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*Original article*

# Efficacy of β-hydroxy-β-methylbutyric acid (HMB) for growing rate and its influence for health indicators in blood test of young early-weaning goats

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## Abstract

The study was conducted on 26 male, 30 days-old goats, separated from their mothers, divided into two equal groups: I - control and II - experimental, consisting of 13 animals each. All animals were fed with milk replacer, experimental group received additionally 50 g/kg body weight, additive of HMB, for 60 days. The following features were analyzed: body weight, daily increases of body weight, as well as hematological and biochemical blood features. Differences in body weight were found, between experimental and control group, after 60 days of experiment 0.57 kg ($p \leq 0.01$). The daily weight gain of experimental animals was higher in comparison with control group. Significant differences were also noted in results of hematological and biochemical blood parameters. Experimental animals showed a higher level of red blood cells as well as number of lymphocytes in comparison with the control group, ($p \leq 0.01$).Significant changes were also observed in the level of triglycerides, inorganic phosphorus and protein between both groups. The acid-base balance parameters and ionogram, showed a higher pH level ($p \leq 0.05$) $HCO_3^-$ act., $HCO_3^-$ std., BE, $ctCO_2$, $O_2$sat, $K^+$, $Cl^-$ ($p \leq 0.01$), while the anion gap (AG) and $Na^+$ were significantly lower in control group ($p \leq 0.01$).

**Key words:** small ruminant, kids, feed additives, muscle mass, hematology, biochemistry

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## Introduction

Production of goats milk is associated with the necessity of early separation of young animals from their mothers and such situation may negatively affect their future growth (Szymanowska et al. 1997). Development of effective breeding technology, demands creating balanced feeding patterns and it is a significant research problem. The substance which can stimulate the animal growth, in such circumstances is β-hydroxy-β-methylbutyric acid (HMB) (Tatara et al. 2007, Tatara 2008, Tatara et al. 2012).

Endogenous HMB is one of the leucine metabolites (Van Koevering and Nissen 1992). There is also a possibility to merge exogenous HMB, produced by chemical or microbiological synthesis into metabolic pathways (Van Koevering and Nissen 1992, Vukovich et al. 2001). In humans, HMB is used as a diet supplement, which supports the muscular cells activity and strength – in case of intense workouts or in patients suffering from cachexia, AIDS or terminal cancer (Fitschen et al. 2013). The inhibitory effect of HMB on apoptotic processes, has also been demonstrated in human and chicken muscle cells (Kornasio et al. 2009). Muscle mass gain, improving the nitrogen balance and hematological parameters, was observed after using HMB in diet (Jowko et al. 2001, Portal et al. 2002, Nissen and Sharp 2003, Flakoll et al. 2004, Rathmacher et al. 2004, Kuhls et al. 2007).

HMB plays also an important role in protein metabolism by inhibiting proteolysis, supporting protein and cholesterol synthesis (Bloch et al. 1954, Adamson and Greenberg 1955, Bachhawat et al. 1955, Gey et al. 1957, Ostaszewski et al. 2000, Smith et al. 2005).

Scientific research demonstrated the stimulating effect of HMB in muscle development in turkeys (Moore et al. 2005). One of the theories says, that these changes are associated with stimulation of intestinal villi development (Tako et al. 2004), which leads to increased forage intake. Research conducted by Tatara et al. (2007) indicate another mechanism that may be associated with neuroendocrine regulators, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1), both in humans and animals. An increased level of these factors was observed in the offspring of sows receiving HMB during their pregnancy (Tatara et al. 2007, 2012). On the other hand, such changes were not observed in broilers (Qiao et al. 2013). Previous studies indicated that HMB takes part in increasing the level of certain amino-acids in organism of young lambs (Papet et al. 1997), as well as the improvement of bone system of lambs (Krupski et al. 2011).

In addition to the positive effect of HMB on increasing the muscle mass, the immunomodulatory effect of HMB should also be noted. Stimulating effect of this compound was observed in cellular and humoral defense of broilers chicken (Peterson et al. 1999) and fish (Siwicki et al. 2000, 2001, 2003). The authors of mentioned studies noticed on increase of phagocytic capacity of macrophages, lymphocyte proliferation both by ConA (concanavalin A) and LPS (lipopolysaccharide), and higher level of ASC (antibody secreting cells).

HMB plays a positive role in animal organism. Considering fact that the influence of HMB on growth and health of early-weaned goats has not been studied so far, the aim of this research was to investigate HMB as a diet supplement, its effect on health status and growth of early-weaned goats.

## Materials and Methods

The study was planned and conducted in accordance to Ethical Committee Approval, decision No. 42/2014, 25.06.2014. The experiment was conducted on 26 male, Alpine goats, from single births.

### Experimental design

Goats weaned from mothers at the age of 30 ± 3 days were divided into two groups, consisting 13 animals in each: I - control and II - experimental. Animals from both groups had a similar mean body weight at the beginning of the study (6,40 kg for control group and 6,43 for experimental group). During the 60 days of research, all animals received the same diet: the milk replacer WITAMILK 2 (Table 1), produced by Wipasz Olsztyn (Poland) at a dose of 1.5 l /for one animal/day, with water and grass haylage *ad libitum*. Animals from experimental group additionally received, β-hydroxy--β-methylbutyric acid (HMB), with a supplemental mixture, at dose of 50 mg/kg of body weight per day. Throughout the experiment, the amount of consumed and non-consumed forage was controlled. Chemical composition was determined using standard methods (AOAC 2005). The individual food intake, in both groups, was determined during the entire experiment on basis of the results obtained.

The animals were kept in accordance with the guidelines included in the animal protection act, were under the constant veterinarian supervision and they didn't show any symptoms that could suggest illness.

Table 1. Components of WITAMILK 2 produced by Wipasz Olsztyn (Poland).

| Ingredients | | WITAMILK 2 |
|---|---|---|
| Whole protein | % | 20.00 |
| Crude fat | % | 10.00 |
| Crude fiber | % | 0.80 |
| NaCl | % | 1.40 |
| Na | % | 0.60 |
| Ca | % | 0.70 |
| P org. | % | 0.60 |
| K org. | % | 1.50 |
| Methionine | % | 0.40 |
| Lactose | % | 30.00 |
| Vit A | Ul | 50 000.00 |
| Vit D3 | Ul | 8 000.00 |
| Vit E | mg | 100.00 |
| Vit K3 | mg | 4.00 |
| Vit B1 | mg | 10.00 |
| Vit B2 | mg | 8.00 |
| Nicotinic acid | mg | 40.00 |
| Pantothenic acid | mg | 30.00 |
| Vit B6 | mg | 8.00 |
| Vit B12 | mg | 0.05 |
| Biotin | mg | 0.10 |
| Choline chloride | mg | 500.00 |
| Vit C | mg | 150.00 |
| Folic acid | mg | 0.95 |
| Mg | % | 0.20 |
| Fe | mg | 120.00 |
| Mn | mg | 50.00 |
| Cu | mg | 15.00 |
| Zn | mg | 90.00 |
| I | mg | 1.20 |
| Co | mg | 2.00 |
| Se | mg | 0.30 |
| Probiotic | -/+ | + |

## Analyzed parameters

### The growth rate

Analyses of lambs growth included determinations of their body weight at the beginning of the experiment, after 30 and 60 days, as well as daily body weight gains and their growth rates in the periods of: 1-30, 31-60 and 1-6 days.

The relative growth rate (RGR) index of the lambs in selected rearing periods was determined from the formula:

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

### Hematological and biochemical blood analyses

Blood for analyses was sampled from external jugular vein three times: before the beginning of the experiment (day 0) and on the 30$^{th}$ and 60$^{th}$ day.

Hematological tests included: white blood cell count (WBC), leucogram determination (including: percentage of basophils, eosinophils, granulocytes and segmented granulocytes, lymphocytes and monocytes), red blood cell count (RBC), hematocrit value (HCT), hemoglobin level (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT). These parameters were determined in whole blood using a Siemens hematology analyzer ADVIA 2120.

Biochemical tests included: glucose, total proteins, cholesterol, triglycerides (TG), inorganic phosphorus ($P_{inorg}$), calcium (Ca), magnesium (Mg), ionogram – $Na^+$, $K^+$, $Cl^+$, acid – base balance – pH, partial pressure of carbon dioxide and oxigen ($pCO_2$ and $pO_2$), bicarbonate concentration ($HCO_3^-$), base excess (BE), oxygen saturation ($O_2$sat), $CO_2$ concentration ($ctCO_2$) and anion gap. This parameters were determined using following methods: enzymatic method (glucose, cholesterol level) colorimetric method (total protein, triglycerides, Ca, $P_{inorg}$, Mg level ). Parameters were obtained with the Cormay ACCENT 200, using Cormay diagnostic kits.

Parameters of acid-base balance and electrolytes in venous blood were determined using the RapidLab 348 from Siemens.

Hematological and biochemical blood parameters were analyzed in laboratories of the Faculty of Veterinary Medicine University of Warmia and Mazury, Olsztyn, Poland.

### Statistical analyzes

The results of the study were statistically analyzed using the analysis of variance (ANOVA) for univariate systems (body mass and daily increases) and two-factor systems (blood indicators). Significance of differences between groups was verified using the post-hoc Duncan test. The mean values presented in Tables 4-6 are given together with the standard error (± SE). The calculation was made using Statistica 13.0 (Soft Incorp).

Table 2. Total nutrient intake per group during the experiment (60 days).

| Specification | Group | |
|---|---|---|
| | I | II |
| DM – dry matter (kg) | 316.31 | 329.22 |
| UFV – feed unit for meat production | 0.42 | 0.43 |
| CP – crude protein (kg) | 38.94 | 40.79 |
| PDIN (kg) | 41.80 | 43.74 |
| PDIE (kg) | 37.57 | 39.13 |
| CF - crude fibre (kg) | 59.36 | 62.17 |

Table 3. Total growth rate per group during the experiment (60 days).

| | Group | | | |
|---|---|---|---|---|
| | I | | II | |
| | Mean | SD | Mean | SD |
| Body weight (kg): | | | | |
| - at the beginning of the experiment | 6.40 | 0.99 | 6.43 | 1.24 |
| - after 30 days of the experiment | 9.04 | 2.22 | 9.38 | 2.28 |
| - after 60 days of the experiment | 11.73[b] | 2.58 | 12.30[a] | 3.07 |
| Daily gains (g) in the period (days): | | | | |
| 1 – 30 | 87.69 | 48.16 | 98.06 | 45.00 |
| 31 – 60 | 89.81 | 21.60 | 97.50 | 31.88 |
| 1 – 60 | 88.75 | 29.49 | 97.78 | 35.92 |
| Relative growth rate (%) in the period (days): | | | | |
| 1 – 30 | 32.21 | 14.14 | 35.92 | 12.42 |
| 31- 60 | 26.49 | 5.76 | 26.78 | 5.70 |
| 1 – 60 | 57.47 | 10.99 | 61.12 | 12.32 |

a, b – $p \leq 0.01$

## Results

### Nutrient intake

The nutrient intake is presented in Table 2.

Experimental group of animals ate 3.92% more DM (dry matter) and consequently UFV (feed unit for meat production), CP (crude protein) and CF (crude fibre), respectively: 2.33; 4.53 and 4.52%.

### The growth rate

Results referring to body weights, daily body weight gains and growth rate of the lambs were presented in Table 3. The body weight after 30 and 60 days of the experiment was higher in comparison to the goats of the control group. The difference of 0.57 kg were statistically significant ($p \leq 0.01$). The experimental animals characterized by higher total growth rate in all analyzed periods.

### Blood parameters

The results of hematological analyses are presented in Table 4.

Goats receiving HMB showed a higher number of RBC in comparison with the control group. This increase was noticed after 30 and 60 days of the experiment. A higher hematocrit value was revealed. Throughout the whole period of the study, HCT was approximately at the same level in the experimental group, while in the control group it decreased.

The results of the experiment showed that WBC in the II group was lower in comparison with the control group, throughout the entire experiment. The leucogram showed a higher total number and percentage of lymphocytes in the II (experimental) group of animals, despite the decrease noted on 30$^{th}$ and 60$^{th}$ day of the study. The number of monocytes increased after 30 days of the study, and later decreased after 60 days in both groups. However, it was higher in control animals.

The group of goats fed with the addition of HMB had a lower PLT number. In both groups PLT decrease was observed throughout the entire experiment.

Table 4. Blood hematological parameters.

| Group | WBC $10^3/\mu L$ | RBC $10^3/\mu L$ | HGB g/dL | HCT % | MCV fL | MCH pg | MCHC g/dL | RDW % | PLT $10^3/\mu L$ | MPV fL | NEUT $10^3/\mu L$ | LYMPH $10^3/\mu L$ | MONO $10^3/\mu L$ | EOS $10^3/\mu L$ | BASO $10^3/\mu L$ | LUC $10^3/\mu L$ | NEUT % | LYMPH % | MONO % | EOS % | BASO % | LUC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E 0 | 12.38 ±2.31 | 14.28 ±0.50 | 8.20 ±0.27 | 28.82 ±0.78 | 20.27 ±0.41 | 5.76 ±0.13 | 28.43 ±0.20 | 33.18 ±1.28 | 1007.6 ±127.6 | 7.29 ±0.07 | 30.24 ±3.39 | 61.60 ±2.76 | 1.12 ±0.16 | 1.34 ±0.27 | 0.78 ± 0.06 | 0.29 ±0.03 | 4.43 ±0.60 | 7.51 ± 0.31 | 0.14 ± 0.02 | 0.17 ±0.03 | 0.10 ± 0.01 | 0.04 ±0.01 |
| E 30 | 12.81 ±2.15 | 16.33 ±0.56 | 8.53 ±0.30 | 28.84 ±0.99 | 17.69 ±0.40 | 5.22 ±0.11 | 29.53 ±0.10 | 21.51 ±2.62 | 1243.3 ±120.6 | 7.01 ±0.07 | 34.68 ±3.20 | 60.93 ±3.06 | 2.49 ±0.56 | 0.83 ±0.08 | 0.76 ±0.08 | 0.32 ±0.05 | 4.59 ±0.65 | 7.66 ±0.25 | 0.32 ±0.08 | 0.10 ±0.01 | 0.10 ±0.01 | 0.04 ±0.01 |
| E 60 | 13.27 ±3.19 | 16.70 ±0.45 | 8.91 ±0.19 | 28.82 ±0.66 | 17.29 ±0.34 | 5.34 ±0.09 | 30.96 ±0.10 | 22.33 ±0.38 | 952.3 ±43.2 | 7.10 ±0.07 | 35.85 ±1.71 | 60.00 ±1.54 | 1.69 ±0.43 | 1.32 ±0.60 | 0.88 ±0.11 | 0.26 ±0.04 | 4.86 ±0.55 | 7.87 ±0.47 | 0.23 ±0.06 | 0.16 ±0.02 | 0.12 ±0.02 | 0.03 ±0.01 |
| C 0 | 13.82 ±4.01 | 14.28 ± 0.43 | 7.92 ± 0.26 | 28.43 ±0.87 | 19.97 ± 0.52 | 5.56 ±0.15 | 27.89 ±0.14 | 36.56 ± 1.11 | 1022.4 ± 60.5 | 7.53 ±0.09 | 40.00 ±1.80 | 55.07 ±1.91 | 2.05 ±0.61 | 1.89 ±0.23 | 0.78 ±0.17 | 0.22 ±0.02 | 5.13 ±0.47 | 7.47 ±0.58 | 0.33 ±0.12 | 0.24 ±0.02 | 0.11 ±0.02 | 0.03 ±0.01 |
| C30 | 13.74 ±1.97 | 15.31 ± 0.71 | 7.74 ± 0.37 | 26.32 ±1.08 | 17.23 ± 0.94 | 5.06 ±0.10 | 29.42 ±0.26 | 26.53 ± 0.69 | 1298.6 ± 76.2 | 7.18 ±0.05 | 43.18 ±2.28 | 50.98 ±1.73 | 3.66 ±2.90 | 1.02 ±0.28 | 0.77 ±0.08 | 0.34 ±0.06 | 5.98 ±0.49 | 6.99 ±0.36 | 0.49 ±0.12 | 0.14 ±0.02 | 0.10 ±0.01 | 0.04 ±0.01 |
| C 60 | 12.43 ±2.36 | 16.41 ± 0.46 | 8.56 ± 0.25 | 27.91 ±0.79 | 17.03 ± 0.27 | 5.22 ±0.08 | 30.65 ±0.15 | 23.25 ±0.40 | 980.0 ± 52.9 | 7.24 ±0.08 | 40.74 ±1.85 | 55.61 ±1.81 | 0.87 ±0.24 | 1.76 ±0.21 | 0.79 ±0.08 | 0.22 ±0.02 | 5.11 ±0.47 | 6.87 ±0.36 | 0.12 ±0.04 | 0.22 ±0.02 | 0.10 ±0.01 | 0.04 ±0.02 |
| SEM | 0.36 | 1.85 | 0.91 | 2.70 | 1.78 | 0.41 | 1.23 | 6.93 | 287.0 | 0.28 | 8.47 | 7.53 | 1.85 | 0.69 | 0.24 | 0.13 | 1.68 | 1.27 | 0.27 | 0.08 | 0.05 | 0.03 |
| **Group (0-60)** | | | | | | | | | | | | | | | | | | | | | | |
| E | 12.82 ±2.54 | 15.75± 0.35 | $8.55^{a}$ ± 0.15 | 28.83 ±0.45 | 18.44 ± 0.33 | 5.44± 0.08 | $29.64^{a}$ ± 0.21 | $25.82^{B}$ ±1.35 | 1061.7 ± 62.1 | $7.14^{B}$ ±0.04 | $33.56^{B}$ ±1.65 | 60.84 ± 1.40 | 1.74 ± 0.25 | $1.18^{b}$ ±0.12 | 0.81 ± 0.05 | 0.29 ±0.02 | 4.63 ±0.33 | 7.68 ± 0.20 | 0.23 ± 0.03 | $0.15^{B}$ ±0.02 | 0.11 ± 0.01 | 0.03 ±0.01 |
| C | 13.31 ±2.91 | 15.33 ±0.34 | $8.09^{b}$ ±0.18 | 27.60 ±0.53 | 18.10 ±0.33 | 5.29 ±0.07 | $29.32^{b}$ ±0.24 | $28.86^{A}$ ±1.17 | 1093.7 ± 43.8 | $7.32^{A}$ ±0.05 | $41.24^{A}$ ±1.12 | 53.99 ±1.09 | 2.14 ±0.42 | $1.58^{a}$ ±0.13 | 0.78 ±0.04 | 0.26 ±0.02 | 5.39 ±0.28 | 7.11 ±0.26 | 0.30 ±0.06 | $0.20^{A}$ ±0.01 | 0.10 ±0.01 | 0.04 ±0.03 |

a, b - p≤0.05 A, B - p≤0.01
SEM - standard error of the mean

Table 5. Biochemical parameters of blood.

| Group | Cholesterol mmol/l | TG mmol/l | Ca mmol/l | Mg mmol/l | P mmol/l | Gluc mmol/l | Total protein g/l |
|---|---|---|---|---|---|---|---|
| E 0 | 2.02±0.86 | 0.30±0.07 | 2.36±0.10 | 1.13±0.15 | 3.43±0.47 | 4.41±0.76 | $53.34^{Bb}$±3.31 |
| E 30 | 1.71±0.27 | 0.20±0.07 | 2.32±0.21 | 0.98±0.13 | 3.57±0.38 | 5.15±0.51 | $64.89^{A}$±3.12 |
| E 60 | 1.81±0.39 | 0.27±0.07 | 2.48±0.12 | 0.95±0.10 | 3.95±0.60 | 4.07±0.46 | $65.39^{A}$±3.07 |
| C 0 | 1.98±0.40 | 0.28±0.07 | 2.36±0.13 | 0.93±0.22 | 3.11±0.52 | 4.60±0.66 | $52.02^{C}$±3.29 |
| C 30 | 1.40±0.22 | 0.21±0.26 | 2.36±0.09 | 1.04±0.08 | 3.74±0.32 | 4.95±0.49 | $57.31^{Ba}$±4.27 |
| C 60 | 1.77±0.31 | 0.26±0.05 | 2.48±0.12 | 0.94±0.09 | 3.78±0.59 | 4.13±0.39 | $64.36^{A}$±3.10 |
| SEM | 0.06 | 0.02 | 0.02 | 0.02 | 0.07 | 0.09 | 6.48 |
| Group (0-60) | | | | | | | |
| E | 1.85±0.57 | 0.26±0.08 | 2.39±0.16 | 1.02±0.15 | 3.65±0.53 | 4.52±0.73 | $61.21^{A}$±6.64 |
| C | 1.73±0.39 | 0.25±0.15 | 2.40±0.13 | 0.97±0.15 | 3.54±0.57 | 4.55±0.61 | $57.90^{B}$±5.98 |

a, b - p≤0.05 A, B - p≤0.01
SEM - standard error of the mean

Results of biochemical analyses of blood are summarized in Table 5.

Cholesterol level decreased during the first 30 days of the study. However, after 60$^{th}$ day, its increase was observed, in both control and experimental goats. It should be noted that the average value of cholesterol determined throughout the study was higher in the experimental group. The level of triglycerides, after 60 days of the study, did not differ significantly in both groups.

In spite of the fact that in the II group an increase in the calcium level was observed after 60 days, level of this macroelement was similar in both groups. Magnesium level decreased in the supplemented group and its level in the blood after 60 days was higher than in the controlled group. The level of phosphorus, increased 60 days of experiment in all animals.

Glucose level in animals receiving HMB increased during the first 30 days, however it decreased during the next month. After 60 days, the average blood glucose level was comparable in both groups. The level of total protein was higher in the group that received HMB, both after 30 and 60 days of the study.

Acid-base balance and blood ionogram parameters are presented in Table 6.

The blood pH of all young goats was at the same level until 30$^{th}$ day of the study. Higher level of this parameter was observed after 60$^{th}$ day in the group supplemented with HMB.

Level of $pCO_2$ and $pO_2$ showed similar changes: decrease until 30$^{th}$ day of the study, followed by increase after two months. The average level of both parameters was similar in both groups.

During 60 days of the study, a continuous increase

Table 6. Acid-base balance and blood ionogram.

| Group | pH | $pCO_2$ mmHg | $pO_2$ mmHg | $HCO_3$akt mmol/l | $HCO_3$std mmol/l | BE(ecf) mmol/l | BE(B) mmol/l | $ctCO_2$ mmol/l | AG | $O_2$sat % |
|---|---|---|---|---|---|---|---|---|---|---|
| E 0 | 7.32±0.18 | 50.12±14.18 | 37.33±5.60 | 27.35±10.80 | 25.257±9.02 | 1.56±13.58 | 0.67±12.50 | 28.78±11.15 | 6.01±20.69 | 64.93±16.32 |
| E 30 | 7.47±0.09 | 47.80±10.65 | 27.08±4.93 | 33.30±2.69 | 31.11±2.99 | 9.80±2.90 | 8.54±2.77 | 34.73±2.86 | 11.12±4.97 | 51.57±17.37 |
| E 60 | 7.51±0.02 | 49.37±2.03 | 33.48±2.38 | 38.13±3.05 | 36.52±3.21 | 15.48±3.41 | 13.61±2.93 | 39.55±3.10 | -9.06±3.66 | 64.74±5.61 |
| C 0 | 7.32±0.07 | 51.32±6.97 | 40.02±11.72 | 25.69±4.60 | 23.32±4.29 | -0.02±5.40 | -0.57±4.82 | 27.17±4.66 | 32.57±13.31 | 64.13±18.70 |
| C 30 | 7.47±0.07 | 47.91±9.21 | 29.33±3.04 | 33.33±2.77 | 31.47±2.20 | 10.08±2.55 | 8.94±2.14 | 34.72±2.95 | 8.70±5.65 | 53.59±10.38 |
| C 60 | 7.44±0.05 | 48.39±2.17 | 31.88±3.05 | 31.72±3.77 | 29.67±3.97 | 7.90±4.63 | 6.85±4.18 | 33.11±3.81 | 4.68±6.77 | 56.92±6.76 |
| SEM | 0.02 | 1.10 | 0.96 | 0.88 | 0.84 | 1.10 | 1.01 | 0.90 | 2.30 | 1.84 |
| Group (0-60) | | | | | | | | | | |
| E | 7.43±0.14 | 49.14±9.97 | 32.82±6.07 | 32.91±7.96 | $30.96^{a}$±7.40 | 8.92±10.02 | 7.58±9.25 | 34.34±8.13 | $1.74^{B}$±15.09 | 60.72±14.86 |
| C | 7.41±0.09 | 49.25±6.61 | 33.90±8.45 | 30.14±4.98 | $28.04^{b}$±5.01 | 5.84±6.14 | 4.94±5.63 | 31.56±5.01 | $15.54^{A}$±15.54 | 58.37±13.34 |

a, b - p≤0.05 A, B - p≤0.01
SEM - standard error of the mean

of $HCO_3$ level was observed in experimental goats, while in the control group this parameter increased only during first 30 days. Similar trend was noted for BE. Its level in the supplemented group was higher than in the control group. The magnitude of anion gap in the control group was decreasing during the entire experiment. In the experimental group it increased during the first 30 days, but then it started to decrease.

In the II group there was a continuous increase of $ctCO_2$, while in the I group this parameter's level decreased after 30 days. In the case of $O_2$ saturation a decrease was observed in the experimental group for the first 30 days, after that it started to increase (to values comparable to the initial ones). In the control group, a continuous decrease of this parameter was noted through whole time of the experiment.

## Discussion

The results obtained clearly indicate the stimulating effect of HMB on muscle mass of young goats. The low growth rate of experimental animals in the first 30 days of the study is associated with early weaning from their mothers and transition to feeding with milk replacer and solid feed. Similar effects were observed by Szymanowska et al. (1997). It should be noted that body weight gains, demonstrated in that research, were lower in comparison with the results of the present research, which may be connected to the fact, that it was conducted on kids from twin births. The addition of HMB used for kids nutrition significantly compensated the negative effects of early weaning. Experimental group achieved 18.77% higher final body weight than the control group. That was possible mainly because of the higher daily weight increase in the first period of the experiment.

The results indicate the influence of HMB on some hematological parameters. HMB supplementation cause an increase of RBC. Similar results were observed by Rathmaher et al. (2004). HMB may have inhibitory effect on cellular apoptosis, due to the reduction of caspase 3 and 9 (Hao et al. 2011), level of which affects the activity of apoptosis. In the aspect of HMB, the phenomenon of caspase activation by an apoptotic signal was examined mainly in relation to myolysis inhibition (Slater and Jenkins 2000). Influence of HMB for cellular apoptosis of erythrocytes has not been exposed so far and requires further research.

During the experiment, the immunostimulatory effect of HMB on the body of young goats was noted, together with an increase of the number of blood lymphocytes. This observation was confirmed by previous studies, suggesting a positive effect of HMB on lymphocyte blastogenesis (Nissen and Abumrad 1997, Siwicki et al. 2004). The present research indicates that the addition of HMB to forage stimulates humoral and cellular immune response. At low concentrations HMB can act positively on the production of lymphocytes and other cells of the immune system. At higher doses, a reduction in lymphocyte blastogenesis may be observed. This indicates a reverse effect to the intended immunoactivity increase. Administration of HMB at dose of 50 mg/kg resulted in an increase of lymphocyte levels. It can be assumed that this dose stimulates the immune system of young goats.

Some studies (Nissen et al. 1996, Shu-Ling et al. 2006) propose that HMB may be a precursor of cholesterol. On the other hand, other researchers suggest that HMB reduces the level of cholesterol in organism (Ostaszewski et al. 1995). As revealed in the present study, cholesterol blood level fluctuates in both experimental and control group. This suggests that HMB has no effect on the cholesterol blood level in young goats.

High level of triglycerides in the serum of both groups, may be explained by physiological hyperlipidemia in young small ruminants. Barowicz et al. (1994) observed a higher content of fat fraction in the serum of young lambs, compared to older animals. It seems that the reason for this phenomenon may be the higher content of fat fraction in young goats' diet.

The results indicate a higher level of phosphorus in the blood of the experimental animals. This fact can be explained by irregular and individual level of ossification in animals. The level of this parameter stays, however, within the physiological norm (Shiga et al. 1995). The level of calcium and magnesium was similar in both groups, and the results remained within the normal range.

Higher level of total proteins in the experimental group was observed. The blood pH level increased in the supplemented group. Moreover, higher levels of BE and $HCO_3$ were observed in the experimental group. This suggests a slight blood alkalosis in supplemented group. This is confirmed by the magnitude of anion gap. Occurrence of alkalosis, may be related to the stimulatory effect of HMB on protein synthesis (Smith et al. 2005). There are a few theories explaining this effect. According to one, it is related to the influence of HMB on mTOR mammalian target of rapamycin (Zanchi et al. 2008) - kinase involved in the control of cell growth and mRNA translation efficiency. HMB supplementation increases the phosphorylation of mTOR, its S6 kinase substrates (S6K) and the protein-binding factor to the eukaryotic 4E translation initiation factor (Eley et al. 2007, Aversa et al. 2011). According to the other research, this fact is related to HMB influence on the GH/IGF-1 signaling axis (Kornasio et al. 2009, Gerlinger-Romero et al. 2011). The mechanism of the HMB impact on protein increase is not fully-known and requires further research.

During the experiment, a higher $O_2$sat was noted in group of young goat fed with addition of HMB. These values, however, did not exceed physiological norms.

In conclusion, we can say that HMB used as a supplement of the young goats diet has a positive effect on their growth and immunity status. It also reduces the negative effects of early weaning on daily weight gain. In addition, HMB does not show any adverse effect on the health status of animals tested.

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