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Short Communication

Effect of Dietary Administration of the β-Hydroxy-β-Methylbutyrate (HMB) on the Innate Immunity and Protection against Aeromonas Septicaemia in European Catfish (*Silurus Glanis*) Fingerlings

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- Innate immunity
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Abstract

The present study examined the influence of leucine metabolite β-hydroxy-β-methylbutyrate (HMB) on the nonspecific cellular and humoral defence mechanisms in european catfish (Silurus glanis). The fish were fed a commercial feed with 50 mg or 100 mg HMB kg^{-1} feed per day (HMB-50 or HMB-100) for 4 weeks. The control group of fish was fed pellets without HMB (control group). After feeding HMB, 50 healthy european catfish of approximately 40 g from each group were anaesthetised and blood was drawn from the caudal vein into heparinized syringes. Also the pronephros and spleen 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. A disease challenge test using *Aeromonas hydrophila* were conducted after 4 weeks of feeding. Briefly, 100 fish from each group were each given a single intraperitoneal injection of a 48 h growth of *A. hydrophila* (0.2 ml). Mortalities were tabulated and the presence of pathogens was confirmed by isolation from the kidney. The results of this experimental study showed that HMB at a dose of 50 and 100 $mgkg^{-1}$ feed per day statistically stimulated the non-specific cellular and humoral defence mechanisms and protection against *Aeromonas* septicaemia in european catfish.

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 and provides the building blocks of nonspecific defence mechanisms and thus protection against infectious diseases. Certain nutrients can be supplemented in the feed to stimulate or directly modulate 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 used for stimulation of nonspecific defence mechanisms and protection against diseases [1,2]. The substance β-hydroxy-β-methylbutyrate (HMB) is a catabolate of the amino acid leucine. In rainbow trout, pike perch

and tench HMB activated cell-mediated immunity [3-7]. This study continues the examination of the influence of feeding the leucine metabolite HMB at different doses on the nonspecific defence mechanisms and on protection against *Aeromonas hydrophila*, an important bacterial diseases of cultured european catfish.

MATERIAL AND METHODS

The fish were reared at the Inland Fisheries Institute in Olsztyn, Poland. The juvenile european catfish were reared circular fibreglass tanks with a water volume of 200 liters each. The tanks were part of a recirculation system equipped with biological and mechanical filters. The water temperature was maintained at 24°C ± 1°C. The fish were fed for 18 h per day with a commercial feed with the dose recommended by the manufacturer depending on the weight of fish and water temperature using automatic band feeders. The diets were formulated to provide either 0 (control-fed group), 50 mg HMB kg^{-1} feed per day (HMB-50 group) and 100 mg HMB kg^{-1} feed per day (HMB-50 group) for 4 weeks. The leucine metabolite was obtained as a monohydrate calcium salt with a purity > 98 % (Metabolic Technologies, Ames, USA). The fish were observed daily for unusual behaviour, morphological changes and any mortality. Four weeks after feeding HMB-50 or HMB-100, 20 healthy catfish fingerlings of approximately 50 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., [3]. Potential killing activity (PKA) of the pronephric phagocytes was measured by the method presented by Siwicki and Anderson [8]. The lymphocytes proliferation (LP) was determined by the MTT colorimetric assay methods modified by Siwicki et al., [3] 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 [8], and ceruloplasmine activity in the plasma was determined according to Siwicki and Studnicka [9] 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 [8]. A disease challenge test using *Aeromonas hydrophila* were conducted after 4 weeks of feeding. Bacteria were amplified at the TSB media (Sigma-Aldrich) and grown on Aeromonas Ryan Agar (Thermo Fisher Scientific). Briefly, 50 fish from each group were each given a single intraperitoneal injection of a 48 h growth of *A. hydrophila* using a dose of 0.2 ml/fish (2MF). Mortalities were tabulated for 10 days and the presence of pathogens was confirmed by API 20 NE kits (bioMerieux) after isolation from the kidney and growth on Aeromonas Ryan Agar (Thermo Fisher Scientific). 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 $mgkg^{-1}$ of feed and 100 $mgkg^{-1}$ of feed significantly 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-50 and HMB-100 fed european catfish, 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 european catfish fed with HMB-50 and HMB-100, compared to the control group of fish. The analyses of the study results showed that HMB at dose 50 and 100 $mgkg^{-1}$ of feed activated nonspecific humoral-mediated immunity, but we did not observe a dose-related effect. The lysozyme activity in plasma and total Ig levels in serum were statistically significant ($P<0.05$) greater at group fed HMB-50 and HMB-100 that in the control-fed european catfish. The feeding of HMB-50 decrease the mortality to 30 % and feeding of HMB-100 decrease the mortality to 40 % after infection with *A. hydrophila.*

Table 1: The nonspecific cellular and humoral defence mechanisms levels in control-fed and HMB-50 fed or HMB-100 fed in european catfish (n = 20; mean ± SD, *statistically significant $P<0.05$ to control group).

Immunological parameters:	Control group	HMB-fed groups HMB-50 HMB-100
RBA of phagocytes (OD 620 nm)	0.41 0.03	0.62 ± 0.04* 0,64 ± 0.05*
PKA of phagocytes (OD 620 nm)	0.39 ± 0.05	0.58 ± 0.03* 0,60 ± 0.04*
LP stimulated by ConA(OD 620 nm)	0.45 ± 0.04	0.59 ± 0.05* 0,58 ± 0.03*
LP stimulated by LPS (OD 620 nm)	0.32 ± 0.03	0.54 ± 0.05* 0,53 ± 0.04*
Lysozyme activity (mg l^{-1})	39.5 ± 2.8	47.5 ± 2.9* 48,1 ± 3.4*
Ceruloplasmine activity (IU)	56.8 ± 6.5	57.5 ± 8.5 55.9 ± 7.5
Total protein in serum (g l^{-1})	62.5 ± 5.2	63.2 ± 3.9 62.2 ± 4.9
Total Ig in serum (g l^{-1})	14.0 ± 1.5	19.5 ± 3.0* 18.9 ± 2.5*

The current study indicates that feeding HMB to european catfish in intensive culture can improve the innate immunity and decrease mortality after experimental infection with pathogenic bacteria *A. hydrophila.* The application of HMB by feed demonstrated to have a practical and economical impact in intensive european catfish culture.

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