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Short communication

The effect of β -hydroxy- β -methylbutyrate (HMB) on the proliferative response of blood lymphocytes and the phagocytic activity of blood monocytes and granulocytes in calves

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Abstract

The objective of this study was to evaluate the effect of HMB on selected indicators of immunity in calves. The experiment was performed on 14 calves aged 30 ± 2 days, divided into two equal groups of control (group I) and experimental (group II) animals. The feed administered to experimental group calves was supplemented with HMB at 40 mg/kg BW, whereas 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 parameters of immunity: proliferative response of LPS- and ConA-stimulated lymphocytes (MTT), respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes. The results revealed a significant increase in RBA and MTT values in calves administered HMB in comparison with the control group throughout the experiment. In the group of animals receiving HMB, an increase in PKA values was noted only on day 30.

Key words: calves, HMB, phagocytic activity of blood granulocytes and monocytes, proliferative response of blood lymphocytes

Introduction

The preventive and therapeutic efficacy of substances modulating the immune system of different animal species has been investigated in laboratory and clinical studies for many years (Nartowska et al. 2004, Wójcik et al. 2010). An example of an immune stimulator is β -hydroxy- β -methylbutyric acid (HMB) which

occurs naturally in humans, animals and plants. The results of research investigating various animal species (fish, birds, mammals), including limited studies of cattle (single experiments performed on older animals) have demonstrated that HMB plays an important role in stimulating the immune system and preventing disease (Talleyrand et al. 1994, Peterson et al. 1999, Puchajda-Skowrońska et al. 2006). The objective of this study was to evaluate the effect of in-feed

Table 1. The effect of HMB on the phagocytic activity of blood monocytes and granulocytes and the proliferative response of blood lymphocytes in calves.

Parameter	Group	Experimental day							
		0		15		30		60	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
RBA (OD 620 nm)	K	0.37	0.07	0.33	0.03	0.40	0.04	0.29	0.06
	H	0.36	0.03	0.37 [*]	0.02	0.46 ^{*,C}	0.05	0.39 ^{**}	0.05
PKA (OD 620 nm)	K	0.17	0.04	0.19	0.03	0.15	0.04	0.13	0.01
	H	0.17	0.02	0.19	0.04	0.27 ^{***,C}	0.04	0.14	0.01
MTT-ConA (RI)	K	1.21	0.18	0.86	0.12	1.04	0.22	0.96	0.10
	H	1.51	0.48	1.76 ^{***}	0.33	1.47 [*]	0.32	1.20 ^{**}	0.12
MTT-LPS (RI)	K	0.94	0.2	0.81	0.04	0.96	0.24	0.93	0.08
	H	1.03	0.19	1.33 ^{***}	0.26	1.41 [*]	0.46	1.29 ^{***}	0.12

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 to day 0.

administered HMB on the proliferative response of blood lymphocytes and the phagocytic activity of blood monocytes and granulocytes in calves.

Materials and Methods

Experimental design

The experiment was performed on 14 Polish Holstein-Friesian calves aged 30 ± 2 days, divided into two equal groups of control (group I) and experimental (group II) animals. The feed administered to experimental group calves was supplemented with β -Hydroxy- β -Methylbutyrate (HMB, Metabolic Technologies Inc. Ames, IA, USA) at 40 mg/kg BW, whereas 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 immunity.

Evaluation of non-specific cellular immunity parameters

The metabolic activity of blood phagocytic cells was determined based on the measurement of intracellular:

Respiratory burst activity (RBA) after stimulation with PMA (Phorbol Myristate Acetate, Sigma), as described by Chung and Secombes (1988) and adapted by Siwicki et al. (2004); The potential killing activity (PKA) of mononuclear (MN) phagocytes and

polymorphonuclear (PMN) phagocytes was determined in isolated blood leukocytes stimulated with killed microorganisms, according to the method presented by Rook et al. (1995) and adapted by Siwicki et al. (2004).

The proliferative response of blood lymphocytes

Proliferative response of blood lymphocytes after stimulation with mitogens, concanavalin A (ConA) and lipopolysaccharide (LPS), were determined by MTT assay, first described by Mosmann (1983) with some modifications described by Wagner et al. (1999).

Results and Discussion

In evaluations of nonspecific cellular immunity, which involved analyses of respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes, the most significant increase was noted in RBA values which were 12%, 15% and 35% higher in the experimental group (H) than in the control group (C) on days 15, 30 and 60 of the experiment, respectively. PKA values were identical in both groups of animals during the first 15 days of the experiment. An increase in PKA values was noted on days 30 and 60 in the group receiving HMB supplementation, but a significant difference (80%) between groups was observed only on day 30. In the experimental group, RBA and PKA values increased significantly only on day 30 of the experiment in comparison with day 0 (Table 1).

In our previous studies on geese (Puchajda-Skowrońska et al. 2006), where HMB was administered with feed, we observed increased levels of metabolic and phagocytic activity of polymorphonuclear (PMN) and mononuclear cells (MN) as well as intensified lymphocyte proliferation, but differences in the activity of those cellular mechanisms were noted between the sexes. In evaluations of specific cellular immunity, which involved analyses of the proliferative response of LPS- and ConA-stimulated lymphocytes (MTT), both lymphocyte populations (T and B) were characterized by similar sensitivity to HMB. In comparison with control, the proliferative activity of B lymphocytes in experimental calves increased significantly by 64%, 47% and 39%, and the activity T lymphocytes – by 104%, 41% and 25% on days 15, 30 and 60, respectively (Table 1). In an *in vitro* study investigating the responses of the chicken macrophage cell line (MQ-NCSU) to HMB (Peterson et al. 1999), the expression of Fc receptors (FcR) on the surface of macrophages was intensified and significantly higher in comparison with control. HMB was also found to induce macrophage proliferation in cell lines and intensify their effector (cytotoxic) functions, such as nitrite production and the phagocytic activity of macrophages. The results of this experiment indicate that the administration of HMB significantly affects cellular immune response.

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