



Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (*Oncorhynchus mykiss*)

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ARTICLE INFO

Article history:

Received 13 April 2011

Received in revised form 15 June 2011

Accepted 24 June 2011

Available online 1 July 2011

Keywords:

Potato protein concentrate

Fish meal supplementation

Histology

Rainbow trout

Organic aquaculture

Glycoalkaloid

ABSTRACT

A feeding trial was conducted to evaluate the effect of potato protein concentrate (PPC) replacing fishmeal in nutrition of rainbow trout (*Oncorhynchus mykiss*) according to organic aquaculture guidelines. Eight diets were formulated by partial replacement of fishmeal (25%, 50%, 75% and 100%) with PPC of two different qualities (LG-PPC with low glycoalkaloid content of 7.41 mg kg⁻¹ dry weight, HG-PPC with high glycoalkaloid content of 2150 mg kg⁻¹ dry weight) and one experimental diet without PPC served as control. Experimental diets were fed over a period of 84 days to triplicate experimental groups until apparent satiation.

Best growth performance was observed in the control group. The inclusion of LG-PPC resulted in significantly reduced feed intake, feed utilization and growth with increasing replacement of 25% and higher. Increasing amount of dietary LG-PPC inclusion resulted in alterations in liver histology and in anterior intestine indicating an impact of glycoalkaloids on the different gastrointestinal tissues. The HG replacement revealed most prominently a decreased feed intake and growth parameters indicating massive undernourishment. Congruently, hypotrophy of the liver with ceroid pigment accumulation, and villi degeneration in the intestine were observed in all HG groups. Blood parameters were equally affected by an inclusion of PPC (LG and HG) in the diets involving reduced plasma TG (HG75), glucose (HG100) as well as protein (HG100). As a conclusion PPC as alternative protein in fish nutrition is only feasible with LG contents as HG diets resulted in severe undernourishment of fish, lowest growth performance and irreversible intestinal damages.

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1. Introduction

Organic aquaculture is ultimately committed to sustainability, including the sustainable use of resources for feeding. About 3724 thousand tonnes of fishmeal from capture based fisheries (16.6 million tonnes of small pelagic forage fish) were used for aquaculture feed production in the year 2006 mainly originating from decreasing wild stocks (Tacon and Metian, 2008). A sustainable growth and production of aquaculture is aiming for a progressive reduction of fishmeal from wild fisheries used in aqua feeds (Francis et al., 2001). As a consequence, alternative protein sources need to be explored, preferentially plant proteins with huge market supply. Restricted plant protein utilization in fish nutrition is commonly related to the presence of antinutritional ingredients, lower protein quality and palatability (Tacon and Jackson, 1985). But in recent years purification of plant proteins has been applied successfully to eliminate antinu-

trients and produce high quality feed sources for fish nutrition (Adelizi et al., 1998; Francis et al., 2001; Gatlin et al., 2007; Slawski et al., 2011).

In accordance to the European guideline EC 710/2009 for organic aquaculture fish feed ingredients shall be sourced from (a) certified organic aquaculture; (b) certified trimmings (e.g. MSC-certified); (c) sustainable trimmings for human fish consumption or (d) plant derived and other animal materials (listed in ANNEX V, European Union, 2009). In addition utilization of plant derived products should not exceed 70% in the nutrition of carnivorous fish. Background for that is the claim of the legislation and organic movement that a carnivorous fish should be fed species-appropriate (European Union, 2008; IFOAM, 2010). But: A sufficient supply of certifiable animal protein sources as feed is currently not available with regard to an increasing demand for organic fish products (Bergleiter, 2009; IFOAM, 2010). Thus, an increased demand of alternative protein and fat sources compliant to the ANNEX V of the Commission Regulation (EC) 710/2009 is beneficial for the development of organic aquaculture production (European Union, 2009).

The use of protein concentrates from plant origin – with crude protein contents often achieving 70–90% DM – are restricted to

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concentrates produced without the application of solvent extractors or other chemical agents (IFOAM, 2005). Consequently, the use of protein concentrates and isolates from e.g. soybean and rape seed meals (both hexane or methanol extracted for oil separation) and lupine seed (hydrochloric acid extraction for protein recovery) is thereby excluded from organic aquaculture (Sironi et al., 2005; Xu and Diosady, 1994; Zhang and Liu, 2005). In addition, the use of synthetic AA used to compensate for AA deficient plant protein profiles in organic feeds is prohibited (IFOAM, 2005).

Potato protein concentrates (PPC) are produced by thermal coagulation as a by-product of the industrial production of potato starch (Friedman, 2006; Refstie and Tiekstra, 2003; Strolle et al., 1973). This process has been approved by the European Union and organic agricultural associations for organic feed including aqua feed production (European Union, 2008; IFOAM, 2005; Naturland, 2010). Furthermore, PPCs are well balanced in the AA profile and of higher protein quality than commonly used soy and rape seed products which do not require any supplementation of free (synthetic) AA. Still, methionine has been reported as a potentially limiting AA in PPC-containing diets in trout nutrition (Refstie and Tiekstra, 2003; Xie and Jokumsen, 1997a, 1998).

Nevertheless, remaining antinutritional substances predominantly glycoalkaloids may impede both growth performance and the well-being of farmed fish (Friedman, 2006; Glencross et al., 2006; Tacon and Jackson, 1985). In addition to the impact on growth performance, glycoalkaloids and their metabolites may adversely affect gastrointestinal tract and liver. It has been reported that the inclusion of freeze-dried potato berries in mice diets (glycoalkaloid contents of 50–1600 mg kg⁻¹) resulted in histopathological abnormalities in the stomach and liver tissue (Friedman, 2006). To our knowledge, no data on health impact of potato proteins (including glycoalkaloids) are available for fish.

The aim of the present study was to explore the replacement of fishmeal by PPC with varying glycoalkaloid contents on growth

Table 1

Nutrient and amino acid composition (g kg⁻¹ dry matter) and concentrations of antinutritional factors of the used main protein sources in the experimental diets.

	Diet ingredients		
	Fish meal	LG-PPC	HG-PPC
<i>Nutrient composition (g kg⁻¹)</i>			
Dry matter	918	955	919
Crude protein	689	859	841
Crude lipid	69	32	29
Crude fibre	5	0.5	6
NfE ^a	31	24	34
Crude ash	206	85	90
Phosphorus	25	22	21
<i>Essential amino acids (g kg⁻¹)</i>			
Arginine	37.0	43.2	40.1
Histidine	12.7	19.1	17.3
Isoleucine	23.0	47.8	44.2
Leucine	40.9	90.5	82.2
Lysin	41.5	71.2	64.0
Methionine + Cysteine	20.1	32.9	30.2
Phenylalanine	22.3	57.8	52.6
Threonine	24.7	52.6	47.1
Valine	28.2	57.0	52.2
<i>Glycoalkaloids^b (mg kg⁻¹)</i>			
Solanin		4.40	1060
Chaconin		3.01	1090
Phytic acid (g kg ⁻¹)		<0.1	<0.1

^a NfE, Nitrogen-free extract = 1000 – (Crude protein + Crude lipid + Ash + Crude fibre).

^b Analyzed at LUFA-ITL, Kiel, Germany, according to LC-MS.

Table 2

Ingredients and proximate composition (g kg⁻¹ dry matter) of the experimental diets.

	Diets							
	control	LG	LG	LG	LG	HG	HG	HG
	25	50	75	100	25	50	75	100
<i>Ingredients (g kg⁻¹)</i>								
Fish meal ^a	686	514	343	171	–	514	343	171
LG-PPC ^b	–	132	265	397	529	–	–	–
HG-PPC ^b	–	–	–	–	–	140	281	421
Fish oil ^a	100	107	114	121	129	107	115	122
Wheat starch ^c	145	137	129	120	112	135	125	116
Wheat gluten ^c	20	20	20	20	20	20	20	20
vitamin ^d + mineral mixture ^e	20	20	20	20	20	20	20	20
Cellulose ^f	20	20	20	20	20	20	20	20
Magnesium silicate ^g	–	40	80	120	160	33	66	99
<i>Nutrient composition (g kg⁻¹)</i>								
Dry matter	929	936	944	952	959	930	932	935
Crude protein	488	490	486	483	477	492	491	488
Crude lipid	145	158	158	156	145	156	153	158
Crude ash	183	179	186	192	199	178	177	179
Phosphorus	14.4	13.7	13.0	12.3	11.6	13.7	13.1	12.4
NfE + crude fibre	166	141	116	100	97	152	116	110
Gross energy (MJ kg ⁻¹ DM)	20.4	20.3	20.6	20.4	20.4	20.3	20.8	21.1
<i>Essential amino acids (g kg⁻¹)</i>								
Arginine	28.2	27.3	26.3	25.4	24.5	27.4	26.6	25.8
Histidine	9.8	10.1	10.4	10.6	10.9	10.1	10.4	10.6
Isoleucine	17.7	20.1	22.4	24.7	27.0	20.2	22.7	25.1
Leucine	31.7	36.6	41.5	46.4	51.3	36.6	41.5	46.4
Lysin	31.3	33.4	35.5	37.6	39.7	33.3	35.3	37.3
Methionine + Cysteine	15.6	16.4	17.2	18.0	18.8	16.5	17.3	18.2
Phenylalanine	17.5	21.3	25.2	29.0	32.8	21.4	25.2	29.1
Threonine	18.9	21.5	24.2	26.9	29.5	21.4	24.0	26.6
Valine	21.7	24.3	27.0	29.6	32.2	24.4	27.1	29.8
<i>Glycoalkaloids (mg kg⁻¹)</i>								
Solanine	–	0.4	0.8	1.2	1.6	166	306	459
Chaconine	–	0.6	1.2	1.7	2.3	149	298	446

Tryptophane was not analysed.

^a VFCUX, Cuxhaven, Germany.

^b Emsland-Stärke GmbH, Emlichheim, Germany.

^c Cargill Deutschland GmbH, Krefeld, Germany.

^d Vitamin-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: vitamin A, 1000,000 IU kg⁻¹; vitamin D3, 200,000 IU kg⁻¹; vitamin E (as α-tocopherol-acetate), 40,000; vitamin K3 (as menadione), 4000; vitamin B1, 4000; vitamin B2, 8000; vitamin B6, 4000; vitamin B12, 8; vitamin C (as monophosphate), 60,000; pantothenic acid, 8000; nicotinic acid, 40,000; folic acid, 1600; biotin, 100; inositol 40,000.

^e Mineral-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: cobalt (as cobaltsulphate), 400; manganese (as manganese sulphate), 2500; iodine (as calciumiodine), 500; copper (as copper sulphate), 2500; selenium, 25; zinc (as zincsulphate), 28,000.

^f Pellan, Mikro-Technik, Bürgstadt, Germany.

^g Magnesia 4371, Magnesia GmbH, Lüneburg, Germany.

performance, feed intake, homeostasis of plasma parameters and intestinal histopathology of rainbow trout (*Oncorhynchus mykiss*).

2. Materials and methods

2.1. Experimental diets

Eight isonitrogenous and isoenergetic experimental diets were formulated by partial replacement of fishmeal (FM) at approximately 25%, 50%, 75%, 100%, with potato protein concentrate (PPC) of two qualities, LG with low glycoalkaloid content (7.41 mg kg⁻¹ dry weight; K5, Emsland Group, Emlichheim, Germany) and HG with high glycoalkaloid content (2150 mg kg⁻¹ dry weight; IG, Emsland Group, Emlichheim, Germany) as documented in Tab. 1. and 2. One diet with low temperature (LT) fish meal (VFCUX, Cuxhaven, Germany) as main protein source served as control diet. In order to

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