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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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 septicemia 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 catabolite 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 were obtained by using 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 pronephros 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 plasma and total Ig levels in serum were statistically significant (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)

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HMB-50 HMB-100</th>
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<tbody>
<tr>
<td>RB of phagocytes (OD 620 nm)</td>
<td>0.41 ± 0.03</td>
<td>0.62 ± 0.04* 0.64 ± 0.05*</td>
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<tr>
<td>PKA of phagocytes (OD 620 nm)</td>
<td>0.39 ± 0.05</td>
<td>0.58 ± 0.03* 0.60 ± 0.04*</td>
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<tr>
<td>LP stimulated by ConA (OD 620 nm)</td>
<td>0.45 ± 0.04</td>
<td>0.59 ± 0.05* 0.58 ± 0.03*</td>
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<tr>
<td>LP stimulated by LPS (OD 620 nm)</td>
<td>0.32 ± 0.03</td>
<td>0.54 ± 0.05* 0.53 ± 0.04*</td>
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<tr>
<td>Lysozyme activity (mg l⁻¹)</td>
<td>39.5 ± 2.8</td>
<td>47.5 ± 2.9* 48.1 ± 3.4*</td>
</tr>
<tr>
<td>Ceruloplasmin activity (IU)</td>
<td>56.8 ± 6.5</td>
<td>57.5 ± 8.5 55.9 ± 7.5</td>
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<tr>
<td>Total protein in serum (g l⁻¹)</td>
<td>62.5 ± 5.2</td>
<td>63.2 ± 3.6 62.2 ± 4.9</td>
</tr>
<tr>
<td>Total Ig in serum (g l⁻¹)</td>
<td>14.0 ± 1.5</td>
<td>19.5 ± 3.0* 18.9 ± 2.5*</td>
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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.

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.  

REFERENCES


8. Siwicki, AK, Anderson DP. Nonspecific defence mechanisms assay in fish. Fish Disease Diagnosis and Prevention’s Methods. FAO-Project GCP/INT/526/JPN, IFI Olsztyn. 1993; 105-112.