Twin-Screw Extrusion Processing of Rainbow Trout (*Oncorhynchus mykiss*) Feeds Using Various Levels of Corn-Based Distillers Dried Grains with Solubles (DDGS)

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ABSTRACT

Increasing demand for seafood products and rising demand for fish meal for commercial fish feeds is driving the search for effective alternative protein sources. Twin-screw extrusion trials were conducted to study the production of nutritionally balanced feeds for rainbow trout fingerlings (*Oncorhynchus mykiss*). Six isocaloric (≈4.61 kcal/g) ingredient blends with a target protein content of >45% db were formulated with 0, 10, 20, 30, 40, and 50% distillers dried grains with solubles (DDGS) and other feed ingredients. The moisture contents of the diets were initially adjusted to 5–7% db, and then extruded at 250 rpm using dual 1.9 mm dies with varying amounts of steam (7.2–7.7 kg/hr) injected into the conditioner and water (4.3–6.5 kg/hr) into the extruder. Mass flow rates, moisture contents, and temperatures were measured during processing and moisture content, water activity, unit density, bulk density, expansion ratio, compressive strength, compressive modulus, pellet durability index, water stability, and color were analyzed to quantify the effects of varying DDGS content on the extrudate physical properties. Significant differences ($P < 0.05$) among the blends were observed for color and bulk density for both the raw and extruded materials, respectively, and for the unit density and pellet durability index of the extruded products. There were also significant changes in redness and yellowness, but only minor changes in brightness, among the final products with increasing DDGS content. The compressive strength of the extrudates increased significantly with increasing DDGS. Expansion ratio of all pellets was low. All extruded diets achieved very good water stability.

The world's rising demand for seafood has increasingly depleted wild fish stocks through capture fisheries and made aquaculture one of the fastest growing sectors in the food industry (FAO 2009). For instance, the production of farm-raised rainbow trout (*Oncorhynchus mykiss*) has grown exponentially from 4,400t in 1950 to 576,289t in 2008 (FAO 2010). In intensive aquaculture farming, fish meal is a major ingredient in many feeds, particularly for carnivorous species such as trout (Sugiura et al 2001). It provides proteins that are essential for fish metabolism. Carnivorous species require 2.5–5 times more of fish meal biomass input than farmed fish biomass ultimately harvested, whereas other commonly farmed species require only an average of 1.9 kg of fish meal input for every kg of fish raised on compound feeds. Only three out of every 10 food fish species consume less fish meal in their feed than live weight of fish produced (Naylor et al 2000). Fish meal, the main protein source in aquatic feed, has become limited in supply, which has resulted in increasing expense of aquaculture feeds (Zhu et al 2004). Diet costs can often represent 40–70% of aquaculture operating expenses (Thompson et al 2008). Hence, these effects can be diminished by searching for alternative, lower priced sources for protein. Numerous studies have been pursued to find reasonable, cost-efficient, and compatible alternatives for protein for fish feed.

In recent years, much research has focused on plant protein sources that are relatively easy to obtain, mostly affordable, and have a consistent composition compared to animal sources such as meat or poultry by-products. A negative aspect of plant products is that they often contain antinutritional factors, adventitious toxins, lower protein levels, and unbalanced and lower levels of essential amino acids compared to fish meal (Adelizi et al 1998). Therefore supplementations of essential amino acids such as methionine or lysine have been used in the majority of cases required for a partial or total replacement of fish meal. For example, Gomes et al (1995) observed a decrease in growth performance of rainbow trout due to a depression in voluntary feed intake with diets containing 100% plant proteins such as lupin seed meal, faba bean meal, maize gluten, and full-fat soybean. Numerous other studies have shown that partial replacement of fish meal in rainbow trout diets were possible using lupin, rapeseed, pea, wheat gluten, peanut meal, cottonseed meal, canola meal, and many other plant materials. Minerals and vitamins were added to all of those diets and, in addition, some diets were supplemented with lysine and methionine (Gomes et al 1993; Davies et al 1997; Adelizi et al 1998; Burel et al 2000; Luo et al 2006; Shafaeipour et al 2008).

Soybean meal (SBM) is one of the most widely used substitutes in fish feed because of high protein content, stability on the market, and low price (Refstie et al 2000; Thompson et al 2008). Difficulties with this protein source are lower protein digestibility, less accessible energy, nondigestible oligosaccharides, mineral and amino acid deficiencies, and antinutritional factors (Burrells et al 1999). Therefore, total replacement of fish meal by SBM is often only possible for some species by preprocessing (heat treatment, fermentation) (Arndt et al 1999) and amino acid supplemented blends to ensure feed efficiency, palatability, digestibility, and optimal growth (Viola et al 1981; Floreto et al 2000; El-Saidy and Gaber 2002). Studies on total replacement of fish meal for rainbow trout were successfully achieved for growth performance and nutrient utilization with soy protein concentrate supplemented with L-methionine (Kaushik et al 1995). Other plant protein sources have resulted in lower growth performance and lower weight gain (Luo et al 2006).

Distillers dried grains with solubles (DDGS) is another potential protein replacement. It is a coproduct of corn fermentation for fuel ethanol production and usually contains moderate amounts of protein (28–33%) (Rosentrater and Muthukumarappan 2006; Thompson et al 2008), crude fiber (5–11%) (Rosentrater and Muthukumarappan 2006; Thiex 2009), and low levels of starch (4–6%) (Rosentrater and Muthukumarappan 2006). Changes in domestic

*The *e*-**X**tra logo stands for "electronic extra" and indicates that Figures 2, 3, 4, and 5 appear in color online.

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energy policies have resulted in the rapid growth of the ethanol industry (ACE 2010), thus increasing the availability of DDGS. These high quantities can be a sustainable resource of alternative protein for fish feed. So far, DDGS has been traditionally fed to ruminants (USDA 2006) and at low inclusion levels in poultry and swine feed (Whitney et al 2006; Świątkiewicz and Koreleski 2008). In contrast to corn, DDGS contains approximately three times the amount of most nutrients such as fat, protein, and minerals as a result of the fermentation process (Jacques et al 2003). In addition, DDGS contains none of the antinutritional factors of SBM (U.S. Grains Council 2008) and has less phosphorus than fish meal. Hence, it can reduce the total phosphorus levels of the diet and diminish the amount of phosphorus excreted by fish into water, reducing water pollution (Cheng and Hardy 2004). Preceding research with diets containing DDGS as a partial replacement for fish meal showed that economical growth and feed utilization for different species of fish such as hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), sunshine bass (*Morone chrysops* × *M. saxatilis*), and channel catfish (*Ictalurus punctatus*) could be achieved (Coyle et al 2004; Thompson et al 2008; Lim et al 2009).

Rainbow trout have been domesticated and cultured since the late 19th century (Sedgwick 1995). They are the most cultured salmonids for food, fishing, and stocking of public water in North America, with an annual total public production of ≈200 million fish (Lovell 2002). Generally, trout species are carnivorous fishes that prey on other living organisms (Sedgwick 1995). Salmonid diets contain relatively large amounts of high-quality and high-priced fish meal. These diets are typically high in protein, energy, and lipid, and low in ash (Hardy 1996). Salmonids utilize carbohydrates poorly (Pierce et al 2008), so <9% of digestible carbo-

TABLE I
Ingredient Components (g/100 g) in Feed Blends and Compositions

	Dry Weight of Ingredients (g/100 g)					
Ingredients (% db)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
DDGS	0.00	10.00	20.03	30.09	40.16	50.27
Fish meal (herring)	50.58	40.51	30.42	20.30	10.17	0.00
Corn gluten meal	15.49	15.51	15.53	15.55	15.57	15.59
Whole wheat flour	15.03	15.05	15.07	15.08	15.10	15.12
Menhaden oil	11.30	11.79	12.27	12.67	13.16	13.55
Celufil	5.18	4.71	4.25	3.88	3.41	3.03
Vitamin/mineral premix	1.95	1.96	1.96	1.96	1.96	1.97
Vitamin C mix	0.47	0.47	0.47	0.47	0.47	0.47
Total	100.00	100.00	100.00	100.00	100.00	100.00
	Diet Composition (% db)					
Protein	60.23	57.27	54.31	51.35	48.39	45.43
Fat	17.26	17.30	17.34	17.28	17.32	17.26
Neutral detergent fiber (NDF)	40.45	41.21	41.97	42.73	43.49	44.25
Ash	5.95	5.11	4.27	3.43	2.59	1.75

TABLE II
Physical Properties of Raw Feed Blends[a]

	Diet (% DDGS)					
Properties	**0**	**10**	**20**	**30**	**40**	**50**
MC raw (% db)	5.70ab (0.88)	4.97a (0.40)	6.01ab (0.90)	6.69b (0.60)	6.53b (0.97)	6.43b (0.48)
a_w (-)	0.36a (0.00)	0.36b (0.00)	0.39c (0.00)	0.40d (0.00)	0.40d (0.00)	0.42e (0.00)
BD (kg/m^3)	365.93a (3.73)	393.36b (7.57)	403.09b (6.86)	428.07c (5.49)	443.83d (6.34)	471.31e (11.94)
k [W/(m·°C)]	0.07bc (0.01)	0.07a-c (0.00)	0.07ab (0.01)	0.06a (0.01)	0.07bc (0.01)	0.08c (0.01)
α (mm^2/sec)	0.13ab (0.00)	0.13a-c (0.01)	0.14bc (0.01)	0.15c (0.02)	0.12a (0.01)	0.12a (0.01)
Hunter L (-)	32.07a (1.77)	35.84b (1.36)	36.00b (1.01)	39.26c (0.17)	42.66d (0.38)	43.38d (0.35)
Hunter a (-)	6.04a (0.16)	6.65b (0.13)	7.55c (0.23)	8.82d (0.06)	10.43e (0.04)	12.27f (0.20)
Hunter b (-)	13.76a (0.61)	15.61b (0.44)	16.57c (0.43)	18.79d (0.10)	21.27e (0.16)	22.73f (0.21)

[a] Mean values followed by the same letter for a given dependent variable are not significantly different at $P < 0.05$. Values in parentheses are standard deviation. MC, moisture content; a_w, water activity; BD, bulk density; k, thermal conductivity; α, thermal diffusivity.

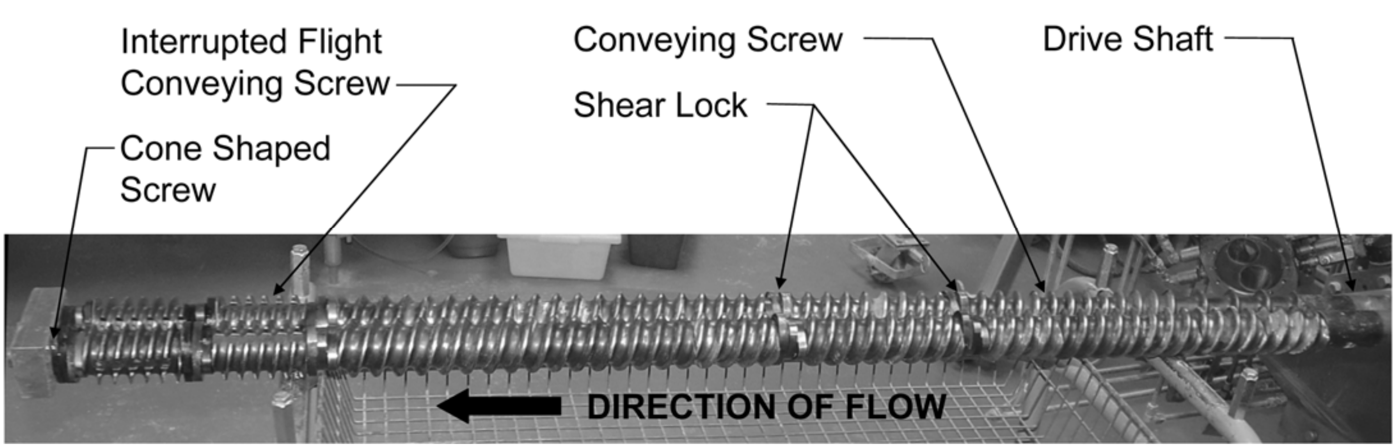

Fig. 1. Extruder screw profile.

hydrate should be fed to trout to avoid negative effects on growth and weight gain (Sedgwick 1995). Salmonids obtain ≈ 50% of their energy requirement from dietary protein (Cho 1992). So, feed for rainbow trout should contain 400–500 g of protein/kg (Kim and Kaushik 1992; Sedgwick 1995; Hardy 1996). Cheng and Hardy (2004) reported that diets including 22.5% DDGS, in combination with SBM and corn gluten meal and supplemented with lysine and methionine, could replace ≤75% of fish meal for rainbow trout while maintaining weight gain and feed conversion ratio.

Some studies have also been conducted on the processing of DDGS-based feeds. For example, Chevanan et al (2007a,b,c, 2008, 2009, 2010), Rosentrater et al (2009a,b), and Kannadhason et al (2009a,b, 2010) investigated how die dimensions, screw speeds, and barrel temperatures of the extruder, ingredient moisture, and DDGS content of the blends affected extrudate physical properties and extrusion processing parameters for single and twin-screw extruders. Chevanan et al (2008) determined that ingredient moisture content and screw speed had significant effects on extrudate durability and color, extruder throughput, and also that DDGS could be included in the diet successfully at ≤40%. In twin-screw studies, Chevanan et al (2007) achieved a DDGS inclusion level at ≤60%; this resulted in increased moisture content, unit density, fiber, and fat content but decreased durability and expansion ratio.

Industrial-scale extrusion of animal feed began between 1955 and 1960 (Moscicki and van Zuilichem 1983). Extrusion is a continuous process that is performed at high temperatures for a short residence time, under high pressures, with high shear forces, and results in modifying, cooking, and texturizing the food ingredients (Cheftel 1986; Lai and Kokini 1991). Additionally, at appropriate temperatures, extrusion inactivates several antinutritional factors, as well as oxidative and other deterioration enzymes (Cheftel 1986); it can enhance the final product in terms of durability, water stability, palatability, digestibility, and animal performance (Chang and Wang 1998; Cheng and Hardy 2003). Compared to single-screw extruders, twin-screw extruders use either two co-rotating or counterrotating screws instead of one screw, inside the barrel and are more suitable for processing high-moisture extrudates (Noguchi 1989). In single-screw extruders, the conveying mechanism is based on frictional forces in the conveying zone and viscous forces in the melt conveying zone. Frictional and viscous properties of the material have less effect on the conveying behavior in the twin-screw extruder due to the intermeshing of the two screws (Rauwendaal 2004). Hence, some of the basic advantages of twin-screw extruders are better feeding, more positive conveying, self-wiping of the screws, short residence times, better mixing, larger heat transfer area, easier scale-up, large output (Shi and Utracki 1992), and ability to handle sticky materials that are difficult to convey through the barrel (Harper 1989).

Starch is gelatinized with water when heated and this is primarily responsible for the expansion in extruded products upon exiting the die (Lue et al 1991; Kannadhason et al 2010). Proteins (polypeptides consisting of amino acids) are responsible for the resulting meat-like texture in protein-based products after extrusion at high temperatures (150–200°C) (Noguchi 1989; Stanley 1989). Feed for carnivorous species require at least 40% protein (Cho et al 1976). Therefore, ingredient blends often contain higher amounts of protein and very small amounts of starch; the final products will more likely exhibit a less expanded and less crispy structure. Accordingly, less expansion may result in lower or no floatability.

A primary goal in fish feed processing is to obtain floating and water-stable pellets that contribute few losses of feed and nutrients, and thus minimize water pollution (Vens-Cappell 1984). In domesticated trout culture, floatability of feed is not a decisive factor as the fish are grown in tanks at high densities; generally fish are trained to take feed immediately and fed at rates commensurate with consumption demand. To date, however, no studies have examined the processing of DDGS-based trout feeds. The objectives of this study were 1) to produce viable extruded feed for juvenile rainbow trout (*Oncorhynchus mykiss*) using DDGS as an alternative protein source, and 2) to examine the effects of varying DDGS content on the resulting physical properties of the extrudates and on extruder processing behavior.

MATERIALS AND METHODS

Feed Blend Preparation

Six isocaloric (≈4.61 kcal/g) ingredient blends, with a target protein content of >45% db each, with increasing contents of DDGS (0, 10, 20, 30, 40, and 50% db) and decreasing amounts of herring fish meal, varying amounts of Celufil, and menhaden oil, but a nearly constant ratio of whole wheat flour, corn gluten meal, and vitamin and mineral mix (Table I) were used to prepare nutritionally balanced diets for rainbow trout fingerlings. Approximately 200 lb (90.7 kg) of each blend were prepared and extruded. DDGS (≈42% crude protein, ≈4% crude lipid) was provided by Poet Nutrition (Sioux Falls, SD) and was ground with a pilot-scale mill (model DAS06, Fitzpatrick, Elmhurst, IL) to a fine particle size (500 μm). Herring fish meal was obtained from Lortscher Agri Service (Bern, KS). The Celufil (used as a nonnutritive filler) was purchased from USB Corporation (Cleveland, OH); menhaden fish oil from Omega Protein (Houston, TX); whole wheat flour from Bob's Red Mill Natural Foods (Milwaukie, OR); corn gluten meal from Consumers Supply Distributing (Sioux City, IA); vitamin C from DSM Nutritional Products France SAS (Village-Neuf, France); vitamin mix and mineral mix from Lortscher Agri Service (Bern, KS). At first, the corn gluten meal, the menhaden fish oil, and the whole wheat flour were combined in a mixer (model 600, Hobart, Troy, OH) for ≈3 min until the fish oil was well dispersed in the mixture; then the mineral and vitamin mix were added. This premix was added to the rest of the ingredients and mixed in a twin-shell dry blender (Patterson-Kelley, East Stroudsburg, PA) to produce a homogeneous bulk. Table II summarizes the physical properties of the raw feed blends.

To balance all of the nutrients, the protein content declined as DDGS level increased. The values for fat content showed negligible variations. The fiber content increased from 40.45 to 44.25% with increasing DDGS content; this was due to the higher fiber in the DDGS compared to the remaining ingredients.

Extrusion Processing

Extrusion was performed using a co-rotating, fully-intermeshing, self-wiping, twin-screw extruder (Wenger TX-52, Sabetha, KS), with a feed hopper and preconditioner. The extruder had 52 mm diameter twin screws, a barrel length of 1,340 mm, with a 25.5:1 length-to-diameter ratio. The screw speed of the extruder could operate from 100 to 1,800 rpm, and the temperatures could be adjusted at 60–150°C. The screw had 25 individual sections (Fig. 1), and the configuration from the feeding section down the length of the barrel to the die section consisted of four conveying screws, three shear locks, one conveying screw, one conveying screw backward, three conveying screws, one conveying screw backward, four conveying screws, one shear lock, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one shear lock, and one cone-shaped screw at the end. The raw material was scooped into the feed hopper then conveyed by screws into the preconditioner where steam at rates of 7.19–7.70 kg/hr were added to adjust the blends to specific temperature and moisture contents. The conditioned ingredients were conveyed into the extruder at a feeder speed between 11 and 12 rpm. The extruder screw speed was maintained at 250 rpm (26.2 rad/sec). From the feeding zone to the die section, the barrel was divided into eight different temperature zones that were maintained at specific temperature levels: 25°C for Head 2 Zone 1, 15°C for Head 3, 15°C for Head 4 Zone 2, 15°C for Head 5 Zone 3, 15°C for Head 6 Zone 4, 15°C for Head 7 Zone 5, 75°C for Head 8, and

75°C for Head 9 Zone 6. Depending upon the extrudates' final physical characteristics (cohesiveness), the amount of water added to the extruder was between 4.32 and 6.45 kg/hr. Table III lists these conditions during processing. The two dies each had circular openings of 1.9 mm. A rotating cutter with three knife blades was positioned at the end of the dies and was adjusted to specific speeds to cut the exiting extrudates to desired lengths. During extrusion processing, moisture content (% db) was monitored at the conditioner exit and the die exit. Temperature (°C) of the raw material, the blend in the preconditioner, down the extruder barrel, and exiting the extruder die was measured with an infrared thermometer (model 42540, Extech Instruments, Waltham, MA).

After the prepared blends were processed in the extruder, they were cooled for 72 hr at room temperature (24 ± 1°C), then dried in a laboratory oven (model TAH-500, The Grieve Corporation, Round Lake, IL) for 24 hr at 45°C, and then subjected to extensive physical property testing.

Processing Behavior

Mass flow rate (MFR) was determined by collecting two (n = 2) samples of extrudates upon exiting the extruder die at intervals of 30 sec during extrusion processing, and then weighing on an electronic balance (Defender 3000 Series, Ohaus, Pine Brook, NJ).

According to Approved Method 44-19.01 (AACC International 2010), moisture content (MC, % db) changes were monitored by taking three (n = 3) samples from the raw blends, exiting the preconditioner (i.e., entering the extruder), exiting the die, as well as dried extrudates for each blend. Moisture contents were determined using a laboratory oven (Thelco Precision, Jovan, Wincester, VA) at 135°C for 2 hr.

Physical Properties of Raw Blends and Extrudates

After drying and cooling, triplicates (n = 3) were then analyzed for water activity (-), length and diameter (mm), expansion ratio (-), unit density (kg/m^3), bulk density (kg/m^3), pellet durability index (%), water stability (min), and color (-); compressive strength (MPa), and compressive modulus (MPa) were determined with n = 10 replications.

The raw blend and extrudate samples from each treatment were analyzed for water activity (a_w) using an a_w measuring system (a_w Sprint TH-500, Novasina, Pfäffikon, Switzerland). The sample bowl was filled with each sample and then placed in the measuring chamber of the instrument for analyzing the water activity.

Bulk density (BD) was determined as the ratio of the mass of extrudates or raw blend, respectively, that could fill a given bulk container and was measured using a standard bushel tester (Seedburo Equipment, Chicago, IL) following the method recommended by USDA (1999).

Each raw blend was analyzed for thermal conductivity (k) and thermal diffusivity (α). A raw blend was placed in a 250 mL beaker, the sensor needle of a thermal properties analyzer (KD2

TABLE III
Steam and Water Conditions During Extrusion of Each Diet[a]

	Diet (% DDGS)					
Parameters	**0**	**10**	**20**	**30**	**40**	**50**
Conditioner steam (kg/hr)	7.19 (0.45)	7.47 (0.02)	7.64 (0.23)	7.65 (0.25)	7.53 (0.05)	7.70 (0.14)
Extruder water (kg/hr)	6.45 (2.59)	5.88 (0.00)	5.10 (1.00)	4.32 (0.00)	4.32 (0.00)	4.32 (0.00)

[a] Values in parentheses are standard deviation.

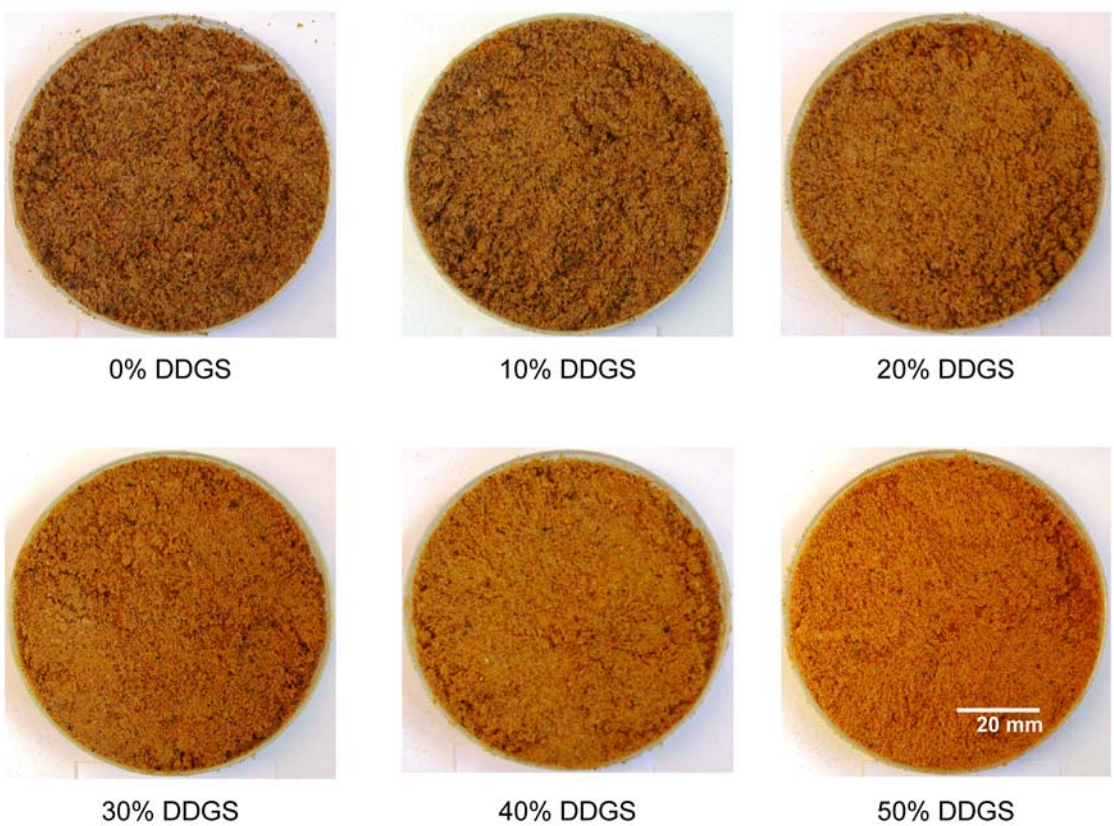

Fig. 2. Raw blends.

Thermal Properties Analyzer, Decagon Devices, Pullman, WA) was inserted into the medium, and the thermal conductivity and thermal diffusivity were measured three times on three different positions in the material at room temperature (24 ± 1°C).

A spectrophotocolorimeter (LabScan XE, HunterLab, Reston, VA) was used to determine the color of the raw blends as well as that of extrudates, where Hunter *L* quantifies brightness/darkness, Hunter *a* quantifies redness/greenness, and Hunter *b* quantifies yellowness/blueness of the samples. Likewise, color was tested on the raw material blends.

Extrudates were cut to approximate lengths of 25.4 mm, weighed on an analytical balance (Adventurer, item no. AR 1140, Ohaus, Pine Brook, NJ) and measured with a digital caliper (Digimatic model no. CD-6''C, Mitutoyo, Tokyo, Japan) to determine diameter. According to Rosentrater et al (2005), the unit density (UD) was calculated as the ratio of the mass M (kg) to the volume V

Fig. 3. Resulting extrudates.

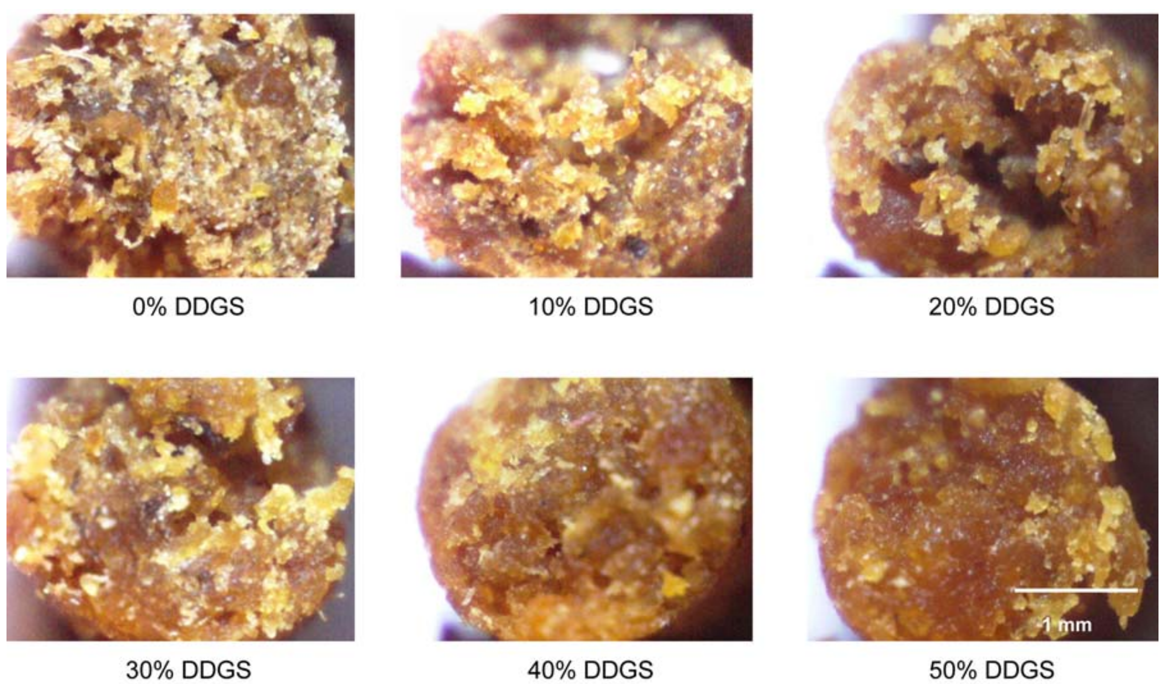

Fig. 4. Cross-sections of resulting extrudates (60×).

(m^3) of each measured and weighed sample, assuming a cylindrical shape for each extrudate: UD = *M*/*V*.

The ratio of the diameter of the dry extrudates, measured with a digital caliper (Digimatic, model no. CD-6''C, Mitutoyo, Tokyo, Japan), to the diameter of the die nozzle (1.9 mm) was used to determine the expansion ratio (ER). The results were displayed as the mean of 10 measurements.

Extruded samples were then tested for compressive strength and modulus (i.e., stiffness) using a dual column universal materials testing machine (model no. 5564, Instron, Canton, MA).

The pellet durability index (PDI) was determined according to Method S269.4 (ASAE 2004). Approximately 100 g of extrudate sample from each blend was manually sieved (Standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL) for ≈10 sec and then tumbled in a pellet durability tester (model PDT-110, Seedburo Equipment, Chicago, IL) for 10 min. Afterwards, the samples were again sieved for ≈10 sec and weighed on an electronic balance (Explorer Pro, model EP4102, Ohaus, Pine Brook, NJ). For blends 1 through 4, sieve No. 7 (2.80 mm) was used but for blends 5 and 6 sieve No. 8 (2.36 mm) was used (this was due to the difference in extrudate diameter). Relating extrudate sample weights before and after tumbling, the PDI was calculated as: PDI (%) = $(M_a/M_b) \times 100$, where M_a was the mass (g) after tumbling and M_b was the sample mass (g) before tumbling.

For extrudates of each blend, a 1-g sample was placed in 200 mL of distilled water and stirred with a magnet stirrer (PMC no. 524C, Barnstead International, Dubuque, IA) until the extrudates began to disintegrate to determine stirred water stability (WS_{stir}). In still water stability (WS_{still}), the same process was used but without stirring. Water stability of aquafeeds indicates the overall performance of the extrudates in an aquatic setting. It represents one of the most important properties of the feed because water stability determines how much time it takes before an extrudate breaks, and therefore is no longer available for the fish to eat. In addition, disintegration of extrudates leads to leaching of nutrients into water.

Data Analysis

All data were analyzed with software (SAS Institute, Cary, NC) using a Type I error rate (α) of 0.05 by analysis of variance (ANOVA) to determine whether there were significant differences between treatments and, if differences existed, post hoc LSD tests were used to determine where they occurred. Pearson linear correlation analyses were also performed among all independent and dependent variables to test for linear relationships.

RESULTS AND DISCUSSION

Extrudate Properties

DDGS level influenced both the processing behavior as well as the resulting extrudate characteristics, both externally and internally. Figure 2 illustrates the differences in color of raw blends with increasing amounts of DDGS: the higher the DDGS the content, the brighter and yellower the color of the initial diets. Figure 3 shows the resulting extrudates and demonstrates that the samples increased in length and appeared to be more cohesive, reddish, and lighter in color. Figure 4 displays cross-sections of the final extrudate samples at 60× magnification; it is evident that the extrudates increased in homogeneity with higher amounts of DDGS.

Moisture Content

Moisture content has an important influence on extrusion processing and on the properties of the resulting extrudates (Table IV). Most processing parameters such as pellet durability, mass flow rate, and color are ultimately affected by the moisture content of the dough and the changes that occur during processing. Moreover, MC affects cohesiveness and water stability of extrudates when exiting the die. Modifications in the amount of steam and water added during processing determine whether the final product is soft, brittle, or cohesive. Due to plasticization and denaturization of protein and interactions with water, the texture of the extrudates becomes less brittle and less fragile when appropriate amounts of moisture are applied.

The MC of the raw materials (Table II) was 4.97–6.69% and showed only minor differences among the blends. This was as expected due to the design of the composition of the diets. No clear pattern of changes in MC could be observed for samples taken at the conditioner or directly at the die (Table V). Differences in MC at the conditioner and die were related to injected steam and water (Table III), respectively, which were modified to improve the cohesiveness of the extrudates of each diet. Analyzing the final extrudates (Table IV), MC was much lower than the MC of the die samples (Table V) due to moisture evaporation and drying. The significant differences among the blends were similar to the MC of raw blends, except for the blend with 50% DDGS. These differences among the final extrudates and that taken at the die were due to extensive drying after processing. Another factor in the lower MC of the extrudates was due to the flashing-off of internal moisture at the die exit, which was caused by the sudden drop of pressure from high values inside the extruder to atmospheric level outside the die.

Water Activity

In contrast to moisture content, water activity detects the unbound water in a material and is defined as the ratio between the water pressure of the solution to that of pure water under the same condition (Koop et al 2000). Free water is available for microorganisms like molds, yeast, and bacteria, and enables growth and, consequently, spoilage of a product. Water activity determines the shelf life of a product and is generally considered to guarantee a stable product with long storage stability at levels <0.6 (Lowe and Kershaw 1995). The values of a_w for the raw material (Table II) increased significantly with higher DDGS levels at 0.36–0.42. The water activity for the extrudates (Table IV) resulted in values of 0.10–0.20. These low values can be ascribed to the extensive air and oven drying postextrusion. The raw and the final products exhibited very low levels of water activity and yielded a very dry product that may allow long storage times without the risk for fast spoilage.

Expansion Ratio

Radial expansion is calculated as the ratio of the diameter of an extrudate to that of the die. Longitudinal and volumetric indices were neglected. During expansion, water is nucleated and forms bubbles in the extruded material that expands during exit from the die (Arhaliass et al 2009). The internal structure of the expanding melt is affected by the radial expansion that occurs at the die exit and results in different textures of the extrudates (Arhaliass et al 2003). Generally, ER is inversely related to the unit density (Bhatnagar and Hanna 1996) and depends on the starch content, flashing of water vapor and flow properties of the molten mass (Colonna et al 1989).

In this study, ER was very low (Table IV) and yielded values of 1.40–1.61. No clear pattern could be observed relating to changes in DDGS levels. But the assumption that ER is inversely related to UD could not be observed. This can be traced back to the fact that the composition of the blends was based on producing extrudates with high protein levels using DDGS and fish meal. DDGS naturally has a low starch content of 4.7–5.9% (Rosentrater and Muthukumarappan 2006) but a high amount of protein, as does fish meal. Therefore, high expansion was not expected.

Unit Density

The difference between unit density (UD) and bulk density (BD) is that UD is mass density for a single extrudate, whereas BD relates to the mass of extrudate in a given volume. Generally, UD depends on the expansion that occurs during extrusion processing. Values for unit density can be an indicator for floatability of the extrudate, that is, if the unit density is below the density of water (1,000 kg/m^3). In this study, no relationship could be observed between the levels of expansion ratio and UD of the extrudates (Table IV). The values for UD increased from 887.74 kg/m^3 to 988.97 kg/m^3 with increasing DDGS levels (all of which were less than water), which was an increase of 11.40%. In a similar study of DDGS-based feed for yellow perch using a single-screw extruder, Ayadi et al (2009) found an increase of 17% in UD with increasing DDGS levels of 10–50%; unit density was also not affected by the expansion ratio. Though values for UD lay below the density of water, none of the extrudate samples floated when placed in water. Ayadi et al (2009) observed similar behavior for DDGS-based extrudates that did not float in water although the density lay near the density of water. This was due to the porous texture of the extrudates that absorbed water quickly and caused them to sink.

Bulk Density

Bulk density is defined as the weight per unit volume of a material, including the void spaces between particles. This includes voids between irregular shaped extrudates and the pores that were formed during expansion. Therefore, the BD provides important information for the design and sizing of storage spaces that are necessary for extrudates or raw materials. Increasing the amount of DDGS in raw blends resulted in a significant increase (28.8%) of BD from 365.95 kg/m^3 (control diet) to 471.31 kg/m^3 (50% DDGS). The control diet (Table IV) showed one of the highest levels of BD with 468.05 kg/m^3. A similar steady increase in BD for the DDGS blends occurred at 10–50%. As for the raw materials, BD increased significantly (8.25%) at 434.44–470.29 kg/m^3.

Similar conclusions were made by Chevanan et al (2007b), who determined a bulk density increase of 61.4% for extrudates when raising the DDGS content from 20 to 60% for twin-screw extrusion of tilapia feeds.

The increase in BD for both the raw materials and the final products in this study can be caused by the increase in DDGS and the increase in fish oil because amount of fish meal was reduced and the remaining ingredients remained constant.

Compressive Strength and Compressive Modulus

Compression tests indicate an extrudate's ability to withstand forces without deforming or breaking. Testing was performed until the stress-strain curve reached its first peak (the yield point) when the deformation of the material became irreversible. Compressive strength values increased significantly from 0.59 to 2.65 MPa (Table IV) with increasing DDGS levels. However, these values were very low and described relatively fragile products that did not resist high forces, and deformed easily.

Compressive modulus is a measure of product stiffness. The values increased significantly from 5.72 to 35.25 MPa (Table IV) with increasing amounts of DDGS; extrudates became stiffer with higher amounts of DDGS. With increasing values for compressive modulus, the standard deviation increased from 1.54 to 20.42 MPa. These diverging values can be can be ascribed to the heterogeneous texture of the extrudates that react differently when forces are applied. It can be assumed that with increasing DDGS levels, the heterogeneity of the product increased.

PDI

Pellet durability index is a measure of the physical quality of extrudates. The tumbling test simulates the mechanical handling of extrudates during storage and transport, and predicts the possible fines that may be produced due to abrasion. In the feed industry, high durability means high quality pellets (Kaliyan and Morey 2009). PDI values were 82.95% for the control diet and 95.39% for the diet containing 50% (Table IV). The blend with 30% DDGS had the lowest PDI, of 87.74%. The blends with 10, 40, and 50% reached high PDI >91%. Generally, the extrudates exhibited good PDI that indicated products with good resistance to abrasive forces.

TABLE IV
Treatment Effects on Extrudate Physical Properties[a]

	Diet (% DDGS)					
Properties	**0**	**10**	**20**	**30**	**40**	**50**
MC extrudate (% db)	1.84a (0.31)	2.33a (0.77)	2.16a (0.22)	3.71b (0.89)	4.25b (0.67)	2.33a (0.39)
a_w (-)	0.11b (0.00)	0.10a (0.00)	0.14c (0.00)	0.15c (0.01)	0.20d (0.01)	0.14c (0.00)
ER (-)	1.49c (0.05)	1.61d (0.04)	1.45bc (0.04)	1.41a (0.05)	1.40a (0.04)	1.42ab (0.04)
UD (kg/m^3)	927.02ab (55.01)	887.29a (38.02)	890.87a (47.62)	938.29a-c (90.80)	971.06bc (56.71)	988.97c (54.56)
BD (kg/m^3)	468.05de (2.62)	434.44a (0.34)	444.27b (1.90)	456.27c (0.56)	466.67d (0.76)	470.29d (2.41)
Compressive strength (MPa)	0.59a (0.11)	0.75b (0.13)	0.81b (0.19)	1.04c (0.13)	1.53d (0.17)	2.65e (0.28)
Compressive modulus (MPa)	5.72a (1.54)	6.53a (2.83)	9.97ab (4.37)	13.44ab (4.17)	16.78b (11.56)	35.25c (20.41)
PDI (%)	82.95a (1.25)	91.01c (0.40)	88.54b (0.15)	87.74b (1.60)	91.54c (0.45)	95.39d (0.60)
WS stir (min)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)
WS still (min)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)	>30 (0.00)
Hunter *L* (-)	26.04a (0.28)	26.05a (0.28)	26.25ab (0.50)	26.77ab (0.55)	26.28ab (0.51)	26.87b (0.23)
Hunter *a* (-)	4.52a (0.03)	4.79b (0.06)	5.58c (0.17)	6.35d (0.16)	7.51e (0.13)	9.65f (0.02)
Hunter *b* (-)	10.52a (0.13)	10.78a (0.12)	11.55b (0.32)	12.03c (0.30)	12.29c (0.22)	13.16d (0.16)

[a] Means followed by the same letter for a given dependent variable are not significantly different at $P < 0.05$. Values in parentheses are standard deviation. MC, moisture content; a_w, water activity; ER, expansion ratio; UD, unit density; BD, bulk density; PDI, pellet durability index; WS, water stability.

TABLE V
Treatment Effects on Measured Processing Behavior[a]

	Diet (% DDGS)					
Parameters	**0**	**10**	**20**	**30**	**40**	**50**
MC conditioner (% db)	19.68c (1.30)	17.40b (1.26)	14.80a (0.87)	16.92b (0.19)	16.79b (0.41)	15.95ab (0.75)
MC die (% db)	35.77c (0.94)	47.48d (1.03)	26.47ab (1.04)	25.34a (1.57)	25.21a (0.29)	27.50b (0.20)
MFR (kg/min)	0.80ab (0.06)	0.90b (0.03)	0.86ab (0.03)	0.90b (0.08)	0.66a (0.20)	0.86ab (0.03)

[a] Means followed by the same letter for a given dependent variable are not significantly different at $P < 0.05$. Values in parentheses are standard deviation. MC, moisture content; MFR, mass flow rate.

Water Stability

Similar to PDI, water stability plays a decisive role in the quality of fish feed because it is the time that extrudates resist dissolution when they are placed in water. When pellets dissolve in an aquatic environment, this leads to loss of nutrients, nonavailability of feed for fish, and potential water pollution.

Therefore, high WS values infer that extrudates are very stable. All measured times for WS (Table IV) were >30 min, irrespective of stirring or not stirring. Hence, all extrudates were of high quality. Similar observations were made by Ayadi et al (2009), who observed WS values at 24–30 min for DDGS-based feed extrudates for yellow perch.

Color

Formulation changes can lead to extrudate color changes. Processing conditions can also result in extrudate color alterations. Color is an important physical property of feeds in that color changes can also be an indication of the loss of lysine in the final product. Lysine is an essential amino acid in aquaculture feed. The Maillard reaction, favored by high temperatures in combination with low water contents, can induce reduction of sugars and free amino groups in proteins and decrease the protein digestibility and availability of amino acids, particularly lysine (Björck et al 1985). Some studies observed that darker-colored DDGS tended to have reduced concentrations and lower digestibility of lysine

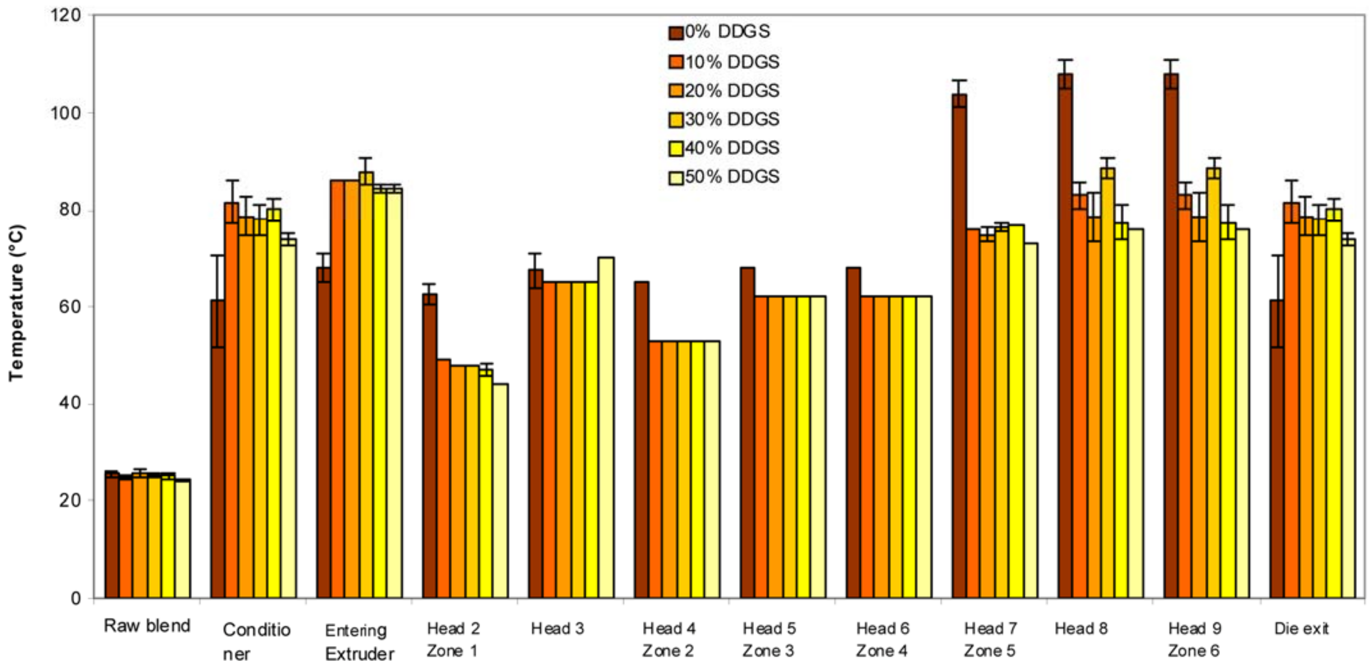

Fig. 5. Temperature distribution of extrusion process for each ingredient blend. Error bars ± 1 standard deviation.

TABLE VI *(continued on facing page)*
Linear Correlations Among All Independent and Dependent Variables[a]

	Extrudate Properties							
	DDGS	MC	a_w	ER	UD	BD	C Strength	C Modulus
Extrudate properties								
DDGS	1							
MC	0.5382	1						
a_w	0.6983	0.8420	1					
ER	–0.6909	–0.5386	–0.7917	1				
UD	0.7886	0.4405	0.5983	–0.7018	1			
BD	0.4395	0.2150	0.4455	–0.6942	0.8685	1		
Compressive strength	0.8943	0.1886	0.4024	–0.5017	0.8407	0.5529	1	
Compressive modulus	0.8890	0.1450	0.3679	–0.5400	0.8202	0.5510	0.9914	1
PDI	0.8023	0.2353	0.3260	–0.1395	0.4720	0.0167	0.8166	0.7781
L	0.7986	0.3127	0.3372	–0.6460	0.6528	0.4051	0.7408	0.8020
a	0.9584	0.3341	0.5504	–0.6358	0.8632	0.5686	0.9811	0.9775
b	0.9886	0.4473	0.6397	–0.7276	0.7941	0.4811	0.9103	0.9222
Processing properties								
MFR	–0.2232	–0.4993	–0.7116	0.4122	–0.4639	–0.5410	–0.1391	–0.0542
Raw properties								
MC	0.7476	0.6310	0.7838	–0.9766	0.7230	0.6439	0.5333	0.5724
a_w	0.9571	0.4476	0.6622	–0.8092	0.7416	0.4741	0.8367	0.8670
BD	0.9941	0.5052	0.6305	–0.6224	0.7807	0.4064	0.9140	0.9072
k	0.3381	–0.4502	–0.0898	0.0401	0.3886	0.3041	0.6618	0.6307
α	–0.3201	0.0406	–0.1943	–0.0289	–0.5247	–0.4319	–0.5601	–0.4661
L	0.9800	0.6315	0.7220	–0.6043	0.7781	0.3907	0.8594	0.8318
a	0.9828	0.4490	0.6367	–0.6677	0.8655	0.5500	0.9541	0.9440
b	0.9938	0.5611	0.7038	–0.6612	0.8271	0.4755	0.9064	0.8889

[a] MC, moisture content; a_w, water activity; ER, expansion ratio; UD, unit density; BD, bulk density; C strength, compressive strength; C modulus, compressive modulus; PDI, pellet durability index; *L, a, b,* Hunter color parameters; MFR, mass flow rate; *k*, thermal conductivity; α, thermal diffusivity.

than lighter-colored DDGS (Cromwell et al 1993; Fastinger and Mahan 2006).

Regarding the changes in color of the raw material (Table II) by increasing DDGS content from 0% to 50%, brightness (Hunter *L*), redness (Hunter *a*), and yellowness (Hunter *b*) increased significantly by 35.3, 103.1, and 65.2%, respectively. The DDGS used in this study was light golden yellow in color after grinding and was considerably lighter than the fish meal that was used. Hence, due to the increasing amounts of DDGS and decreasing amounts of fish meal, the raw blends became lighter, yellower, and reddish in color. This could be also confirmed visually (Fig. 2). After extrusion, there were minor differences in brightness between the blends (Fig. 3); only the blend containing 50% DDGS was significantly different than all other blends. On the other hand, all extruded blends increased significantly in redness with increasing DDGS content (Table IV). Comparing the yellowness, there were significant increases with higher DDGS levels; only the blends containing 30 and 40% DDGS were not significantly different from each other. All in all, only changes in redness in the final product conformed to changes in redness of the raw material. Comparing the changes in brightness between the raw material and the final product, all blends showed decreases in brightness of 18.8, 27.3, 27.2, 31.8, 38.4, and 38.1% for blends containing 0, 10, 20, 30, 40, and 50%, respectively. It can be assumed that processing conditions related to the Maillard reaction could have induced a darker product. Because the control diet with 0% DDGS yielded the highest decrease in brightness, this assumption remains somewhat disputable and should be evaluated by additional laboratory analyses in future studies.

Thermal Conductivity and Thermal Diffusivity

Thermal properties affect the behavior of a material during processing. Thermal conductivity is the measure of a material's availability to conduct heat. The values were 0.06–0.08 W/(m°C) and showed no clear pattern with changes in DDGS levels. These low values describe the feedstock as a material with relatively poor heat transfer properties. Thermal diffusivity is defined as the ratio of the thermal conductivity to the volumetric heat capacity, and governs the heating time of a material throughout its volume. The values for all blends were 0.12–0.15 mm^2/sec and thus were very low. This indicated that more time was required to heat up the diets and also for them to cool down, which can have negative effects on the temperature adjustment of the extrusion process due to additional time required for heating or cooling. On the other hand, it can be assumed that these low thermal diffusivities affect the dough's frictional heating ability during extrusion processing and may affect physical-chemical reactions that occur during processing.

Processing Behavior

The mass flow rate (MFR) (Table V) quantifies the amount of extrudates being produced during a given time period (kg/min), and is also known as material throughput. Hence, it describes the efficiency of the extruder and its production capacity. MFR varied between 0.66 kg/min for the blend containing 40% DDGS and 0.90 kg/min for the blends containing 10 and 30% DDGS. Generally, MFR is affected by screw speed, diameter of the die (Kannadhason et al 2010), shear rate, levels of DDGS, moisture content and viscosity of the dough (Chevanan et al 2008). In this case, no clear pattern could be observed with different DDGS levels. Thus, it can be assumed that other factors such as moisture content, viscosity of the dough, and shear rate affected the mass flow rate because screw speed and diameter of the die were not modified.

Figure 5 illustrates the temperature distribution throughout the extrusion process for each ingredient blend at the different head zones. The temperature in the conditioner increased, because steam was injected to adjust the blends to the desired temperature; temperatures in the Head 2 Zone 1 decreased due to water injection in the extruder. Higher temperature values for the blend containing 30% DDGS could be ascribed to the highest thermal conductivity recorded for this blend. Even though the temperature zones were maintained at the same temperature settings for each blend, obvious differences can be detected in the bar graph, particularly in Head 7 to Head 8. It can be assumed that these variations in temperature were not only caused by friction but also related to the ingredient particle characteristics and the nutrient

TABLE VI ***(continued from facing page)***
Linear Correlations Among All Independent and Dependent Variables[a]

Extrudate Properties				Proc Prop	Raw Properties							
PDI	*L*	*a*	*b*	MFR	MC	a_w	BD	*k*	α	*L*	*a*	*b*
1												
0.5114	1											
0.7949	0.7918	1										
0.7601	0.8549	0.9697	1									
–0.0104	0.2585	–0.2001	–0.1493	1								
0.20.64	0.7516	0.6706	0.7761	–0.3129	1							
0.6569	0.8744	0.9190	0.9831	–0.1190	0.8475	1						
0.8409	0.8142	0.9632	0.9819	–0.1567	0.6941	0.9372	1					
0.5765	0.0881	0.5411	0.3629	–0.1390	–0.1276	0.2634	0.3616	1				
–0.5071	0.1256	–0.4684	–0.2714	0.5828	0.0996	–0.1306	–0.3326	–0.8115	1			
0.8322	0.7200	0.9205	0.9409	–0.2891	0.6787	0.8852	0.9811	0.2976	–0.3799	1		
0.7968	0.7852	0.9918	0.9798	–0.2556	0.7119	0.9314	0.9827	0.4584	–0.4411	0.9599	1	
0.8115	0.7634	0.9620	0.9727	–0.2818	0.7202	0.9250	0.9916	0.3617	–0.3959	0.9906	0.9883	1

[a] Proc Prop, processing properties; MC, moisture content; a_w, water activity; ER, expansion ratio; UD, unit density; BD, bulk density; PDI, pellet durability index; *L, a, b*, Hunter color parameters; MFR, mass flow rate; *k*, thermal conductivity; α is thermal diffusivity.

composition of the blends. As observed in Fig. 3, the control blend without DDGS, appeared to have the most heterogeneous texture, which is reflected in the greatest bar heights. The temperature fluctuations in the final zones can also be attributed to the higher friction caused by less available free water due to the processing and cooking.

Property Correlations

Pearson linear correlation analysis results are listed in Table VI. Among the various relationships, $r > 0.80$ for 62 correlations and $r \leq 0.8$ for four correlations. Many of these strong linear relationships were anticipated based on previous research (Chevanan et al 2007a,b; Kannadhason et al 2009a). In this study, many properties, as well as color parameters, were highly correlated as discussed above. Additionally, compressive strength showed strong positive relationships with unit density and bulk density with $r > 0.87$. Many color parameters of raw blends were highly positively correlated to extrudate properties such as unit density, bulk density, compressive strength, and compressive modulus. This can generally be ascribed to the quantity of DDGS in the blends, which showed strong relationships to these parameters. Chinnaswamy and Hanna (1990) had similar conclusions with corn starch blends.

CONCLUSIONS

The goal of this twin-screw extrusion study was to investigate the effect of increasing levels of DDGS when producing nutritionally balanced feed for rainbow trout fingerlings. Increasing the amount of DDGS in the raw feed blend resulted in significant increases in blend water activity, bulk density, and color (Hunter *L, a, b*). For the resulting extrudates, significant increases in unit density, compressive strength, and color (Hunter *a* and *b*) were detected with increasing amounts of DDGS. The changes in color for both the raw and extruded material can be ascribed to the composition of the blends themselves (i.e., varying amounts of DDGS and fish meal), as well as the processing conditions during extrusion. Extrudate compressive strength values were all very low and the extrudates broke relatively easily, whereas pellet durability indices were high and indicated durable products that had high resistance against abrasive and destructive forces. Regarding high PDI and low compressive strength, a final feed product with good transportation and storage characteristics but one that is easily consumable by fish is beneficial. Extrudates had very low water activity values, which indicates long shelf life. High water stabilities (>30 min) indicated water-resistant products when placed in an aquatic setting. In summary, this study resulted in viable DDGS-based extrudates with good physical properties. Future studies should focus on the availability and digestibility of lysine and other essential amino acids in DDGS-based feed and should identify how changes in color, particularly brightness, are related to the loss of lysine during extrusion processing.

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