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Effect of β-hydroxy-β-methylbutyrate acid on meat performance traits and selected indicators of humoral immunity in goats

Katarzyna Ząbek[1,*], Roman Wójcik[2], Stanisław Milewski[1], Joanna Małaczewska[2], Zenon Tański[1] and Andrzej Krzysztof Siwicki[2]

[1]University of Warmia and Mazury in Olsztyn, Department of Sheep and Goat Breeding, Faculty of Animal Bioengineering, Olsztyn, Poland

[2]University of Warmia and Mazury in Olsztyn, Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, Olsztyn, Poland

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Abstract

The aim of this study was to determine the effect of β-hydroxy-β-methylbutyrate acid, on parameters of meat performance in goats as well as on selected parameters of non-specific humoral defense. An experiment was performed on 24 Alpine kids divided into two equal groups: I - control and II - experimental. Over a period of 60 days, the animals were fed an HMB-supplemented diet. The following meat performance parameters were determined: body weight, daily gains, growth rate, the dimensions of *musculus longissimus dorsi* (m.l.d.) sections and fat thickness over the loin "eye". Selected indicators of non-specific humoral immunity were determined in the blood serum of kids: lysozyme activity, ceruloplasmin activity and gamma globulin content. It was found that the kids administered HMB had a significantly higher body weight on days 30 and 60 of the experiment compared to the control group. The kids in this group also had a significantly more favorable musculature development. Simultaneously, a significant impact of HMB on the examined immunological indices was found. The significance of differences in relation to the control group was confirmed statistically for lysozyme activity and ceruloplasmin activity on days 30 and 60, while the content of gammaglobulins was confirmed statistically on days 15, 30 and 60 of the study. It was also found that the addition of HMB had a stimulating impact on immunity and growth rate as well as on the development of muscles. It is thus justified to administer HMB to early-weaned kids to enhance their rearing parameters.

Key Words: goat, HMB, humoral immunity, meat performance

*Corresponding author: dr Katarzyna Ząbek, UWM, ul. Oczapowskiego 5, 10–719 Olsztyn, Poland
Phone: +48895233806. Fax: +48895245105. E-mail: khoik@uwm.edu.pl
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Introduction

Rational kid feeding is a crucial consideration, as it may contribute to improving the meat performance of goats. An end product of optimal quality is obtained through the application of traditional feeding systems. It should be noted, however, that traditional systems are characterized by low production effectiveness. Since goats are mainly reared in order to produce milk, it is necessary to wean kids as soon as possible, although this may negatively impact their subsequent growth and development[30]. Therefore, attempts have been made to develop new solutions to improve the use of feed, while ensuring production profitability. One of the recommended solutions involves the supplementation of goat diets with stimulators such as β-hydroxy-β-methyl-butyric acid (HMB). In the body, leucine is a precursor of HMB[33], but exogenous HMB produced with chemical or microbiological synthesis may, to a large degree, be incorporated into metabolic processes[33,35]. Since both the amount of HMB that is produced in the body and that supplied with food is insufficient, it is therefore essential to supplement it from external sources.

It has been shown that HMB used as a food supplement generates many beneficial effects, such as the improvement of nitrogen balance[8] and blood morphology indices[23] and an increase in muscle gains[1,3,6,13]. Experiments by Ostaszewski *et al.*[18] conducted on muscles in rats and chickens demonstrated a suppressive effect of HMB on muscle protein degradation. A beneficial effect of HMB on the development of skeletal muscles and weight gains in turkeys was reported by Moore *et al.*[11]. Tako *et al.*[31] showed that HMB used in poultry nutrition stimulated the development of intestinal villi. Studies on pigs have demonstrated that this substance administered to sows during pregnancy generated, among others, an increase in growth hormone in the blood of newborn piglets and an improvement of fleshiness of their carcasses at slaughter[32]. Many publications on immunity have reported a beneficial impact of HMB on the defense mechanisms in poultry[4,12,20,21,22]. Fuller *et al.*[4] and Nissen *et al.*[12] recorded a significant reduction in the mortality of chicken broilers that were administered this additive. Studies by Peterson *et al.*[20,21] carried out with chicken broilers demonstrated a stimulating effect of HMB on both the humoral and cellular immunity. A similar growth in the immunity resulting from HMB administration and leading to a reduction in morbidity and mortality has been also reported in studies conducted with fish[26,27,28]. Experiments on calves have confirmed the significant impact of HMB on the cellular[36] and humoral[37] immune response.

Considering the above, it is justified to pursue further studies on the efficacy of livestock diet supplementation with HMB. In particular, it is important to determine the application potential of this stimulator in the nutrition of animals with lower slaughter value, such as goats. This matter was undertaken in the present publication to demonstrate the impact of HMB used in diets of early-weaned kids on meat performance traits and blood morphological and biochemical parameters.

Materials and methods

The experiment was conducted on 24 buck goats originating from Alpine race productive herd goats. All procedures related to the animals in this study were approved by Local Ethical Committee for animal experiments in Olsztyn (18/2013).

Experimental design: Goat kids aged 30 ± 3 days were divided into two groups of 12 animals each: I - control and II - experimental. The two groups were similar in body weight. During the 60-day experimental period, the animals in both groups were fed at an identical level using the same feed compositions: a WITAMILK 2 milk replacer (Wipasz, Olsztyn), a CIELAK complimentary feed mixture (Wipasz, Olsztyn) and grass haylage.

WITAMILK 2 was administered throughout the whole period at a dose of 1.5 L/animal/day diluted at 1 : 10 whereas the other feedstuffs were fed *ad libitum*. From the very beginning of the study, the experimental group was administered β-hydroxy-β-methylbutyric acid (HMB, Metabolic Technologies Inc. Ames, IA, USA). This was supplied with a complimentary feed mixture at a dose of 50 mg/kg BW according to experimental results obtained by Siwicki *et al.*[28]. Throughout the study, the amount of administered feed and leftovers was controlled. Their chemical composition was determined with standard methods[2]. Based on the results, the amount of nutrients ingested by kids was determined for both groups throughout the experimental period.

At the beginning of the study (day 0) and on day 15, 30 and 60 of the experiment, blood was sampled from the jugular vein to determine the selected indicators of humoral immunity in goats.

Evaluation of meat performance parameters: The following meat performance traits were investigated: body weight at the beginning of the study and on day 30 and 60 of the experiment, daily gains, growth rate in the following time intervals: days 1–30, 31–60 and 1–60 of the experiment, meatiness and fatness indices determined *in vivo* by ultrasound examination at the beginning and after 30 and 60 days of the experiment. Growth rate (GR) was computed based on the following formula[10]:

$$GR = \frac{\text{final body weight} - \text{initial body weight}}{1/2\,(\text{initial body weight} + \text{final body weight})} \times 100\,(\%)$$

Meatiness indices - dimensions of *musculus longissimus dorsi* (m.l.d.) sections, including: depth, width and area and fatness indices - skin and fat thickness and fat thickness over the loin "eye", were determined by ultrasonography. The measurements were performed behind the last rib[9] using a Mindray DP-50 ultrasonograph with a 7.5 MHz linear probe (Mindray Medical International Limited, Shenzhen, China).

Evaluation of humoral immunity indicators: The humoral immunity of lambs was determined based on lysozyme activity, ceruloplasmin activity, total protein and gamma globulin levels. Lysozyme activity in blood serum was determined by the turbidimetric method proposed by Parry *et al.*[19] and modified by Siwicki and Anderson[25], ceruloplasmin activity in blood serum—as described by Siwicki and Studnicka[24] and serum concentrations of gamma globulins—by the precipitation method, as described by Siwicki and Anderson[25].

Lysozyme activity: whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The serum was diluted 1 : 1 with phosphate buffer, and 0.1 ml of the solution was placed in the wells of microplates. 0.5 ml of *Micrococcus lysodeikticus* bacterial suspension (25 mg bacteria/100 ml phosphate buffer) (Sigma Chemical Co.) was added. Absorbance was measured directly after the addition of bacteria (E_0) and after 1, 2, 3 and 30 min (final E). The final absorbance was subtracted from the initial absorbance (E_0) to determine lysozyme activity with the use of a standard curve. The standard curve was plotted based on the optical density values for known lysozyme concentrations.

Ceruloplasmin activity: whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The following buffers were prepared: 1) acetate buffer (pH 5.2, containing crystalline acetic acid, sodium acetate trihydrate and 15 mg EDTA), 2) buffered substrate solution (0.2% p-phenyldiamine (PPD) in acetic buffer), 3) sodium azide solution (0.02% sodium azide solution in deionized water). 0.5 ml of buffered solution was added to each of two 16 × 100 mm test tubes immersed in a water bath at a temperature of 37°C. One test tube served as an experimental sample, and the other as control. 50 µl of serum was added to the experimental sample which was incubated for 15 min at 37°C. Next 2 ml of a sodium azide solution was added to the experimental and control sample. 50 µl of serum was added to the

control sample, and both samples were mixed. The absorbance of the experimental sample was measured at a wavelength of 540 nm, using the control sample as a blind test. Ceruloplasmin activity was determined with the use of the standard curve. The standard curve was plotted based on the optical density values for known ceruloplasmin concentrations.

Gamma globulins level: whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The optical density of total protein was determined in blood serum following the above procedure. 0.1 ml of serum was placed in the wells of microplates and 0.1 ml 12% polyethylene glycol (10,000 kD) (Sigma Chemical Co.) suspended in deionized water was added. The microplates were incubated at room temperature for 2 h, and well contents were stirred continuously. The microplates were centrifuged for 10 min at 5,000 g to separate the γ-globulin fraction bound by polyethylene glycol (plate sediment) from the remaining total protein fraction which constituted the supernatant. The optical density of supernatant was measured in a microplate reader at 620 nm. The optical density of supernatant was subtracted from the optical density of total protein. γ-globulin content was determined using a standard curve (plotted earlier for total protein) as a reference, based on the ability of gamma globulins to bind with polyethylene glycol and precipitate.

Statistical analysis: The results of intake of nutrients and meat performance parameters were processed statistically by a single-factorial analysis of variance in an orthogonal design. The significance of differences between groups was verified with Student's t-test. The results of humoral immunity parameters of the goats were processed by one-way ANOVA, and the significance of differences between groups was verified with Duncan's test.

Results

During the 60 days of the study, no great differences in the intake of feed by the kids were recorded. The volume of ingested nutrients is presented in Table 1. The experimental kids consumed more dry matter (DM) by 4.08% and, consequently, more feed unit for meat production (UFV), crude protein (CP) and crude fibre (CF) by 2.86, 4.75 and 4.73%, respectively. It may be thus assumed that it was comparable in both groups.

The administered HMB supplement had a significant effect on the growth rate of kids (Table 2). The body weight of experimental kids (group II) was higher compared to the control kids, both after 30 and 60 days of the experiment, and the observed differences were 1.96 kg and 2.75 kg, respectively ($p \leq 0.01$) which resulted from higher daily gains. The differences between the groups were statistically significant in the first 30 days ($p \leq 0.01$) as well as throughout the entire period of the experiment ($p \leq 0.01$). This also affected the growth rate of kids, which was significantly higher in group II in both experimental periods, but the resulting differences were statistically significant only in the first 30 days of the experimental period ($p \leq 0.01$).

Ultrasonography examinations showed that all parameters of m.l.d. (Table 3) were significantly higher in experimental kids than in control group animals, both after 30 and 60 days of the experiment. In this aspect, the advantage of experimental kids was more pronounced in the second half of the experiment ($p \leq 0.01$). However, no significant differences in the thickness of subcutaneous fat and the thickness of fat over loin "eye" were recorded between the groups (Table 4).

In comparison with the control group that was not fed any additives, the experimental kids administered HMB in their diets showed an increase in the levels and activities of the examined humoral immunity parameters on days 15, 30 and 60 of the study (Table 5). The

Table 1. Total nutrient intake per group in the investigated period (60 days)

Specification	Group	
	I	II
DM - dry matter (kg)	263.59	274.34
1–30	80.96	86.25
31–60	182.63	188.09
UFV - feed unit for meat production	0.35	0.36
1–30	0.11	0.11
31–60	0.24	0.25
CP - crude protein (kg)	32.45	33.99
1–30	9.86	10.53
31–60	22.59	23.46
PDIN (kg)	34.83	36.45
1–30	10.60	11.32
31–60	24.23	25.13
PDIE (kg)	31.3	32.61
1–30	9.60	10.24
31–60	21.70	22.37
CF - crude fibre (kg)	49.47	51.81
1–30	15.04	16.06
31–60	34.43	35.75

PDIN: protein digestible in the small intestine when rumen fermentable nitrogen, PDIE: protein digestible in the small intestine when rumen fermentable energy.

significance of differences in the experimental group as compared to the control group was confirmed statistically for lysozyme ($p \leq 0.05$) and ceruloplasmin ($p \leq 0.01$) activities on days 30 and 60 of the experiment, whereas for the level of gammaglobulins the increase was confirmed at all time points when the immune parameters were measured. Moreover, the activity of ceruloplasmin and the level of gammaglobulins in the animals that were supplemented with HMB increased statistically and significantly on days 15, 30 and 60 in relation to the starting day of the study (day 0) ($p \leq 0.01$). The activity of lysozyme in the same group of animals was statistically significantly higher on days 30 and 60 of the experiment. In the group of control kids, an increase in the activity of ceruloplasmin was recorded on days 15, 30 and 60 in relation to day 0, although this increase was statistically significant ($p \leq 0.01$) only on day 60.

Discussion

The results of the study unambiguously indicate a stimulating effect of HMB on the growth of kids, as the differences in the intake of nutrients were minor. A low growth rate of kids, regardless of the group, during the first 30 days of the experiment is associated with earlier weaning and transition to a milk replacer and solid feed regime. Similar consequences were reported by Szymanowska *et al.*[30] in an experiment on White Improved milk goats. The growth rates reported in that study were lower compared to the results of own studies, which may result from the fact that the former study was conducted on kids from sibling pregnancies.

Table 2. Meat performance of kids

Traits	Group			
	I		II	
	Mean	Sd	Mean	Sd
Body weight (kg):				
- at the beginning of the experiment	6.32	0.82	6.69	0.76
- after 30 days of the experiment	8.62^{B}	1.24	10.58^{A}	1.01
- after 60 days of the experiment	14.65^{B}	1.86	17.40^{A}	1.89
Daily gains (g) in the period (days):				
1–30	76.57^{B}	30.39	129.67^{A}	32.71
31–60	201.10	29.92	229.67	53.99
1–60	138.83^{B}	22.53	179.67^{A}	32.92
Growth rate (%) in the period (days):				
1–30	30.44^{B}	10.95	45.13^{A}	11.04
31–60	52.05	5.37	48.29	9.06
1–60	79.31	7.84	88.91	12.13

a, b-P $\leq$ 0.05; A, B-P $\leq$ 0.01

Table 3. Dimensions of m.l.d

	Group			
	I		II	
	Mean	Sd	Mean	Sd
- at the beginning of the experiment				
Depth (cm)	1.41	0.10	1.42	0.12
Width (cm)	3.77	0.41	3.83	0.33
Area (cm^2)	4.66	0.74	4.65	0.99
- after 30 days of the experiment				
Depth (cm)	1.72^{b}	0.17	1.95^{a}	0.21
Width (cm)	4.23^{B}	0.27	4.69^{A}	0.19
Area (cm^2)	6.53^{b}	1.23	7.81^{a}	0.68
- after 60 days of the experiment				
Depth (cm)	2.06^{B}	0.19	2.40^{A}	0.14
Width (cm)	4.68^{B}	0.17	5.01^{A}	0.07
Area (cm^2)	8.03^{B}	0.47	9.38^{A}	0.73

a, b-P $\leq$ 0.05; A, B-P $\leq$ 0.01

HMB used in the kid diets reduced, to a large extent, the negative effects of a shortened mothering period. The obtained results suggest better feed efficiency in the kids administered HMB, in the first part of experiment, which was consistent with results demonstrated in studies with steers[34]. HMB supplementation improved daily gains, feed intake and efficiency in a group of animals slaughter at 105 day of age, but in steers slaughtered in 147 day these indicators were worse than in the control group. A lack of increase in the fat cover of carcasses of the kids administered HMB (as indicated by the fat and fat-plus-skin thickness measurements that were

Table 4. Fatness indices of kids

	Group			
	I		II	
	Mean	Sd	Mean	Sd
Skin and fat thickness over the loin "eye" (cm):				
- at the beginning of the experiment	0.58	0.04	0.61	0.11
- after 30 days of the experiment	0.66	0.05	0.65	0.11
- after 60 days of the experiment	0.80	0.09	0.84	0.09
Fat thickness over the loin "eye" (cm)				
- at the beginning of the experiment	0.075	0.016	0.067	0.016
- after 30 days of the experiment	0.082	0.010	0.080	0.010
- after 60 days of the experiment	0.101	0.015	0.094	0.010

Table 5. Humoral immunity parameters of the kids

Parameters	Group	Immunological investigation days							
		0		15		30		60	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Lysozyme activity (mg/l)	I	1.98	0.72	1.75	0.50	1.57^{b}	0.21	1.52^{b}	0.59
	II	2.10	0.42	1.94	0.67	1.88^{a}	0.37	1.80^{a}	0.42
Ceruloplasmin activity (mg/l)	I	48.23	4.34	51.28	3.43	51.23^{B}	2.34	49.77^{B}	3.06
	II	48.23	3.89	53.36^{**}	4.33	55.51^{A**}	3.78	59.77^{A**}	4.11
Gamma globulin content (g/l)	I	13.57	3.95	13.86^{B}	2.73	16.03^{B}	2.95	19.73^{b**}	1.31
	II	13.52	3.33	19.26^{A**}	5.05	23.05^{A**}	4.86	23.78^{a**}	4.61

a, b-$P \leq 0.05$; A, B-$P \leq 0.01$; *-$p \leq 0.05$ in relation to day 0; **-$p \leq 0.01$ in relation to day 0

comparable to the control values) is thought to be a desirable effect from a consumer's point of view. Van Koevering *et al.*[34], in their experiment on cattle recorded a reduction in the fat thickness tissue as a result of HMB supplementation (1.07 v 0.99 cm). Such an increase in the lean body mass impacted by HMB has also been reported in studies on humans[5].

Although the role of HMB in enhancing the immune system and preventing cattle disease has been intensively investigated in both humans and in different animal species[12,14,16,22,28] such data for goats is missing in the available literature.

During own studies on lysozyme as one of the most important factors of the non-specific humoral immunity, an increase in its activity was reported in the experimental group compared to the controls and, surprisingly, a reduction of its activity was also reported in both the experimental and control group, if the results for individual days are compared to day 0. A similar increase in the activity of lysozyme in the blood of chicken broilers was reported by Ostaszewski *et al.*[17] after a 7-week period of HMB supplementation in drinking water. Krakowski *et al.*[7] observed an improvement in colostral parameters, including an increase in the activity of lysozyme, in pregnant sows administered HMB at 4–6 weeks before weaning. Ostaszewski *et al.*[16] demonstrated that HMB supplementation in piglet diets during the first three weeks after weaning generated an increase in the activity of lysozyme in the blood serum. For ceruloplasmin, which is one of the acute phase proteins, a statistically significant increase in its activity in the blood serum was recorded in

the experimental group compared to the control group, which had not been reported in previous studies on geese[22] although it was observed by Ostaszewski *et al.*[17] in hens. The observed increased activity of ceruloplasmin after goat diet supplementation preparation of HMB may be due to an induction by HMB for the production of proinflammatory cytokines (IL-1, IL-6, TNF-α) and stimulate genes of acute phase proteins in hepatocytes, including ceruloplasmin.[15] This effect is significant, because the mobilization of acute phase proteins, determining humoral innate immune mechanisms, may indicate a rapid return of the body to homeostasis. The level of gammaglobulins, one of the total protein fractions, increased statistically significantly in the blood serum in the group of kids that were HMB-supplemented as compared to the control group as well as in the experimental and control groups on the individual days of the experiment in relation to day 0, which correlated with the results of studies on geese[22], pigs[7] and fish[29].

It may be concluded that the administration of HMB to kids has a stimulating effect on their meat performance traits and selected indicators of humoral immunity.

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