Spanish Journal of Agricultural Research
21 (4), e0611, 9 pages (2023)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2023214-20367
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Effects of dietary mannan oligosaccharides and coated calcium butyrate on performance, carcass parameters, blood biochemistry and meat quality of growing Japanese quails

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Abstract

Aim of the study: Despite previous research into mannan oligosaccharides (MOS) and calcium butyrate coated with palm oil (CCB) in poultry, there is a notable gap in the literature regarding the effects of these feed additives, either individually or in combination, on the growth performance, carcass values, blood biochemistry, immune response, and meat quality of growing Japanese quails.

Area of study: Türkiye.

Material and methods: A total of 168 mixed-sex one-day-old quails were randomly allocated to one of four treatment groups, with each group containing 7 birds per cage. The control group was fed a basal diet, while the treatment groups received the following additives: 1 g/kg of CCB, 2 g/kg of MOS, and a combination of MOS+CCB added to the basal diet, respectively.

Main results: None of the treatments had a significant impact on performance, relative organ weights, total protein, albumin, globulin, total cholesterol, high-density lipoprotein cholesterol, and lipoprotein lipase concentrations in blood serum, or humoral immunity on day 28. However, the inclusion of MOS and CCB in the diet, either individually or in combination, increased carcass yield and reduced low-density lipoprotein cholesterol and triglyceride levels in the blood serum. Additionally, these additives helped maintain optimal pH levels and lower malondialdehyde concentrations in the breast meat. Moreover, the combination of MOS + CCB significantly improved water holding capacity and antibody titers against the Newcastle Disease vaccine on day 42 in Japanese quails.

Research highlights: The natural feed additives CCB and MOS contribute to enhanced carcass yield, improved meat quality, and strengthened humoral immunity, while simultaneously lowering lipid values in the bloodstream.

Additional key words: antioxidants; butyrate; humoral immunity; lipid homeostasis; malondialdehyde.

Abbreviations used: BF (Bursa of Fabricius); CCB (calcium butyrate coated with palm oil); HCY (hot carcass yield); MDA (malondialdehyde); ME (metabolic energy); MOS (mannan oligosaccharides); NDV (Newcastle Disease Virus); TBARS (thiobarbituric acid reactive substances); WHC (water holding capacity).

Citation: Gumus, E; Sevim, B; Olgun O; Kucukersan, S (2023). Effects of dietary mannan oligosaccharides and coated calcium butyrate on performance, carcass parameters, blood biochemistry and meat quality of growing Japanese quails. Spanish Journal of Agricultural Research, Volume 21, Issue 4, e0611. https://doi.org/10.5424/sjar/2023214-20367

Received: 02 Apr 2023. **Accepted:** 25 Oct 2023.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Ethics approval statement: The animal study adhered to approved animal care and use procedures as stipulated by the Animal Use and Care Committee of the Faculty of Veterinary Medicine, Selcuk University (Approval No: 2021/80), and was in accordance with the EU Directive 2010/63/EU for animal experiments.

Introduction

Japanese quails (*Coturnix coturnix japonica*) hold significant prominence in the realm of poultry nutrition research, primarily due to their manageable size, making them well-suited for easy handling and confinement in limited spaces (Sarmiento-García et al., 2023). Furthermore, their applicability as a laboratory animal model is noteworthy. The anticipation of increased demand for quail meat stems from its nutritional advantages, characterized by low fat and cholesterol content and rich amino acid profiles (Farag et al., 2021). Consequently, a pivotal facet of Japanese quail research revolves around evaluating dietary supplementation with diverse additives to enhance growth performance and carcass attributes.

Recent years have witnessed a burgeoning interest in poultry nutrition studies that prioritize feed additives known for their sustainable and environmentally friendly qualities, driven by mounting concerns over antibiotic resistance (Abd-Allah & Abdel-Raheem, 2012; Sikandar et al., 2017; Abd El-Wahab et al., 2019; Yang et al., 2022). This surge in interest has led to the proliferation of alternative feed additives, including organic acids, prebiotics, synbiotics, probiotics, enzymes, and phytogenic supplements (Makled et al., 2019). Notably, prebiotics and organic acids have emerged as increasingly prevalent growth promoters in poultry production.

One such organic acid, butyrate, characterized by its concise molecular structure, has emerged as a viable option for enhancing the performance and carcass characteristics of poultry. Its capacity to enhance feed efficiency by positively impacting intestinal histomorphology and microflora is well-documented (Sikandar et al., 2017). Furthermore, the incorporation of butyrate into poultry diets serves as a strategy to foster the growth of immune organs and enhance immunomodulatory and antioxidant capabilities (Zhang et al., 2011; Sikandar et al., 2017; Yang et al., 2022). Notably, uncoated butyrate salts, once prevalent in poultry nutrition, have progressively given way to coated butyrate salts. These coated salts are designed to release butyrate in the distal regions of the small intestine, where its efficacy is most pronounced (Eshak et al., 2016; Gümüş et al., 2021).

Mannan oligosaccharides (MOS), a class of polysaccharides inherent in the cell walls of yeast *Saccharomyces cerevisiae*, have demonstrated their potential to support the growth of intestinal microflora and exhibit immunomodulatory properties in poultry. These properties hold the promise of improved growth performance (Abd-Allah & Abdel-Raheem, 2012). Additionally, MOS may contribute to averting tissue damage arising from oxidative stress by fostering a healthy gut microbiota that, in turn, releases antioxidants (Biswas et al., 2021).

While MOS promote the growth of beneficial bacteria in poultry intestines (Bonos et al., 2011), butyric acid serves to acidify the gut environment and stimulate villus proliferation (Elnesr et al., 2019; Gümüş et al., 2021). In light of these considerations, this study postulates that the combined use of both additives could potentially yield a synergistic effect on the intestinal environment, translating into a favorable impact on performance and other parameters. Although a multitude of studies have individually investigated the influence of MOS or butyrate on the performance, immune response, and meat quality of poultry, to our knowledge, no studies have undertaken a comparative analysis of their effects in growing quails. Thus, the objective of this study is to assess the impact of dietary supplementation with MOS and calcium butyrate coated with palm oil (CCB), either individually or in tandem, on the growth performance, carcass characteristics, blood biochemistry, immune response, and meat quality of Japanese quails.

Material and methods

Experimental animals, diets, and design

A total of 168 mixed-sex one-day-old Japanese quails (*Coturnix coturnix japonica*) with an initial average weight of 9.23 ± 0.31 g were included in this study. They were randomly assigned to one of four treatment groups, each comprising six replicate pens measuring 30 × 45 cm. Within each pen, seven birds were accommodated, and the study period spanned from day 1 to day 42 of age. Each pen was equipped with a tray feeder and two nipple drinkers to provide ad libitum access to both feed and fresh water.

Temperature management was a critical aspect of the experimental conditions. During the initial week, the ambient temperature inside the housing facility was maintained at 33°C, with a gradual reduction of 3°C per week. This temperature regimen continued until it reached 25°C by the third week of the study. Throughout the experiment, the quails were exposed to a consistent lighting schedule.

All experimental groups received a basal diet (as outlined in Table 1). This basal diet was carefully formulated to maintain an isonitrogenous composition, containing 24% crude protein, and to possess isoenergetic properties with a metabolizable energy content of 2900 kcal/kg. The formulation was tailored to align with the dietary requirements established for growing Japanese quails, as stipulated by NRC (1994). The chemical composition of the basal diet was assessed in compliance with AOAC (2006) procedures.

In the control group, the basal diet was administered without any modifications. In contrast, the treatment groups received the same basal diet, but with the inclusion of specific additives. These additives comprised 1 g/kg of calcium butyrate coated with palm oil (CCB) and 2 g/kg of

Table 1. Composition of basal diet of growing Japanese quail.

Ingredients	%	Chemical composition	%
Maize	45.50	Dry matter	88.74
Soybean meal	40.00	Crude protein	24.02
Barley	5.00	Crude fat	6.26
Soybean oil	3.26	Crude ash	7.11
Corn gluten meal	2.20	Crude fiber	4.93
Dicalcium phosphate	1.88	**Calculated composition**	**%**
Limestone	1.10	ME (kcal/kg)[2]	2,907
Salt	0.35	Sodium	0.23
Premix[1]	0.25	Calcium	1.02
DL-methionine	0.24	Available phosphorus	0.50
Sodium bicarbonate	0.10	Digestible lysine	1.31
Coccidiostat	0.08	Digestible Met+Cys	0.91
L-lysine hydrochloride	0.04	Digestible threonine	0.85
		Digestible tryptophan	0.24
		Digestible arginine	1.48

[1] Premix per kg of diet: 8,800 IU vitamin A, 2,200 IU vitamin D3, 11 mg vitamin E, 44 mg nicotinic acid, 8.8 mg calcium D-pantothenate, 4.4 mg riboflavin, 2.5 mg thiamin, 6.6 mg vitamin B12, 1 mg folic acid, 0.11 mg D-biotin, 220 mg choline, 80 mg manganese, 60 mg iron, 5 mg copper, 60 mg zinc, 0.20 mg cobalt, 1 mg iodine, 0.15 mg selenium. [2] Metabolizable energy content of diets was calculated according to Carpenter & Clegg´s (1956) equation.

mannan oligosaccharides (MOS). Additionally, there was a combination treatment group where both CCB and MOS were added at levels of 1 g/kg and 2 g/kg, respectively. Both MOS and CCB were sourced from a local feed additive provider (Sanita Sağlık, Türkiye).

Performance

The weight of individual quails was meticulously measured by cage in the morning following a 12-hour period of feed deprivation, both at 21 and 42 days of age. Precision weighing scales with a sensitivity of ±0.01 g were employed for this purpose. Simultaneously with quail weighing, the feed consumption for each cage was recorded. This was achieved by subtracting the remaining feed amount from the initially provided quantity.

The recorded data were then divided by the average number of birds in each cage, with due consideration for any adjustments related to mortality. This division facilitated the computation of essential variables, including body weight gain (in grams), feed intake (in grams), and body weight gain/feed ratio for the specified time intervals.

Mortality rates were vigilantly recorded on a daily basis, and the cumulative mortality rate was determined for the entire duration of the experimental period.

Carcass and organ traits

Upon reaching 42 days of age, two male Japanese quails were randomly selected from each pen, weighed, and humanely euthanized through cervical dislocation. The weight of the hot carcass was meticulously recorded to calculate the hot carcass yield (HCY). The heart, liver, proventriculus, gizzard, spleen, and bursa of Fabricius were weighed for each quail. The relative organ weights were calculated based on the live weights of the quails and expressed as percentages.

Antibody titer count

The quails involved in the experiment received vaccinations against *Newcastle disease virus* (NDV) strains B1 and Newcastle La Sota. These vaccinations occurred at 10 and 21 days of age, respectively, utilizing a live vaccine (Fatro, Bologna, Italy) and the spray method.

On day 29, blood samples were collected from one bird per pen through venipuncture, specifically from the brachial vein. Additionally, on day 42, blood samples were obtained from selected quails designated for sacrifice. These samples were procured from the jugular vein using 10 mL sterile vacuum blood tubes.

Table 2. Effects of treatments on performance traits of Japanese quail at different phases.

Growth traits	Treatments[1]				SEM	p values
	Control	CCB	MOS	CCB+MOS		
Average body weight (g)						
d 1	9.18	9.38	9.17	9.17	0.06	0.591
d 21	96.08	93.43	93.97	93.12	0.65	0.389
d 42	203.46	195.67	193.71	199.95	2.18	0.413
Average body weight gain (g)						
d 0-21	86.90	84.05	84.80	83.95	0.65	0.354
d 22-42	107.38	102.24	99.74	106.84	2.13	0.550
d 0-42	194.28	186.28	184.55	190.79	2.17	0.399
Average feed intake (g/bird-period)						
d 0-21	215.27	212.93	221.99	213.99	2.10	0.443
d 22-42	515.25	497.88	510.05	479.30	7.10	0.296
d 0-42	730.52	710.81	732.04	693.28	8.19	0.301
Feed conversion ratio						
d 0-21	2.48	2.54	2.62	2.55	0.03	0.444
d 22-42	4.82	4.89	5.23	4.51	0.13	0.280
d 0-42	3.76	3.82	3.99	3.64	0.06	0.176
Mortality rate (%)						
d 0-42	7.14	4.76	7.14	7.14	2.10	0.975

[1] CCB = coated calcium butyrate. MOS = mannan oligosaccharide.

The collected blood samples underwent centrifugation at 5,000 × g for a duration of 5 minutes to separate the serum from other components.

The serum's antibody responses to NDV on days 29 and 42 were quantified via a microtiter hemagglutination inhibition assay, in accordance with the protocol outlined by the Food and Agriculture Organization (FAO, 2023).

Blood serum analysis

Commercial kits (Beckman Coulter OSR) were employed for the analysis of serums collected on day 42. The parameters analyzed included glucose, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, lipase, total protein, and albumin.

Globulin levels were determined by subtracting the value of albumin from the total protein values.

Analysis of meat quality

Following the humane euthanization of two birds from each pen, the breast meat was carefully extracted from each bird, minced, and subsequently divided into four pieces for the evaluation of quality characteristics.

The pH of the breast meat was measured 24 hours post-mortem using a portable pH meter (Milwaukee MW102, USA). The electrode was inserted into the meat to obtain the pH value.

To assess the WHC, 1 gram of minced breast meat was placed on a segment of tissue paper within a centrifugation tube. It was then centrifugated for 4 minutes at 1500 × g. The residual water remaining after the process was quantified through overnight drying of the samples at 70°C. The WHC of the samples was calculated using the formula: (Weight after centrifugation – Weight after drying) / Initial weight × 100.

The level of lipid oxidation in breast meat was measured using reactive thiobarbituric acid substances (TBARS), following the protocol described by Zeb & Ullah (2016). In this method, 1 gram of the sample was mixed with 5 mL of glacial acetic acid, shaken for 1 hour in a cold-water bath, and subsequently centrifuged and filtered. Afterward, 1 mL of the extract was mixed with 1 mL of TBARS, heated at 100°C for 1 hour, and cooled to room temperature. The absorbance values were determined at 532 nm using a spectrophotometer device (Genesys™ 10S UV-Vis, Thermo Scientific, USA) and expressed as mg of malondialdehyde (MDA) per kg of breast meat.

Statistical analysis

All collected data underwent one-way analysis of variance (ANOVA) using IBM SPSS Statistics (version 22, IB Corp.,

Table 3. Effects of treatments on carcass characteristics and internal organs of Japanese quail (n = 48).

Variables[1]	Treatments[2]				SEM	p values
	Control	CCB	MOS	CCB+MOS		
HCY (%)	61.191[b]	64.063[a]	63.045[a]	63.005[a]	0.310	0.007
Liver (%)	1.789	1.579	1.670	1.636	0.042	0.356
Heart (%)	0.918	0.942	0.934	0.892	0.015	0.698
Gizzard (%)	1.747	1.717	1.794	1.819	0.031	0.655
Proventriculus (%)	0.413	0.410	0.429	0.443	0.008	0.460
Spleen (%)	0.053	0.062	0.060	0.044	0.062	0.754
BF (%)	0.110	0.130	0.118	0.112	0.005	0.487

[1] HCY = hot carcass yield. BF = Bursa of Fabricious. [2] CCB = coated calcium butyrate. MOS = mannan oligosaccharide. [a-b] Means within the same row with no common superscripts differ significantly ($p < 0.05$).

Armonk, NY, USA). Dietary treatments served as the primary source of variation among the measured values.

For growth performance, the unit of measurement was the pen, and for all other parameters, the unit of measurement was the individual bird.

The data were presented using the mean and standard error of the mean (SEM). Differences were considered statistically significant at $p < 0.05$.

Results

Table 2 presents the productive performance, feed intake, feed conversion ratio, and mortality rate of Japanese quail from 1 to 42 days of age. Across all phases of the experiment, no significant differences were observed between the mean values of the treatment groups for average body weight, body weight gain, feed intake, feed conversion ratio, and mortality rate ($p > 0.05$).

The relative weights of the liver, heart, gizzard, proventriculus, bursa of Fabricius, and spleen of Japanese quails fed experimental diets are depicted in Table 3. The results indicate that the feed additives used in this study did not have a significant effect on the relative weights of these organs ($p > 0.05$). However, hot carcass yield (HCY) exhibited a statistically significant increase in birds fed with CCB and MOS additives, either individually or in combination, compared to the control group ($p < 0.05$).

Table 4 provides insight into the impact of the experimental diets on serum biochemical metabolites. The dietary supplements tested did not exert a significant impact ($p > 0.05$) on the concentrations of total cholesterol, HDL-cholesterol, lipoprotein lipase, total protein, albumin, and globulin. However, the addition of MOS and CCB, whether individually or in combination, led to a reduction in LDL-cholesterol and triglyceride levels in the serum of Japanese quails in comparison to the control group ($p < 0.05$).

Table 5 offers data regarding changes in antibody titer with the addition of MOS and CCB, either alone or in combination, to the diets of growing Japanese quails. The dietary supplementation with MOS and CCB did not significantly affect antibody titer at 21 days of age ($p > 0.05$). However, at d-42, the antibody titer was statistically higher in the treatment group fed with CCB+MOS supplemented diet compared to the control and other treatment groups ($p < 0.05$).

The data in Table 6 illustrate the effects of the treatments on pH, water-holding capacity (WHC), and malondialdehyde (MDA) levels in the breast meat of Japanese quails. In contrast to the control group, both CCB and MOS led to decreased pH values 24 hours after postmortem, both individually and in combination ($p < 0.05$). Similarly, all treatments had a positive effect on MDA levels in the meat of the birds ($p < 0.05$). Furthermore, the combination of MOS and CCB improved the breast meat WHC in the quails ($p < 0.05$).

Discussion

Mannan oligosaccharides and butyrate salts have emerged as promising alternatives to antibiotics as growth-promoting feed additives in poultry nutrition (Abd El-Wahab et al., 2019; Mátis et al., 2019; Hazrati et al., 2020). Numerous studies have documented the positive impacts of both MOS (Brzóska et al., 2007; Abd-Allah & Abdel-Raheem, 2012; Hazrati et al., 2020) and butyrate (Abd El-Wahab et al., 2019; Elnesr et al., 2019) on the performance of Japanese quails. MOS and butyrate are believed to enhance poultry performance by regulating the intestinal microflora against pathogens while fortifying mucosal protection (Brzóska et al., 2007; Abd El-Wahab et al., 2019). Butyrate, serving as an energy substrate for colonocytes and enterocytes, also bolsters resistance to bacterial challenges and modulates

Table 4. Effects of treatments on blood serum biochemical status of Japanese quail (n = 48).

Variables[1]	Treatments[2]				SEM	p values
	Control	CCB	MOS	CCB+MOS		
HCY (%)	61.191[b]	64.063[a]	63.045[a]	63.005[a]	0.310	0.007
Liver (%)	1.789	1.579	1.670	1.636	0.042	0.356
Heart (%)	0.918	0.942	0.934	0.892	0.015	0.698
Gizzard (%)	1.747	1.717	1.794	1.819	0.031	0.655
Proventriculus (%)	0.413	0.410	0.429	0.443	0.008	0.460
Spleen (%)	0.053	0.062	0.060	0.044	0.062	0.754
BF (%)	0.110	0.130	0.118	0.112	0.005	0.487

[1] TC = total cholesterol. LDL = low-density lipoprotein. HDL = high-density lipoprotein. LL = lipoproteine lipase. [2] CCB = coated calcium butyrate, MOS = mannan oligosaccharide. [a-b] Means within the same row with no common superscripts differ significantly ($p < 0.05$).

immune cell responses (Abd El-Wahab et al., 2019; Elnesr et al., 2019).

However, the data from this study indicate that both MOS and CCB supplementation in quail diets did not result in statistically significant improvements in performance traits. Similar studies have reported no impact of MOS (Şahin et al., 2008; Yalçınkaya et al., 2008) or butyrate (Zhang et al., 2011) on performance values in poultry. The variation in results observed in different studies may be attributed to differences in the microbial environment of the birds, influenced by factors such as management practices, environmental conditions, and the presence of pathogens. Birds raised in low-pathogen or high-health status environments may not exhibit a growth-enhancing response to butyrate (Zhang et al., 2011).

In the present study, higher HCY was observed in all treatment groups compared to the control. This aligns with experiments conducted by Bonos et al. (2010) and Abd-Allah & Abdel-Raheem (2012), where higher carcass characteristics were reported in birds fed diets containing MOS. The hypothesis put forth is that MOS may reduce the presence of harmful bacteria in the intestines, facilitating better nutrient absorption and utilization, ultimately leading to increased lean meat production. Additionally, butyrate is known to influence the development of the bursa of Fabricius (BF), which is a critical immune organ in poultry (Sikandar et al., 2017; Elnesr et al., 2019). The increase in HCY observed in this study could be attributed to the development of the BF due to dietary CCB and MOS, which could indirectly affect carcass yield.

The serum biochemistry data in this study suggest that the dietary inclusion of MOS and CCB, either individually or in combination, led to reduced levels of LDL-cholesterol and triglycerides. This reduction in serum lipids is consistent with previous findings in chickens, where dietary butyrate was associated with lower cholesterol and triglyceride levels (Elnesr et al., 2019; Yang et al., 2022). Such effects on lipid metabolism might be attributed to the modulatory impact of butyrate on the expression of genes related to fatty acid metabolism in the liver (Liu et al., 2019; Elnesr et al., 2021).

The increased antibody titer against NDV observed at day 42 in the quails fed with the combination of CCB and MOS suggests an improved humoral immune response. This aligns with findings in chickens, where dietary butyrate improved immune responses (Bortoluzzi et al., 2021; Yang et al., 2022). The modulatory effect of butyrate on immune organs like the bursa of Fabricius might be the underlying cause (Sikandar et al., 2017; Elnesr et al., 2019).

Regarding meat quality, the lower pH values in breast meat found in this study for the treated groups may be indicative of a more acidic environment in the muscle tissue. This may promote protein denaturation, leading

Table 5. Effects of dietary treatments on antibody titre (log2) of Japanese quails.

Variables	Treatments[1]				SEM	p values
	Control	CCB	MOS	CCB+MOS		
d-29 (n = 24)	3.71	4.00	3.43	4.14	0.15	0.387
d-42 (n = 48)	3.67[b]	3.67[b]	3.25[b]	4.33[a]	0.11	0.002

[1] CCB = coated calcium butyrate. MOS = mannan oligosaccharide. [a-b] Means within the same row with no common superscripts differ significantly ($p < 0.05$).

Table 6. Effects of dietary treatments on meat quality of Japanese quails (n = 48).

Variables	Treatments[1]				SEM	p values
	Control	CCB	MOS	CCB+MOS		
WHC (%)	61.58^{b}	61.10^{b}	61.87^{b}	64.74^{a}	0.46	0.016
pH_{24h}	5.47^{a}	5.28^{b}	5.30^{b}	5.36^{b}	0.02	0.001
MDA (μM/g)	1.09^{a}	0.46^{b}	0.42^{b}	0.76^{b}	0.07	0.002

[1] WHC = water holding capacity, MDA = malondialdehyde. [2] CCB = coated calcium butyrate, MOS = mannan oligosaccharide. $^{a-b}$ Means within the same row with no common superscripts differ significantly ($p < 0.05$).

to firmer meat (Gümüş et al., 2021). Additionally, lower pH values can discourage microbial growth, contributing to the shelf life of the meat. The observed improvement in meat quality due to CCB and MOS is consistent with previous studies that reported the benefits of butyrate supplementation on meat quality parameters (Tavaniello et al., 2017; Bortoluzzi et al., 2021).

Lower levels of MDA in the breast meat of quails fed with CCB and MOS suggest reduced lipid oxidation, indicative of enhanced meat quality. Lipid oxidation generates off-flavors, discoloration, and decreased nutritional value (Xu et al., 2007; Gümüş et al., 2021). The antioxidant potential of MOS is well-documented (Biswas et al., 2021), and butyrate has also been shown to have antioxidant properties (Sikandar et al., 2017).

The significant increase in WHC in quail breast meat for the combination treatment (CCB + MOS) suggests an improvement in meat quality. WHC is an essential indicator of meat quality, as it influences sensory attributes such as juiciness and tenderness (Sikandar et al., 2017). The enhancement in WHC observed in this study may be attributed to the water-binding properties of MOS (Zhang et al., 2011) and the potential of butyrate to regulate the pH and water-binding capacity in the meat (Gümüş et al., 2021).

In conclusion, the results of this study suggest that dietary supplementation with Mannan Oligosaccharides (MOS) and Calcium Butyrate Coated with Palm Oil (CCB), either individually or in combination, has no significant impact on the growth performance, relative organ weights, blood serum biochemistry, or humoral immunity of growing Japanese quails. However, these feed additives positively influenced carcass yield, lipid metabolism, meat quality, and water-holding capacity in the breast meat. The combination of MOS and CCB, in particular, was associated with increased antibody titers against Newcastle Disease Virus (NDV) and improved water-holding capacity (WHC) of the meat.

These findings contribute to the evolving understanding of the impact of natural feed additives like MOS and CCB on Japanese quail production. Further research is necessary to elucidate the underlying mechanisms behind these observed effects and to explore the potential of these additives for improving poultry production under different environmental and health conditions.

Acknowledgments

The authors extend their appreciation to Sanita Sağlık (Türkiye) for generously supplying the mannan oligosaccharides and calcium butyrate coated with palm oil used in this study.

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Software: Not aplicable.
Supervision: O Olgun, S Kucukersan
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Writing – review & editing: E Gumus, B Sevim, O Olgun, S Kucukersan

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