Twin Screw Extrusion of DDGS-Based Aquaculture Feeds

S. Kannadhason, South Dakota State University
Kurt A. Rosentrater, United States Department of Agriculture
Kasiviswanathan Muthukumarappan, South Dakota State University
Micahel L. Brown, South Dakota State University
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S. Kannadhason, ASABE Member
Graduate Research Assistant, South Dakota State University, Brookings, SD 57007

Kurt A. Rosentrater, ASABE Member Engineer
Bioprocess Engineer, USDA, ARS, North Central Agricultural Research Laboratory, Brookings, SD 57006

K. Muthukumarappan, ASABE Member Engineer
Professor, South Dakota State University, Brookings, SD 57007

Michael L. Brown
Professor, South Dakota State University, Brookings, SD 57007

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Abstract. Six isocaloric (3.65 kcal/g), isonitrogenous (35% db protein), ingredient blends were prepared with 0, 17.5, 20, 22.5, 25, and 27.5% DDGS and other common ingredients, including soybean meal, corn, fish meal, whey, soybean oil, and vitamin and mineral mix, in order to produce balanced rations appropriate for tilapia grower diets. The blends were moisture balanced to 15% db, and then extruded in a pilot-scale twin screw extruder. The extrusion process was carried out using two processing conditions: a 2 mm die at 190 rpm (with temperatures ranging from 80 to 110°C, depending on barrel location), and a 3 mm die at 348 rpm (with barrel temperatures ranging from 60 to 100°C). Analyses of the resulting 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. Processing parameters such as mass flow rate, and moisture content at the die were measured to quantify the behavior of the extruder. Increasing the DDGS level from 0 to 17.5% db resulted in decreased expansion ratio values 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, however. The WSI of the extrudates were found to increase substantially (25.2 and 24.0%) as the proportion of DDGS was increased from 0 to 27.5% db for each die, respectively. The extrudates that contained 0% DDGS (i.e. the control blend) had the highest expansion ratio and the lowest unit density, bulk density, and sinking velocity values, and could be used as a floating feed. The extrudates that contained 20 and 27.5% DDGS, on the other hand, had the highest pellet durability and sinking velocity values, which indicates that they could better resist mechanical damage during transportation and storage, but are more suitable for sinking feed, respectively.

Keywords. Distiller’s dried grains with solubles (DDGS), twin screw extrusion, physical properties, processing parameters, proximate composition
Introduction

Aquaculture is one of the fastest growing food-producing sectors in the world, growing at approximately 8.8% annually since 1950, and far out-performing other livestock food sectors, which have grown only by 1% for beef and veal, between 2 and 3% for lamb, mutton, and pig meats, and 4.9% for chicken meat (Tacon, 2004; FAO, 2006). For most aquaculture systems, the cost of feed constitutes between 30 and 60% of total operational costs of the farm, among which protein is the most costly dietary component (Keong, 2003). In general, fish exhibit better efficiency to convert feed to body weight compared to other land-based animals, such as chickens, pigs, sheep, and beef cattle (New, 1986). Among nutrients, protein requirements and recommendations for optimal dietary levels for various feed ingredients are inconsistent, even though much literature is available in these fields (Cho and Kaushik, 1985). Generally, aquaculture feeds require 26 to 50% protein, depending upon the species to be fed (Lovell, 1988). The research literature is unclear on the efficacy of supplementing fish diets with dietary proteins. Fish can be fed higher percentages of protein in their diets than land-based animals, because fish typically have low energy requirements in general (Lovell, 1991). The protein conversion efficiency of fish is very high compared to other animals.

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 destroy 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 that is composed of two types of macro molecules, namely amylose and amylopectin (Kokini et al., 1992; 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%) (Johnson and Bucholtz, 1997). 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 Janda, 1983; Schweizer et al., 1986). On the other hand, the largest branched molecules of amylopectin were found to breakdown through mechanical forces, due to the shearing by the extruder (Cai et al., 1995; Davidson et al., 1984; Diosady, 1985).

The expansion volume of starch is mainly dependent on its degree of gelatinization within the extruder (Stanely, 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. Distillers 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 metabolized energy, and various amounts of fat, fiber, and minerals (Pagon, 1991; Spiehs, 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 (approximately $1000/ton) has encouraged the evaluation of other alternate protein sources (USDA, 1988). Due to its moderately high protein content and lower cost in comparison with fish meal, there is an increasing interest in using DDGS in aquaculture diets (US Grains Council, 2008).

Nile Tilapia (Oreochromis niloticus) is an increasingly popular warm water fish grown throughout the world, and it is an extremely suitable fish for farming due to the fact they are fast growing and very tolerant to various water conditions (US Grains Council, 2008). There is a growing consensus that tilapia could become one of the most important cultured fishes. It has been observed that the production of tilapia increased by 25% annually from 1984 to 2002 (FAO, 1980). Tilapia may be an appropriate species to test the efficacy of DDGS as a protein source. In fact, some researchers have been successful. 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 unpeletted DDGS in polyculture of tilapia with fresh water prawns, and found that pelleted DDGS resulted in a better growth rate compared to unpeletted DDGS. The current recommendations for maximum dietary inclusion rates of DDGS for tilapia are up to 35% without synthetic lysine and supplementation in high protein diets (i.e., 40% crude protein) (US Grains Council, 2008).

Even though some feeding work has been carried out, only limited reports are available that discuss the actual processing of DDGS feed blends. The effect of DDGS (20 to 40% wb), moisture content (15 to 25% wb), and screw speeds (100 to 160 rpm) on the physical properties of extrudates were studied by Chevanan et al. (2007c), 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. (2006). This study was accomplished by varying the inclusion levels of DDGS (20 to 40% wb), moisture contents (15 to 25% wb), barrel temperatures (100 to 160 °C), and different screw speeds (80 to 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 to 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 in order 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 (3.65kcal/g) were formulated to 35% (db – dry basis) protein using various 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 3. 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 (approximately 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, 27.5% db) and two different dies (2 and 3 mm), which resulted in 12 total treatment combinations (6x2=12) which was implemented using a randomized block design (each die size required a separate screw speed in order to properly form final products (190 rpm for the 2 mm die, and 348 rpm for the 3 mm die). Thus screw speed/die size was the blocking factor. Table 2 shows the temperature profile and operating conditions used for the two different dies (2 and 3mm) during each extruder run.

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 screw had 25 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). A rotating cutter assembly, which had 3 blades, was placed at the end of the die and had various adjustable speeds, which allowed the production of extrudates of specific lengths.

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).

Extrudate Properties

Extrudates were analyzed following the procedures described previously by Rosentrater et al. (2005).

Moisture Content (MC)
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 ± 1°C). Moisture content of the extrudates were determined following AACC method 44-19 (1995), using a forced-convection laboratory oven (Thelco Precision, Jovan Inc., Winchester, VA) at 135°C for 2 h.

**Expansion Ratio (ER)**

The radial expansion ratio was obtained as the ratio of the diameter of the extrudates to the diameter of the die (Faubion and Hoseney, 1982).

**Unit Density (UD)**

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; \( V = \pi r^2 h \)) following the procedure of Jamin and Flores (1998).

**Bulk Density (BD)**

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**

Color (L*, a*, and b*) of the extrudates were determined using a calibrated spectrophotometer (portable model CM 2500d, Minolta Corporation, Ramsey, NJ) using the Hunterlab 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 (SV)**

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 measuring cylinder, which was 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 hit the bottom of the container.

**Water Absorption and Solubility Indices (WAI & WSI)**

Water absorption and solubility indices are often used as indicators of volume of swollen gelled particles that maintain integrity in aqueous dispersion (Mason and Hoseney, 1986) and degradation of molecular components (Kirby et al., 1988), respectively.

The extrudates were ground to fine powder (approximately 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 placed in a desiccator for 20 minutes before weighing the dry solids of supernatant. The gel remaining in the centrifuge tube was weighed, and WAI was calculated as follows:
Water absorption index was calculated as:

\[ \text{WAI} = \frac{W_g}{W_{ds}} \]  

(1)

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

Water solubility index was calculated as:

\[ \text{WSI} = \left( \frac{W_{ss}}{W_{ds}} \right) \times 100 \]  

(2)

where \( \text{WSI} \) 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).

**Pellet Durability Index (PDI)**

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 200g 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 eqn. (3)

\[ \text{PDI} = \left( \frac{M_{at}}{M_{bt}} \right) \times 100 \]  

(3)

where \( \text{PDI} \) 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).

**Nutrient Analysis**

Proximate composition was also determined for all of the samples. 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**

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 and the extruder processing parameters, for each treatment combination; duplicate measurements (n=2) were measured for all nutrient properties, for each treatment combination. 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 (\( \alpha \)) of 0.05, with SAS v.9 software (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 and 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). Table 4 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 6), we observed that increasing the DDGS levels from 0 to 17.5% resulted in a decreased the extrudate moisture content of 23.90%. 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 20.28%. In contrast, Chevanan et al. (2007a) found an increase in extrudate moisture content as DDGS level increased 20 to 60%.

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 showed 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). Tables 4 and 6 shows 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 19.62 and 23.31% 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. 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 is key to expansion (Nielsen, 1976). In general, products with higher amounts of starch expand better. 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. 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 (~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). Overall, the unit density values for the extrudates extruded using the 2 mm die increased by 36.03% as the DDGS changes from 0 to 20% (Table 4); increasing the DDGS levels from 0 to 17.5% resulted in a 38.27% increase in unit density values for the extrudates extruded from the 3 mm die (Table 6). 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 kg/m$^3$ 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. 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 and 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. Overall, the bulk density values showed a significant increase by 16.9% for the change in DDGS level from 0 to 27.5% using the 2 mm die (Table 4); 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 6). These increases in the bulk density values 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). 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. 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. All the extrudates, for both dies, except the control samples (0% DDGS) were found to sink (Tables 4 and 6). 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 signified that DDGS contained lesser amount of starch, which hampered the expansion, and increased the propensity of the extrudates to sink. Similar results were observed from 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. Water absorption index (WAI) is the amount of gel obtained per gram of dry sample and is a measure of the swelling power of the starch (Anderson et al., 1969; Kite et al., 1957). 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). 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 4 and 6). In contrast, Chevanan et al. (2007a) and Kannadhason et al. (2007a) reported that WAI values followed a decreasing trend with an increase in DDGS levels.

The water solubility index (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. In our study, the control diet (containing no DDGS) exhibited the lowest WSI value for both the 2 and 3 mm die (Tables 4 and 6). 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. Similar 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. 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**

Pellet Durability Index is a direct measurement of a pellet’s quality to withstand breakage and disintegration during handling and transport (Chang and Wang, 1998). Tables 4 and 6 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. Our results are similar to the findings of Chevanan et al. (2007a) and Kannadhason et al. (2007a and 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 due to 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. Table 4 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 6 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). 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. It could be clearly observed that there were changes in the color parameters while changing the die conditions from 2mm to 3 mm (Tables 4 and 6).
**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. In general, it appeared that an increase in the mass flow rate occurred as the DDGS level increased (Tables 5 and 7) for a given die/speed combination. The highest mass flow rates (1.24 g/min and 0.91 g/min) were observed at the highest DDGS inclusion level, for the 3 mm and 2 mm die, respectively. 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 due to 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**

From tables 5 and 7, we observe the trend in extrudate moisture content at the die for the two treatment conditions using the 2 and 3 mm die, respectively. The ranges of moisture content were higher than compare to die with 3 mm diameter. For the 2 mm die, the highest moisture content at 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). 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.

**Nutrient Analysis**

Table 8 and 9 summarizes the effect of varying the proportions of DDGS in the diets on the nutritional composition of the extrudates for the 2 and 3 mm die, respectively. Increasing the DDGS levels from 0 to 25% (db) resulted in a differing trends for the 2 and 3 mm die: decreased and increased the moisture content of the extrudates by 36.8 and 3.14%, respectively. The highest moisture content was 20.7% db, and was observed with diet 4, which (25% DDGS) for the 3 mm die; the second highest was 19.2% (db) for diet 5 for the 3 mm die. These levels were much higher than the other extrudates. The ingredient blends were formulated for 15% moisture content (db), though. This increase in moisture content was due to a higher amount of water and steam that was added to the pre-conditioner to produce extrudates with better expansion and other functional properties for these blends, at this die/speed combination. No significant difference in the extrudates’ crude protein was found among the diets for either the 2 mm or the 3 mm die, since the ingredient blends were targeted to achieve a protein level of at least 35% (db) during formulation. A significant increase in Neutral Detergent Fiber, by 36.1 and 52.7%, were observed for the change in DDGS levels from 0 to 25% db, for the 2 and 3 mm die, respectively. Furthermore, it is interesting to point out that the crude fat content showed a substantial increase of 200 and 175.5% for the extrudates extruded using the 2 and 3 mm die, respectively. Thus 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, for both dies.

**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.

Acknowledgements
The authors wish to thank the financial support provided by the Agricultural Experiment Station, South Dakota State University, Brookings, SD, and the North Central Agricultural Research Laboratory, USDA-ARS, Brookings, SD, for performing this project. The authors also thankfully acknowledge Dr. Mehmet Tulbek (Pulse and Oilseed Specialist, NDSU), Mr. Rilie Morgan (Processing Technician, NDSU), Jenna Carsrud, Sharon Nichols, Rumela Bhadra, and Travis Schaeffer for their valuable help throughout the extrusion run.

References


Table 1. Experimental design used in the study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diet</th>
<th>Control</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Screw speed (rpm) / Die size (mm)</td>
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<td>190/2</td>
<td>190/2</td>
<td>190/2</td>
<td>190/2</td>
<td>190/2</td>
</tr>
<tr>
<td>2</td>
<td>Screw speed (rpm) / Die size (mm)</td>
<td>348/3</td>
<td>348/3</td>
<td>348/3</td>
<td>348/3</td>
<td>348/3</td>
<td>348/3</td>
</tr>
</tbody>
</table>

*6 diets x 2 (screw speeds (rpm)/die size (mm)) = 12 treatment combinations*

Table 2. Operating conditions of the extruder during processing

<table>
<thead>
<tr>
<th>Die size (mm)</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

**Feeder Information**

- Feeder screw speed (rpm): 10 15

**Preconditioner information**

- Water flow to preconditioner (kg/min): 0 0
- Conditioning Steam (kg/min): 0.2152 0.2255

**Extrusion Information**

- Extruder shaft speed (rpm): 190 348
- Extruder motor load (%): 15 10
- Water flow to extruder (kg/min): 0 0
- Extruder steam (kg/min): 0.0844 0.1200
- Knife drive speed (%): 100 90
- Number of knife blades: 3 3
- Temperature – 2nd head (°C): 80-80 60-65
- Temperature – 3rd head (°C): 80-80 80-82
- Temperature – 4th head (°C): 80-80 80-80
- Temperature – 4th head (°C): 80-80 80-80
- Temperature – 4th head (°C): 107-110 100-100
- Temperature – 4th head (°C): 110-110 110-110
- Temperature – 4th head (°C): 110-110 110-110
- Pressure at head 9 (psi): 600 400
Table 3. Dry ingredient composition in the experimental feed blends

<table>
<thead>
<tr>
<th>Feed Ingredient (% db)</th>
<th>Dry weight of ingredients (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>DDGS</td>
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</tr>
<tr>
<td>Soybean meal, solvent extracted</td>
<td>52.7</td>
</tr>
<tr>
<td>Corn</td>
<td>24.5</td>
</tr>
<tr>
<td>Fishmeal, Menhaden</td>
<td>15.0</td>
</tr>
<tr>
<td>Vitamin premix # 30</td>
<td>0.60</td>
</tr>
<tr>
<td>Rovimix Stay-C</td>
<td>0.20</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.00</td>
</tr>
<tr>
<td>Whey</td>
<td>5.00</td>
</tr>
</tbody>
</table>
Table 4. Main effects of diet blends on extrudates properties (die=2 mm/rpm=190)\(^a\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>MC (%db)</th>
<th>ER (-)</th>
<th>UD (kg/m(^3))</th>
<th>BD (kg/m(^3))</th>
<th>SV (m/s)</th>
<th>WAI (%)</th>
<th>WSI (%)</th>
<th>PDI (%)</th>
<th>L* (-)</th>
<th>a* (-)</th>
<th>b* (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.80(^a)</td>
<td>1.76(^a)</td>
<td>734.9(^b)</td>
<td>396.4(^d)</td>
<td>0.000(^c)</td>
<td>3.02(^a)</td>
<td>19.4(^d)</td>
<td>92.8(^c)</td>
<td>34.5(^c)</td>
<td>4.68(^c)</td>
<td>12.9(^c)</td>
</tr>
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<td>(0.00)</td>
<td>(0.14)</td>
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<td>(0.26)</td>
<td>(0.48)</td>
<td>(0.04)</td>
<td>(0.17)</td>
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</tr>
<tr>
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<td>1.50(^b)</td>
<td>901.6(^ab)</td>
<td>458.0(^a)</td>
<td>0.092(^a)</td>
<td>3.03(^a)</td>
<td>22.5(^b)</td>
<td>96.6(^a)</td>
<td>34.5(^c)</td>
<td>5.10(^b)</td>
<td>12.7(^c)</td>
</tr>
<tr>
<td>(0.08)</td>
<td>(0.06)</td>
<td>(64.1)</td>
<td>(0.78)</td>
<td>(0.00)</td>
<td>(0.21)</td>
<td>(0.35)</td>
<td>(0.12)</td>
<td>(0.38)</td>
<td>(0.09)</td>
<td>(0.13)</td>
<td></td>
</tr>
<tr>
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<td>6.69(^a)</td>
<td>1.42(^b)</td>
<td>999.7(^a)</td>
<td>448.7(^b)</td>
<td>0.092(^a)</td>
<td>2.78(^a)</td>
<td>22.1(^c)</td>
<td>97.0(^a)</td>
<td>34.6(^c)</td>
<td>5.13(^b)</td>
<td>13.1(^c)</td>
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<td>(0.04)</td>
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<td>(0.11)</td>
<td>(0.53)</td>
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<td>(0.16)</td>
<td>(0.06)</td>
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<tr>
<td>3</td>
<td>5.12(^b)</td>
<td>1.46(^b)</td>
<td>876.5(^ab)</td>
<td>432.6(^c)</td>
<td>0.094(^a)</td>
<td>2.79(^a)</td>
<td>23.2(^ab)</td>
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<td>15.8(^b)</td>
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<td>(0.10)</td>
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</tr>
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<td>1.44(^b)</td>
<td>872.6(^ab)</td>
<td>432.1(^c)</td>
<td>0.080(^b)</td>
<td>2.72(^a)</td>
<td>23.8(^a)</td>
<td>96.9(^a)</td>
<td>34.7(^c)</td>
<td>5.26(^b)</td>
<td>13.1(^c)</td>
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<td>916.4(^ab)</td>
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<td>(0.65)</td>
<td>(0.10)</td>
<td>(0.25)</td>
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</tbody>
</table>

\(^a\)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)
MC is moisture content; ER is expansion ratio; UD is unit density; BD is bulk density; SV is sinking velocity; WAI is water absorption index; WSI is water solubility index; L* is brightness/luminosity; a* is redness/greenness; b* is blueness/yellowness.
Table 5. Main effects of diet blends on extruder processing parameters (die=2 mm/rpm=190)<sup>a</sup>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Moisture content at die (db)</th>
<th>Mass flow rate (g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>1</td>
<td>49.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>2</td>
<td>42.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>36.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>48.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>42.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means with similar letters within a given property are not significantly different at α=0.05; n=3 for each property, for each diet. (Values in parentheses are ± 1 standard error of the mean)
Table 6. Main effects of diet blends on extrudates properties (die=3 mm/rpm=348)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Diet</th>
<th>MC (%db)</th>
<th>ER (-)</th>
<th>UD (kg/m\textsuperscript{3})</th>
<th>BD (kg/m\textsuperscript{3})</th>
<th>SV (m/s)</th>
<th>WAI (%)</th>
<th>WSI (%)</th>
<th>PDI (%)</th>
<th>L* (-)</th>
<th>a* (-)</th>
<th>b* (-)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>6.21\textsuperscript{a}</td>
<td>1.96\textsuperscript{a}</td>
<td>583.4\textsuperscript{b}</td>
<td>426.9\textsuperscript{a}</td>
<td>0.00\textsuperscript{d}</td>
<td>2.94\textsuperscript{a}</td>
<td>19.33\textsuperscript{c}</td>
<td>90.3\textsuperscript{c}</td>
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<td>4.40\textsuperscript{d}</td>
<td>14.4\textsuperscript{c}</td>
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<td></td>
<td>(0.12)</td>
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<td>(52.9)</td>
<td>(2.90)</td>
<td>(0.00)</td>
<td>(0.08)</td>
<td>(0.20)</td>
<td>(0.12)</td>
<td>(0.32)</td>
<td>(0.005)</td>
<td>(0.10)</td>
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<tr>
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<td>4.72\textsuperscript{d}</td>
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<td>492.6\textsuperscript{d}</td>
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<td>43.7\textsuperscript{a}</td>
<td>5.50\textsuperscript{b}</td>
<td>17.9\textsuperscript{a}</td>
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<td>(0.02)</td>
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<td>(0.07)</td>
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<td>(0.12)</td>
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<td>(0.07)</td>
<td>(0.23)</td>
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<td>1.79\textsuperscript{d}</td>
<td>22.9\textsuperscript{b}</td>
<td>94.1\textsuperscript{a}</td>
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<td>16.6\textsuperscript{b}</td>
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<tr>
<td></td>
<td>(0.02)</td>
<td>(0.01)</td>
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<td>(0.51)</td>
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<td>(0.03)</td>
<td>(0.06)</td>
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<td>493.0\textsuperscript{d}</td>
<td>0.11\textsuperscript{c}</td>
<td>2.94\textsuperscript{a}</td>
<td>23.3\textsuperscript{ab}</td>
<td>90.3\textsuperscript{c}</td>
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<td>5.49\textsuperscript{b}</td>
<td>16.8\textsuperscript{b}</td>
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<td>(0.17)</td>
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<td>3.00\textsuperscript{a}</td>
<td>23.6\textsuperscript{a}</td>
<td>91.5\textsuperscript{b}</td>
<td>40.8\textsuperscript{cd}</td>
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<td>(0.03)</td>
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<td>739.7\textsuperscript{a}</td>
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<td>2.90\textsuperscript{a}</td>
<td>24.0\textsuperscript{a}</td>
<td>88.5\textsuperscript{d}</td>
<td>42.5\textsuperscript{b}</td>
<td>5.66\textsuperscript{a}</td>
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</tr>
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<td>(0.15)</td>
<td>(0.02)</td>
<td>(0.14)</td>
<td>(0.01)</td>
<td>(0.06)</td>
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</table>

\textsuperscript{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)
MC is moisture content; ER is expansion ratio; UD is unit density; BD is bulk density; SV is sinking velocity; WAI is water absorption index; WSI is water solubility index; L* is brightness/luminosity; a* is redness/greenness; b* is blueness/yellowness.
Table 7. Main effects of diet blends on extruder processing parameters (die=3 mm/rpm=348)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Diet</th>
<th>Moisture at die (% db)</th>
<th>Mass flow rate (g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33.3\textsuperscript{c}</td>
<td>1.14\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
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<td>(0.01)</td>
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<td></td>
<td>(0.25)</td>
<td>(0.03)</td>
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<tr>
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<td>1.13\textsuperscript{b}</td>
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<tr>
<td></td>
<td>(0.26)</td>
<td>(0.02)</td>
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<tr>
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<td>(0.03)</td>
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<td>35.8\textsuperscript{b}</td>
<td>1.24\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.03)</td>
</tr>
</tbody>
</table>

\textsuperscript{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 \( \pm 1 \) standard error of the mean)
<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% wb)</td>
<td>5.30a</td>
<td>4.70a</td>
<td>4.80a</td>
<td>3.90b</td>
<td>3.35b</td>
<td>3.85b</td>
</tr>
<tr>
<td></td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.40)</td>
<td>(0.20)</td>
<td>(0.05)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Dry Matter (% wb)</td>
<td>94.7b</td>
<td>95.3b</td>
<td>95.2b</td>
<td>96.1a</td>
<td>96.6a</td>
<td>96.1a</td>
</tr>
<tr>
<td></td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.40)</td>
<td>(0.20)</td>
<td>(0.05)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Crude Protein (% db)</td>
<td>40.3a</td>
<td>39.3abc</td>
<td>39.1abc</td>
<td>40.1ab</td>
<td>38.2bc</td>
<td>38.1c</td>
</tr>
<tr>
<td></td>
<td>(0.45)</td>
<td>(0.40)</td>
<td>(1.05)</td>
<td>(0.45)</td>
<td>(0.15)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>Neutral Detergent Fiber (% db)</td>
<td>9.70c</td>
<td>11.6ab</td>
<td>11.0bc</td>
<td>12.9a</td>
<td>13.2a</td>
<td>12.9a</td>
</tr>
<tr>
<td></td>
<td>(0.30)</td>
<td>(0.35)</td>
<td>(1.05)</td>
<td>(0.00)</td>
<td>(0.15)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Crude Fat (% db)</td>
<td>2.05f</td>
<td>3.65e</td>
<td>4.60d</td>
<td>5.00c</td>
<td>5.50b</td>
<td>6.15a</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.15)</td>
<td>(0.10)</td>
<td>(0.10)</td>
<td>(0.00)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Ash (% db)</td>
<td>7.50a</td>
<td>6.60b</td>
<td>6.45b</td>
<td>6.45b</td>
<td>6.50b</td>
<td>6.55b</td>
</tr>
<tr>
<td></td>
<td>(0.10)</td>
<td>(0.00)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.00)</td>
<td>(0.05)</td>
</tr>
</tbody>
</table>

Means with similar letters within a given property are not significantly different at α=0.05; n=2 for each component in each diet (Values in parentheses are ± 1 standard error of the mean)
Table 9. Proximate composition of resulting extrudates (die=3 mm/rpm=348)\(^a\)

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% wb)</td>
<td>5.00c (0.60)</td>
<td>3.75d (0.25)</td>
<td>4.25cd (0.05)</td>
<td>4.15cd (0.05)</td>
<td>20.7a (0.30)</td>
<td>19.2b (0.15)</td>
</tr>
<tr>
<td>Dry Matter (% wb)</td>
<td>95.0b (0.60)</td>
<td>96.2a (0.25)</td>
<td>95.7ab (0.05)</td>
<td>98.8ab (0.05)</td>
<td>79.3d (0.30)</td>
<td>80.7c (0.15)</td>
</tr>
<tr>
<td>Crude Protein (% db)</td>
<td>39.8a (0.65)</td>
<td>40.1a (0.55)</td>
<td>39.4a (0.55)</td>
<td>39.1a (0.25)</td>
<td>38.9a (0.05)</td>
<td>39.1a (0.75)</td>
</tr>
<tr>
<td>Neutral Detergent Fiber (% db)</td>
<td>9.30e (0.40)</td>
<td>11.6d (0.25)</td>
<td>12.4cd (0.05)</td>
<td>13.0bc (0.25)</td>
<td>14.2a (0.55)</td>
<td>13.8ab (0.20)</td>
</tr>
<tr>
<td>Crude Fat (% db)</td>
<td>2.25e (0.05)</td>
<td>4.40d (0.10)</td>
<td>4.85c (0.05)</td>
<td>5.60b (0.10)</td>
<td>5.45b (0.05)</td>
<td>6.20a (0.00)</td>
</tr>
<tr>
<td>Ash (% db)</td>
<td>7.65a (0.05)</td>
<td>6.45b (0.05)</td>
<td>6.45b (0.05)</td>
<td>6.45b (0.05)</td>
<td>6.40b (0.00)</td>
<td>6.55b (0.05)</td>
</tr>
</tbody>
</table>

\(^a\) Means with similar letters within a given property are not significantly different at \(\alpha=0.05\); \(n=2\) for each component in each diet (Values in parentheses are ± 1 standard error of the mean)