastrointestinal tract by implementing an osmoregulatory activity over epithelial cells and microflora (Abudabos et al. 2021). Dietary Bet could increase lean meat and reduce the fat ratio in poultry due to its methionine-sparing activity in the metabolism (El-Bahr et al. 2021, Liu et al. 2022). As an essential organic osmolyte and antioxidant, Bet could be utilized as a meat quality promoter by safeguarding the muscle tissue against dehydration and oxidative stress (Chen et al. 2022).

Although they have similar effects on the poultry metabolism and meat quality; based on our best knowledge, no study has been found on the investigation of the combined effect of butyrate and Bet in the quail nutrition. This study aimed to determine the effect of protected calcium butyrate (PCB) and Bet supplementation separately and together on growing quail performance, carcass traits, blood biochemical status and meat quality in 1 to 42-day-old Japanese quails.

Materials and Methods

A total of 144 one-day-old unsexed Japanese quails (*Coturnix coturnix Japonica*) were randomly selected and divided into four dietary treatments with six replicates. The quail chicks were macroscopically examined before the experiment began, and only those birds without physical abnormalities were chosen to participate in the study. Management methods and treatment protocol were approved by the Animal Use and Care Committee of the Faculty of Veterinary Medicine, Konya Selcuk University (Protocol No: 2021/95). The study also complied with EU Directive 2010/63/EU for animal experiments. Japanese quails in each replication were housed in battery cages. All animals were fed a maize-soybean meal basal diet, which was formulated to meet the nutritional needs of growing Japanese quails according to the National Research Council (1994), for a period of 42 days (Table 1). The control group did not receive any feed additives, while the treatment groups were supplemented with 1.2 g/kg Betaine-HCL (Bet), 1 g/kg calcium butyrate salt protected with palm oil (PCB), and a combination of the two (Bet+PCB) at a dose of 1.2 g/kg and 1 g/kg feed, respectively, in their diets. The PCB used in the trial included 70% butyrate and was protected with palm oil by the microencapsulation method. The feed additive levels were determined according to the studies conducted by Chen et al. (2022), and Elnesr et al. (2019). Water and feed were available, *ad-libitum*.

The quails from each replicate were weighed individually at the beginning, middle, and the end of the experiment for monitoring of live weight gain with a digital scale accurate to 0.01 g. The provided and remaining feed was similarly weighed and subtracted to determine the feed consumption of each study group. The feed conversion rate was estimated by dividing the feed intake by the average live weight gain of each replicate in the study periods.

At the end of the experimental period, two male quails from each replicate were randomly selected,

Table 1. Feed ingredients and calculated composition of the basal feed for Japanese quail.

Feed Composition			
Ingredients (%)		Chemical Analysis (%)	
Maize	53.70	Dry Matter	90.72
Soybean meal	40.00	Crude Protein	20.56
Sunflower oil	2.50	Crude Oil	2.91
Grid	1.06	Crude Fiber	5.93
Dicalcium phosphate	1.90	Crude Ash	6.44
Salt	0.35	Calculated Composition (g/kg)	
Mineral-Vitamin Premix[1]	0.25	Ca	10.00
DL-Methionine	0.24	P	13.15
TOTAL	100.00	Lysin	5.25
		Met+Cys	9.64
		Cystine	4.39
		Metabolic Energy, kcal/kg[2]	2.633

[1] Vitamin mineral premix supplied per kg diet: Vitamin A (trans-retinol) – 3.6 mg, vitamin D3 (cholecalciferol) – 0.1 mg, vitamin E (α-tocopherol acetate) – 75 mg, vitamin K3 (menadione) – 5 mg, vitamin B1 (thiamin) – 3 mg, vitamin B2 (riboflavin) – 6 mg, vitamin B6 (pyridoxine) – 5 mg, vitamin B12 (cyanocobalamin) – 0.03 mg, niacin – 40 mg, pantothenic acid – 10 mg, folic acid – 0.75 mg, D-biotin – 0.075 mg, choline chloride – 375 mg, manganese – 80 mg, iron – 40 mg, zinc – 60 mg, copper – 5 mg, iodine – 0.15 mg, selenium – 0.3 mg.
[2] Metabolizable energy content of diets calculation was conducted according to the equation of Carpenter and Clegg (1956)

weighed, and sacrificed. The carcass, heart, liver, gizzard, proventriculus, bursa of Fabricius (BF), and spleen were removed from the birds and weighed, divided by slaughter weight and multiplied to 100 to determine carcass yield and relative organ weights. Blood and liver samples were collected, and biochemical analysis was carried out later.

Blood samples from the slaughtered quails were centrifuged at 5,000 rpm for 10 minutes to separate the serum. Glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST) and alkaline phosphatase levels were determined using commercial test kits, using an autoanalyzer.

The breast meat samples taken from the carcasses were divided into four pieces. After all pieces were grinned into mince, the pH values of the meat on the 1st and 30th day after slaughter were detected with a portable pH/temperature meter (Milwaukee MW102, USA). Briefly, a 1 g of breast meat sample was placed in a centrifuge tube, covered with a piece of filter paper, and centrifuged. After drying the sample at 70°C, the final weight was recorded. Water holding capacity (WHC) was calculated using the following formula: (weight after centrifugation – weight after drying) / initial weight × 100. The malondialdehyde (MDA) levels from liver and meat tissues were detected with the method described by Zeb and Ullah (2016). The absorbance values were determined at 532 nm using a spectrophotometer device (Genesys™ 10S UV-Vis, Thermo Scientific, USA).

The data obtained from performance, carcass parameters, selected blood biochemical levels, meat quality and liver MDA levels underwent a Shapiro-Wilk test of normality to assess their normal distribution. For normally distributed data, Duncan's Multiple Range Tests of one-way analysis of variance (ANOVA) were conducted to assess the variance between groups. Moreover, non-normally distributed data were analyzed using the Kruskal-Wallis test, a nonparametric statistical test. The analysis was conducted using the SPSS 22.0 software package. (SPSS Inc., Chicago, IL, USA). A probability value of $p<0.05$ was statistically significant.

Results

Table 2 demonstrates the effect of Bet and PCB supplementation in growing Japanese quails on growth performance during the experiment. No significant difference in growth performance was associated with feed additives in the current study in terms of LWG, FI and FCR ($p>0.05$).

The effect of Bet and PCB inclusion in growing quails' diet on carcass parameters are presented in Table 3. No significant difference ($p>0.05$) was recorded between groups in terms of relative weights of heart, gizzard, proventriculus, bursa of Fabricius and spleen. However, dietary PCB and PCB+Bet combination improved carcass yield in comparison to the control and Bet, and Bet supplementation

Table 2. Growth performance of Japanese quail supplemented with betaine and protected calcium butyrate.

Paramaters	C	Bet	PCB	Bet+PCB	p
0 to 21 d					
LWG (g/b)*	80.49±5.48	83.07±2.85	82.20±3.20	81.54±4.22	0.735
FI (g/b)*	277.84±23.60	269.23±10.04	262.22±20.07	263.24±12.30	0.405
FCR (g/g)*	3.45±0.22	3.24±0.15	3.19±0.26	3.24±0.29	0.251
22 to 42 d					
LWG (g/b)*	88.35±43.47	91.32±16.93	110.18±17.04	101.56±15.78	0.474
FI (g/b)**	395.06±191.70	404.98±28.08	440.14±65.83	413.67±34.56	0.459
FCR (g/g)**	4.74±1.19	4.54±0.76	4.01±0.19	4.13±0.55	0.259
0 to 42 d					
LWG (g/b)*	168.84±44.82	174.39±18.93	192.38±17.54	183.10±14.46	0.467
FI (g/b)**	672.90±189.02	674.21±34.21	702.36±71.67	676.91±36.91	0.745
FCR (g/g)*	3.99±0.28	3.89±0.30	3.65±0.15	3.71±0.31	0.135

* Means analyzed by Duncan test
** Means analyzed by Kruskal-Wallis test
LWG – Live weight gain, FI – Feed intake, FCR – Feed conversation rate

Table 3. Carcass and relative organ weight traits of Japanese quail supplemented with betaine and protected calcium butyrate.

Parameters	Control	Bet	PCB	Bet+PCB	p
Carcass Yield (%)	$59.35{\pm}1.59^{b}$	$59.99{\pm}1.54^{b}$	$62.67{\pm}2.89^{a}$	$62.68{\pm}2.48^{a}$	0.001
Heart (%)	0.97±0.09	0.99±0.07	0.98±0.13	0.92±0.09	0.311
Liver (%)	$1.58{\pm}0.09^{b}$	$1.80{\pm}0.11^{a}$	$1.55{\pm}0.26^{b}$	$1.65{\pm}0.13^{b}$	0.005
Gizzard (%)	1.57±0.14	1.63±0.29	1.57±0.30	1.50±0.18	0.630
Proventriculus (%)	0.43±0.05	0.47±0.07	0.45±0.05	0.43±0.06	0.237
Bursa of Fabricius (%)	0.12±0.02	0.13±0.03	0.11±0.03	0.14±0.03	0.088
Spleen (%)	0.05±0.02	0.05±0.03	0.06±0.03	0.06±0.01	0.468

BF – Bursa of Fabricius
^{a,b} Means in the same column and treatment with no common superscript differ significantly (p<0.05).

increased the relative weight of the liver in comparison to the other groups (p<0.05).

Dietary Bet and PCB supplementation did not affect (p>0.05) blood cholesterol, HDL, LDL AST and ALP levels in serum and MDA values in liver (Table 4). However, glucose levels were decreased in the group fed with PCB supplemented diets, and significantly lower triglyceride levels were observed in the treatment groups than in the control (p<0.05).

As indicated in Table 5, Bet and PCB significantly lowered meat pH values both on the 1st day and 30th day after slaughter, and meat lipid peroxidation levels (p<0.05). Lowest MDA value was observed in the group fed with Bet+PCB supplemented diets. However, no significant difference was observed between groups in terms of WHC and CL traits (p>0.05).

Discussion

Bet and butyrate are two feed additives that have shown potential for improving the performance of poultry (Abd El-Wahab et al. 2019, Al-Abdullatif et al. 2021). The data of the present study indicated that Bet and PCB supplementation in quails' diet did not improve LWG, FI and FCR values in performance. Similarly to our research results, previous studies on growing quails (El-Bahr et al. 2021) and broiler chickens (Esteve-Garcia and Mack 2000, Pillai et al. 2006, Uzunoğlu and Yalçın 2019) have also found that diets supplemented with Bet did not have a statistically significant effect on performance. Moreover, butyrate supplementation in quails (Shihab et al. 2020) and broilers (Zhang et al. 2011a, Czerwiński et al. 2012) showed no statistical difference between the control and the treatment groups in the other studies. Other studies

Table 4. Blood biochemical profile of Japanese quail supplemented with betaine and protected calcium butyrate.

Parameters	Control	Bet	PCB	Bet+PCB	p
Glucose (mg/dl)*	333.46±28.51[a]	338.75±20.28[a]	314.50±15.91[b]	343.92±18.36[a]	0.010
Triglyceride (mg/dl)*	80.58±8.14[a]	68.45±6.56[b]	73.82±9.29[b]	73.09±7.37[b]	0.007
Cholesterol (mg/dl)*	196.54±40.75	208.67±33.68	190.58±24.05	197.92±19.51	0.549
HDL (mg/dl)**	138.53±15.99	139.89±11.55	134.44±13.21	138.88±10.96	0.721
LDL(mg/dl)*	37.99±6.85	43.01±6.18	41.89±8.19	40.34±5.78	0.317
AST (U/L)*	272.38±45.89	285.58±70.29	243.25±35.61	271.92±33.81	0.197
ALP (U/L)*	750.54±148.49	704.58±93.17	678.08±182.52	705.33±185.19	0.708
Liver MDA (μM/g)**	2.50±1.15	2.32±0.60	1.89±0.58	2.51±0.71	0.182

* Means analyzed by Duncan test
** Means analyzed by Kruskal-Wallis test
HDL – High-density lipoprotein, LDL – Low density lipoprotein, AST – Aspartate aminotransferase, ALP – Alkaline phosphatase
[a,b] Means in the same column and treatment with no common superscript differ significantly ($p<0.05$).

Table 5. Meat quality parameters of Japanese quail supplemented with betaine and protected calcium butyrate.

Parameters	Control	Bet	PCB	Bet+PCB	p
pH, 1st Day*	5.42±0.15[a]	5.22±0.09[b]	5.15±0.10[b]	5.20±0.14[b]	0.000
pH, 30nd Day*	5.36±0.17[a]	5.30±0.15[ab]	5.20±0.19[b]	5.19±0.16[b]	0.007
CL (%)*	19.94±5.50	22.78±7.69	20.77±6.16	25.14±7.81	0.295
WHC (%)*	61.95±3.12	59.82±1.83	60.37±0.95	60.06±2.87	0.072
MDA (μM/g)**	0.19±0.16[a]	0.17±0.12[ab]	0.09±0.08[bc]08[abc]	0.07±0.05[c]	0.024

* Means analyzed by Duncan test
** Means analyzed by Kruskal-Wallis test
CL – Cooking loss, WHC – Water holding capacity, MDA – Malondialdehyde
[a,b] Means in the same column and treatment with no common superscript differ significantly ($p<0.05$).

have reported that the addition of the two feed additives has a substantial influence on growth performance, particularly in adverse conditions such as the presence of a negative factor such as stress or pathogens (Zhang et al. 2011, Czerwiński et al. 2012, El-Bahr et al. 2021).

Another important finding of the current study was the improvement of carcass yield by dietary supplementation of PCB and PCB+Bet combination. In support of our findings, other authors have reported that greater carcass and breast meat weight could be obtained from the birds fed with butyrate supplemented diets (Leeson et al. 2005, Su et al. 2009, Abd El-Wahab et al. 2019, Mátis et al. 2019, Gümüş et al. 2021). Mátis et al. (2019) have stated that one of the reasons for this may be that dietary butyrate stimulates muscle development by regulating insulin homeostasis in the metabolism. According to our findings, relative liver weights of quails fed with Bet enriched diets were statistically higher than the control and other treatment groups. One possible explanation for this situation is that lipid metabolism regulation activity of Bet might result in an increase in the number of hepatocytes and their size due to the accumulation of triglycerides in liver cells (Liu et al. 2022).

In the present study, blood serum glucose level of the quails fed with 1 g/kg PCB enriched diets was significantly lower than that in the control and other test groups. Similarly to our findings, Dawood et al. (2020) reported that dietary sodium butyrate supplementation decreased blood glucose levels in Nile tilapias exposed to heat stress. Rice et al. (2019) also observed that the addition of increased amounts of sodium Bet in the diets of weaned calves caused a linear decrease in plasma glucose levels. The regulation mechanism of butyrate on insulin homeostasis and glycolytic enzyme activity which might affect gluconeogenesis in the quails (Mátis et al. 2019, Rice et al. 2019). When compared to the control group, it was found that triglyceride levels were low in all of the treatment groups in our study. Likewise, other studies also noted that the use of dietary Bet (Rama Rao et al. 2011, Uzunoğlu and Yalçın 2019, El-Bahr et al. 2021) and butyrate (Yin et al. 2016, Deepa et al. 2017, Elnesr et al. 2019) in poultry nutrition leads to a decrease in blood lipid values, similar to our findings. Both Bet and PCB contribute to the fat accumulation to the metabolism in poultry (Liu et al. 2022).

Meat quality can be described as the combination of characteristics that determine the overall appeal of meat to consumers, including pH, water-holding capacity, texture, nutrient content, and oxidative status (Chen et al. 2022). One aspect of evaluating meat quality is measuring the pH of the meat, which can affect the texture, color, and water holding capacity of the muscle tissue during rigor mortis (Abudabos et al. 2021). In the current study, both Bet and PCB supplementation in growing Japanese quail diets decreased meat pH on the 1st and 30th day after slaughter. Similarly, Al-Sagan et al. (2021) also showed dietary Bet supplementation in broilers could decrease pH levels of the meat 24 h postmortem. Moreover, Al-Abdullatif et al. (2021) found no effect of dietary Bet on breast meat pH values in broilers. In contrast to our findings, Abudabos et al. (2021) and Chen et al. (2022) reported Bet supplementation increased meat pH at 24 hours post mortem in broilers. Moreover, the pH value of the breast meat in the present study was significantly decreased in the PCB and PCB+Bet supplemented groups. Similar to our results, a study conducted on broilers showed that butyrate and butyrate + forskolin supplementation decreased breast meat pH at day 28 (Yang et al. 2022a). The pH of meat can vary due to a number of factors such as the ambient temperature at the time of slaughter, stress, the dose or type of feed additive used, and other factors (Al-Abdullatif et al. 2021, Al-Sagan et al. 2021).

Under typical physiological conditions, there is a delicate equilibrium between pro- and antioxidant systems that maintains normal cellular activity in muscle tissues. However, when the antioxidant system is compromised, lipid oxidation of muscle may occur, resulting in the production of excessive amounts of free radicals (Yang et al. 2022b). Antioxidant feed additives can aid in neutralizing free radicals in muscles in such cases. MDA, a metabolite produced from lipid peroxidation, is widely utilized as a marker of oxidative damage (Chen et al. 2022). In the current study, Bet, PCB and their combination depressed the lipid peroxidation in breast meat, yet no effect was observed in liver MDA levels. It has been reported that both dietary supplementation of butyrate (Zhang, et al. 2011a, Jiang et al. 2015, Gümüş et al. 2021) and Bet (Egbuniwe et al. 2021, El-Bahr et al. 2021, Chen et al. 2022, Yang et al. 2022b) exhibit protective effects against lipid peroxidation in poultry. According to our results, the lowest MDA levels were recorded in the treatment groups supplemented with PCB and Bet+PCB. It has been stated that butyric acid suppresses the production of i-kappa-b kinase, a cell-secreted compound involved in inflammation, by inhibiting oxidative stress in the cells, and that butyric acid also indirectly reduces MDA levels in the tissues by stimulating the activity of antioxidant enzymes such as superoxide dismutase and catalase (Zhang, et al. 2011b, Gümüş et al. 2021). Additionally, it has been noted that Bet increases the amount of glutathione and the activity of glutathione peroxidase in the cells, and also exhibits antioxidant activity by affecting the Nrf2 signaling pathway which protects the cells from oxidative damage (Chen et al. 2022, Yang et al. 2022b). The different free radical scavenging activity of two feed additives might have caused a combined effect on the MDA status of the breast meat in our study.

Conclusion

In conclusion, Bet supplementation significantly increased relative liver weights, whereas PCB addition decreased glucose levels in blood serum in growing Japanese quails. Moreover, both feed additives decreased triglyceride levels in blood serum and pH values in meat on the 1st and 30th days of postmortem in the current study. Consequently, according to our results, Bet and PCB combination in Japanese quail diet improves both carcass yield and meat quality. Further research is required to investigate the impact of dietary Bet and butyrate on the structure of amino acids in meat, antioxidant enzyme activity, and the immune system in poultry.

Acknowledgements

We would like to express our deepest gratitude and appreciation to Yamtar Tarim for invaluable contributions to this study.

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