2-2010

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S. Kannadhason
South Dakota State University

Kurt A. Rosentrater
USDA, Agricultural Research Service

K. Muthukumapappan
South Dakota State University

Michael L. Brown
South Dakota State University

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 41, No. S1
February, 2010

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S. Kannadhason

Agricultural & Biosystems Engineering, South Dakota State University, Brookings, SD, USA, 57007

Kurt A. Rosentrater[2]

USDA, ARS, North Central Agricultural Research Laboratory, Brookings, SD, USA, 57006

K. Muthukumarappan

Agricultural & Biosystems Engineering, South Dakota State University, Brookings, SD, USA 57007

Michael L. Brown

Wildlife and Fisheries Sciences, South Dakota State University, Brookings, SD, USA 57007

Abstract

Six isocaloric (3.65 kcal/g), isonitrogenous (35% dry-basis [db] protein), ingredient blends were prepared with 0, 17.5, 20, 22.5, 25, and 27.5% distiller's dried grains with solubles (DDGS) and other ingredients (soybean meal, corn, fish meal, whey, soybean oil, vitamin and mineral mix). The blends were moisture balanced to 15% db, then extruded in a twin screw extruder using a 2 mm die at 190 rpm, and a 3 mm die at 348 rpm. Analyses of the extrudates included moisture content, expansion ratio, unit density, bulk density, sinking velocity, color (L*, a*, and b*), water absorption, water solubility, and pellet durability indices. Increasing the DDGS level from 0 to 17.5% db resulted in decreased expansion ratios by 14.8 and 23.5% for the products extruded using a 2 and 3 mm die, respectively. No significant difference in expansion ratio existed for DDGS levels between 17.5 and 27.5% db for either die. The water solubility index (WSI) of the extrudates increased (25.2 and 24.0%) as the DDGS increased from 0 to 27.5% db for each die. The 0% DDGS had the highest expansion ratio and the lowest unit density, bulk density, and sinking velocity. The extrudates that contained 20 and 27.5% DDGS had the highest durability and sinking velocity values.

Extrusion cooking is a process by which moistened, expansive, starchy, and proteinaceous materials can be plasticized and cooked by a combination of moisture, pressure, temperature, and mechanical shear (Hauck and Huber 1989). Done properly, extrusion can preserve the nutrient composition of the ingredients, and at the same time abate pathogenic microorganisms and anti-nutritional factors. In some cases, extrusion processing enhances the feeding value of ingredients since it makes the nutrients more digestible (Castaldo 1998). Cooking, as in extrusion processing of fish feeds, has been shown to increase the digestibility of starch (Cruz 1975) and other ingredients. Aqua feed and pet food manufacturers are using better tools to control the extrusion process to help in that regard (Henry 2006).

Starch is a major functional ingredient for extrusion processing, and is primarily responsible for the expansion of extruded products. It is a biopolymer which is composed

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[2] Corresponding author.

of two types of macro molecules, namely amylose and amylopectin (Kokini et al. 1992; Brouillet-Fourmann et al. 2003). Corn is valued as a feed ingredient because of its high proportion of starch and its higher amylopectin content (76%) versus amylose content (24%) (Swindels 1985). The ratio of amylose–amylopectin is very important in predicting the properties of starch-based extruded products. Amylopectin is responsible for the expansion of starch during extrusion, and the higher the amylopectin in the blend results in a light, elastic, and homogeneous texture with a smooth and sticky external structure. In contrast, blends that contain higher proportions of amylose result in harder and less-expanded products (Mercier and Feillet 1975). Starchy materials are known to undergo substantial changes in the physical constitution of the starch granules during the process of extrusion cooking (Charbonniere et al. 1973). Changes in the properties of starchy foods caused by the addition of lipids are attributed to the formation of complexes between amylose and lipids (Mercier et al. 1980; Colonna and Mercier 1983; Stute and Konieczny-Janda 1983; Schweizer et al. 1986). On the other hand, the largest branched molecules of amylopectin were found to break down through mechanical forces, due to the shearing by the extruder (Davidson et al. 1984; Diosady 1985; Cai et al. 1995).

The expansion volume of starch is highly dependent on its degree of gelatinization within the extruder (Stanley 1986). Pressure and shear developed during extrusion determine the degree of gelatinization of starch (Diosady et al. 1985). Extrusion variables, namely barrel configuration, temperature, screw design, and moisture of the starch, control the pressure and shear within the extruder and, subsequently, the expansion of the starch (Anderson et al. 1969; Colonna et al. 1983; Fletcher et al. 1985; Bhattacharya and Hanna 1987).

The USA's rapidly growing fuel ethanol industry produces large quantities of corn-based feed ingredients. Distiller's dried grains with solubles (DDGS), a co-product of dry grind ethanol manufacturing, is a valuable ingredient for cattle, swine, and poultry feeds, and typically contains nearly 30% crude protein, 2500 kcal/kg of metabolizable energy, and various amounts of fat, fiber, and minerals (Pagon 1991; Spiehs et al. 2002; Shurson 2006). It is an excellent source of energy and protein for animal feeds. Although fish meal is the major protein source for many fish diets, the high cost (ca $1000/ton) has encouraged the evaluation of other alternate protein sources (USDA 1988), including DDGS. Due to its moderately high protein content and lower cost (current market price of ca $150–$200/ton) in comparison with fish meal, there is an increasing interest in using DDGS in aquaculture diets (US Grains Council 2008). As the USA ethanol industry continues to grow, there will be an increasing supply of DDGS for years to come.

Over the last decade, several investigations have examined the use of DDGS in various aquaculture diets. For example, Wu et al. (1994) reported that diets formulated to 36% protein containing 29% DDGS resulted in higher weight gains for tilapia than fish fed with commercial fish feed containing the same amount of crude protein (but using fish meal as a protein source). Tidwell et al. (2000) evaluated the growth, survival, and body composition of tilapia fed pelleted and unpelleted DDGS in polyculture of tilapia with freshwater prawns, and found that pelleted DDGS resulted in a better growth rate compared to unpelleted DDGS. Other studies have also examined DDGS in tilapia diets (Coyle et al. 2004a; Wu et al. 1996, 1997). Additionally, the use of DDGS has been investigated in diets for rainbow trout (Cheng et al. 2003, 2004a, 2004b), freshwater prawn, *Macrobrachium rosenbergii* (Coyle et al. 2003, 2004b; Tidwell et al. 1993, 1998), and channel catfish (Webster et al. 1991, 1992, 1993).

Although some feeding work has been pursued, only limited reports are available, which discuss the actual processing of DDGS feed blends. The effect of DDGS (20–40% wb), moisture content (15–25% wb), and screw speeds (100–160 rpm) on the physical properties of extrudates were studied by Chevanan et al. (2008a), and results indicated that DDGS could be successfully incorporated up to 40% in tilapia feeds. The effect of die dimensions with varying nozzle diameter, length of nozzle, and L/D ratio (ranging from 3.3 to 10.0) on extrusion processing parameters and resulting properties of 40% DDGS-based aquaculture feed blends were studied by Chevanan et al. (2007b). The effect of processing conditions on single screw extrusion of feed ingredients containing DDGS was also studied by Chevanan et al. (2008b, 2009). These studies were accomplished by varying the inclusion levels of DDGS (20–40% wb), moisture contents (15–25% wb), barrel temperatures (100–160 C), and different screw speeds (80–160 rpm).

Twin screw extrusion has numerous advantages over single screw, like production of novel products, high productivity, high product quality, versatility, and energy efficiency. Chevanan et al. (2007a) produced twin screw extruded tilapia feed using DDGS as a base material and studied the effect of various levels of DDGS (20–60%), extruder screw speeds (350 and 420 rpm), and feed moisture content (15 and 19% wb) on the physical properties of the extrudates. Even so, more work remains to be done to optimize the processing of DDGS into viable feed blends. Therefore, this study was undertaken using a twin screw extruder with the following objectives:

(1) Production of a floating feed for tilapia using DDGS as a base material.
(2) Examine the effects of various levels of DDGS, screw speeds, and die dimensions on extrudate properties and on extruder processing parameters.

Materials and Methods

Feed Blend Preparation

Six isocaloric feed blends (3.65 kcal/g) were formulated to 35% db protein using graded levels of DDGS (0, 17.5, 20.0, 22.5, 25.0, and 27.5% db), with appropriate quantities of soybean meal, ground corn, whey, Menhaden fish meal, soybean oil, vitamin, and mineral mix, as shown in Table 1. The DDGS was provided by Dakota Ethanol LLC (Wentworth, SD); whole corn was procured from market, and soybean meal from Dakota Land Feeds Inc. (Huron, SD). These ingredients were ground to a fine particle size (ca 425 μm) using a laboratory grinder (s500 disc mill, Genmills, Clifton, NJ). Mineral mix, vitamin mix (Vitapak, Land O' Lakes Feed, St. Paul, MN), soybean oil, whey (Bongards Creameries, Perham, MN), and fish meal (Consumer Supply Distribution Company, Sioux City, IA) were incorporated into the feed blends at proportions of 0.2, 0.6, 2, 5, and 5%, respectively, on a dry basis. These ingredient blends were mixed in a laboratory-scale mixer (N50 Mixer, Hobart Corporation, Troy, OH) for 30 min to produce a homogeneous mixture. The moisture content of the ingredient blends were then adjusted to 15% db by adding appropriate quantities of water and then mixed thoroughly.

Experimental Design and Extrusion Processing

Extrusion experiments were conducted using six levels of DDGS (0, 17.5, 20.0, 22.5, 25.0, and 27.5% db) and two different dies (2 and 3 mm), which resulted in 12 total treatment combinations ($6 \times 2 = 12$), which were implemented using a randomized block design (each

TABLE 1. *Ingredient composition (dry basis) in the experimental feed blends.*

	Dry weight of ingredients (g/100 g)					
Feed Ingredient (% db)	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
DDGS	0.00	17.5	20.0	22.5	25.0	27.5
Soybean meal, solvent extracted	52.7	59.0	57.7	56.5	55.2	54.0
Corn	24.5	10.7	9.50	8.20	7.00	5.70
Fishmeal, Menhaden	15.0	5.00	5.00	5.00	5.00	5.00
Vitamin premix # 30	0.60	0.60	0.60	0.60	0.60	0.60
Rovimix Stay-C	0.20	0.20	0.20	0.20	0.20	0.20
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Whey	5.00	5.00	5.00	5.00	5.00	5.00

die size required a separate screw speed to properly form final products [190 rotations per min (rpm)] for the 2 mm die, and 348 rpm for the 3 mm die). Thus screw speed/die size was the blocking factor.

Extrusion experiments were conducted using a co-rotating fully intermeshing, self-wiping twin screw extruder (Wenger TX-52, Sabetha, KS), which had a 52 mm diameter screw, and the barrel had a length-to-diameter ratio of 25.5/1. The extruder had a barrel length of 1340 mm, operating screw speeds from 100 to 1800 rpm, and a temperature range of 60–150 C depending on the product and mix configuration. The extruder screw had 23 individual sections, and the configuration of the screw from the feeding section to the die section was comprised of 4 conveying screws, 3 shear locks, 1 conveying screw, 1 conveying screw backward, 3 conveying screws, 1 conveying screw backward, 4 conveying screws, 1 shear lock, 1 interrupted flight conveying screw, 1 conveying screw, 1 interrupted flight conveying screw, 1 shear lock, and 1 final screw (cone shaped). The feeder screw speed was maintained at 10 and 15 rpm for a 2 and 3 mm die, respectively. Also, the conditioning steam was applied at the rate of 0.2152 and 0.2255 kg/min during the operation using a 2 and 3 mm die, respectively. The application of extruder steam was at the rate of 0.08 and 0.12 kg/min for the die/screw speed combination of 2 mm/190 rpm and 3 mm/348 rpm, respectively. Varying temperature combinations (80–110 C) were maintained at head 2, 3, and 4 depending on the final product characteristics and the die/screw speed combination. A rotating cutter assembly, which had three blades, was placed at the end of the die and had various adjustable speeds, which allowed the production of extrudates of specific lengths. More details regarding processing conditions of the extruder can be found in Kannadhason et al. (2008).

Processing Properties

During extrusion processing, extrudate samples were collected at 30-s intervals. These samples were weighed on an electronic balance, and moisture content was determined, so that a dry matter mass balance could be determined for the extrusion process (i.e., steam evaporation could be accounted for at the die exit). Triplicates ($n = 3$) were measured for the extruder processing parameters studied for each treatment combination.

Extrudate Properties

At least 40 kg were produced for each of the 12 treatment combinations. Triplicates ($n = 3$) were measured for all physical properties of the extrudates for each treatment combination and the extrudates were analyzed following the procedures described previously by Rosentrater et al. (2005).

After processing, the extrudates were allowed to cool under ambient conditions for at least 30 min, and were then placed in sealed polyethylene bags, which were then stored at ambient conditions (25 $\pm$ 1 C). Moisture content of the extrudates were determined following American Association of Cereal Chemistry (AACC) method 44-19 (1995), using a forced-convection laboratory oven (Thelco Precision, Jovan Inc., Wincester, VA) at 135 C for 2 h.

The radial expansion ratio was obtained as the ratio of the diameter of the dried extrudates to the diameter of the die (Faubion and Hoseney 1982). Unit density of the extrudates was calculated as the ratio of the mass to the volume of the extrudates. This was achieved by cutting extrudates to lengths approximately 25.4 mm using a razor blade, determining their mass using a laboratory balance, and then calculating the volume (assuming the extrudates were right circular cylinders; volume $= \pi * (\text{radius})^2 *$ height) following the procedure of Jamin and Flores (1998). Bulk density of the extrudates is a measure of how dense and tightly packed the extrudates will be in storage. It was determined as the mass of the extrudates that fit within a given bulk volume, and was measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (1999).

Color (L∗, a∗, and b∗) of the extrudates were determined using a calibrated spectrophotometer (portable model CM 2500d, Minolta Corporation, Ramsey, NJ) using the Hunter Lab color space, where L∗ refers to luminosity/brightness of the extrudates, a∗ refers to redness/greenness of the extrudates, and b∗ refers to yellowness/blueness of the extrudates.

Sinking velocity was calculated using the method developed by Himadri et al. (1993). Sample extrudates were cut into small pieces approximately 25.4 mm in length, using a razor blade, and then dropped into a 2-L volumetric cylinder filled with distilled water. The time taken for each piece to reach the bottom was recorded. Sinking velocity was then calculated as the ratio of the height of the measuring cylinder to the time taken by the extrudates to reach the bottom of the cylinder.

Water absorption and solubility indices are often used as indicators of volume of swollen gelled particles that maintain integrity in aqueous suspension (Mason and Hoseney 1986) and degradation of molecular components (Kirby et al. 1988), respectively. The extrudates were ground to fine powder (ca 150 μm) using a laboratory mill (Smart Grind, Black & Decker Corporation, Towson, MD). Water absorption index (WAI) and water solubility index (WSI) were determined according to the method described by Anderson et al. (1969): 2.5 g of the finely ground sample was suspended in 30 mL of distilled water in a tarred 50-mL centrifuge tube, stirred intermittently, placed in an oven at 30 C for a period of 30 min, and centrifuged at 3000 rpm for 10 min. The supernatant liquid was transferred carefully into an aluminum dish, placed in the oven for 2 h at 135 C (AACC method 44-19, 1995), and then cooled in a desiccator for 20 min before weighing the dry solids of supernatant. The gel remaining in the centrifuge tube was weighed, and WAI was calculated as follows:

$$\mathrm{WAI} = \frac{W_g}{W_{ds}} \tag{1}$$

where WAI is water absorption index (−), W_g is the weight of gel (g), and W_{ds} is the weight of dry sample (g).

WSI was calculated as:

$$\mathrm{WSI} = \left(\frac{W_{ss}}{W_{ds}}\right) \times 100 \tag{2}$$

where WSI is the water solubility index (%), W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of dry sample (g).

The durability of the extrudates was determined using a pellet durability tester (model PDT-110, Seedburo Equipment Company, Chicago, IL) following Method S269.4 (ASAE 2004). About 200 g of extrudates were cut into pieces of approximately 25.4 mm in length, and were divided into two batches of 100 g each. Each batch was placed in the pellet durability tester for a period of 10 min. The sample was placed on a No. 6 sieve before and after tumbling, and measured for the mass retained on the screen. The pellet durability was then calculated using Eq. 3:

$$\mathrm{PDI} = \left(\frac{M_{at}}{M_{bt}}\right) \times 100 \tag{3}$$

where PDI is the pellet durability index (%), M_{at} is the mass of the pellets retained on the screen after tumbling (g), and M_{bt} is the mass of the pellets retained on the screen before tumbling (g).

Extrudate nutrient analysis was also determined for all of the samples. Duplicates ($n = 2$) were measured for all the nutrient properties. Crude protein, neutral detergent fiber, fat, and ash contents were determined following official Method 990.03, 2002.04, 920.39, and 942.05, respectively (AOAC 2003).

Statistical Analysis

The collected data were analyzed with the Proc GLM (general linear models) procedure to determine main effects (i.e., DDGS level), and to test for differences between these levels using the least significant difference (LSD) test using a Type I error rate (α) of 0.05, with SAS (2004), version 9 (SAS Institute, Cary, NC), for each level of the blocking variable (i.e., processing condition: 2 mm die at 190 rpm and 3 mm die at 348 rpm).

Results

Moisture Content

Table 2 summarizes the main effects of varying the levels of DDGS on the resulting extrudate properties for the 2 mm die. Overall, increasing the DDGS level from 0 to 27.5% resulted in a 23.79% decreased extrudate moisture content. But, no significant difference existed for the change in DDGS levels from 0 to 20%, or from 22.5 to 27.5%. For the 3 mm die (Table 3), we observed that increasing the DDGS levels from 0 to 17.5% resulted in a decreased the extrudate moisture content of 23.9%. Changing the levels of DDGS from 20 to 25% did not result in a significant effect on extrudate moisture content. Overall, increasing the DDGS level from 0 to 27.5% had a significant effect on the moisture content of the extrudates, which decreased by 20.3%. Most of the

TABLE 2. *Main effects of diet blends on extrudate properties (die = 2 mm/rpm = 190).*[a]

Diet	MC (% db)	ER (–)	UD (kg/m^3)	BD (kg/m^3)	SV (m/s)	WAI (–)	WSI (%)	PDI (%)	L* (–)	a* (–)	b* (–)
Control	6.80^{a}	1.76^{a}	734.9^{b}	396.4^{d}	0.00^{c}	3.02^{a}	19.4^{d}	92.8^{c}	34.5^{c}	4.68^{c}	12.9^{c}
	(0.13)	(0.14)	(89.0)	(1.27)	(0.00)	(0.14)	(0.33)	(0.26)	(0.48)	(0.04)	(0.17)
1	6.78^{a}	1.50^{b}	901.6^{ab}	458.0^{a}	0.09^{a}	3.03^{a}	22.5*b*^{c}	96.6^{a}	34.5^{c}	5.10^{b}	12.7^{c}
	(0.08)	(0.06)	(64.1)	(0.78)	(0.00)	(0.21)	(0.35)	(0.12)	(0.38)	(0.09)	(0.13)
2	6.69^{a}	1.42^{b}	999.7^{a}	448.7^{b}	0.09^{a}	2.78^{a}	22.1^{c}	97.0^{a}	34.6^{c}	5.13^{b}	13.1^{c}
	(0.05)	(0.04)	(66.9)	(2.46)	(0.00)	(0.11)	(0.53)	(0.26)	(0.16)	(0.06)	(0.18)
3	5.12^{b}	1.46^{b}	876.5^{ab}	432.6^{c}	0.09^{a}	2.79^{a}	23.2^{ab}	95.0^{b}	38.0^{b}	6.16^{a}	15.8^{b}
	(0.21)	(0.09)	(20.0)	(3.57)	(0.00)	(0.06)	(0.24)	(0.07)	(0.16)	(0.05)	(0.10)
4	5.16^{b}	1.44^{b}	872.6^{ab}	432.1^{c}	0.08^{b}	2.72^{a}	23.8^{a}	96.9^{a}	34.7^{c}	5.26^{b}	13.1^{c}
	(0.06)	(0.03)	(26.6)	(3.16)	(0.00)	(0.06)	(0.20)	(0.02)	(0.59)	(0.16)	(0.40)
5	5.19^{b}	1.41^{b}	916.4^{ab}	463.4^{a}	0.10^{a}	2.68^{a}	24.3^{a}	93.3^{c}	40.3^{a}	6.03^{a}	16.8^{a}
	(0.18)	(0.01)	(11.3)	(1.25)	(0.00)	(0.05)	(0.15)	(0.12)	(0.65)	(0.10)	(0.25)

MC = moisture content; ER = expansion ratio; UD = unit density; BD = bulk density; SV = sinking velocity; WAI = water absorption index; WSI = water solubility index; L∗ = brightness/luminosity; a∗ = redness/greenness; b∗ = blueness/yellowness.

[a]Means with similar letters within a given property are not significantly different at $\alpha = 0.05$; $n = 3$ for each property, for each diet. (Values in parentheses are ±1 standard error of the mean.)

TABLE 3. *Main effects of diet blends on extrudate properties (die = 3 mm/rpm = 348).*[a]

Diet	MC (% db)	ER (–)	UD (kg/m^3)	BD (kg/m^3)	SV (m/s)	WAI (–)	WSI (%)	PDI (%)	L* (–)	a* (–)	b* (–)
Control	6.21^{a}	1.96^{a}	583.4^{b}	426.9^{e}	0.00^{d}	2.94^{a}	19.33^{c}	90.3^{c}	39.9^{d}	4.40^{d}	14.4^{c}
	(0.12)	(0.08)	(52.9)	(2.90)	(0.00)	(0.08)	(0.20)	(0.12)	(0.32)	(0.005)	(0.10)
1	4.72^{d}	1.50^{b}	806.8^{a}	492.6^{d}	0.12^{ab}	2.87^{a}	23.3^{ab}	91.9^{b}	43.7^{a}	5.50^{b}	17.9^{a}
	(0.02)	(0.02)	(37.2)	(0.07)	(0.004)	(0.12)	(0.13)	(0.15)	(0.58)	(0.07)	(0.23)
2	5.35^{b}	1.52^{b}	781.8^{a}	531.6^{a}	0.12^{bc}	2.79^{a}	22.9^{b}	94.1^{a}	41.7^{bc}	5.34^{c}	16.6^{b}
	(0.02)	(0.01)	(19.9)	(1.21)	(0.001)	(0.004)	(0.51)	(0.13)	(0.09)	(0.03)	(0.06)
3	5.31^{b}	1.51^{b}	732.4^{a}	493.0^{d}	0.11^{c}	2.94^{a}	23.3^{ab}	90.3^{c}	41.5^{bc}	5.49^{b}	16.8^{b}
	(0.03)	(0.02)	(17.7)	(2.69)	(0.001)	(0.01)	(0.14)	(0.26)	(0.33)	(0.04)	(0.17)
4	5.44^{b}	1.49^{b}	770.6^{a}	514.2^{b}	0.12^{abc}	3.00^{a}	23.6^{ab}	91.5^{b}	40.8^{cd}	5.66^{a}	16.7^{b}
	(0.01)	(0.03)	(30.1)	(0.20)	(0.001)	(0.14)	(0.13)	(0.38)	(0.36)	(0.04)	(0.10)
5	4.95^{c}	1.50^{b}	739.7^{a}	506.0^{c}	0.13^{a}	2.90^{a}	24.0^{a}	88.5^{d}	42.5^{b}	5.66^{a}	17.6^{a}
	(0.06)	(0.00)	(47.5)	(1.50)	(0.00)	(0.03)	(0.15)	(0.02)	(0.14)	(0.01)	(0.06)

MC = moisture content; ER = expansion ratio; UD = unit density; BD = bulk density; SV = sinking velocity; WAI = water absorption index; WSI = water solubility index; L∗ = brightness/luminosity; a∗ = redness/greenness; b∗ = blueness/yellowness.

[a]Means with similar letters within a given property are not significantly different at $\alpha = 0.05$; $n = 3$ for each property, for each diet. (Values in parentheses are ±1 standard error of the mean.)

TABLE 4. *Comparison of extrudate properties between processing conditions (2 mm/190 rpm vs. 3 mm/348 rpm).*[a]

Diet	Die/RPM	MC (% db)	ER (–)	UD (kg/m^3)	BD (kg/m^3)	SV (m/s)	WAI (–)	WSI (%)	PDI (%)	L* (–)	a* (–)	b* (–)
Control	2/190	6.81^a	1.76^a	734.9^a	396.4^b	0.00^a	3.02^a	19.4^a	92.8^a	34.5^b	4.68^a	14.5^a
	3/348	6.21^b	1.96^a	583.4^a	426.9^a	0.00^a	2.94^a	19.3^a	90.3^b	39.9^a	4.40^b	12.9^b
1	2/190	6.78^a	1.51^a	901.6^a	458.0^b	0.09^b	3.03^a	22.6^a	96.6^a	34.5^b	5.10^b	12.7^b
	3/348	4.72^b	1.50^a	806.8^a	492.6^a	0.12^a	2.87^a	23.3^a	91.9^b	43.7^a	5.50^a	17.9^a
2	2/190	6.69^a	1.43^a	999.7^a	448.7^b	0.09^b	2.78^a	22.1^a	97.0^a	34.6^b	5.13^b	13.1^b
	3/348	5.36^b	1.52^a	781.8^b	531.6^a	0.12^a	2.79^a	22.9^a	94.1^b	41.7^a	5.34^a	16.6^a
3	2/190	5.12^a	1.46^a	876.5^a	432.6^b	0.09^b	2.79^b	23.3^a	95.0^a	38.0^b	6.16^a	15.9^b
	3/348	5.31^a	1.51^a	732.4^b	493.0^a	0.12^a	2.94^a	23.3^a	90.3^b	41.6^a	5.49^b	16.8^a
4	2/190	5.16^b	1.44^a	872.6^a	432.1^b	0.08^b	2.72^a	23.8^a	96.9^a	34.7^b	5.26^a	13.2^b
	3/348	5.44^a	1.49^a	770.6^a	514.2^a	0.12^a	3.00^a	23.6^a	91.5^b	40.8^a	5.66^a	16.7^a
5	2/190	5.19^a	1.42^b	916.4^a	463.4^b	0.10^b	2.68^b	24.3^a	93.3^a	40.3^b	6.03^a	16.8^a
	3/348	4.95^a	1.50^a	739.7^b	506.0^a	0.13^a	2.90^a	24.0^a	88.4^b	42.5^a	5.66^b	17.6^a

MC = moisture content; ER = expansion ratio; UD = unit density; BD = bulk density; SV = sinking velocity; WAI = water absorption index; WSI = water solubility index; L∗ = brightness/luminosity; a∗ = redness/greenness; b∗ = blueness/yellowness.

[a]Means with similar letters for a given diet formulation, for a given property, are not significantly different at $\alpha = 0.05$ between the two processing conditions (i.e., die size/screw speed); $n = 3$ for each property for each diet for each processing condition.

diet blends exhibited significant differences due specifically to processing condition (Table 4).

Expansion Ratio

Tables 2 and 3 show the main effects of changing the DDGS levels on the expansion ratios of the extrudates extruded using the 2 and 3 mm die, respectively. For the 2 mm die, the highest (1.96) and the lowest (1.49) expansion ratio were found for the control diet (which contained no DDGS) and the diet which contained 25% DDGS, respectively. Overall, increasing the DDGS level from 0 to 27.5% resulted in a decreased expansion ratio by 19.6 and 23.3% for the extrudates using the 2 and 3 mm die, respectively. But no significant differences could be discerned for DDGS levels from 17.5 to 27.5%, for either the 2 or the 3 mm die. In our study, the control diet (0% DDGS) expanded better than all other diets; this was due to the fact that the control diet had a higher proportion of starch (24.5%) compared to the other diets. None of the diet blends exhibited significant differences due to processing condition (Table 4).

Unit Density

Overall, the unit density values for the extrudates extruded using the 2 mm die increased by 36.0% as the DDGS changes from 0 to 20% (Table 2); increasing the DDGS levels from 0 to 17.5% resulted in a 38.3% increase in unit density values for the extrudates extruded from the 3 mm die (Table 3). But, changing the levels of DDGS from 17.5 to 27.5% did not have any significant effect on the unit density values of the extrudates extruded using either the 2 or the 3 mm die. The lowest unit density (734.89 and 583.43 kg/m^3) values were observed for the control diets extruded using the 2 and 3 mm die, respectively. In our experiment, the extrudates obtained from the control samples (which had no DDGS) were found to float for a substantially longer period of time compared to the other diets. Most of the diet blends exhibited significant differences due to processing condition (Table 4).

Bulk Density

Overall, the bulk density values exhibited a significant increase by 16.9% for the change in DDGS level from 0 to 27.5% using the 2 mm die (Table 2); for the products extruded using the 3 mm die, increasing the DDGS levels from 0 to 27.5% resulted in increased bulk density values by 18.5% (Table 3). No trends could be discerned as the DDGS level increased, using either the 2 or the 3 mm die, but the control diet was significantly lower for each case. Most of the diet blends exhibited significant differences due to processing condition (Table 4).

Sinking Velocity

All the extrudates, for both dies, except the control samples (0% DDGS) were found to sink (Tables 2 and 3). Overall, increasing the levels of DDGS from 0 to 27.5% resulted in a significant increase in sinking velocity values for the extrudates extruded using the 2 and 3 mm die, respectively. The highest sinking velocity values were found for the highest level of DDGS addition (27.5%), which indicated that DDGS contained a lesser amount of starch, hampering the expansion and increasing the propensity of the extrudates to sink. Most of the diet blends exhibited significant differences due to processing condition (Table 4).

Water Absorption and Solubility Indices

In our experiment, increasing the DDGS levels from 0 to 27.5% did not produce a significant change in the WAI values of the extrudates for either the 2 or the 3 mm die (Tables 2 and 3). In our study, the control diet (containing no DDGS) exhibited the lowest WSI value for both the 2 and 3 mm die (Tables 2 and 3). Increasing the DDGS levels from 0 to 27.5% resulted in 25.2 and 24.0% increase in WSI values for the 2 and 3 mm die, respectively. For WAI, most of the diet blends exhibited significant differences due to processing condition (Table 4); for WSI, however, none of the diet blends exhibited significant differences due to processing condition.

Pellet Durability Index

Tables 2 and 3 illustrates the main effect of varying the levels of DDGS on the pellet durability values for the extrudates resulting from the 2 and 3 mm die, respectively. In our study, the highest pellet durability value (97.0%) was observed for the blend that had 20% DDGS and was extruded using the 2 mm die; for the 3 mm die, this blend also had the highest PDI for that treatment (94.1%). Increasing DDGS level from 20 to 27.5% resulted in decreased pellet durability values by 3.41 and 3.76% for the 2 and 3 mm die, respectively. Most of the diet blends exhibited significant differences due to processing condition (Table 4).

Color

Table 2 provides the results for color parameters for the extrudates produced by the 2 mm die. There was an increase in L∗, a∗, and b∗ values in the diet formulations with DDGS compared to the control sample (0% DDGS). This is quite logical, because DDGS is slightly brown in color, and will impart additional dark color to the formulation diets. The values of L∗ and b∗ were highest in diet 5 (27.5% DDGS) while the highest a∗ value was obtained for diet 3 (22.5% DDGS). Table 3 provides color results for the extrudates produced by the 3 mm die. The highest L∗ value was observed in diet 1 with only 17.5% DDGS addition, while the highest a∗ and b∗ values were found in diet 5 (22.5% DDGS). It could be clearly observed that there were changes in the color parameters while changing the die conditions from 2 to 3 mm (Tables 2 and 3). For all color parameters, most of the diet blends exhibited significant differences due to processing condition (Table 4).

Mass Flow Rate

In general, it appeared that an increase in the mass flow rate occurred as the DDGS level increased (Table 5) for a given die/speed combination. The highest mass flow rates (1.24 and 0.91 kg/min) were observed at the highest DDGS inclusion level, for the 3 and 2 mm die, respectively. Most of the diet blends exhibited significant differences due to processing condition (Table 6).

Moisture Content at Die

We observed a trend in extrudate moisture content at the die for the two treatment conditions using the 2 and 3 mm die (Table 5). The ranges of moisture content were higher than compared to die with 3 mm diameter. For the 2 mm die, the highest moisture content at

TABLE 5. *Main effects of diet blends on extruder processing parameters.*[a]

Die/screw speed combination	2 mm/190 rpm		3 mm/348 rpm	
Diet	Moisture content at die (% db)	Mass flow rate (kg/min)	Moisture content at die (% db)	Mass flow rate (kg/min)
Control	42.1[b] (0.12)	0.83[ab] (0.04)	33.3[c] (0.42)	1.14[b] (0.01)
1	49.1[a] (0.33)	0.75[b] (0.02)	38.6[a] (0.25)	1.15[b] (0.03)
2	42.7[b] (0.34)	0.79[ab] (0.01)	38.8[a] (0.26)	1.13[b] (0.02)
3	36.0[c] (0.21)	0.80[ab] (0.00)	32.5[c] (0.24)	1.17[ab] (0.03)
4	48.8[a] (1.45)	0.88[a] (0.08)	38.2[a] (0.28)	1.16[ab] (0.04)
5	42.1[b] (0.86)	0.91[a] (0.03)	35.8[b] (0.06)	1.24[a] (0.03)

[a]Means with similar letters within a given property are not significantly different at $\alpha = 0.05$; $n = 3$ for each property, for each diet, for each processing condition. (Values in parentheses are ± 1 standard error of the mean.)

the die (49.1% db) was observed for diet 1 (17.5% DDGS), while the least moisture content at the die (36.0% db) occurred for diet 3 (22.5% DDGS). For the 3 mm die, the highest moisture content at the die (38.8% db) was observed for diet 2 (20.0% DDGS). Most of the diet blends exhibited significant differences due to processing condition (Table 6).

Extrudate Nutrient Analysis

Table 7 summarizes the effect of varying the proportions of DDGS in the diets on the nutritional composition of the extrudates. Crude protein and ash were found to have no significant effect for the change in DDGS levels from 17.5 to 27.5% db (which was anticipated a priori because the diets were formulated to be isonitrogenous), whereas the same decreased by 3.74 and 13.5% as the DDGS levels were changed from 0 to 27.5% db, respectively. However, increasing the proportion of DDGS from 0 to 27.5% db resulted in a substantial increase in NDF and crude fat values by 41 and 187%, respectively.

TABLE 6. *Comparison of extruder processing parameters between processing conditions (2 mm/190 rpm vs. 3 mm/348 rpm).*[a]

Diet	Die/RPM	Moisture content at die (% db)	Mass flow rate (kg/min)
Control	2/190	42.1[a]	0.83[b]
	3/348	33.3[b]	1.14[a]
1	2/190	49.1[a]	0.75[b]
	3/348	38.6[b]	1.15[a]
2	2/190	42.7[a]	0.79[b]
	3/348	38.8[b]	1.13[a]
3	2/190	36.0[a]	0.80[b]
	3/348	32.5[b]	1.17[a]
4	2/190	48.8[a]	0.88[b]
	3/348	38.2[b]	1.16[a]
5	2/190	42.1[a]	0.91[b]
	3/348	35.8[b]	1.24[a]

[a]Means with similar letters for a given diet formulation, for a given property, are not significantly different at $\alpha = 0.05$ between the two processing conditions (i.e., die size/screw speed); $n = 3$ for each property for each diet for each processing condition.

TABLE 7. *Nutrient analysis of extrudates.*[a]

Property (% db)	Diet: Control	1	2	3	4	5
Crude protein	40.1[a]	39.7[ab]	39.7[ab]	39.6[ab]	38.7[b]	38.6[b]
	(0.35)	(0.37)	(0.40)	(0.36)	(0.38)	(0.43)
Neutral detergent fiber	9.50[c]	11.6[b]	11.6[b]	13.0[a]	13.9[a]	13.4[a]
	(0.23)	(0.17)	(0.82)	(0.11)	(0.50)	(0.26)
Crude fat	2.15[e]	4.02[d]	4.73[c]	5.30[b]	5.50[b]	6.17[a]
	(0.09)	(0.23)	(0.12)	(0.18)	(0.03)	(0.02)
Ash	7.57[a]	6.52[b]	6.47[b]	6.45[b]	6.43[b]	6.55[b]
	(0.06)	(0.05)	(0.03)	(0.03)	(0.03)	(0.03)

[a]Means with similar letters within a given property are not significantly different at $\alpha = 0.05$; $n = 4$ for each component in each diet. (Values in parentheses are ± 1 standard error of the mean.)

Discussion

Moisture Content

Moisture content of extrudates is a very important parameter that affects several other extrudate properties, such as pellet durability, water absorption, and solubility indices

(Rolfe et al. 2001). Moisture of the resulting extrudates was impacted primarily by the steam and water added during the process of extrusion in order to obtain properly formed final products. Water content has been found to affect the cellular structure (Harper 1981) and mechanical properties (Mercier and Feillet 1975) of extruded products, which ultimately influences their resulting densities. Additionally, it has been shown that efficient mixing throughout the screw length has the advantage that water, or any other liquid, in fact, can be added directly to the extruder barrel. In twin screw extruders, because of the efficient mixing effect of the screws, moisture content of the ingredient mass can be readily adjusted by injecting water into the barrel. Direct water addition is essential for regulating the extrusion process, because its effects are of a similar magnitude to the effects caused by changes in the screw speed or the feed rate of dry ingredients. In general, to maintain stable running conditions and produce a uniform product, it is necessary to limit moisture changes during extrusion to less than five percentage points (Mercier et al. 1989).

Expansion Ratio

The degree of expansion of extrudates is closely related to the size, number, and distribution of air cells within the cooked material (Lue et al. 1990). High temperatures, shear stresses, and shear strains produced during the extrusion process can also affect the complex interactions between the chemical constituents, and alter the resulting internal cellular structures that occur during the evaporation of water upon die exit (Miller 1985), all of which impact the expansion of the product as it passes through the extruder die (Moore et al. 1990). Results of Chevanan et al. (2007a) reported a 36.7% decrease in expansion ratio values when DDGS levels were increased from 20 to 60%, for tilapia diets using DDGS. In our experiments, extrudates obtained from the control diet (0% DDGS) were the only ones able to float, due to the higher expansion ratio compared to the other treatments.

Radial expansion is highly dependent on the composition of the extruded material, and is starch gelatinization which is the key to expansion (Nielsen 1976). In general, products with higher amounts of starch expand better. The amylose–amylopectin ratio is a critical factor that affects the properties of extrudates. Previous research has shown that working with blends that contain higher proportions of amylose have resulted in a decrease in expansion (Launay and Lisch 1983). The amylopectin component present in corn starch (ca 72%) is largely responsible for its expansion. The higher the amylopectin content, the greater the expansion of the starch.

Unit Density

Unit density is another measure of the internal structure of extrudates, and it quantifies the mass of the material per unit volume of each extrudate, and includes the air entrapped within interior pores (Cumming et al. 1972; Badrie and Mellowes 1991). The unit density is directly related to the degree of expansion obtained during processing (Colonna et al. 1989). Results reported by Chevanan et al. (2007a) for extruded tilapia blends which incorporated DDGS, showed a great increase in unit density values (159%) with change in DDGS levels from 20 to 60%. In contrast, no significant differences in unit density values were noticed by Kannadhason et al. (2007a, 2007b) for a change in DDGS levels. This contradiction was probably due to differences in the feed compositions used in the studies.

Bulk Density

Bulk density is another very important dependent variable (Mercier et al. 1989), as it determines the space required for the storage of the extruded materials, both at feed production plants and on farms. The increase in the bulk density values with corresponding increase in DDGS levels were anticipated, because as the percentage of DDGS in the blend increased, the expansion ratio was found to decrease, and hence the extrudates were denser (i.e., had less internal pore spaces). Another factor influencing bulk density (and therefore whether a product floats or sinks) is length of the die opening. Long lengths can cause the product to be denser and, therefore, more likely to sink (Riaz 2000).

Sinking Velocity

The extent of biochemical changes during processing affects the water-absorption capacity and structural integrity of the extrudates, which in turn affect product expansion, unit density, and thus the sinking velocity. Similar relations of sinking velocity with DDGS were observed by Chevanan et al. (2007a). Sinking velocity is also related to the density of the extrudates, which often means the lower the density values; the better the extrudates will float.

Water Absorption and Solubility Indices

When extruded starches are dispersed in an excess of water, their main functional properties can be quantified by water absorption and water solubility. WAI is the amount of gel obtained per gram of dry sample and is a measure of the swelling power of the starch (Kite et al. 1957; Anderson et al. 1969). In general, WAI and WSI are inversely proportional to each other and have been examined by many authors (Kirby et al. 1988; Ng et al. 1999). Chevanan et al. (2007a) and Kannadhason et al. (2007a) reported that WAI values followed a decreasing trend with an increase in DDGS levels.

The WSI, on the other hand, expresses the percentage of dry matter recovered after the supernatant is evaporated from the water absorption determination (Anderson et al. 1969). WSI is related to the quantity of soluble molecules, which is related to starch dextrinization. Increasing trends were reported by Chevanan et al. (2007a) and Kannadhason et al. (2007a) in their twin screw and single screw extrusion studies of DDGS, respectively, which are in agreement with our findings. Often, the water solubility of starch increases with expansion (Mercier et al. 1989). We observed that the expansion ratio was the highest for the control diets (containing no DDGS), but had the lowest WSI value.

Pellet Durability Index

PDI is a direct measurement of a pellet's quality to withstand breakage and disintegration during handling and transport (Chang and Wang 1998). Our results are similar to the findings of Chevanan et al. (2007a) and Kannadhason et al. (2007a, 2007b). All conditions led to fairly high PDI values, and were thus resistive to the destructive forces commonly encountered by feed materials during handling and storing, and are thus important to maintaining the quality and value of the feed product.

Color

Color is an important physical property which is often used by feed customers to assess product quality (Turner 1995). In aquaculture feeds, color *per se* is not considered an important factor, but changes in color because of high temperatures and other reactions (e.g., Maillard) during processing can be a sign of alteration or loss of lysine (0–40%), which is an important amino acid needed in diets (Bjorck and Asp 1983), or the degradation of protein digestibility. Color indicates to some extent the nutritional quality of product. Significant changes in color parameters indicate differences in the nutritional properties and therefore can affect the quality of fish growth. There were statistical significant differences found among the three color parameters for each diet formulation for both die conditions.

Mass Flow Rate

The amount of extrudate produced is quantified by mass flow rate: the higher the mass flow rate value, the higher the yield. Moreover, the higher the screw speed, the higher the mass flow rate. It can be observed that the mass flow rate generally decreased as the die diameter decreased from 3 to 2 mm; this was primarily because of the decrease in screw speed from 348 to 190 rpm (thus the speed/die combination was used as a blocking factor for the experiment).

Moisture Content at Die

The differences in the moisture content at the die with change in die conditions suggests that die conditions and the various levels of DDGS are both vital for moisture content at the die, which in turn impacts expansion, and final product quality.

Extrudate Nutrient Analysis

Since the diet formulations were isonitrogenous by design, any differences in crude protein content of the extrudates were because of formulation errors alone. An increase in DDGS level produced extrudates with higher fiber and fat contents concurrent with the level of DDGS, due to the higher amount of fiber and fat in the DDGS in comparison with the other ingredients. Similar results were discussed by Chevanan et al. (2007a). However, adding DDGS resulted in a significant decrease in ash content.

Conclusions

The aim of this pilot-scale experimental study was to investigate the effect of various levels of DDGS, at a constant feed moisture content (15% db) and net protein content (35% db), using two different die/screw speed combinations, on resulting extrudate properties and extruder processing parameters. Changing the levels of DDGS produced significant effects on moisture content, expansion ratio, unit density, bulk density, sinking velocity, color (L∗, a∗, and b∗), water absorption, and pellet durability indices. Floatability of extrudates is a key factor for aquaculture feeds; control diets, which possessed no DDGS, resulted in extrudates with good floatability and low unit density, bulk density, and sinking velocity, but high expansion ratio. Extrudates which contained 20 and 27.5% DDGS, on the other hand, had high pellet durability, which indicates that they could resist mechanical damage during transportation and storage, but these also had high sinking velocities, which suggests that they were more suitable for sinking feed applications.

Acknowledgments

The authors wish to thank the financial support provided by the Agricultural Experiment Station, South Dakota State University, Brookings, and the North Central Agricultural Research Laboratory, USDA-ARS, Brookings, South Dakota, for performing this project. The authors also thankfully acknowledge Dr. Mehmet Tulbek (Pulse and Oilseed Specialist) and Mr. Riley Morgan (Processing Technician) at North Dakota State University, as well as Rumela Bhadra, Jenna Carsrud, Sharon Nichols, and Travis Schaeffer for their valuable assistance during the extrusion runs and data collection.

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