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In ovo feeding with β-hydroxy β-methylbutyrate and broiler performance, intestinal health, and immunity status

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Abstract: The effects of in ovo administration of β-hydroxy-β-methylbutyrate (HMB) on broiler performance, intestinal health, and immunity were investigated. At day 18 of incubation, fertile eggs were divided into negative (no injection) and positive (physiologic serum injection) controls and three HMB treatment groups. HMB solution was injected at 0.1% (Group 1), 0.2% (Group 2), and 0.3% (Group 3) concentrations. There were no significant differences between the groups for live weight gain, feed conversion rate, and hot carcass, breast meat, liver, and heart weight. However, the gizzard weight in Group 1 was significantly higher than those in the other groups ($P < 0.05$). Although the intestinal villi lengths in the treatment Groups 1 and 3 on day 20 were greater than those in the control groups ($P < 0.01$), at the end of the experiment there were no significant differences among the groups. For antibody levels, the differences between groups on days 4 and 42 were highly significant ($P < 0.01$), and the antibody levels in Group 2 were higher than those in the other groups. Separately, growth performance and antibody levels were positively correlated with the length of villi in Group 2 (HMB 0.2%).

Key words: β-Hydroxy-β-methylbutyrate, in ovo feeding, broiler, immunity, performance

1. Introduction

The aim of in ovo feeding studies is the enhancement of intestinal development by enteric modulators such as β-hydroxy-β-methylbutyrate (HMB). In ovo administration of 3 mg of ascorbic acid on days 11–15 of incubation caused a reduction in the rate of embryonic death and cull chicks (1). In a similar study the control and experimental groups were not affected differently by the same experiment (2). Conversely, on day 18 of incubation hatchability and chick weight were substantially reduced by the administration of 0.5 g of ascorbic acid (3).

In ovo administration of vitamin E and thiamine had a positive impact on growth in the early stages of life (4). In addition, in ovo HMB application resulted in 45% higher villus surface area at 3 days of age and 5% higher body weight at 10 days of age (5), and the peptide YY positively affected the feed conversion rate (FCR), live weight, and performance (6).

In a study of in ovo feeding with egg white protein, HMB, and carbohydrate in turkey eggs, the difference in body weight of the HMB group continued up to 7 days and had a positive impact on the humoral immune response (7). Furthermore, it was reported that in ovo L-carnitine feeding increased villus length and maltose activity (8).

In the present study, the effects of in ovo HMB injection at different concentrations on the growth performance, intestinal health, and immunity were investigated.

2. Materials and methods

The study was approved by the Local Ethics Committee on Animal Experiments at Ondokuz Mayıs University (2011/40, 27.06.2011).

2.1. In ovo application time and in ovo feeding

Eggs collected from a 48-week-old breeder flock (Ross 308) were incubated under optimal conditions. Eggs of average weight (58 g ± 1.3 g) and containing live embryos on day

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18 of incubation were divided into three experimental and two control groups, each containing 120 eggs. In the treatment groups, HMB solution (Sigma 55453) (0.5 mL) was injected in ovo at 0.1% (Group 1), 0.2% (Group 2), or 0.3% (Group 3). Nothing was administered to the positive control group and saline solution (0.9% NaCl) was administered to the negative control group. In ovo feeding was performed with an automatic syringe (Socorex, Cat. # 187.2.0501) and a 21 G needle introduced into the amniotic sac. To reduce the effects of environmental conditions in the coop, 114 chicks of each experimental group were divided into four subgroups. Broiler feed was used in three periods (0–10 days CP: 23%, ME: 3100 kcal/kg; 11–21 days CP: 22%, ME: 3180 kcal/kg; and 22–42 days CP: 20%, ME: 3250 kcal/kg).

2.2. Histomorphology

Seven animals from each group were sacrificed on days 4, 20, and 42 of the study. Small intestinal (mid-ileum) villi lengths and crypt depths were measured (9).

2.3. Vaccination, serological monitoring, and microbiology

Per os Newcastle HB vaccine (Hipraviar Clone) was administered on days 4, 12, and 28 of the study. Maternal antibody levels (ELISA test) were determined from blood samples (serum) on day 4 and changes in the humoral immune response (Newcastle specific antibodies) were determined serologically on days 20 and 42 according to the manufacturer's instructions (BIOCHECK Antibody Test Kit).

Total and coliform bacterial counts were performed on small intestine samples (mid-jejunum) taken on days 4, 20, and 42 of the study. The small intestines were removed from each bird and 1 g of the content was diluted 1:9 (wt/vol) with physiological buffer solution (log 10). Samples were serially diluted from 10^{-1} to 10^{-8}. Using these samples, total aerobic bacteria was enumerated on tryptic soy agar plates following incubation at 37 °C for 24 h; coliform was counted on MacConkey agar plates blue agar incubated at 37 °C for 24 h (10).

2.4. Statistics analysis

One-way ANOVA was performed to compare live weight, carcass characteristics, intestinal villi, crypt measurements, total bacteria and coliform, and antibody levels of the groups at different times; Duncan's multiple range tests were used to determine the significance of the variances between the groups. Colony forming unit data transformed to log and ANOVA were used to compare the means of the groups for transformed data. The data were presented with descriptive statistics.

3. Results

Live weight changes are provided in Table 1. Weekly average feed intakes, live weight gains, and feed conversion efficiencies are provided in Table 2. Hot carcass, breast meat, gizzard, liver, and heart weights are given in Table 3. Intestinal villi lengths are given in Table 4 and intestinal crypt depths are provided in Table 5. Serological results are presented in Table 6, and total intestinal bacterial and coliform counts are given in Table 7.

4. Discussion

The effects of in ovo injection of different concentrations of HMB on the growth performance, intestinal health, and immunity of broilers were investigated. Firstly, no negative effects of HMB on hatching were observed (hatching 95% in all groups). Although there were no significant differences in weekly weight changes (Table 1), weight gains, and FCR (Table 2) between the groups at the end of the fattening period, treatment Group 2 performed better. In a different study, the in ovo administration of 30 mg of threonine resulted in a better FCR until day 7 (11). In other studies using different amino acids (12) and royal jelly (13), FCR was better in the early period of growth (0–3 weeks), but there were no differences between the groups at the end of the experimental period. In contrast, according to our results, in ovo HMB at 0.2% had a positive effect on growth performance.

Table 1. Average live weights (mean ± SE) (g).

Groups	Day 4	Day 11	Day 18	Day 25	Day 32	Day 42
PC	81.18 ± 2.38	268.62 ± 14.73	566.31 ± 25.69^{b}	948.78 ± 41.62	1622.28 ± 57.28	2597.85 ± 107.32
NC	86.74 ± 1.19	298.10 ± 5.38	615.80 ± 14.0^{a}	1053.00 ± 19.54	1723.01 ± 27.56	2841.27 ± 56.67
Treatment Group 1	88.55 ± 2.01	286.10 ± 6.01	541.42 ± 12.96^{b}	1027.18 ± 27.14	1683.56 ± 52.67	2780.63 ± 76.73
Treatment Group 2	85.61 ± 2.35	281.75 ± 5.46	531.33 ± 11.39^{b}	1010.53 ± 20.49	1648.50 ± 31.26	2845.00 ± 73.48
Treatment Group 3	87.75 ± 2.14	285.72 ± 5.85	549.88 ± 13.35^{b}	992.64 ± 19.10	1692.81 ± 25.83	2727.63 ± 72.89
P value	0.202	0.073	0.000***	0.065	0.407	0.285

***: $P < 0.001$; ^{a,b}: means with different superscripts in the same column are significantly different.

Table 2. Average feed intake (g), live weight gain (g), and feed conversion ratio.

	Control groups		Treatment groups		
	PC	NC	Group 1	Group 2	Group 3
Feed intake					
Day 11	234.7	272.2	343.5	363.0	370.0
Day 18	515.5	512.1	477.7	477.6	551.7
Day 25	680.1	745.6	737.3	741.1	715.9
Day 32	804.2	1054.5	951.6	897.0	1049.4
Day 42	1840.2	1702.5	1727.0	1688.7	1709.5
Live weight gain					
Day 11	193.8	211.4	198.4	196.1	197.2
Day 18	306.8	317.7	264.2	249.6	255.3
Day 25	354.9	437.2	442.8	479.2	485.8
Day 32	506.2	670.0	700.2	638.0	1656.4
Day 42	1080.4	1118.3	1034.8	1197.0	1132.4
Feed conversion ratio (feed to gain ratio)					
Day 11	1.21	1.29	1.74	1.85	1.86
Day 18	1.68	1.61	1.81	1.91	2.16
Day 25	1.92	1.71	1.67	1.55	1.47
Day 32	1.59	1.57	1.36	1.41	1.60
Day 42	1.70	1.52	1.67	1.41	1.51

Table 3. Hot carcass, breast meat, gizzard, liver, and heart weights (mean ± SE) (g).

Groups	Hot carcass	Breast meat	Gizzard	Liver	Heart
PC	2019.55 ± 105.51	621.90 ± 23.88	24.11 ± 1.40^{b}	53.44 ± 3.70	21.55 ± 1.78
NC	2086.77 ± 49.78	685.55 ± 21.60	25.75 ± 0.72^{b}	55.63 ± 1.76	18.05 ± 0.79
Treatment Group 1	2094.40 ± 61.93	603.80 ± 32.21	28.70 ± 1.02^{a}	50.62 ± 1.98	17.90 ± 0.71
Treatment Group 2	2099.38 ± 65.32	668.00 ± 29.32	24.86 ± 0.67^{b}	54.86 ± 1.67	18.24 ± 0.52
Treatment Group 3	2054.87 ± 99.24	673.70 ± 14.88	24.75 ± 1.27^{b}	54.18 ± 1.75	19.18 ± 1.03
P value	0.967	0.109	0.011*	0.372	0.169

*$P < 0.05$; ^{a,b}: means with different superscripts in the same column are significantly different.

In ovo glutamine, sucrose, and maltose had no effect on gizzard, forestomach, and liver weights (14), but the administration of dextrin and HMB increased body weight and pectoral muscle weight (15). In our study, carcass, breast meat, liver, and heart weight differences between the control groups and the treatment groups were not significant (Table 3); however, gizzard weight was higher in treatment Group 1 ($P < 0.05$).

Changes in villus morphology affect nutrient absorption and production. Histopathologic examination

Table 4. Intestinal villi lengths (mean ± SE) (µm).

Groups	Day 4	Day 20	Day 42
PC	325.22 ± 24.16	816.19 ± 7.38^{c}	1094.82 ± 8.85
NC	436.31 ± 58.51	817.19 ± 9.34^{c}	1081.98 ± 98.78
Treatment Group 1	471.15 ± 52.24	903.59 ± 11.75^{a}	1089.38 ± 6.59
Treatment Group 2	553.92 ± 89.59	728.23 ± 19.83^{b}	1095.06 ± 8.90
Treatment Group 3	477.55 ± 58.98	856.98 ± 24.01^{ac}	1086.44 ± 6.94
P value	0.150	0.000***	0.450

***P < 0.001; ^{a,b,c}: means with different superscripts in the same column are significantly different.

Table 5. Intestinal crypt depths (mean ± SE) (µm).

Groups	Day 4	Day 20	Day 42
PC	56.89 ± 9.24	96.46 ± 0.88	127.89 ± 1.19
NC	71.50 ± 12.73	99.76 ± 7.88	127.48 ± 1.16
Treatment Group 1	79.94 ± 7.93	85.79 ± 2.89	126.75 ± 1.29
Treatment Group 2	86.25 ± 8.17	97.12 ± 3.33	127.34 ± 1.22
Treatment Group 3	92.74 ± 8.64	86.18 ± 3.87	127.44 ± 2.03
P value	0.113	0.105	0.746

Table 6. Antibody levels (sample to positive control ratio) (mean ± SE).

Groups	Day 4 (maternal)	Day 20	Day 42
PC	2.58 ± 0.16^{b}	1.15 ± 0.08	4.19 ± 0.04^{b}
NC	3.59 ± 0.27^{a}	1.09 ± 0.09	2.81 ± 0.17^{c}
Treatment Group 1	2.50 ± 0.27^{b}	0.84 ± 0.10	4.69 ± 0.14^{ab}
Treatment Group 2	4.11 ± 0.16^{a}	1.28 ± 0.14	4.76 ± 0.20^{a}
Treatment Group 3	2.32 ± 0.13^{b}	0.99 ± 0.10	4.32 ± 0.20^{ab}
P value	0.000***	0.335	0.000***

***P < 0.001; ^{a,b,c}: means with different superscripts in the same column are significantly different.

Table 7. Total intestinal aerobic and coliform bacteria counts.

Groups		Days		
		4	20	42
NC	Total bacteria (¥cfu/mL)	9.53 ± 0.091^{d}	24.40 ± 0.073^{a}	21.41 ± 0.037^{c}
	Coliform bacteria (cfu/mL)	100.00 ± 4.472^{c}	1000.00 ± 109.544^{ab}	10,000.00 ± 829.993^{b}
PC	Total bacteria (cfu/mL)	9.67 ± 0.080^{d}	20.24 ± 0.042^{e}	20.62 ± 0.009^{d}
	Coliform bacteria (cfu/mL)	4000.00 ± 130.384^{b}	200.00 ± 13.416^{ab}	600.00 ± 9.428^{d}
Treatment Group 1	Total bacteria (cfu/mL)	16.80 ± 0.0603^{b}	21.82 ± 0.044^{c}	25.10 ± 0.012^{a}
	Coliform bacteria (cfu/mL)	600.00 ± 122.474^{c}	140.00 ± 8.944^{b}	6000.00 ± 163.299^{c}
Treatment Group 2	Total bacteria (cfu/mL)	12.18 ± 0.124^{c}	21.63 ± 0.079^{d}	22.33 ± 0.015^{b}
	Coliform bacteria (cfu/mL)	600.00 ± 104.881^{c}	3000.00 ± 216.795^{a}	14,000.00 ± 745.355^{a}
Treatment Group 3	Total bacteria (cfu/mL)	19.51 ± 0.051^{a}	22.11 ± 0.038^{b}	22.29 ± 0.017^{b}
	Coliform bacteria (cfu/mL)	20,000.00 ± 2213.59^{a}	2620.00 ± 1597.62^{a}	6000.00 ± 129.099^{c}
P value	Total bacteria (cfu/mL)	<0.0001***	<0.0001***	<0.0001***
	Coliform bacteria (cfu/mL)	<0.0001***	0.0264*	<0.0001***

*P < 0.05; ***P < 0.001; ¥: colony forming units; ^{a,b,c,d,e}: means with different superscripts in the same column are significantly different.

of the samples obtained after the sacrifices on days 4, 20, and 42 of the study revealed no significant differences between the groups in terms of villi length; however, the villi of the treatment groups were longer (Table 4). There were no differences between the groups for the depth of intestinal crypts (Table 5). Villi length increased, but crypt depth was not affected by the in ovo administration of butyric acid (16,17). HMB administration had a positive effect on villus length, crypt depth, and live weight gain (5), and villi surface area and immune response increased (18). The early differences between the treatment groups and the controls in the present study that paralleled those of other studies (16–18) disappeared by the end of the study.

In ovo 10 IU vitamin E treatment increased antibody and macrophage response and increased anti-SRBC antibody titers and the amount of phagocytic macrophages (19), while different amounts of threonine significantly increased humoral immune response (11); however, royal jelly administration had no effect on antibody response (Newcastle vaccine) (13). In the present study, serologic examination of blood and intestinal samples (Table 6) and microbiological examinations (Table 7) revealed no significant differences in antibody levels between the treatment groups, but on days 4, 20, and 42 of the study the antibody level of Group 2 was higher than in all other groups (Table 6). The concentrations of total aerobic bacteria and coliform in the small intestine were affected ($P < 0.001$) by the in ovo administration of HMB in the present study (Table 7). During the first days after hatching, the chicks were considered to be sensitive to infectious diseases because intestinal flora bacteria had not colonized the cecum and small intestine yet. The present study showed that the chicks became more resistant to infectious diseases because bacteria settled in the intestinal flora in the first 4 days after hatching from the in ovo administration of HMB (especially treatment Group 3).

Overall, the in ovo 0.2% HMB administration positively affected the growth performance, villi length, and antibody levels of 8-week-old broilers.

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