Aquaculture 354–355 (2012) 121–127

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Albumin and globulin rapeseed protein fractions as fish meal alternative in diets fed to rainbow trout (*Oncorhynchus mykiss* W.)

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ARTICLE INFO

Article history:
Received 17 January 2012
Received in revised form 15 March 2012
Accepted 21 March 2012
Available online 29 March 2012

Keywords:
Feed evaluation
Growth trial
Digestibility
Plant protein
Aqua feeds
Animal welfare

ABSTRACT

The potential of two rapeseed protein concentrates partitioned in albumin and globulin fractions as fish meal alternative was evaluated. In a digestibility experiment with juvenile rainbow trout apparent digestibility coefficients (ADCs) were determined by indirect marker method with feces collected by continuous sieving. ADCs of protein from fish meal (89.2 ± 1.1%) and globulin concentrate (88.8 ± 0.6%) were significantly higher than ADCs from albumin concentrate (77.7 ± 1.4%). ADCs of dietary dry matter were similar between the control diet (62.5 ± 4.7%) and the globulin concentrate diet (62.3 ± 0.5%), but significantly lower in the albumin concentrate diet (56.2 ± 1.5%). In a subsequent growth trial, each of 21 experimental tanks of a freshwater flow-through system was stocked with ten rainbow trout (initial average weight 31.5 ± 0.5 g). Fish were organized in triplicate groups and received experimental diets with 0, 50, 75, or 100% of fish meal replaced with albumin (A50–100) or globulin (G50–100) concentrate on the basis of digestible protein. At the end of a 70 day feeding period feed conversion ratio in the albumin treatment groups was not significantly affected at all substitution levels. But due to lowered feed intake at higher inclusion levels growth performance decreased at A 75 and A 100. Dietary globulin inclusion influenced growth performance by reduced feed intake and utilization in all groups negatively due to higher levels of glucosinolates and sinapinic acid. Significant lower fish survival rates were observed when fish received diets A75, A100, G50, G75, or G100 compared to the control diet or diet A50. For the whole body composition, the crude protein content was significantly lower in fish fed diet G75 or G100 compared to the control diet, while fish fed on diet A50, A75, or A100 were lower in body fat content than fish fed on the control diet. Thus, we demonstrated that the used albumin concentrate can effectively replace 50% of dietary fish meal in rainbow trout diets, whereas the application of the globulin concentrate negatively influenced diet palatability, thereby reducing diet intake and subsequently fish growth.

1. Introduction

Fish meal, the most important source of animal protein for fish diets, is a limited resource (Tacon and Metian, 2008). Due to increasing prices for fish meal together with environmental concerns the aquaculture sector is forced to find alternative protein sources to be included in fish feeds (Josupeit, 2011; Naylor et al., 2009). Wide availability, relatively high protein contents and a desirable amino acid profile have caused an interest in rapeseed products as ingredients for fish feed production (Enami, 2011; Slawski et al., 2011a,b,c). Yet, the nutritional quality of rapeseed (*Brassica napus* L.) products largely depends on their levels of antinutritional factors (ANFs), particularly glucosinolates, phytic acid, sinapinic acid, phenolic constituents and indigestible carbohydrates, as it was found in feeding trials with several fish species (Burel et al., 2000a,b,c, 2001; Glencross et al., 2004; Shafaeipour et al., 2008; Slawski et al., 2011a,b,c; Thiessen et al., 2003, 2004; Webster et al., 1997). It was generally observed, that the nutritional quality of simple rapeseed products was below that of fish meal, mainly due to antinutritional factors present in rapeseed products (Francis et al., 2001; Mawson et al., 1995). By several processing techniques the level of antinutrients in rapeseed products can be decreased and their value for fish nutrition improved (Anderson-Hafermann et al., 1993; Fenwick et al., 1986; Tripathi et al., 2000). Protein extraction from meals by hexane treatment increased protein level and ethanol-extractions effectively removed glucosinolates, phenolic compounds, soluble sugars and some oligosaccharides (Chabanon et al., 2007; Naczk and Shahidi, 1990).

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doi:10.1016/j.aquaculture.2012.03.024

However, particularly ethanol-treatments are costly and time consuming (Slawski et al., 2011a,b) and reduce solubility and yield of purified proteins (Bérot et al., 2005).

The predominant storage proteins of *rapeseed* are albumin (napin) and globulin (cruciferin). The 12 S globulin is a 300 kDa glycoprotein with an isoelectric point of 7.25. It is composed of six subunits with large (52–60 kDa) and small polypeptides (29–37 kDa; 22–24 kDa), which are partly linked by disulfide bonds (Dalgalarrondo et al., 1986). The lower molecular mass of the strongly alkaline 2S albumin comprises closely related water soluble proteins with an isoelectric point above 10 and a molar mass in the range of is 12–14 kDa (Krause and Schwenke, 2001). Different properties and functions of the two predominant storage proteins also suggest different potentials of globulin and albumin as ingredient in aqua feeds.

Albeit rapeseed have been indicated as alternative protein source for animal and human nutrition (reviewed by Enami et al., 2011; Wanasundra, 2011) and detailed information about protein purification, protein structure and biochemical composition as well as amino acid profile of rapeseed proteins (Bérot et al., 2005; Dalgalarrondo et al., 1986; Krause and Schwenke, 2001; reviewed in Tan et al., 2011) have been published, no reports about utilization of purified rapeseed protein fractions in fish nutrition are available.

Therefore, the aim of the present study was to evaluate, if size-fractionized protein concentrates produced with different protocols and less effort than high quality protein concentrates or isolates are valuable fish meal alternatives and protein source in the nutrition of rainbow trout. We hypothesized, that single rapeseed protein fractions are optimized in terms of ANF content, palatability and digestibility to maintain growth performance and health status of fish compared to fish fed with a fish meal based diet.

2. Materials and methods

2.1. Manufacturing of rapeseed protein fractions

The rapeseed albumin and globulin concentrates were produced by the Pilot Pflanzenöltechnologie Magdeburg e.V., Germany. For the production of the concentrates a batch of rapeseed (variety Lorenz, Norddeutsche Pflanzenzucht, Hohenlieth, Germany) was conditioned in a vacuum dryer for 15 minutes at 70–80 °C to inactivate the enzyme myrosinase. Then rapeseed was cold pressed. To remove residual oil from the oilcake (12.9% crude lipid, 31.3% crude protein) it was crushed into 1–5 mm particle size followed by a hexane treatment. The treatment lasted for two hours and the incubation temperature was 60 °C. Hexane was removed from rapeseed meal extract by ventilation until the material contained not more than 251 ppm hexane. Then rapeseed meal extract was further crushed to a particle size of 0.5 to 0.2 mm. In the following, protein was gained through liquid water extraction (rapeseed meal extract 1:10 water). For this, the suspension was heated to 40–45 °C followed by one hour of constant agitation. Afterwards the suspension was decanted. Following decantation the solvent was collected and residue material was secondly extracted (residue 1:10 water, 0.5 mol NaCl) at 40–45 °C and one hour contact time under constant agitation. Following extraction the suspension was decanted. The separated residual meal was disposed. The protein solutions of extraction 1 and 2 were treated separately during the next processing steps. They were concentrated using the membrane separation technique (membrane size: 10 kDa, SPIRA-CEL® DS UP010 3838 G 1). In diafiltration procedures, the concentrated protein solution of extraction 2 was "washed", i.e. de-salted. During diafiltration conductivity was checked. Protein washing ended, when conductivity was 5–6 mS cm^{-1}. The gained protein materials of extraction 1 and 2 were separately spray dried at T_{Inlet}/T_{Outlet} of 150–160/70–80 °C, which led to a globulin and an albumin concentrate with a crude protein content of 56.3 and 70.1%, respectively (Table 1).

Table 1
Nutrient composition (g kg^{-1} dry matter), amino acid profiles (g kg^{-1} crude protein) and concentration of antinutritional factors of fish meal, albumin concentrate and globulin concentrate.

	Fish meal	Albumin	Globulin
Nutrient Composition			
Dry matter (%)	916	946	948
Crude protein	690	701	563
Crude fat	70	3.8	3.7
Ash	207	208	86
NfE[a]	33	87	295
Gross energy[b] (MJ kg^{-1})	199	184	188
Amino acids			
Arginine	58.4	65.0	62.4
Histidine	20.0	32.8	26.2
Isoleucine	36.2	40.0	38.7
Leucine	64.5	75.5	70.4
Lysine	65.5	69.5	50.7
Methionine	23.7	18.6	20.2
(+ cysteine)	31.7	52.2	41.8
Phenylalanine	35.2	38.9	41.6
Threonine	39.0	42.4	42.0
Valine	44.5	53.1	50.0
Antinutritional factors			
Phytic acid (g 100 g^{-1})[c]		2.04	1.53
Glucobrassicanapin			0.29
Glucobrassicin			
Gluconapin			0.86
Gluconapoleiferin			
Progoitrin			1.16
4-Hydroxyglucobrassicin			
∑ glucosinolates (μmol g^{-1})[d]		<0.10	2.31
∑ sinapinic acid and sinapinic acid ester (g kg^{-1})[c]		0.37	3.53

[a] Nitrogen free extract = 100 − (%crude protein + %crude fat + %ash).
[b] Calculated by: crude protein = 23.9 MJ kg^{-1}; crude fat = 39.8 MJ kg^{-1}; NfE, fiber: 17.6 MJ kg^{-1}.
[c] Analyzed by the A.C.T. FOODS GmbH, Kiel, Germany, according to HPLC analysis.
[d] Analyzed by the LUFA-ITL GmbH, Kiel, Germany, according to LC-MC.

2.2. Digestibility trial

Three diets were produced for the digestibility trial. A quantity of 10 g kg^{-1} of titanium oxide was added to a control diet mixture as inert marker for determination of apparent digestibility coefficients (ADCs) of ingredients protein. To determine the ADCs of rapeseed albumin and rapeseed globulin concentrate diets were formulated that consisted of 700 g kg^{-1} of the control diet and of 300 g kg^{-1} rapeseed albumin or globulin concentrate, respectively in accordance to Glencross et al. (2010). Diet mixes were manufactured to give pellets 4 mm in diameter (L 14-175, AMANDUS KAHL, Reinbek, Germany). Diet formulations, nutritional compositions and amino acid profiles are presented in Table 2. The digestibility trial was conducted in the experimental facilities of the Gesellschaft für Marine Aquakultur mbH in Büsum, Germany. Fifty rainbow trout (average weight 41.9 ± 3.3 g), obtained from Fischzucht Reese (Sarlhusen, Germany) were stocked into each of nine cylindrical tanks (350 L) of a freshwater recirculation system. Photoperiod was artificially controlled (6.00 a.m. to 6.00 p.m.). Tank water was exchanged two times per hour and chemico-physical water parameters were: temperature: 15.5 ± 0.7 °C; O_2: 9.2 ± 0.5 mg L^{-1}; pH: 7.2 ± 0.5; NH_4^+: <1.0 mg L^{-1}; NO_2^-: <0.5 mg L^{-1}). Fish were fed at a restricted level of 2% of their body weight per day in three portions to assure effective nutrient utilization. Beside an overflow, tanks had a funnel shaped bottom were feces accumulated. The funnel was connected to a pipe outlet. Feces were obtained by continuous sieving of pipe outlet water. After a one week adaptive period to the experimental diets readily excreted feces were collected for seven days. Fecal samples were freeze dried before analysis. According to DIN EN ISO 11885 (DIN, 2009) the amount of titanium in the feces was determined by the LUFA-ITL