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Short communication

# The effect of $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) on selected parameters of humoral immunity in calves

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## Abstract

The objective of this study was to evaluate the effect of HMB on selected parameters of the humoral immunity in calves. The experiment was performed on 14 calves aged  $30 \pm 2$  days, divided into two equal groups of control (group K) and experimental (group H) animals. The feed administered to the experimental calves was supplemented with HMB at 40 mg/kg BW, whereas the control calves were administered standard farm-made feed without supplementation. Blood was sampled from the jugular vein immediately before the experiment (day 0) and on experimental days 15, 30 and 60 to determine the following immunological parameters: total protein levels, gammaglobulin levels, lysozyme activity and ceruloplasmin activity. An analysis of the results obtained revealed a significant increase ( $p < 0.05$ ;  $p < 0.01$ ;  $p < 0.001$  respectively) in gammaglobulin levels and lysozyme activity throughout the entire experimental period, an increase ( $p < 0.05$ ;  $p < 0.01$  respectively) in ceruloplasmin activity on experimental days 15 and 30, but no changes in serum total protein levels of calves administered HMB as compared to those found in the control group.

**Key words:** HMB, calves, total protein, ceruloplasmin, lysozyme, gammaglobulin

## Introduction

$\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) is naturally synthesized in the body from the oxidation of approximately 5% of dietary leucine, whereas the remaining 95% of the product of leucine transamination, 2-ketoisocaproate ( $\alpha$ -KIC), is converted to isovaleryl-CoA in the liver (Van Koeving and Nissen 1992). Small quantities of HMB are found in corn,

milk, cheese, citrus fruit, selected fish species, red wine and red meat. The quantity of HMB which occurs naturally in the body and is supplied with food is insufficient and, therefore, has to be supplemented from external sources. Dietary supplementation is safe because even if the recommended dose is exceeded, there are no side effects, and excessive HMB is excreted with urine (Vukovich et al. 2001). HMB has been found to stimulate the immune system of

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Table 1. The effect of HMB on selected parameters of humoral immunity in calves.

Parameter	Group	Experimental day							
		0		15		30		60	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Total protein levels (g/l)	K	48.71	1.99	49.12	3.24	50.43	4.05	55.09	4.16
	H	48.99	5.55	50.61	5.12	53.94	1.77	57.51A	5.69
Gammaglobulin levels (g/l)	K	7.01	0.67	8.96	0.72	8.89	0.59	10.11	0.53
	H	7.06	0.94	10.37 <sup>*,C</sup>	1.34	11.56 <sup>***,C</sup>	0.83	13.40 <sup>***,C</sup>	0.35
Lysozyme activity (mg/l)	K	0.42	0.11	0.34	0.07	0.37	0.09	0.39	0.10
	H	0.37	0.15	0.51 <sup>**</sup>	0.10	0.53 <sup>**A</sup>	0.08	0.56 <sup>**A</sup>	0.05
Ceruloplasmin activity (mg/l)	K	47.67	3.42	44.85	2.67	52.88	3.97	48.93	3.01
	H	51.20	3.25	50.04 <sup>**</sup>	2.55	57.69 <sup>*,B</sup>	3.52	52.14	2.83

Key: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; SD – standard deviation; A –  $p \leq 0.05$ ; B –  $p \leq 0.01$ ; C –  $p \leq 0.001$  in comparison with day 0.

many animal species (Gatnau et al. 1995, Peterson et al. 1999, Puchajda-Skowrońska et al. 2006, Siwicki et al. 2006), but very few studies investigating its beneficial effects have been conducted on cattle (Talleyrand et al. 1994, Wójcik et al. 2013). The objective of this study was to evaluate the effect of HMB supplementation on selected parameters of the humoral immunity in calves.

## Materials and Methods

### Experimental design

The experiment was performed on 14 Polish Holstein-Friesian calves aged  $30 \pm 2$  days, divided into two equal groups of control (group K) and experimental (group H) animals. The feed administered to the experimental calves was supplemented with  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB, Metabolic Technologies Inc. Ames, IA, USA) at 40 mg/kg BW, whereas the control calves were administered standard farm-made feed without supplementation. Blood was sampled from the jugular vein prior to HMB supplementation of feed and on days 15, 30 and 60 of the experiment to determine and compare selected indicators of the immunity.

### Evaluation of biochemical parameters

Total serum protein levels were determined with spectrophotometry using method proposed by Lowry et al. (1951) and modified by Siwicki and Anderson (1993).

### Evaluation of non-specific humoral immunity parameters

Serum gammaglobulin levels were determined by the precipitation method modified by Siwicki and Anderson (1993), plasma lysozyme activity was determined by the turbidimetric method (Parry et al. 1965) modified by Siwicki and Anderson (1993) and plasma ceruloplasmin activity by the method developed by Siwicki and Studnicka (1986).

### Statistical analysis

The results obtained were processed statistically by one-way ANOVA with an orthogonal design. The significance of differences between groups was verified by the Student's *t*-test and the Bonferroni test with the use of GraphPad Prism 5 software.

## Results and Discussion

No significant differences in serum total protein levels were observed between calves whose feed was supplemented with HMB (H) and control group animals (K). A significant increase ( $p \leq 0.05$ ) in serum total protein levels was observed only on day 60 in the experimental group in comparison with day 0 (Table 1). Similar results were reported in our previous study of geese (Puchajda-Skowrońska et al. 2006), whereas Krakowski et al. (2002) observed a significant increase in the total protein content of colostrum in pregnant sows after the administration of HMB.

A significant increase ( $p < 0.05$ ;  $p < 0.001$ ) in concentrations of gammaglobulins, one of the protein fractions investigated, was observed throughout the entire experiment in the group of calves administered HMB in comparison with those found in the control and in the experimental animals on different days of the experiment relative to day 0 (Table 1). Similar results were reported in studies of geese (Puchajda-Skowrońska et al. 2006), pigs (Krakowski et al. 2002) and fish (Siwicki et al. 2006). Lysozyme activity, one of the key indicators of non-specific humoral immunity, continued to increase ( $p < 0.01$ ) in the experimental group throughout the entire experiment in comparison with the control, and higher levels of lysozyme activity were observed in the experimental group on days 30 and 60 relative to day 0 (Table 1). The above findings are consistent with the results reported by Puchajda-Skowrońska et al. (2006), Krakowski et al. (2002) and Siwicki et al. (2006). In comparison with the control values, the activity of ceruloplasmin, an acute phase protein, increased significantly ( $p < 0.05$ ;  $p < 0.01$ ) on days 15 and 30 in the experimental group, which was not found in geese (Puchajda-Skowrońska et al. 2006), but was reported in a study of chickens (Ostaszewski et al. 1998). The results of this experiment indicate that the administration of HMB significantly ( $p < 0.05$ ;  $p < 0.01$ ;  $p < 0.001$  respectively) affects the non-specific humoral immune response.

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