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The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets

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Abstract

Plant protein sources normally used in aquaculture feed contain by nature low levels or no hydroxyproline. To evaluate the potential effect of dietary inclusion of hydroxyproline (hyp) in high plant protein diets, a regression design was carried out in an 88 days growth experiment with salmon. All experimental diets were equal with regard to chemical composition and level of the various ingredients, the only difference was the addition of crystalline hyp. Fish meal inclusion was 185 g kg^{-1}. Five different inclusion levels of hyp were used in duplicate tanks; no addition, 0.7, 1.4, 2.8 and 5.6 g kg^{-1}. One control diet was run in triplicate, added 2.8 g kg^{-1} crystalline proline (pro). Growth was significantly improved by dietary hyp addition in a polynomial regression, indicating maximum growth at 2.9 g kg^{-1} added hyp in which weight gain was improved by 14%. No effect was observed on feed intake or feed efficiency. Analysis of the whole vertebral column showed significantly increased hyp content, and lower ash content of vertebrae in fish fed increased dietary hyp inclusion. No difference was observed in fish fed diets with no addition and those fed dietary supplementation of pro. The results indicate that the anabolism of hyp may be limiting and that dietary hyp supplementation improved growth and vertebrae development or that hyp has some specific physiological or biochemical function. This information may be important in work to find replacement of fish meal in a sustainable growing global aquaculture industry and in work to prevent vertebrae disorders in farmed fish. This work suggests that a focus on hyp should be included in studies to evaluate amino acid requirements and studies of the impact of various protein sources for fish feeds.

Keywords: Atlantic salmon; Fish meal; Plant proteins; Hydroxyproline; Proline; Growth performance; Feed utilization; Vertebra

1. Introduction

In work to find replacements of fish meal for a growing global farming of carnivorous fish species, much work has been carried out on various plant protein sources to increase the quality of these by removal or inactivation of anti-nutritional factors. Another approach may be to identify beneficial properties of marine protein sources and to identifying essential components in marine resources that are missing in plant sources. New knowledge of any specific growth promoting marine component may open the possibility to improve these specific beneficial properties of marine protein sources. Such knowledge may give increased utilisation of limiting marine protein and also to ease the scientific challenge to find replacements for fish meal. Protein sources from plant and marine origin differ significantly in other components than gross chemical composition and thus may be expected to perform differently when used as ingredients in fish feed (Aksnes, 2005).

Significant dietary use of plant protein sources often result in reduction in growth and/or feed efficiency (Watanabe et al., 1993; Olli et al., 1994; Sanz et al., 1994; Gomes et al., 1995; Refstie et al., 2001; Opstvedt et al., 2003; Mundheim et al., 2004; Morris et al., 2005). The reduced growth performance by fish fed plant protein sources is partially explained by the presence of anti-nutritional factors (Olli et al., 1994; Francis et al., 2001). However other differences between protein sources of plant and marine origin could also be of importance. For instance, taurine has been shown to improve growth in Japanese

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flounder (Park et al., 2001; Kim et al., 2005) and nucleotides have been shown to improve growth and survival in salmon (Burrells et al., 2001). Both taurine and free nucleotides are present in marine and animal feed stuffs, but mainly absent in plant resources (Aksnes, 2005). Further, Aksnes et al. (2006b,c) have shown that small molecular weight compounds in marine raw material improve growth and feed efficiency in Atlantic salmon and cod. Hyp is present in fish meal, but only low levels are present in most plant sources used in animal feeds (Aksnes et al., 2006a). Several works have described increased growth performance when using hyp rich by-products such as fish silage or fish hydrolysate in diets for fish. In exchange of fish meal, fish hydrolysates generally show a beneficial effect on growth performance and feed utilisation at low inclusion levels, but decreased performance exceeding a specific dietary level. This has been shown in Atlantic salmon for fish silage (Espe et al., 1999) and for enzymatically produced fish hydrolysates (Refstie et al., 2004, Hevrøy et al., 2005). The improved performance was postulated to be due to the balance of free amino acids, peptides and proteins in digestion, absorption and utilisation from the plasma pool (Espe et al., 1999; Hevrøy et al., 2005), but no convincing evidence is presented for this explanation. Furthermore, some work testing the hyp rich ingredient fish bone in feed for fish, has shown beneficial effects on growth (Toppe et al., 2005) and nutrient digestibility (Toppe and Albrektsen 2006, unpublished). Theoretical explanations other than dietary content of hyp, such as impacts on mineral balance and intestinal transit time have been discussed (Toppe et al., 2005) to explain these effects, however. In finding replacement for fish meal in aquaculture feeds, the possible impact of small nitrogen compounds in marine materials on growth and normal performance of fish has to be taken into consideration, and hyp is a potential candidate. The above beneficial biological performance of silage, fish hydrolysates and fish bone, may theoretically also be explained by different dietary hyp levels.

In the work by Aksnes et al. (2006b), the retention of essential amino acids were determined, showing the highest values for lysine and methionine of 71 and 63%, respectively. The six experimental diets in that experiment differed significantly in hyp, from 0.8 to 5.6 g kg^{-1} diet (Aksnes et al., 2006a). Calculating a retention of hyp from data in that experiment showed differences in retention varying from 25 to 95%, and significantly inversely related to the dietary level of hyp (retention of hyp$=-13.7*(\text{hyp})+101$ ($r^2=0.64$, $n=18$). Even if hyp is a non-essential amino acid and can be synthesized *in vivo*, these data indicate a strong statistical inverse relationship between the dietary content and retention of hyp, and that dietary hyp may have an impact on biological performance.

The functional properties and mechanical strength of the connective tissue in bone and other tissues are dependent on the amount of hydroxylated proline and lysine for optimal formation of the triple helix structure of collagen (Blanck and Peterkofsky, 1975; Brinckmann et al., 2005). To our knowledge, no studies seem to be available concerning the possible dietary impacts of hyp. However, the ratio between hydroxylated and non-hydroxylated pro may indicate early signs of ascorbic acid deficiency, previous to development of more advanced deficiency signs such as lordosis and scoliosis in the bone of rainbow trout (Sato et al., 1978).

Hyp is described as a non-essential amino acid and is known to be produced by post-hydroxylation of pro after protein synthesis. There is therefore no known reason why dietary hyp should have any impact on fish performance. On the other hand hyp seems to be a scientifically low prioritised amino acid and is normally not included in amino acid data reported even in papers evaluating amino acids in fish feed (Anderson et al., 1995; Wilson, 2002). Protein diets for requirement studies normally contain some hyp. However, in the classic work by Halver et al. (1957) using crystalline amino acids for requirement studies in fish, hyp was not included. This work is often used as a basis for later studies designed to determine amino acid requirements in fish (Wilson, 2002). To our knowledge no work is described for the effect of dietary hyp inclusion in fish diets. Based on this background, we designed a simple regression growth experiment in Atlantic salmon to evaluate the possible effect of dietary supplementation of hyp in high plant protein and thus low hyp diets.

2. Materials and methods

To investigate the possible effect by dietary supplementation of hydroxyproline (hyp) a regression design was used by adding crystalline hyp to a basal diet low in hyp. To obtain this, the basal diet low in fish meal was chosen and the remaining protein source was of plant origin. The plant protein sources were chosen to be well accepted in commercial feeds.

2.1. Experimental diets

The experimental diets were formulated to be equal in crude protein, crude lipid and gross energy, and to vary only in the level of hyp. The amount of hyp

Table 1
Formulation of the experimental diets (g kg^{-1} diet)

Diet no	1	2	3	4	5	6
Ingredients						
Hydroxyproline	0	0.7	1.4	2.8	5.6	0
Proline	0	0	0	0	0	2.8
Fish meal[a]	185	185	184	182	178	182
Soy protein concentrate	67	67	67	67	67	67
Full fat soy bean meal	70	70	70	70	70	70
Extracted soy bean meal	50	50	50	50	50	50
Wheat gluten	30	30	30	30	30	30
Corn gluten	160	160	160	160	160	160
Raw wheat[b]	103	103	103	104	105	104
Fish oil[c]	290	290	290	290	290	290
Vitamins[d]	10	10	10	10	10	10
Minerals[e]	5	5	5	5	5	5
Carophyll Pink (CWS 10%)	0.5	0.5	0.5	0.5	0.5	0.5
Lysine HCl	4.0	4.0	4.0	4.0	4.0	4.0
Monocalcium phosphate	22	22	22	22	22	22

[a]Norse-LT 94, Norsildmel, Norway.
[b]Whole wheat, Norgesmøllene, Bergen, Norway.
[c]NorSalmOil. Norsildmel, Norway.
[d]Provided per kg of feed: vitamin D_3, 3000 I.E.; vitamin E, 160 mg; thiamin, 20 mg; riboflavin, 30 mg; pyrodoxine-HCl, 25 mg; vitamin C, 200 mg; calcium pantothenate, 60 mg; biotin. 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B_{12}, 0,05 mg; menadion bisulphite, 20 mg.
[e]Provided per kg of feed: magnesium 600 mg; potassium, 480 mg; zinc, 96 mg; iron, 60 mg; manganese, 12 mg; copper, 6 mg.