INFLUENCE OF DIETARY ADMINISTRATION OF THE β-HYDROXY-β-METHYLBUTYRATE (HMB) ON THE INNATE IMMUNITY AND RESISTANCE AGAINST BACTERIAL INFECTIONS IN PIKEPERCH (*SANDER LUCIOPERCA*)

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UTICAJ β-HIDROKSI-β-METILBUTIRATA (HMB) DODAVANOG U HRANU NA IMUNITET I OTPORNOST NA BAKTERIJSKE INFEKCIJE KOD SMUĐA (*SANDER LUCIOPERCA*)

Abstrakt

U ovoj studiji je ispitivan uticaj metabolita leucina β-hidroksi-β-metilbutirata (HMB) na imunitet i zaštitu od enterične bolesti crvenih usta (ERM) i furunkuloze kod smuđa (*Sander lucioperca*). Ribe su bile hranjene 18 sati dnevno komercijalnom hranom za pastrmke. Hrana je formulisana tako da obezbedi ili 0 (kontrolna grupa) ili 50 mg HBM/kg telesne težine na dan (grupa hranjena sa HBM) tokom 4 nedelje. Posle hranjenja sa HBM 20 zdravih smuđeva prosečne težine 35 g je anestezirano i uzeta je krv iz kaudalne vene u heparizovane špriceve. Takođe je aseptično izdvojen pronefros svake ribe i dobijena suspenzija pojedinačnih ćelija za izolovanje ćelija koristeći ili Gradisol (Polfa) ili Percoll (Pharmacia) gradijent. Test na izazivanje bolesti upotrebom *Yersinia ruckeri* ili *Aeromonas salmonicida* je obavljen 4 nedelje posle hranjenja. Ukratko, svakoj od 20 riba svake grupe data je jedna intraperitonealna injekcija 48 sati kulture *Y.ruckeri* ili *A. salmonicida* (0,2 ml). Zabeležen je mortalitet, a prisustvo patogena potvrđeno izolacijom iz bubrega. Rezultati ovog ogleda pokazuju da HBM u dozi od 50 mg/kg telesne težine statistički značajno stimuliše nespecifične ćelijske i humoralne odbrambene mehanizme i zaštitu od *Yersinia ruckeri* i *Aeromonas salmonicida*.

Ključne reči*: smuđ, HBM, specifični imunitet, enterična bolest crvenih usta (ERM), furunkuloza*

INTRODUCTION

The interaction between nutrition, defence mechanism and protection against diseases in fish has long been known, but this relation is far more complex than originally thought. Nutritional support is important for optimum health by providing the building blocks of nonspecific cellular and humoral defence mechanisms and thus protection against infectious diseases. Certain nutrients can be supplemented in the feed to stimulate or modulate directly host defence mechanisms. Several natural and synthetic drugs and biological modifiers have been tested in fish *in vitro* and *in vivo* and many of these products were applicated for stimulation of nonspecific defence mechanisms and protection against diseases (Siwicki et al. 1998). The substance β-hydroxy-β-methylbutyrate (HMB) is a catabolate of the amino acid leucine. In rainbow trout and tench HMB activated cell-mediated immunity (Siwicki et al. 2001; Siwicki et al. 2006). This study continues the examination of the influence of feeding the leucine metabolite HMB on the nonspecific defence mechanisms and on protection against bacterial diseases of pikeperch (*Sander lucioperca*) grown in an intensive system of culture.

MATERIAL AND METHODS

The fish were reared at the Department of Aquaculture, Inland Fisheries Institute in Olsztyn, Poland. The juvenile pikeperch were reared in circular fibreglass tanks with a water in volume of 200 litres each. The tanks were part of a recilculation system equipped with biological and mechanical filters. The water temperature was maintained at a constant level of about 22°C. Twenty-four hours illumination was applied with the light intensity at 30-80 lux. The fish were fed for 18 h per day with a commercial trout feed (TROUVIT, Nutreco Aquaculture, Holland) using automatic band feeders. The diets were formulated to provide either 0 (control-fed group) or 50 mg HMB kg^{-1} body weight per day (HMB-fed group) for 4 weeks. The leucine metabolite was obtained as a monohydrate calcium salt with a purity > 98 % (Metabolic Technologies, Ames, USA). The feed was distributed *ad libitum*, which was confirmed by observations of feed waste. The fish were observed daily for unusual behaviour, morphological changes and any mortality. Four weeks after feeding HMB, 20 healthy pikeperch of approximately 350 g were anaesthetised in Propiscin (IFI, Poland) and blood was drawn from the caudal vein into heparinized syringes. Also the pronephros of each fish was removed aseptically and single cells suspension were obtained for isolating individual cells using either a Gradisol (Polfa) or Percoll (Pharmacia) gradient. The metabolic activity of pronephros phagocytes by their respiratory burst activity (RBA) stimulated by Phorbol myristate acetate (PMA, Sigma) was measured by the technique presented by Siwicki et al. (2000). Potential killing activity (PKA) of the pronephric phagocytes was measured by the method presented by Siwicki and Anderson (1993). The lymphocytes proliferation (LP) was determined by the MTT colorimetric assay methods modified by Siwicki et al. (2000) for the fish species. The mitogens concanavaline A (ConA, Sigma) or lipopolisaccharide (LPS, Sigma) were used for the stimulation of lymphocytes. The lysozyme activity in the plasma was measured in a turbidimetric assay presented by Siwicki and Anderson (1993) and ceruloplasmine activity in the plasma was determined according

to Siwicki and Studnicka (1986) which was modified for micro-methods. Total protein level in serum was measured by the colorimetric Lowry micro-methods (Sigma, Diagnostic Kits) and total immunoglobulin (Ig) levels in the serum were measured by spectrophotometric methods (Siwicki and Anderson, 1993). A disease challenge test using *Yersinia ruckeri* or *Aeromonas salmonicida* were conducted after 4 weeks of feeding. Briefly, 20 fish from each group were each given a single intraperitoneal injection of a 48 h growth of *Y. ruckeri* or *A. salmonicida* (0.2 ml). Mortalities were tabulated and the presence of pathogens was confirmed by isolation from the kidney. The data were statistically evaluated with the Student's t-test, and the results are presented as mean and standard deviations (SD). The significance level used was $P<0.05$.

RESULTS AND DISCUSSION

The results of this experimental study showed that HMB at a dose of 50 $mgkg^{-1}$ body weight statistically stimulated the non-specific cellular and humoral defence mechanisms (Table 1). The phagocytic ability (RBA) and potential killing activity (PKA) of pronephros phagocytes were statistically significant higher ($P<0.05$) from HMB-fed pikeperch, compared to fish from control group. The similar pattern was observed in proliferative response of pronephros lymphocytes stimulated by mitogens ConA or LPS. The lymphocytes proliferation was statistically significant ($P<0.05$) higher in pikeperch fed with HMB, compared to the control group of fish. The analyses of the study results showed that HMB activated nonspecific humoral-mediated immunity. The lysozyme activity in plasma and total Ig levels in serum were statistically significant ($P<0.05$) greater that in the control-fed pikeperch. The feeding of HMB decrease the mortality to 40 % after infection with *A. salmonicida* and 30 % after infection with *Y. ruckeri.*

The current study indicates that feeding HMB to pikeperch in intensive system of culture can improve the innate immunity and decrease in mortality after experimental infected fish with pathogenic bacteria *Y. ruckeri* and *A. salmonicida.* The application of HMB by feed demonstrated to have a practical and economical impact in intensive pikeperch culture.

Table 1. The nonspecific cellular and humoral defence mechanisms levels in control-fed and HMB-fed pikeperch (n = 20; mean ± SD, * statistically significant $P<0.05$

Immunological parameters:	Control group	HMB-fed group
RBA of phagocytes (OD 620 nm)	0.45 ± 0.04	0.59 ± 0.05*
PKA of phagocytes (OD 620 nm)	0.41 ± 0.04	0.62 ± 0.04*
LP stimulated by ConA (OD 620 nm)	0.49 ± 0.05	0.57 ± 0.05*
LP stimulated by LPS (OD 620 nm)	0.34 ± 0.04	0.51 ± 0.05*
Lysozyme activity (mg l^{-1})	45.5 ± 5.8	57.5 ± 3.9*
Ceruloplasmine activity (IU)	22.8 ± 7.5	21.5 ± 2.5
Total protein in serum (g l^{-1})	64.5 ± 4.0	67.7 ± 3.5
Total Ig in serum (g l^{-1})	18.0 ± 1.4	21.5 ± 2.0*

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