Aquaculture 416–417 (2013) 201–208

Contents lists available at ScienceDirect

Aquaculture

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

Evaluation of distiller's dried grains with solubles (DDGS) and high protein distiller's dried grains (HPDDG) in diets for rainbow trout (*Oncorhynchus mykiss*)

Margareth Øverland [a,*], Åshild Krogdahl [c], Gerald Shurson [b], Anders Skrede [a], Vegard Denstadli [a]

[a] Aquaculture Protein Centre, CoE, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås Norway
[b] Department of Animal Science, University of Minnesota, 1988 Fitch Ave., St. Paul, MN, USA
[c] Aquaculture Protein Centre, CoE, Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, P.O. Box 8146 Dep, N-0033 Oslo, Norway

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 6 September 2013
Accepted 12 September 2013
Available online 18 September 2013

Keywords:
Rainbow trout
DDGS
HPDDG
Growth performance
Nutrient utilization

ABSTRACT

Two different sources of maize distiller's co-products, distiller's dried grains with solubles (DDGS) and high protein distiller's dried grains (HPDDG), were evaluated as dietary ingredients in growth experiments (77 days) with rainbow trout (*Oncorhynchus mykiss*). In Exp. 1, the dietary treatments consisted of a control diet based on fish meal, sunflower meal, rapeseed meal, and field peas, and two diets with 250 or 500 g kg^{-1} DDGS, substituting 50 (DDGS50 diet) or 100% (DDGS100 diet) of the plant protein ingredients, respectively. In Exp. 2, the dietary treatments were a control diet based on fish meal, soy protein concentrate, sunflower meal and rapeseed meal, and two diets with 225 or 450 g kg^{-1} HPDDG, substituting 50 (HPDDG50 diet) or 100% (HPDDG100 diet) of the plant protein sources, respectively. Each experiment was conducted using 9 triplicate fresh water tanks of 20 rainbow trout with an initial weight of 143 g. In Exp. 1, feeding the DDGS50 diet resulted in higher feed intake and weight gain and lower feed conversion ratio (FCR) than in trout fed the control diet, while feeding the DDGS100 diet resulted in a lower FCR compared with the control and the DDGS50 diets. Adding DDGS to diets did not affect the digestibility of protein, most amino acids, or phosphorus, but the DDGS-containing diets tended ($P < 0.07$) to increase energy digestibility. Fish fed the DDGS100 diet had higher ($P < 0.01$) energy and phosphorus retention than those fed the control diet, and had higher ($P < 0.01$) nitrogen retention than those fed the control and DDGS50 diets. In Exp. 2, there was no difference in feed intake, weight gain or FCR of fish fed the control or the HPDDG diets. Rainbow trout fed the HPDDG100 and HPDDG50 diets had higher ($P < 0.05$) energy digestibility compared with those fed the control diet. Feeding the HPDDG100 diet resulted in lower ($P < 0.01$) protein digestibility, but higher ($P < 0.01$) phosphorus digestibility and retention than those fed the control and the HPDDG50 diets. The HPDDG100 diet resulted in lower ($P < 0.05$) digestibility of most amino acids compared with the control diet, except for cysteine digestibility that was significantly higher ($P < 0.05$), but neither of the HPDDG diets affected retention of energy or nitrogen of the fish. Neither the DDGS nor the HPDDG diets affected the relative weight of the distal intestine, intestinal enzyme activity, or plasma metabolites. To conclude, both DGGS and HPDDG were shown to be suitable energy, protein, and phosphorus sources up to the level tested when substituting typical plant ingredients in diets for rainbow trout.

1. Introduction

The extensive growth of the U.S. ethanol industry has led to the production of large quantities (34.4 million metric tons in 2012) of maize co-products from dry-grind ethanol production (Renewable Fuels Association, 2013). Dried distiller's grains with solubles (DDGS) is the predominant maize co-product produced by dry-grind fuel ethanol plants, consisting of distiller's grains combined with the condensed solubles obtained after yeast fermentation to produce ethanol, and typically contain about 27% crude protein (CP), 7% starch, 42% neutral detergent fiber, and 0.6% phosphorus (Stein and Shurson, 2009).

Historically, the majority of distiller's co-products produced in the U.S.A. have been used in ruminant feeds, but because DDGS is high in digestible energy, protein, and phosphorus content, it has also become an economical and widely used ingredient in swine and poultry diets (Stein and Shurson, 2009). At present, less than 1% of the total DDGS produced is being used in aquaculture feeds (Shurson, 2012). The rapid growth in the aquaculture industry (FAO, 2012) has caused increased demands on global feed resources. The limited supply and record high prices of fish meal have created an incentive to use less expensive and abundant alternative energy and protein sources, such as DDGS, in aquaculture feeds. The use of DDGS in diets for salmonids

* Corresponding author. Tel.: +47 64965206; fax: +47 64965101.
E-mail address: margareth.overland@umb.no (M. Øverland).

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.09.016

has, however, given inconsistent results. Cheng and Hardy (2004) showed that DDGS could be used at 150 g kg^{-1} in rainbow trout diets to replace 50% of the fish meal on an isonitrogenous and isoenergetic basis without affecting growth and feed conversion. When the diets were supplemented with lysine and methionine, up to 225 g kg^{-1} DDGS can be added to replace up to 75% of fish meal in the rainbow trout diets without negative effects on weight gain and feed conversion. However, Barnes et al. (2012a) reported reduced gain in hatchery reared juvenile rainbow trout when fed diets containing 100 or 200 g kg^{-1} DDGS, even when supplemented with essential amino acids and phytase.

Distiller's grain co-products may be more attractive for use in aquaculture feeds if the protein level was increased and the indigestible fiber content was reduced (Stone et al., 2005). This can be achieved by use of front-end fractionation technology to separate the fermentable portion of the corn kernel from the non-fermentable portion prior to grinding and further processing by yeast fermentation in dry-grind ethanol plants (Robinson et al., 2008; Singh et al., 2005). One of the resulting co-products produced by this process is a high protein dried distiller's grains (HPDDG) which is higher in crude protein, and lower in fat and fiber than conventional DDGS (Singh et al., 2005). Studies by Barnes et al. (2012b) indicated that at least 200 g kg^{-1} HPDDG, if supplemented with amino acids, may be used to replace fish meal in diets for juvenile rainbow trout. However, Barnes et al. (2012c) concluded that replacement of fish meal with HPDDG in diets for juvenile rainbow trout may only be suitable at levels of less than 100 g kg^{-1}.

Due to the use of maize as a feedstock in ethanol production, and the fermentation and hydrothermal treatment processes inherent in the process, the presence of typical antinutritional factors (ANF) such as phytic acid is lower than in most plant ingredients. Because yeast from *Saccharomyces cerevisiae* is used in the fermentation step, DDGS products are partially made up from yeast remnants. According to Ingledew (1999) the contribution of yeast biomass to the weight of DDGS is at least 3.9%, and the proportion of yeast protein in the total protein content of DDGS could be at least 5.3%. *S. cerevisiae* has recently been evaluated as a potential protein source in aquaculture feeds (Øverland et al., 2013), and yeast cells are sources of nucleic acids, mannan oligosaccharides, and β-glucans that can be used as immunostimulants in fish diets (Li and Gatlin III, 2006; Refstie et al., 2010).

DDGS has been reported to be a suitable feed ingredient to replace plant protein sources in diets for fish species such as tilapia (Coyle et al., 2004; Shelby et al., 2008) and channel catfish (Li et al., 2010, 2011). In previous studies with salmonids, however, DDGS and HPDDG have been used mainly as substitutes for fish meal protein. The recent development towards the reduced supply and high cost of fish meal has led to increased use of plant protein sources in diets for salmonids and the need to evaluate distiller's grain co-products as promising alternatives to commonly used plant protein ingredients, such as soy protein concentrate. Therefore, the aim of the present study was to assess growth performance, digestibility and retention of energy and nutrients, liver and distal intestine weights, blood chemistry, and gut enzyme activity of rainbow trout fed diets containing conventional DDGS or HPDDG produced from maize-based ethanol production.

2. Materials and methods

Two growth performance experiments, Exp. 1 and Exp. 2, evaluated two different sources of distiller's grains co-products, DDGS and HPDDG, in diets for rainbow trout at the fish laboratory of the Norwegian University of Life Sciences, Ås, Norway.

2.1. Diets

In Exp. 1, the dietary treatments were: 1) a control diet (35% CP) based on fish meal, sunflower expeller meal, rapeseed meal, and field peas, 2) a test diet containing 250 g kg^{-1} DDGS, and 3) a test diet containing 500 g kg^{-1} DDGS. The DDGS partly (DDGS50 diet) or fully (DDGS100 diet) replaced a mixture of the plant protein ingredients used in the control diet. In Exp. 2, the dietary treatments were: 1) a control diet (43% CP) based on fish meal, soy protein concentrate, sunflower expeller meal, and rapeseed meal, 2) a test diet containing 225 g kg^{-1} HPDDG, and 3) a test diet containing 450 g kg^{-1} HPDDG. The HPDDG partly (HPDDG50 diet) or fully (HPDDG100 diet) replaced a mixture of the plant protein ingredients. Samples of the DDGS and HPDDG sources used in these experiments were analyzed for chemical composition and mycotoxin concentrations. In both experiments, diets were formulated to have a similar level of crude protein and gross energy based on the analyzed chemical content of the ingredients. All diets contained 0.1 g kg^{-1} yttrium oxide (Y_2O_3) as an indigestible marker for determination of nutrient digestibility (Austreng et al., 2000). The chemical composition of ingredients is shown in Table 1, while the ingredient composition and chemical analysis of the experimental diets used in Exp. 1 and 2 are shown in Table 2.

The diets in Exp.1 and 2 were processed at the feed laboratory of the Norwegian University of Life Sciences, Ås, Norway. Gelatin and pregelatinzed potato starch were used as pellet binders. Except gelatin, the dry ingredients and the fish oil were mixed in a Moretti Foreni kneading machine (Spiry 25, Mondolfo, Italy). Gelatin was dissolved in hot water (50–60 °C) and applied to the rest of the ingredients during mixing. The moist (70–75% DM) dough was subsequently cold pelleted in an Italgi pasta extruder (P35A, Carasco, Italy) equipped with a 3 mm die. The semi-moist pellets were gently dried on large perforated trays in an oven at 55–60 °C to obtain a final DM content of about 90–95%. The diets were stored at −18 °C until feeding.

2.2. Fish husbandry and sampling

In both Exp. 1 and 2, a total of 180 rainbow trout (*Oncorhynchus mykiss*) with an average initial weight of 143 g were randomly

Table 1
Chemical composition of ingredients in Exp. 1 and Exp. 2.

Analyzed composition, g kg^{-1}	FM[1]	SPC[2]	SFM[3]	RSM[4]	Peas[5]	DDGS[6]	HPDDG[6]
Dry matter	911	912	898	903	882	956	957
Crude protein	677	626	342	301	210	275	447
Amino acids, g 16 g N^{-1}[7]							
Essential amino acids							
Arginine	5.2	6.9	7.1	5.7	7.4	5.0	4.4
Histidine	2.0	2.8	2.6	2.8	2.5	3.0	2.8
Isoleucine	3.4	4.5	3.9	3.9	4.1	3.7	4.1
Leucine	6.3	7.0	6.2	6.3	6.5	10.5	9.6
Lysine	6.8	6.1	3.7	5.5	7.0	2.9	4.7
Methionine	2.5	1.3	2.1	1.9	0.9	1.8	2.0
Phenylalanine	3.3	4.7	4.3	3.7	4.5	4.6	4.5
Threonine	4.0	3.7	3.5	4.2	3.5	3.7	4.1
Valine	3.9	4.7	4.7	5.1	4.6	5.1	5.3
Non-essential amino acids							
Alanine	5.0	3.6	3.8	3.7	3.6	6.5	5.7
Aspartic acid	8.2	10.4	8.2	6.9	10.2	6.4	7.3
Cysteine	0.8	1.4	1.5	2.2	1.4	1.9	1.7
Glycine	4.4	3.1	4.1	3.8	3.2	3.4	3.2
Glutamic acid	11.9	16.8	17.5	15.2	15.1	16.6	14.1
Proline	3.3	4.3	3.8	5.0	3.5	7.0	6.0
Serine	4.0	4.7	3.9	4.0	4.3	4.7	4.7
Tyrosine	2.6	3.1	2.5	2.7	2.7	3.2	3.5
Crude fat	114	4	20	103	18	185	54
Starch	10	24.8	85.1	39.1	403	53	62
Neutral detergent fiber	–	64	230	245	103	265	73
Ash	139	63	61	59	25	36	36

[1] NorsECO-LT, Egersund Sildoljefabrikk AS, Egersund, Norway.
[2] Soycomil® R, ADM Specialty Ingredients Europe, Koog aan de Zaan, Holland.
[3] Defatted, 35% crude protein.
[4] Solvent extracted (hexane) double low rapeseed meal, ExPro-00E (Karlshamn AB, Karlshamn, Sweden).
[5] Eldorado, Norway.
[6] Steve Markham at Cenex Harvest States, Inc., Inver Grove Heights, MN, USA.
[7] Water corrected amino acids.

Table 2
Diet formulations (g kg^{-1}) and chemical composition in Exp. 1 and 2.

	DGGS diets, Exp. 1			HPDDG diets, Exp. 2		
	Control	DDGS50	DDGS100	Control	HPDDG50	HPDDG100
Soy protein concentrate	0	0	0	162	81	0
Sunflower meal	125	67.5	0	144	72	0
Rapeseed meal	125	67.5	0	144	72	0
Field peas	250	150	0	0	0	0
DDGS	0	250	500	0	0	0
HPDDG	0	0	0	0	225	450
Fishmeal	189	189	189	213	211	209
Fish oil	160	137	115	166	162	161
Gelatin	70	70	70	70	70	70
Potato starch	62	75	107	82	88	91
Premix[1]	19	19	19	19	19	19
Analyzed composition, g kg^{-1}						
Dry matter	953	949	954	951	961	943
Crude protein	352	352	346	432	432	423
Essential amino acids						
Arginine	22.5	20.5	17.9	26.3	24.6	23.7
Histidine	6.6	6.8	6.7	8.5	8.5	9.5
Isoleucine	10.8	10.5	9.7	13.3	13.2	14.6
Leucine	20.6	23.3	25.3	25.5	27.4	35.2
Lysine	21.7	20.4	18.3	25.4	25.0	26.7
Methionine	8.0	8.2	8.0	9.2	9.4	10.9
Phenylalanine	12.0	12.1	11.9	15.1	15.0	17.0
Threonine	12.8	12.6	12.1	15.5	15.6	17.7
Valine	13.2	13.1	12.5	15.6	15.9	18.5
Non-essential amino acids						
Alanine	19.9	21.6	22.8	22.5	23.8	30.0
Aspartic acid	29.3	27.0	24.3	36.1	34.4	35.1
Cysteine	3.5	3.6	3.6	4.4	4.4	5.1
Glycine	29.6	28.7	27.0	31.6	30.9	33.3
Glutamic acid	50.8	49.6	47.4	63.7	60.7	62.8
Proline	19.9	21.5	22.7	23.2	23.6	28.8
Serine	15.3	15.4	15.3	18.9	18.9	21.5
Tyrosine	8.4	9.0	9.3	10.7	11.2	13.9
Crude fat	173	188	201	182	188	196
Energy, MJ kg^{-1}	21.6	21.9	22.3	22.1	22.6	22.4
Neutral detergent fiber	99	115	117	79	59	35
Ash	63.5	63.8	64.0	74.2	64.4	57.0
Phosphorus	9.9	10.2	10.8	11.2	10.8	10.4
Phytate phosphorus	10.1	5.6	3.0	8.1	5.3	2.9
Calculated values						
CP:GE ratio, g/MJ	16.3	16.1	15.5	19.5	19.116.1	18.8

[1] Provided the following amounts per kg of diet: Mono calcium phosphate 7 g, L-lysine HCL 99% feed grade (CJ Indonesia, Jakarta, Indonesia) 3 g, DL-methionine 99% feed grade (Rhodimet® NP 99, Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil) 2 g, Di-yttrium tri-oxide (Y_2O_3) 2 g, Rare Earth Metal Limited, Shenzhen, Guangdong, China, retinol 2500 IU, thiamine 15 mg, riboflavin 25 mg, pantothenic acid 30 mg, pyridoxine 15 mg, cyanocobalamine 20 μg, niacin 75 mg, biotin 250 μg, folic acid 5 mg, vitamin C 125 mg (Rovimix® Stay-C® 35, DSM Nutritional Products, Basel, Switzerland), cholecalciferol 1500 IU, tocopherol 200 mg, menadione 10 mg, $ZnSO_4$ 120 mg, $CuSO_4$ 5 mg, $MnSO_4$ 15 mg, I 3 mg.

distributed into 9 fiberglass tanks (300 l) with 20 rainbow trout in each tank. The tanks were supplied with recirculated freshwater with temperatures ranging from 9.3 °C at the beginning of the trial in mid March to 13.1 °C in the end of May when the experiment was terminated (77 days). The tanks were illuminated 24 h day^{-1}, with a single light bulb (10 W) attached to the lid in each of the individual tanks. Each of the diets was fed in excess (5–10%) to triplicate tanks twice per day (0700–0830 h and 1300–1430 h) using automatic belt feeders. Uneaten feed was sieved from the outlet water of each tank twice daily. Feed intake was calculated following the method described by Helland et al. (1996). Before starting the experiment, 15 randomly selected fish from the holding tank were anesthetized with MS-222 (60 mg l^{-1}), and sampled for whole body analyses. The intestinal contents were removed, and the fish was stored at −20 °C until analysis. At the end of both experiments (day 77), all fish were anesthetized and weighed individually. Blood was drawn from the caudal vein of six randomly selected fish using heparinized vacutainers (Venoject-Terumo, Leuven, Belgium). The samples were centrifuged and plasma was pipetted into Eppendorf vials and stored at −20 °C until analysis. The gastro-intestinal tract (GIT) from the same six fish was sectioned into pyloric intestine (PI, from the pyloric sphincter to the most distal cecum), mid-intestine (MI), and distal intestine (DI, from the appearance of the visual transverse folding and widening of the intestine to the anus). The viscera fat and connective tissue were removed, and the sections were cut open, rinsed and weighed for calculation of GIT index. Digesta were collected from PI and DI and stored at −80 °C until subsequent analysis of trypsin activity. Samples of PI and DI were taken and stored at −80 °C until subsequent analysis of mucosal enzyme activities. The liver (LI) was removed and weighed individually for determination of liver index. The remaining fish in the tank were mildly anesthetized and stripped for collection of feces according to the procedure described by Austreng (1978). Five fish from each tank, fasted for 48 h, were randomly sampled and stored at −20 °C for whole body analysis.

2.3. Analyses

Ground whole body and fecal samples were freeze-dried prior to analysis. Ingredients, diets, whole body homogenates, and feces were analyzed for DM (Commission dir. 71/393 EEC), and ash (Commission dir. 71/250 EEC). Mineral content of diets and feces was determined by inductively coupled plasma mass spectroscopy (ICP-AES, Thermo Jarrel Ash Polyscan, Thermo Inc., Woburn, MA) after complete digestion of the homogenized and dried sample in HNO_3 after cooking in a microwave oven for 1 h.

Plasma metabolites and enzyme activities in gastrointestinal tract were determined in rainbow trout fed the DDGS100 and HPDDG100 diets. The plasma metabolites were determined according to standard methods at the Central Laboratory of the Norwegian School of Veterinary Science, Oslo, Norway as described by Tietz (1995). Contents from intestinal sections were subjected to colorimetric analysis of trypsin activity according to the method of Kakade et al. (1969). Brush border membrane bound leucine aminopeptidase (LAP) activity was determined in homogenates of intestinal tissue. The tissues were thawed, weighed and homogenized (1:20) in ice-cold 2 mM Tris/50 mM mannitol, pH 7.1, containing the serine protease inhibitor phenyl-methyl-sulphonyl fluoride (Sigma no. P-7626; Sigma Chemical Co., St. Louis, MO, USA). Aliquots of homogenates were frozen in liquid nitrogen and stored at −80 °C until analysis. Enzyme activities after incubation at 37 °C were determined colorimetrically as previously described by Krogdahl et al. (2003).

Crude protein (CP) was determined as Kjeldahl-N × 6.25 on a Kjeltec 2300 (Tecator, Höganäs, Sweden) following Commission dir. 93/28 EEC. Crude lipid was determined after extraction with a mixture of petroleum ether and acetone (4:1, v/v) at 125 °C in an Accelerated Solvent Extractor (ASE200, Dionex, Sunnyvale, CA). Amino acids in ingredients, diets, and feces were analyzed on a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK) following Commission dir. 98/64/EEC. Identification and quantification of mycotoxins in DDGS and HP-DDG were conducted at the Veterinary Diagnostic Laboratory (North Dakota State University, Fargo, ND, USA). Phytate was determined following a method described by Carlsson et al. (2001).

2.4. Calculations and statistical analyses

The apparent digestibility (%) of individual nutrients and minerals was calculated as $100 - [100 \times ((D_i \times F_i^{-1}) \times (F_n \times D_n^{-1}))]$, where D_i and F_i represent the concentration of inert marker in diet and feces, and D_n and F_n represent the concentration of nutrients in diet and

feces, respectively. Feed conversion ratio (FCR) was calculated as feed intake (g as fed)/wet weight gain (g). Retention (%) of nutrients was calculated as 100 × (nutrient gain in fish × nutrient intake^{-1}).

The data were analyzed by SAS statistical software system version 9.2 (SAS Institute, 2002). A one-way ANOVA was used for analysis of the diets, following the model: $y_{ij} = \mu + a_i + e_{ij}$, where y_{ij} was the response, μ was the overall mean, a_i was the effect of the ith diet and e_{ij} was the random error. Duncan's multiple range test was applied in order to rank significantly ($P < 0.05$) different means. The tank mean was used as the experimental unit in the statistical analyses. The results are expressed as least square means for each treatment, and variance was expressed as standard error of the mean (SEM).

3. Results

3.1. Growth performance, nutrient digestibility and retention and relative tissue weights

Fish growth performance, nutrient digestibility and retention, and relative tissue weights in Exp. 1 are shown in Table 3. Feeding with the DDGS diets significantly improved weight gain and FCR of the fish. Fish fed the DDGS50 diet had a higher feed intake and weight gain, and a lower FCR than those fed the control diet. Fish fed the DDGS100 diet had similar feed intake and weight gain as those fed the control diet, but a lower FCR than fish fed the control and the DDGS50 diets. There were no differences among dietary treatments in the digestibility of CP and most amino acids, except for methionine and threonine, where digestibility was lower in fish fed the DDGS100 diet compared with the control and the DDGS50 diets. The digestibility of energy tended ($P < 0.07$) to be higher in the fish fed the DDGS diets compared to those fed the control diet, and energy retention was significantly higher in fish fed the DDGS100 diet compared to those fed the control and DDGS50 diets. Nitrogen retention was also higher in fish fed DDGS100 compared with those fed the control and DDGS50 diets. No differences among diets were found for P digestibility, but fish fed the DDGS50 or the DDGS100 diets had higher P retention than those fed the control diet. Also, fish fed the DDGS50 and DDGS100 diets had higher liver weights as a proportion of body weight than those fed the control diet, whereas there were no differences among the diets in distal intestinal weight relative to body weight.

Fish growth performance, nutrient digestibility and retention, and relative tissue weights in Exp. 2 are shown in Table 4. Feed intake, weight gain, and FCR were not significantly affected by the inclusion of HPDDG to diets. Fish fed the HPDDG50 and HPDDG100 diets had higher energy digestibility than those fed the control diet, while fish fed the HPDDG100 diet had a lower protein digestibility than those fed the control and the HPDDG50 diet. There was also a reduction in the digestibility of most essential and non-essential amino acids when feeding the HPDDG diets compared with the control diet, except for the digestibility of cysteine which was higher. Fish fed the HPDDG100 diet had significantly higher P digestibility than those fed the control

Table 3
Fish performance, nutrient digestibility and retention, and weight of liver and distal intestine in Exp. 1.

	Control	DDGS50	DDGS100	SEM[1]	P-value
Performance[2]					
Feed intake, g[3]	222[a]	249[b]	203[a]	7.4	0.01
Weight gain, g	216[a]	261[b]	230[a]	7.8	0.01
Feed conversion ratio	1.03[a]	0.96[b]	0.88[c]	0.003	<0.0001
Nutrient digestibility					
Energy	66.2	68.8	70.5	1.1	0.07
Phosphorus	48.9	53.7	54.5	1.8	NS
Crude protein	81.3	81.5	80.8	0.6	NS
Essential amino acids					
Arginine	88.8	89.2	88.2	0.4	NS
Histidine	81.5	81.5	79.2	0.7	NS
Isoleucine	81.6	81.8	79.9	0.8	NS
Leucine	83.5	84.8	84.9	0.5	NS
Lysine	86.5	86.4	84.9	0.5	NS
Methionine	89.0[a]	88.9[a]	86.3[b]	0.5	0.01
Phenylalanine	82.2	82.9	82.1	0.6	NS
Threonine	80.9[a]	79.9[a]	76.4[b]	0.6	0.01
Valine	81.5	81.8	80.1	0.7	NS
Non-essential amino acids					
Alanine	87.5	87.3	86.0	0.4	NS
Aspartic acid	78.4	77.9	75.1	0.9	0.09
Cysteine	61.5	63.9	63.8	1.5	NS
Glycine	85.4	85.1	83.3	0.6	NS
Glutamic acid	86.9	87.1	86.1	0.5	NS
Proline	83.4	84.2	84.1	0.7	NS
Serine	80.9	81.3	79.8	0.6	NS
Tyrosine	83.4	84.5	83.2	0.5	NS
Nutrient retention					
Energy	41.1[a]	45.6[ab]	50.2[b]	1.5	0.01
Phosphorus	41.5[a]	46.2[b]	49.3[b]	1.1	0.01
Nitrogen	46.7[a]	48.4[a]	54.5[b]	0.9	0.01
Tissue weight[4]					
Liver	0.99[a]	1.29[b]	1.42[b]	0.08	0.05
Distal intestine	0.76	0.74	0.76	0.04	NS

[1] Standard error of the mean.
[2] Initial weight was 143, 142, and 143 g for the control, DDGS50, and DDGS100, respectively.
[3] Means in a row with no superscripts in common differ (P<0.05).
[4] Tissue weight × whole body weight^{-1} × 100.

Table 4
Fish performance, nutrient digestibility and retention, and weight of liver and distal intestine in Exp. 2.

	Control	HPDDG50	HPDDG100	SEM[1]	P-value
Performance[2]					
Feed intake, g	221	228	220	14	NS
Weight gain, g	254	277	255	20	NS
Feed conversion ratio	0.87	0.83	0.87	0.01	NS
Nutrient digestibility					
Energy[3]	75.2[a]	78.1[b]	77.9[bc]	0.7	0.05
Phosphorus	37.1[a]	44.1[a]	51.5[b]	2.2	0.01
Protein	86.1[a]	84.9[a]	81.0[b]	0.6	0.01
Essential amino acids					
Arginine	93.6[a]	91.1[b]	87.7[c]	0.5	0.001
Histidine	87.3[a]	84.3[b]	81.4[c]	0.7	0.01
Isoleucine	88.0[a]	83.6[b]	79.0[c]	1.0	0.01
Leucine	89.3	87.1	86.0	0.8	0.06
Lysine	90.9[a]	87.1[b]	82.7[c]	0.6	0.001
Methionine	90.3	89.4	88.4	0.7	NS
Phenylalanine	89.2[a]	86.6[a]	83.1[b]	0.8	0.01
Threonine	84.1[a]	81.3[ab]	77.6[b]	1.2	0.05
Valine	86.6[a]	82.9[b]	79.8[bc]	1.0	0.01
Non-essential amino acids					
Alanine	89.6	87.5	86.4	0.8	0.06
Aspartic acid	83.0[a]	81.0[ab]	77.0[c]	0.9	0.01
Cysteine	66.4[a]	74.2[ab]	77.2[b]	2.4	0.05
Glycine	84.9	86.0	85.7	1.0	NS
Glutamic acid	91.1[a]	90.0[ab]	87.6[c]	0.7	0.05
Proline	85.1	86.8	87.7	1.1	NS
Serine	85.9[a]	83.5[ab]	80.7[b]	1.0	0.05
Tyrosine	89.7[a]	86.1[b]	84.3[bc]	0.7	0.01
Nutrient retention					
Energy	46.0	49.5	48.1	3.0	NS
Phosphorus	41.2[a]	49.2[b]	51.1[b]	1.5	0.01
Nitrogen	45.2	46.7	45.7	1.9	NS
Tissue weight[4]					
Liver	1.02	1.10	1.19	0.05	NS
Distal intestine	0.71	0.66	0.62	0.04	NS

[1] Standard error of the mean.
[2] Initial weight was 143, 143, and 141 g for the control, HPDDG50, and HPDDG100, respectively.
[3] Means in a row with no superscripts in common differ (P<0.05).
[4] Tissue weight × whole body weight^{-1} × 100.

diet, and fish fed the HPDDG50 and HPDDG100 diets had higher P retention than those fed the control diet. Feeding HPDDG did not affect the retention of energy and nitrogen of the fish. Neither the HPDDG50 nor the HPDDG100 diet affected the relative weight of liver or distal intestine compared with the control diet.

3.2. Plasma metabolites and enzyme activities

Plasma metabolites and intestinal enzyme activity of the fish fed the highest level of DDGS or HPDDG in Exp. 1 and 2 are shown in Tables 5 and 6, respectively. In both Exp. 1 and 2, the plasma activities of alanine aminotransferase (ALP) and aspartate aminotransferase (ASP), as well as the concentration of total protein, alkaline phosphatase and inorganic phosphate in the plasma were unaffected by dietary treatments. Also, there was no effect of diet on trypsin activity in digesta from PI or DI. In Exp. 1, the activity of LAP expressed as mmol/h/g tissue in PI was lower in fish fed the DDGS100 diet than in fish fed the control diet. These fish also tended ($P < 0.09$) to have lower activity of LAP when expressed as mmol/h/g in DI. In Exp. 2, there were no differences in LAP activity or trypsin activity in PI or DI of fish fed the control and the HPDDG100 diets.

3.3. Mycotoxins in DDGS and HPDDG

Mycotoxin concentration of the distiller's co-products is shown in Table 7. Except for detectable levels of deoxynivalenol (DON) in both DDGS and HPDDG, and 3-Acetyl DON in DDGS, the concentrations were below the detection limits.

4. Discussion

Maize co-products from dry-grind bio-ethanol production, such as DDGS or HPDDG, are attractive ingredients for use in aquaculture feeds because of their contents of energy, protein, and highly digestible phosphorus, and may reduce diet cost compared to conventional plant protein ingredients. In the present study, DDGS and HPDDG were evaluated in diets for rainbow trout by partially or fully replacing mixtures of typical plant protein ingredients, while the fish meal levels were kept constant to avoid confounding effects of differences in fish meal levels. The experiment with DDGS was carried out with a lower CP content and lower CP:energy ratio than the experiment conducted to evaluate HPDDG. This was done to facilitate similar dietary inclusion levels of the two distillers' co-products. Adding DDGS as a partial replacement for plant ingredients in these diets resulted in an increase in feed intake, weight gain, and FCR. Both dietary DDGS inclusion levels resulted in improved FCR, while dietary addition of HPDDG had no effect on feed intake, growth rate or FCR. Researchers have previously shown that DDGS, combined with corn gluten meal, could replace 15 and 22.5% of the fish meal in diets for rainbow trout in unsupplemented and lysine and methionine supplemented diets, respectively (Cheng and Hardy, 2004). In contrast, Barnes et al. (2012a) reported a reduction in growth rate and FCR when 10% DDGS replaced fish meal, corn gluten meal and wheat in diets for rainbow trout, even when the diets were supplemented with essential amino acids and phytase. In the present study, it appears

Table 5
Plasma metabolites, leucine aminopeptidase, and trypsin activity in different sections of the gastrointestinal tract of rainbow trout fed the control and the DDGS100 diets (Exp.1).

	Control	DDGS100	SEM[1]	P-value
Plasma metabolites				
Alanine aminotransferase, U/L	31.3	28.6	1.6	NS
Aspartate aminotransferase, U/L	645	619	32	NS
Total protein, g/L	37.0	37.1	1.0	NS
Triacyl-glycerides, mmol/L	7.7	10.0	1.8	NS
Free fatty acids, mmol/L	0.194	0.183	0.017	NS
Alkaline phosphatase, U/L	205	243	27	NS
Inorganic phosphate, mmol/L	5.11	5.25	0.37	NS
Intestinal enzyme activity				
Pyloric intestine				
Leucine aminopeptidase				
mmol/h/g tissue	11.7	9.0	0.24	0.05
mmol/h/g body weight	186	151	21	NS
μmol/h/mg protein	347	424	47	NS
Trypsin, U mg^{-1} DM	169.0	206.7	53.8	NS
Distal intestine				
Leucine aminopeptidase				
mmol/h/g tissue	13.7	13.7	0.7	NS
mmol/h/g body weight	109	101	4.3	0.09
μmol/h/mg protein	375	417	31	NS
Trypsin, U mg^{-1} DM	9.93	23.0	4.3	NS

[1] Standard error of the mean.

Table 6
Plasma metabolites, leucine aminopeptidase, and trypsin activity in different sections of the gastrointestinal tract of rainbow trout fed the control and the HPDDG100 diets (Exp.2).

	Control	HPDDG100	SEM[1]	P-value
Plasma metabolites				
Alanine aminotransferase, U/L	27.6	26.4	1.7	NS
Aspartate aminotransferase, U/L	578	604	22	NS
Total protein, g/L	38.1	37.9	1.5	NS
Triacyl-glycerides, mmol/L	10.9	7.2	1.3	NS
Free fatty acids, mmol/L	0.228	0.143	0.046	NS
Alkaline phosphatase, U/L	260	202	19	NS
Inorganic phosphate, mmol/L	5.36	5.41	0.19	NS
Intestinal enzyme activity				
Pyloric intestine				
Leucine aminopeptidase				
mmol/h/g tissue	10.7	9.0	0.9	NS
mmol/h/g body weight	168	163	19	NS
μmol/h/mg protein	471	420	34	NS
Trypsin, U mg^{-1} DM	261.0	275.0	70.3	NS
Distal intestine				
Leucine aminopeptidase				
mmol/h/g tissue	13.3	11.7	1.0	NS
mmol/h/g body weight	90	79	9.8	NS
μmol/h/mg protein	390	336	26	NS
Trypsin, U mg^{-1} DM	31.3	22.8	7.1	NS

[1] Standard error of the mean.

Table 7
Mycotoxin concentrations in DDGS and HPDDG.

	DDGS	HPDDG
Mycotoxins, mg kg^{-1}		
Deoxynivalenol	1.1	0.9
T-2 Tetraol	<0.5	<0.5
Fusarenone-X	<0.5	<0.5
3-Acetyl DON	<0.5	<0.5
15-Acetyl DON	0.5	<0.5
DAS	<0.5	<0.5
T-2 Triol	<0.5	<0.5
T-2 Toxin	<0.5	<0.5
Iso T-2 Toxin	<0.5	<0.5
Scirpentriol	<0.5	<0.5
Nivalenol	<0.5	<0.5
15-Acetate-Scirpentriol	<0.5	<0.5
Neosolaniol	<0.5	<0.5
HT-2 Toxin	<0.5	<0.5
Zearalenol	<0.5	<0.5
Zearalenone	<0.5	<0.5
Aflatoxin B1	<0.02	<0.02
Fumonisin B1	<2.0	<2.0

that much higher amounts of DDGS (50%) and HPDDG (45%) can be effectively used in rainbow trout diets, with equal amounts of lysine and methionine supplemented to all diets, without negative effects on fish performance. Differences in growth performance responses among studies may be partly due to differences in experimental design, types of substituted feed ingredients, as well as nutrient content and digestibility of the distiller's co-products added to diets. Unlike previous studies, both DDGS and HPDDG were used as substitutes for plant protein ingredients keeping dietary fish meal levels constant. Previous studies have shown that the concentration and digestibility of protein and amino acids among DDGS sources is quite variable for swine (Urriola et al., 2009) and poultry (Waldroup et al., 2007). Therefore, use of DDGS or HPDDG sources with low amino acid digestibility could also negatively affect growth performance of fish. Working with channel catfish (*Ictalurus punctatus*), Li et al. (2010) reported that 30% DDGS in the diet increased weight gain and FCR compared with an all-plant control diet. Furthermore, Li et al. (2011) attributed the positive effect of DDGS on fish growth to the presence of residual yeast in DDGS.

The present study showed that DDGS could be used to replace a mixture of similar amounts of CP from sunflower meal, rapeseed meal, and field peas without affecting digestibility of CP and only minor effects on amino acid composition and digestibility. Conversely, digestibility of CP and most amino acids decreased with increasing levels of HPDDG as substitution for soy concentrate, sunflower meal and rapeseed meal, where soy concentrate represented about half of the substituted CP and amino acids. Previous studies have shown that soy concentrate is a highly digestible protein source, whereas the CP in sunflower meal, rapeseed meal and field peas is generally less digestible (Aslaksen et al., 2007; Glencross et al., 2004, 2005). This may partially explain the differences in effects of DDGS and HPDDG on CP and amino acid digestibility in our study. Full replacement of the plant protein ingredients in the DDGS100 and HPDDG100 diets resulted in diets with similar digestibilities of CP and amino acids, thus indicating minor differences in digestibility between the two distillers' co-products although the content of fiber was much higher in DDGS than in HPDDG.

The high CP levels and CP:energy ratio in Exp. 2 may be the main reason why the reduction in the digestibility of protein and several amino acids by the addition of HPDDG to diets appeared to cause no adverse effects on growth performance or nitrogen retention of the fish. Moreover, there was no reduction in the digestibility of methionine, and an increase in cysteine digestibility in fish fed the highest HPDDG level. The high digestibility of cysteine in the HPDDG diet indicates lenient heat treatment during production of HPDDG because excessive heat treatment is particularly detrimental to cysteine digestibility (Opstvedt et al., 1984; Skrede and Krogdahl, 1985). Comparing our results with those obtained in other studies that used rainbow trout with lower initial body weight, the requirements of the likely limiting amino acids such as methionine (Rodehutscord et al., 1995), threonine (Bodin et al., 2008), and lysine (Rodehutscord et al., 1997) may have been adequately covered.

Higher weight gain of fish fed the DDGS50 diet compared with the control and DDGS100 diet was most likely a result of higher feed intake. The improved FCR with increasing inclusion of DDGS could be explained by higher energy digestibility and retention of energy and nitrogen. It was interesting to note that when fully replacing the plant protein mixture with DDGS in the low-protein diets in Exp. 1, FCR was similar to that obtained with HPDDG in the high-protein diets in Exp. 2. The effects on FCR may be partially related to much lower content of antinutritional factors in maize co-products compared to the substituted plant ingredients, as well as the yeast fermentation and activation of endogenous enzymes which are capable of degrading inhibitors during the manufacture of DDGS co-products. Total starch content among DDGS sources is low, ranging from 3.8 to 11.4%, and half to two-thirds of total starch is insoluble suggesting that it is relatively indigestible (Stein and Shurson, 2009). In our study, the diets were cold pelleted, implying that non-gelatinized starch from field peas in the control diet in Exp. 1, and to a lesser extent in the DDGS50 diet, may have reduced digestibility of starch and energy. Rainbow trout have limited capacity to digest and utilize starch, especially non-gelatinized starch (Frøystad et al., 2006). This might partially explain the increase in energy digestibility and retention when feeding DDGS at the expense of field peas. The increase in liver index of fish fed the DDGS diets may be related to differences in starch digestibility among these diets. Pregelatinized potato starch, the main starch source in the DDGS100 diet, is known to be highly digestible, and high glucose absorption may have increased liver glycogen in the fish.

Both DDGS and HPDDG contained yeast cells and remnants from *S. cerevisiae* (Ingledew, 1999). Because our diets were cold pelleted, live yeast cells occurring in DDGS and HPDDG may have survived during feed production, which was very different from the common extrusion procedure used by the feed industry. It has been reported that live *S. cerevisiae* yeast can colonize the intestinal mucosa of rainbow trout (Andlid et al., 1995). Øverland et al. (2013) recently reported that the replacement of fish meal by 30% of protein from *S. cerevisiae* in diets for Atlantic salmon reduced growth performance and digestibility of protein. The protein digestibility of *S. cerevisiae* could be lowered by the relatively thick, tough, and rigid cell walls of the whole yeast cells that may resist intestinal digestion as discussed by Rumsey et al. (1991).

The concentration of P in DDGS ranges from 0.6 to 0.7%, and most phytate-bound P is released during the fermentation process to make it highly available for monogastric animals such as pigs (Shurson, 2012). Cheng and Hardy (2004) reported increased P retention in rainbow trout when up to 22.5% DDGS was used in combination with corn gluten meal to replace fish meal and whole wheat. Our study showed that both DDGS and HPPDG had reduced content of phytic acid, which improved digestibility and retention of P in rainbow trout, when replacing mixtures of plant protein ingredients. This can be explained as a result of degradation of phytic acid and liberation of soluble phosphate during HPDDG and DDGS processing. The high concentration of phytic acid in plant protein sources such as soybean, rapeseed meal and sunflower meal (Aslaksen et al., 2007) most likely contributed to the low P digestibility in fish fed the HPDDG control diet. The reason why DDGS affected P digestibility to a lesser extent than HPDDG may be that field peas, which have a low content of phytate-bound P (Aslaksen et al., 2007), were used in the control diet of the DDGS experiment, whereas soy concentrate was used in the HPDDG control diet. Thus, the comparable P digestibility of the DDGS100 and HPDDG100 diets indicates that there were minor differences in P availability between DDGS and HPDDG.

No changes related to gastro-intestinal health or plasma metabolites were detected in our study, indicating that DDGS and HPDDG had no adverse effect on health of the fish, as compared to substituted plant ingredients. The low level of mycotoxins in the distiller's co-products used in the present experiment may have contributed to this. Mycotoxins are a potential risk factor that could limit use of these maize co-products in the fish feed industry because mycotoxin concentrations in DDGS may be concentrated up to three times (Wu and Munkvold, 2008) to 3.5 times (Zhang and Caupert, 2012) in DDGS compared to corn. In the present study, most mycotoxins analyzed in DDGS and HPDDG were at levels below the detection limit. The analyzed concentrations of DON were similar to those reported by Zhang and Caupert (2012), who conducted a comprehensive survey of DDGS samples in 2011 and showed that they all contained <2 mg/kg. Assuming there were no mycotoxins in other ingredients, the highest level of DDGS in our study would correspond to a dietary content of 0.55 mg DON/kg. Studies by Hooft et al. (2011) indicated that rainbow trout are extremely sensitive to low levels of DON from naturally contaminated ingredients, and showed linear or quadratic decreases in feed intake, weight gain, growth rate, FCR, and retained energy and nitrogen with DON levels ranging from 0.3 to 2.6 mg/kg. Working with Atlantic salmon, Döll et al. (2010) reported that feeding a diet containing 3.7 mg DON/kg resulted in decreased feed intake, as well as poorer growth and feed conversion. In Norway, the Scientific Steering Committee of

the Norwegian Scientific Committee for Food Safety has in 2013, recommended a "Lowest Observed Adverse Effect Level" (LOAEL) for DON of 2.6 mg/kg in rainbow trout. This concentration is about five times higher than the calculated level in our DDGS100 diet. The effect of the acetylated versions of DON, such as 15-Acetyl DON, produced during steps along the biosynthetic pathway and detected in the DDGS used in our study, are unknown.

5. Conclusion

Results from this study demonstrated that both DDGS and HPDDG were suitable energy, protein, and phosphorus sources when replacing a mixture of typical plant ingredients in diets for rainbow trout. Feeding DDGS to rainbow trout resulted in improved feed intake, weight gain and FCR when replacing a mixture of pea meal, sunflower meal, and rapeseed meal, while HPDDG resulted in similar growth performance when replacing a mixture of soy protein concentrate, sunflower meal and rapeseed meal. Both DDGS and HPDDG improved P digestibility of the diets, while neither distiller's co-product affected the relative weight of the distal intestine, nor intestinal enzyme activity of rainbow trout.

Acknowledgments

We appreciate the efforts of Steve Markham at Cenex Harvest States, Inc., Inver Grove Heights, MN for providing the distiller's co-products used in this study and coordinating the transportation logistics to the Aquaculture Protein Centre in Ås, Norway. We wish to thank Frank Sundby and Anna Jansen for their valuable assistance during feed production and for helping out with daily feeding and fish husbandry. Thanks are also due to Bjørn Reidar Hansen and Harald Støkken at the UMB Fish Laboratory for their skillful help with all practicalities related to the fish trial. The research was supported by Grant # 417091 from the Research Council of Norway.

References

Andlid, T., Juárez, R.-V., Gustafsson, L., 1995. Yeast colonizing the intestine of rainbow trout (*Salmo gairdneri*) and turbot (*Scophtalmus maximus*). Microb. Ecol. 30, 321–334.

Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V., Jørgensen, H.Y., Hillestad, M., Krogdahl, Å., Storebakken, T., 2007. Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. Aquaculture 272, 541–555.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13, 265–272.

Austreng, E., Storebakken, T., Thomassen, M.S., Refstie, S., Thomassen, Y., 2000. Evaluation of selected trivalent metal oxides as inert markers used to estimate apparent digestibility in salmonids. Aquaculture 188, 65–78.

Barnes, M.E., Brown, M.L., Rosentrater, K.A., 2012a. Juvenile rainbow trout responses to diets containing distiller's dried grain with solubles, phytase, and amino acid supplements. Open J. Anim. Sci. 2 (2), 69–77.

Barnes, M.E., Brown, M.L., Rosentrater, K.A., 2012b. Initial observations on the inclusion of high protein distiller's dried grain into rainbow trout diets. Open Fish Sci. J. 5, 21–29.

Barnes, M.E., Brown, M.L., Rosentrater, K.A., Fletcher, B., 2012c. Replacement of fish meal with high protein distiller's dried grain in juvenile rainbow trout diets. J. Aquac. Feed Sci. Nutr. 4 (3–4), 39–47.

Bodin, N., Mambrini, M., Wauters, J.-B., Abboudi, T., Ooghe, W., Boulenge, E.L., Larondelle, Y., Rollin, X., 2008. Threonine requirements for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at the fry stage are similar. Aquaculture 274, 353–365.

Carlsson, N.-G., Bergman, E.-L., Skoglund, E., Hasselblad, K., Sandberg, A.-S., 2001. Rapid analysis of inositol phosphates. J. Agric. Food Chem. 49, 1695–1701.

Cheng, Z.J., Hardy, R.W., 2004. Nutritional value of diets containing distiller's dried grain with solubles for rainbow trout, *Oncorhynchus mykiss*. J. Appl. Aquac. 15 (3/4), 101–113.

Coyle, S.D., Mengel, G.J., Tidwell, J.H., Webster, C.D., 2004. Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing different protein sources in combination with distiller's dried grains with solubles. Aquac. Res. 35, 365–370.

Döll, S., Baardsen, G., Möller, P., Koppe, W., Stubhaug, I., Dänicke, S., 2010. Effects of Increasing Concentrations of the Mycotoxins Deoxynivalenol, Zearalenone or Ochratoxin A in Diets for Atlantic Salmon (*Salmo salar*) on Growth Performance and Health. The 14th International Symposium on Fish Nutrition & Feeding, May 31-June 4, Qingdao, China.

FAO, 2012. SOFIA: The State of World Fisheries and Aquaculture. Food and Agriculture Organization, Rome, Italy (http://www.fao.org/docrep/013/i1820e/i1820e00.htm).

Frøystad, M.K., Lilleeng, E., Sundby, A., Krogdahl, Å., 2006. Cloning and characterization of α-amylase from Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. 145A, 479–492.

Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Maas, R., Sipsas, S., 2004. A comparison of the digestibility of a range of lupin and soybean products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). Aquaculture 237, 333–346.

Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, M., Sipsas, S., 2005. Evaluation of digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. Aquaculture 245, 211–220.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139, 157–163.

Hooft, J.M., Elmor, A.E.H.I., Encarnacao, P., Bureau, D.P., 2011. Rainbow trout (*Oncorhynchus mykiss*) is extremely sensitive to the feed-borne *Fusarium* mycotoxin deoxynivalenol (DON). Aquaculture 311, 224–232.

Ingledew, W.M., 1999. Yeast — Could you Base a Business on This Bug? In: Lyons, T.P., Jacques, K.A. (Eds.), Under the Microsope – Focal Points for the New Millennium – Biotechnology in the Feed Industry. Proceedings of Alltech's15th Annual Symposium, pp. 27–47.

Kakade, M.L., Simons, N., Liener, I.E., 1969. An evaluation of natural vs. synthetic substrates for measuring the antitryptic activity of soybean samples. Cereal Chem. 40, 518–526.

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 9, 361–371.

Li, P., Gatlin III, D.M., 2006. Nucleotide nutrition in fish: current knowledge and future applications. Aquaculture 251, 141–152.

Li, M.H., Robinson, E.H., Oberle, D.F., Lucas, P.M., 2010. Effects of various corn distillers byproducts on growth, feed efficiency, and body composition of channel catfish, *Ictalurus punctatus*. Aquac. Nutr. 16, 188–193.

Li, M.H., Oberle, D.F., Lucas, P.M., 2011. Evaluation of corn distiller's dried grains with solubles and brewer's yeast in diets for channel catfish *Ictalurus punctatus* (Rafinesque). Aquac. Res. 42, 1424–1430.

Opstvedt, J., Miller, R., Hardy, R.W., Spinelli, J., 1984. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairdneri*). J. Agric. Food Chem. 32, 929–935.

Øverland, M., Karlsson, A., Mydland, L.M., Romarheim, O.H., Skrede, A., 2013. Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). Aquaculture 402–403, 1–7.

Refstie, S., Baeverfjord, G., Seim, R.R., Elvebø, O., 2010. Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. Aquaculture 305, 109–116.

Renewable Fuels Association, 2013. 2013 Ethanol Industry Outlook. Renewable Fuels Association, Washington, DC, USA.

Robinson, P.H., Karges, K., Gibson, M.L., 2008. Nutritional evaluation of four co-product feedstuffs from the motor fuel ethanol distillation industry in the Midwestern USA. Anim. Feed. Sci. Technol. 146, 345–352.

Rodehutscord, M., Jacobs, S., Pack, M., Pfeffer, E., 1995. Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 150 g to supplements of DL-methionine in a semipurified diet containing low or high levels of cystine. J. Nutr. 125, 964–969.

Rodehutscord, M., Becker, A., Pack, M., Pfeffer, E., 1997. Response of rainbow trout (*Oncorhynchus mykiss*) to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. J. Nutr. 126, 1166–1175.

Rumsey, G.L., Hughes, S.G., Smith, R.R., Kinsella, J.E., Shetty, K.J., 1991. Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). Anim. Feed. Sci. Technol. 33, 185–193.

SAS Institute, 2002. SAS/Stats User, s Guide. Version 9.1. SAS Inst. Inc., Cary, NC, USA.

Shelby, R.A., Lim, C., Yildrim-Aksoy, M., Klesius, P.H., 2008. Effects of distiller's grains with solubles-incorporated diets on growth, immune function and disease resistance in Nile tilapia (*Oreochromis niloticus* L.). Aquac. Res. 39, 1351–1353.

Shurson, J., 2012. Maize dried distiller's grains with solubles (DDGS) — a new alternative ingredient in aquafeeds. World Aquac. 43, 54–58.

Singh, V., Johnston, D.B., Naidu, K., Rausch, K.D., Belyea, R.L., Tumbleson, M.E., 2005. Comparison of modified dry-grind corn processes for fermentation characteristics and DDGS composition. Cereal Chem. 82 (2), 187–190.

Skrede, A., Krogdahl, Å., 1985. Heat affects nutritional characteristics of soybean meal and excretion of proteinases in mink and chicks. Nutr. Rep. Int. 32 (2), 479–489.

Stein, H., Shurson, G.C., 2009. The use and application of distiller's dried grains with solubles in swine diets. J. Anim. Sci. 87, 1292–1303.

Stone, D.A.J., Hardy, R.W., Barrows, F.T., Cheng, Z.J., 2005. Effects of extrusion on nutritional value of diets containing corn gluten meal and corn distiller's dried grain for rainbow trout, *Oncorhynchus mykiss*. J. Appl. Aquac. 17 (3), 1–20.

Tietz, N.W., 1995. Clinical Guide to Laboratory Tests, 3rd ed. WB Saunders, Philadelphia (997 pp.).

Urriola, P.E., Hoehler, D., Pedersen, C., Stein, H.H., Shurson, G.C., 2009. Amino acid digestibility of distiller's dried grains with solubles, produced from sorghum, a sorghum–corn blend, and corn fed to growing pigs. J. Anim. Sci. 87, 2574–2580.

Waldroup, P.W., Wang, Z., Coto, C., Cerrate, S., Yan, F., 2007. Development of a standardized nutrient matrix for corn distiller's dried grains with solubles. Int. J. Poult. Sci. 6, 478–483.

Wu, F., Munkvold, G.P., 2008. Mycotoxins in ethanol co-products: modeling economic impacts on the livestock industry and management strategies. J. Agric. Food Chem. 56, 3900–3911.

Zhang, Y., Caupert, J., 2012. Survey of mycotoxins in US distiller's dried grains with solubles from 2009 to 2011. J. Agric. Food Chem. 60, 539–543.