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Bio-Ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, *Hermetia illucens*, along with poultry by-product meal and soybean meal, as total replacement of fish meal in diets for Nile tilapia, *Oreochromis niloticus*

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Abstract

A feeding trial was conducted in a closed system with Nile tilapia, *Oreochromis niloticus,* juveniles (mean initial weight, 2.66 g) to examine total replacement of menhaden fish meal (FM) with distiller's dried grains with solubles (DDGS), which had been used as substrate for the production of black soldier fly larvae, *Hermetia illucens*, in combination with soybean meal (SBM) and poultry by-product meal (PBM), with or without supplementation of the amino acids (AA) DL-methionine (Met), L-lysine (Lys) and a commercial non-amylaceous polysaccharide enzyme (Enz) product. Fish were fed seven isoenergetic [available energy (AE) = 4.0 kcal g^{-1} of diet] and isonitrogenous (350 g kg^{-1} protein as-fed basis) practical diets formulated with equivalent digestible protein levels. Diet 1 was formulated to be similar to a commercial, high-quality, tilapia diet containing 200 g kg^{-1} FM. Diets 2–5 were formulated as a 2 × 2 factorial to replace FM with similar contributions from DDGS (45%), PBM (25%) and SBM (2.1–2.9%), but to differ in supplementation of AA and/or Enz preparation. Diets 6 and 7 were formulated to investigate the effects of a 2/3 and 1/3 reduction, respectively, in DDGS contribution to the replacement protein mix, with concomitant increases in SBM, with respect to diet 3, and were balanced with Lys and Met. After 6 weeks, growth responses were slightly attenuated ($P \leq 0.05$) and average daily intake (ADI) and feed conversion ratio (FCR) were slightly higher in tilapia fed DDGS diets 2–5 compared to those of fish fed the FM control diet 1. Growth responses were not significantly affected by the presence or absence of AA or Enz (diets 2–5), or the level of DDGS (diets 3, 7 and 6). Whole-body proximate composition was not different among treatments. Amino acid profiles of fish fed DDGS diets were not significantly different from those of fish fed the FM control. Evidence of interaction between AA and Enz supplementation was detected in whole-body amino acid concentrations such that AA content was higher with AA or Enz addition alone, but lower when both were added to the diet. Results suggest that DDGS replacement of FM in tilapia diets can be substantial when diets are formulated on a digestible protein basis and DDGS is combined with highly digestible animal (PBM) and plant proteins (SBM).

KEY WORDS: distiller's dried grains with solubles, poultry by-product meal, Nile tilapia, *Oreochromis niloticus*

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Introduction

The black soldier fly, *Hermetia illucens*, larvae has become a popular bio-recycling organism in the United States due to its detritivorous nature (Bondari & Sheppard 1981; Sheppard *et al.* 1994). Black soldier fly larvae (BSFL) are used to compost and sanitize wastes which can include fresh manure, and

animal and vegetable food wastes. Mature larvae and prepupae grown in manure management operations can also be used to supplement livestock diets (Newton *et al.* 1977). Further, the larvae can be processed into a meal that contains a high (>400 g kg^{-1}) protein level and high (>350 g kg^{-1}) lipid level and can be used in fish diets (St-Hilaire *et al.* 2007; Sealey *et al.* 2011; Kroeckel *et al.* 2012). However, use of black soldier fly larvae meal (BSFLM) in aquaculture diets may not be endorsed by consumers if BSFLM is produced from manure management operations. Production of BSFLM grown on plant-based agricultural products may be one method of improving consumer acceptance as well as producing a safe, nutritious fish diet ingredient.

One agricultural by-product that can be used as substrate for BSFL is corn distiller's dried grains with solubles (DDGS), a coproduct of ethanol fuel production and the beverage distilling industry. The nutrient composition of DDGS can vary among sources depending upon geographic location and corn varieties, fermentation time and efficiency, and heating/drying processes; however, DDGS has a typical compositional analysis of 270–280 g kg^{-1} crude protein, 93 g kg^{-1} crude fat and 91 g kg^{-1} crude fibre (NRC 2011). DDGS has been proposed as a protein source for aquaculture diets due to its relatively low cost per unit protein, absence of antinutritional factors found in other plant-based ingredients and increasing availability (Metts *et al.* 2011). Ethanol production in the United States, for example, has undergone significant expansion within the last 10 years as a result of rising energy costs and mandates for biofuel use in the U.S. Energy Acts of 2005 and 2007. Increased ethanol production has subsequently led to increased production of DDGS in the United States, where production has more than tripled since 2005 from 10.4 mmt to an estimated 39.0 mmt for 2014 (Wisner 2014).

Two negative characteristics of DDGS are high levels of cellulose and hemicellulose, like arabinoxylans, which fish cannot easily digest (Krogdahl *et al.* 2005). Additionally, it has been shown that DDGS is first-limiting in lysine (Lys) when included in diets for channel catfish, *Ictalurus punctatus* at high (>35%) levels; however, supplementation with Lys provided similar growth to that of catfish fed a control diet containing FM (Webster *et al.* 1992; Robinson & Li 2008). Work in poultry (EFSA 2013) and swine (EFSA 2013) suggests that dietary inclusion of enzymes that can break down cellulose and hemicellulose improve nutrient digestibility in those animals. One such commercial product is Rovabio Max AP-10 (Adisseo, Antony, France) which has endo-1,4-beta-xylanase (N° EC 3.2.1.8), endo-1,3(4)-beta-glucanase (N° EC 3.2.1.6) and 6-phytase (N° EC 3.1.3.26) obtained from two fermentation broths of *Penicillium funiculosum* and *Schizosaccharomyces pombe*. The enzymes hydrolyse pentosans, beta-glucans and phytates in vegetal materials and have been deemed safe for use in diets for chickens, turkeys, other fowl and pigs by the European Food Safety Authority (EFSA 2013); however, there are no studies that the authors are aware evaluating this enzyme product in fish diets.

Nile tilapia, *Oreochromis niloticus*, are tropical fish endemic to freshwaters in Jordan, Israel and parts of Africa. Because of their rapid growth rates, good quality flesh, disease resistance, adaptability to a wide range of environmental conditions, ability to grow and reproduce in captivity and to feed at low trophic levels, tilapia have become an excellent choice for aquaculture (Lim & Webster 2006). As tilapia are primarily produced in intensive production systems (Ng & Wang 2011), it has become necessary to evaluate practical diets that are economically and environmentally sustainable, as well as nutritionally complete. Fish meal (FM) has customarily been used as a major animal protein source in aquaculture diets and the main source of protein in diets for tilapia fry and juveniles (El-Saidy & Gaber 2003). However, FM is the most expensive macro-ingredient ($US1210-$1540 per mt) and is also highly desired in the diets of other livestock industries. Thus, special attention must be given to tilapia nutrition with emphasis on replacement of FM by less expensive vegetable and animal protein sources (Gonzales *et al.* 2007).

While there have been several studies conducted to evaluate the use of DDGS in Nile tilapia diets (Coyle *et al.* 2004; Lim *et al.* 2007, 2009; Shelby *et al.* 2008), all reports used fish meal (FM) in the diets. No studies have been conducted to evaluate the secondary use of DDGS in diets for an aquaculture species after it has served as a source of nutrients for its primary purpose (bio-ag-reutilization), and there are limited published data on the use of DDGS, in combination with an animal source protein (such as poultry by-product meal, PBM), as total replacement of FM in diets for juvenile Nile tilapia. Hence, a feeding trial was conducted to evaluate growth and body composition of Nile tilapia when fed diets without FM, with and without supplemental methionine and lysine, and with and without a commercial enzyme product.

Materials and methods

Experimental diets

Nile tilapia juveniles were fed one of seven isonitrogenous (350 g kg^{-1} protein, as-fed basis) and isoenergetic (available

Table 1 Nutrient composition (g kg^{-1} dry matter basis) of menhaden fishmeal (FM), poultry by-product meal (PBM), soybean meal (SBM) and distiller's dried grains with solubles (DDGS) initially used as substrate for black soldier fly larvae (BSFL)

	Ingredient			
	FM	PBM	SBM	DDGS
Moisture	71.0	48.0	100.6	85.7
Protein	660.2	658.6	516.2	265.0
Lipid	108.6	154.4	38.1	90.4
Ash	218.2	117.8	73.9	75.1
Arginine	34.4	43.8	37.3	10.2
Cystine	4.9	10.9	6.7	3.8
Histidine	14.2	5.8	12.9	6.1
Isoleucine	26.4	21.3	22.3	9.3
Leucine	32.5	40.4	38.2	26.8
Lysine	48.0	31.0	30.9	10.0
Methionine	16.2	5.1	7.3	4.8
Phenylalanine	25.9	24.3	25.7	10.0
Threonine	29.1	25.4	19.9	8.9
Tryptophan	8.4	6.7	7.0	2.1
Tyrosine	18.9	15.6	17.4	16.0
Valine	31.0	28.5	23.3	11.8

energy, AE = 4.0 kcal g^{-1} of diet) practical test diets (Table 1) containing protein primarily from menhaden fish meal (MFM) and soybean meal (SBM) in diet 1 (control), or a combination of DDGS (Table 1) that had been used as substrate for BSFL (Enviroflight, Yellow Springs, OH, USA), poultry by-product meal (PBM) and SBM in diets 2–7. All diets were formulated on a digestible protein (DP) basis with respect to MFM, PBM, SBM, wheat flour and wheat gluten (Schneider *et al*. 2004; Sklan *et al*. 2004; Koprucu & Ozdemir 2005; Guimaraes *et al*. 2008) and were formulated to meet the known amino acid requirements of Nile tilapia (Table 2). Diet 1 was formulated to be similar to a commercial, high-quality, tilapia diet containing 20% MFM. Diets 2–5 were formulated to replace MFM with similar contributions from DDGS (45%), PBM (25%) and SBM (2.1–2.9%), but to differ in the supplementation of L-lysine (Lys) and DL-methionine (Met) and/or a commercial, non-amylaceous polysaccharide (NSP) enzyme preparation, Rovabio® Max AP-10 (Adisseo). Specifically, diet 3 was formed by supplementing diet 2 with Lys and Met to match available levels found in the control diet (diet 1). Diet 4 was formed by supplementing diet 2 with Rovabio® without amino acid supplementation. Diet 5 was formed by supplementing diet 2 with both amino acids and Rovabio®. Hence, diets 2–5 formed a complete factorial with respect to Lys, Met and enzyme supplementation, and we were interested *a priori* in comparing the responses to these diets to the MFM control (diet 1). In addition, diets 6 and 7 were formulated to investigate the effects of a 2/3 and 1/3 reduction, respectively, in DDGS contribution to the replacement protein mix, with concomitant increases in SBM contribution, with respect to diet 3, and were similarly balanced with supplemental Lys and Met. Hence, diets 3 (45% DDGS), 7 (30% DDGS) and 6 (15% DDGS) formed a dose–response series with respect to dietary DDGS/SBM ratio. The amino acid composition of the test diets is presented in Table 3.

Preparation of diets

Dry ingredients were mixed together for 1 h using a Hobart mixer (A-200 T; Hobart, Troy, OH, USA), and warm tap water was added to obtain a 35% moisture level. Diets were then passed through a meat grinder with a 0.5-cm die two times to form 'spaghetti-like' strands and air-dried. After drying, diets were ground into pellets of appropriate size using a S.500 disc mill (Glen Mills Inc., Clifton, NJ, USA). Diets were sieved (2-mm opening mesh and 0.5-mm mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburg, PA, USA). After sieving, soybean oil (volume of 3.9% for the control) and menhaden fish oil (volume of 1.0% among diets 2–7) were slowly added to diets until all pellets were uniformly coated. The oils were added after pelletizing to avoid destruction of essential fatty acids (highly unsaturated fatty acids) during processing (Thompson *et al*. 2003a,b). Diets were stored at −20 °C in plastic containers until used for feeding.

Diet analysis

Diets were analysed for proximate composition based on standard procedures (AOAC 1998) to determine per cent moisture, protein, lipid, fibre and ash (Table 1). Moisture was determined by Association of Official Analytical Chemists (AOAC) procedure 930.15; protein was determined by combustion method, AOAC procedure 990.03; lipid was determined by the acid hydrolysis method, AOAC procedure 954.02; fibre was determined by AOAC procedure 962.09; and ash was determined by AOAC procedure 942.05. The nitrogen-free extract (NFE, i.e. carbohydrate) was determined by difference: NFE = 100 − (% protein + % lipid + % fibre + % ash). The AE was calculated from physiological fuel values of 4.0, 4.0 and 9.0 kcal g^{-1} for protein, carbohydrate (NFE) and lipid, respectively (Garling & Wilson 1977; Webster *et al*. 1999). Amino acid compositions were determined by a commercial

Table 2 Ingredient and analysed chemical composition (% of diet) of seven practical diets containing distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with and without amino acid and/or enzyme supplementation, as total replacement of menhaden fish meal (MFM) fed to juvenile (initial mean weight, 2.66 g) Nile tilapia

	Diet						
Ingredient	1 (control)	2	3	4	5	6	7
DDGS[1]	0.00	45.00	45.00	45.00	45.00	15.00	30.00
SBM (52%)	32.00	2.85	2.05	2.85	2.85	18.00	11.95
MFM (64%)	20.00	0.00	0.00	0.00	0.00	0.00	0.00
PBM (feed-grade) (57%)	0.00	25.00	25.00	25.00	25.00	25.00	25.00
Wheat flour (12%)	37.95	20.00	19.80	19.95	18.95	34.35	25.30
Soybean oil	3.90	0.00	0.00	0.00	0.00	0.00	0.00
Menhaden fish oil	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Wheat gluten (86%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Mineral mix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stay C (35%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-lysine[4]	0.00	0.00	0.70	0.00	0.70	0.40	0.50
DL-methionine[4]	0.00	0.00	0.30	0.00	0.30	0.10	0.10
Rovabio[5]	0.00	0.00	0.00	0.055	0.055	0.00	0.00
Analysed composition							
Moisture (%)	7.55	7.72	7.76	7.65	8.00	8.13	7.84
Protein (%)[6]	41.06	41.46	41.90	41.27	42.65	41.92	42.73
Lipid (%)[6]	9.57	9.55	9.37	9.34	9.33	7.88	8.68
Ash (%)[6]	8.00	9.94	10.19	10.03	10.03	9.46	10.25
NFE (%)[7]	41.36	39.06	38.54	39.36	37.99	40.74	38.34
Available energy (kcal g^{-1})[8]	4.16	4.08	4.06	4.07	4.07	4.02	4.02
E,P[9]	10.13	9.84	9.69	9.85	9.53	9.58	9.42

[1] Distiller's dried grains with solubles (DDGS) used as black soldier fly substrate Enviroflight.
[2] Vitamin mix supplied the following (mg or IU kg^{-1} of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadione sodium bisulphate complex), 3.7 mg; niacin, 280 mg; D-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamine, 35.8 mg; D_1, 440 IU; and A (as vitamin A palmitate), 6607 IU.
[3] Mineral mix supplied the following (g kg^{-1} of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; and iodine, 0.010 g.
[4] Amino acids, DL-methionine, minimum 99% by thin layer chromatography (TLC); L-lysine, 98% TLC; Sigma-Aldrich, St. Louis, Missouri.
[5] Adisseo, Antony, France.
[6] Dry-matter basis.
[7] NFE = nitrogen-free extract.
[8] Available energy was calculated as 4.0, 4.0 and 9.0 kcal g^{-1} for protein, carbohydrate and lipid, respectively.
[9] E,P = calculated available energy (AE), protein ratio of each diet.

analytical laboratory (Table 3; Texas A&M University, College Station, TX, USA).

Experimental system, stocking, and feeding

The feeding trial was conducted in 21 aquaria (120-L) at the *Aquacult. Res.* Center, Kentucky State University, Frankfort. Dechlorinated city (tap) water was recirculated through a 350-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bioballs (Red Ewald, Karnes City, TX, USA) and then passed through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, LA, USA) to remove nitrogenous wastes and provide substrates for nitrifying (*Nitrosomonas* and *Nitrobacter*). Water was supplied to each aquarium at a rate of 0.65 L min^{-1}. Water temperature was maintained at 27–28 °C by the use of an immersion heater, and continuous aeration was provided. Approximately 5% of the total water volume was replaced daily. Lighting was provided by overhead fluorescent ceiling lights with a 14 h light: 10 h dark cycle. Sodium bicarbonate was added to the recirculating system to maintain alkalinity levels near 100 mg L^{-1}. All tanks were siphoned daily to remove faeces.

Water quality conditions were checked three times weekly. Dissolved oxygen, pH and water temperature were

Table 3 Analysed amino acid composition (% of diet; per cent of protein in parentheses) of seven practical diets containing distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with and without amino acid and/or enzyme supplementation, as total replacement of menhaden fish meal (MFM) fed to juvenile (initial mean weight, 2.66 g) Nile tilapia. Values are means of two determinations per diet

	Diet						
Amino acid	1 (control)	2	3	4	5	6	7
Alanine	1.5 (3.62)	2.5 (5.92)	2.0 (4.78)	2.3 (5.66)	2.2 (5.14)	1.5 (3.67)	2.1 (4.81)
Arginine	1.6 (3.79)	1.5 (3.71)	1.2 (2.96)	1.4 (3.45)	1.3 (3.13)	1.3 (3.10)	1.6 (3.70)
Aspartic acid	1.9 (4.73)	1.9 (4.64)	1.6 (3.72)	1.8 (4.48)	1.7 (4.05)	1.5 (3.54)	1.8 (4.21)
Cystine	0.1 (0.26)	0.1 (0.33)	0.1 (0.26)	0.1 (0.30)	0.1 (0.28)	0.1 (0.28)	0.1 (0.33)
Glutamic acid	4.2 (10.21)	4.8 (11.49)	3.9 (9.24)	4.6 (11.04)	4.2 (9.86)	3.6 (8.59)	4.3 (10.02)
Glycine	1.5 (3.71)	2.4 (5.79)	2.0 (4.65)	2.2 (5.39)	2.1 (4.93)	1.9 (4.50)	2.4 (5.51)
Histidine	0.7 (1.60)	0.7 (1.64)	0.5 (1.30)	0.6 (1.52)	0.6 (1.40)	0.5 (1.28)	0.7 (1.58)
Isoleucine	1.3 (3.15)	1.4 (3.28)	1.1 (2.64)	1.3 (3.15)	1.2 (2.88)	1.1 (2.51)	1.3 (3.08)
Leucine	2.3 (5.53)	3.2 (7.72)	2.6 (6.26)	3.0 (7.36)	2.9 (6.74)	2.1 (4.96)	2.8 (6.51)
Lysine	1.8 (4.39)	2.0 (4.91)	2.1 (4.96)	1.9 (4.62)	2.4 (5.56)	1.7 (4.11)	2.3 (5.28)
Methionine	0.4 (0.96)	0.5 (1.30)	0.6 (1.40)	0.5 (1.25)	0.7 (1.64)	0.4 (1.03)	0.6 (1.42)
Phenylalanine	1.3 (3.24)	1.4 (3.31)	1.1 (2.67)	1.3 (3.09)	1.2 (2.84)	1.1 (2.67)	1.4 (3.26)
Proline	2.0 (4.79)	3.0 (7.19)	2.4 (5.80)	2.8 (6.80)	2.6 (6.18)	2.1 (5.08)	2.7 (6.28)
Serine	1.3 (3.19)	1.5 (3.60)	1.2 (2.89)	1.4 (3.40)	1.3 (3.03)	1.1 (2.71)	1.4 (3.22)
Taurine	0.1 (0.24)	0.1 (0.25)	0.1 (0.31)	0.1 (0.23)	0.1 (0.16)	0.1 (0.16)	0.1 (0.17)
Threonine	1.2 (2.89)	1.3 (3.24)	1.1 (2.62)	1.3 (3.06)	1.2 (2.80)	1.0 (2.35)	1.2 (2.92)
Tyrosine	0.9 (2.22)	0.8 (1.82)	0.8 (1.86)	0.8 (1.95)	0.8 (1.83)	0.7 (1.60)	0.7 (1.69)
Valine	1.5 (3.56)	1.7 (4.02)	1.4 (3.25)	1.6 (3.85)	1.5 (3.51)	1.2 (2.90)	1.6 (3.63)

measured using a Hydrolab Quanta Water Quality Monitoring System, Model QD 02152 (Hydrolab, Loveland, CO, USA). Alkalinity and chloride were measured by titration method (HACH digital titrator, Hach, Loveland, CO, USA); total ammonia and nitrite levels were measured using a HACH DR 2800 spectrophotometer (Hach). During the study, average values (±SE) for water quality parameters averaged (±SE): water temperature, 28.9 ± 1.2 °C; dissolved oxygen, 5.0 ± 0.2 mg L^{-1}; total ammonia nitrogen, 0.62 ± 0.2 mg L^{-1}; nitrite, 0.11 ± 0.03 mg L^{-1}; total alkalinity, 105 ± 25.7 mg L^{-1}; chloride, 94.6 ± 0.03 mg L^{-1}; and pH, 8.86 ± 0.09. All parameters were within acceptable limits for fish growth and health (Boyd 1979).

Nile tilapia juveniles were obtained from Til-Tech Aquafarm (Robert, LA, USA) and were stocked at an average weight of 2.66 g each at a rate of 15 fish per aquarium, with three replicate aquaria per treatment. Fish stocked into each aquarium were batched-weighed using an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland). Mortalities were monitored daily and were removed and replaced during the first week of the study, with no replacements thereafter. All tilapia in each aquarium were fed four times daily (0800, 1045, 1330 and 1600 h) to excess, regardless of treatment, during a 30-min period for 60 days.

Data collection

At the conclusion of the study, fish in each tank were batched-weighed on an electronic scale (AB54-S; Mettler Toledo, Columbus, OH, USA) to determine total weight and hand-counted to determine per cent survival.

Per cent weight gain (WG) was calculated as follows:

$WG = 100 \times [(W_t - W_i)/W_i]$, where W_t is weight after time t and W_i is the initial weight.

Specific growth rate (SGR) was calculated as follows:

$SGR = [100 \times \text{Ln } W_f - \text{Ln } W_0)]/t$, where W_f is final body weight, W_0 is initial body weight, and t is experimental duration.

Feed conversion ratio (FCR) was calculated as follows:

FCR = total dry weight of diet fed (g)/total wet weight gain (g).

Protein efficiency ratio (PER) was calculated as follows:

PER = weight gain (g)/protein fed (g).

Sample analysis

After weighing and counting, all fish within each treatment were chill-killed using an ice water bath. There were three replicate samples for whole-body analysis per treatment for proximate analysis (moisture, protein, lipid and ash analysis) that was performed at the Abernathy Fish Technology

Center (Longview, WA, USA). Tissue samples were analysed as described for the diet analysis with the exception of protein and lipid. Protein in whole bodies of fish was determined by LECO FP-528 (LECO Corporation, St. Joseph, MI, USA) protein/nitrogen determinator (AOAC procedure 968.06 1998), while lipid was determined by extracting with 2 : 1 chloroform: methanol at 100 °C (AOAC procedures 991.36 and 960.39 1998). In addition, there were three replicate samples of fillet per treatment used for the amino acid analysis that was performed at Texas A&M University, College Station, TX, USA.

Statistical analysis

Responses to diets 2–5 were analysed as a 2 (with or without amino acid supplementation) × 2 (with or without enzyme supplementation) mixed model factorial analysis of variance (ANOVA) using PROC MIXED in SAS version 9.1.3 (SAS Institute 2003; SAS Institute, Inc., Cary, NC, USA). Similarly, responses to DDGS diets 2–5 were compared to those of the MFM control (diet 1) via directed contrasts, while responses to diets 3 (45% DDGS), 7 (30% DDGS) and 6 (15% DDGS) were subjected to linear and quadratic contrasts within the context of PROC MIXED. Mean responses and *a priori* targeted contrasts were declared significant at $P \leq 0.05$ using the Tukey adjustment of P values in SAS (Zar 1984).

Results

Growth performance

Growth responses of tilapia fed DDGS diets 2–5 with or without amino acid (AA) and/or Rovabio (Enz) supplementation were slightly attenuated ($P \leq 0.05$) compared to those of fish fed the MFM control diet 1 (Table 4). Specifically, final weight (FW), weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) in fish fed DDGS diets 2–5 were slightly less than those observed in fish fed diet 1. Additionally, both average daily intake (ADI) and feed conversion ratio (FCR) were slightly higher in fish fed diets 2–5 when compared to fish fed diet 1 (Table 4). In contrast, growth responses were not significantly affected by the presence or absence of AA or Enz supplements in diets 2–5 (Table 4), or the level of DDGS in diets 3, 6 and 7 (Table 5). Similarly, survival was unaffected by dietary treatments (Tables 4 & 5).

Table 4 Factorial analysis of variance of final weight (FW; g per fish), weight gain (WG; % of initial weight), specific growth rate (SGR; %/d), average daily intake (ADI; %), feed conversion ratio (FCR; g dry feed per g wet gain), protein efficiency ratio (PER) and survival (S; %) of juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets (1–5) containing distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) and without (−) amino acid (AA) and/or enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of $N = 3$ replicate tanks per treatment. Means within a column followed by an asterisk are significantly ($P < 0.05$) different from the MFM control diet (1), whereas main effect means for diets 2–5 were not different

Diet	Protein	AA	Enz	FW	WG[1]	SGR[2]	ADI[3]	FCR[4]	PER[5]	S
1	MFM	−	−	62.2	2040	7.29	5.27	1.10	2.62	95.6
2	DDGS	−	−	51.4*	1643*	6.79*	6.64*	1.39*	2.05*	97.8
3	DDGS	+	−	53.2*	1681*	6.85*	6.58*	1.38*	2.07*	97.8
4	DDGS	−	+	52.2*	1548*	6.67*	6.62*	1.39*	2.07*	97.8
5	DDGS	+	+	51.9*	1533*	6.65*	6.56*	1.38*	2.10*	97.8
Pooled SEM				2.54	65	0.09	0.36	0.07	0.13	2.8
Main effect means, Diets 2–5										
AA −				51.8	1595	6.73	6.63	1.39	2.06	97.8
AA+				52.6	1607	6.75	6.57	1.38	2.09	97.8
Enz −				52.3	1662	6.82	6.61	1.39	2.06	97.8
Enz +				52.1	1541	6.66	6.59	1.38	2.09	97.8
ANOVA Source, Pr > F										
AA				0.768	0.876	0.850	0.877	0.873	0.841	1.000
Enz				0.933	0.122	0.132	0.965	0.959	0.847	1.000
AA x Enz				0.684	0.714	0.700	0.996	0.998	0.956	1.000

[1] Weight gain (WG; %) = (final weight − initial weight)*100/initial weight.
[2] Specific growth rate (SGR; %/d) = (Ln final weight − Ln initial weight)*100/*t*, where *t* = number of days in the feeding trial.
[3] Average daily intake (ADI; %) = g dry feed consumed/average fish biomass (g)/culture days *100.
[4] Feed conversation ratio (FCR) = g dry feed consumed/g wet weight gained.
[5] Protein efficiency ratio (PER) = weight gain (g)/protein fed (g).

Table 5 Final weight (FW; g per fish), weight gain (WG; % of initial weight), specific growth rate (SGR; %/d), average daily intake (ADI; %), feed conversion ratio (FCR; g dry feed per g wet gain), protein efficiency ratio (PER) and survival (S; %) in juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets containing graded levels of distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) amino acid (AA) and without (−) enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of $N = 3$ replicate tanks per treatment. Linear or quadratic effects of DDGS level were not significant at $P \leq 0.05$

Diet	DDGS	AA	Enz	FW	WG[1]	SGR[2]	ADI[3]	FCR[4]	PER[5]	S
3	45	+	−	53.2	1680	6.85	6.58	1.38	2.07	97.8
7	30	+	−	58.6	1875	7.10	6.25	1.31	2.18	93.3
6	15	+	−	53.6	1728	6.90	6.08	1.28	2.26	97.8
Pooled SEM				2.7	94	0.13	0.32	0.07	0.12	3.1
ANOVA contrast, Pr > F										
Linear				0.923	0.735	0.782	0.326	0.312	0.298	1.000
Quadratic				0.227	0.257	0.268	0.867	0.839	0.957	0.363

[1] Weight gain (WG; %) = (final weight − initial weight)*100/initial weight.
[2] Specific growth rate (SGR; %/d) = (Ln final weight − Ln initial weight)*100/t, where t = number of days in the feeding trial.
[3] Average daily intake (ADI; %) = g dry feed consumed/average fish biomass (g)/culture days *100.
[4] Feed conversation ratio (FCR) = g dry feed consumed/g wet weight gained.
[5] Protein efficiency ratio (PER) = weight gain (g)/protein fed (g).

Whole-body composition

Whole-body moisture, protein, lipid and ash concentrations were unaffected by fishmeal, AA or Enz content of the diet (Table 6) or the level of DDGS in the diet (Table 7), which averaged 707 g kg^{-1}, 159 g kg^{-1}, 82 g kg^{-1} and 44 g kg^{-1}, respectively. Whole-body amino acid profiles of fish fed DDGS diets 2–5 were not significantly different from those of fish fed the FM control (Table 8). A significant interaction ($P = 0.031$) between amino acid (AA) and enzyme (Enz) supplementation was detected in final whole-body His concentrations of fish fed diets 2–5 such that His content was higher with AA or Enz addition alone, but lower when both supplements were included in the diet (Table 8). Additionally, concentrations of all the amino acids measured in whole bodies showed a similar trend ($P = 0.052$ to 0.103) in interaction between AA and Enz supplementation. Linear and/or quadratic contrasts of whole-body AA content with respect to DDGS level in the diet (diets 3, 6 and 7) were statistically significant such that whole-body AA increased as dietary DDGS level decreased (Table 9).

Table 6 Factorial analysis of variance of final whole-body composition (percentage fresh-weight basis) of juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets (1–5) containing distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) and without (−) amino acid (AA) and/or enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of three fish samples per tank, where $N = 3$ replicate tanks per treatment. There were no significant ($P < 0.05$) differences from the MFM control diet (1), or among main effect means for diets 2–5

Diet	Protein	AA	Enz	Moisture	Protein	Lipid	Ash
1	MFM	−	−	70.5	16.3	8.1	4.2
2	DDGS	−	−	71.0	15.4	8.5	4.3
3	DDGS	+	−	70.4	16.0	8.4	4.7
4	DDGS	−	+	70.1	15.6	8.7	4.6
5	DDGS	+	+	70.6	15.8	8.3	4.6
Pooled SEM				0.9	0.3	0.4	0.2
Main effect means, Diets 2–5							
AA −				70.5	15.5	8.6	4.5
AA+				70.5	15.9	8.3	4.6
Enz −				70.3	15.7	8.4	4.5
Enz +				70.7	15.7	8.5	4.6
ANOVA Source, Pr > F							
AA				0.960	0.267	0.587	0.493
Enz				0.699	0.929	0.891	0.678
AA x Enz				0.581	0.539	0.733	0.414

Table 7 Final whole-body composition (percentage fresh-weight basis) in juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets containing graded levels of distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) amino acid (AA) and without (−) enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of $N = 3$ replicate tanks per treatment. Linear or quadratic effects of DDGS level were not significant at $P \leq 0.05$

Diet	DDGS	AA	Enz	Moisture	Protein	Lipid	Ash
3	45	+	−	70.4	16.0	8.4	4.7
7	30	+	−	71.0	15.7	7.6	4.7
6	15	+	−	70.9	16.5	7.7	4.1
Pooled SEM				0.9	0.4	0.4	0.3
ANOVA Source, Pr > F							
Linear				0.739	0.445	0.217	0.218
Quadratic				0.793	0.341	0.389	0.464

Table 8 Factorial analysis of variance of whole-body amino acid composition (percentage wet weight) of juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets (1–5) containing distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) and without (−) amino acid (AA) and/or enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of $N = 3$ replicate tanks per treatment. There were no significant ($P < 0.05$) differences from the MFM control diet (1), or among main effect means for diets 2–5

Diet	Protein	AA	Enz	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Tau	Thr	Tyr	Val
1	MFM	−	−	1.02	0.044	0.42	0.57	1.16	1.12	0.40	0.69	0.23	0.73	0.49	0.73
2	DDGS	−	−	0.96	0.041	0.37	0.54	1.12	1.14	0.37	0.64	0.22	0.71	0.46	0.70
3	DDGS	+	−	1.04	0.046	0.44	0.60	1.24	1.23	0.43	0.72	0.26	0.78	0.53	0.76
4	DDGS	−	+	1.09	0.047	0.46	0.62	1.28	1.26	0.43	0.74	0.25	0.80	0.52	0.79
5	DDGS	+	+	0.88	0.041	0.36	0.53	1.08	1.08	0.37	0.62	0.23	0.66	0.45	0.67
Pooled SEM				0.09	0.003	0.03	0.04	0.09	0.10	0.03	0.05	0.02	0.06	0.03	0.05
Main effect means, Diets 2–5															
AA −				1.02	0.044	0.41	0.58	1.20	1.20	0.40	0.70	0.24	0.75	0.49	0.74
AA+				0.96	0.043	0.40	0.56	1.16	1.16	0.40	0.67	0.25	0.72	0.49	0.72
Enz −				1.00	0.044	0.41	0.57	1.18	1.19	0.40	0.68	0.24	0.74	0.49	0.73
Enz +				0.98	0.044	0.41	0.58	1.18	1.17	0.40	0.68	0.24	0.73	0.49	0.73
ANOVA Source, Pr > F															
AA				0.389	0.741	0.747	0.646	0.635	0.638	0.968	0.734	0.592	0.515	0.935	0.556
Enz				0.788	0.949	0.909	0.931	0.992	0.851	0.865	0.963	0.944	0.799	0.882	0.978
AA x Enz				0.067	0.088	0.031	0.103	0.080	0.186	0.068	0.070	0.133	0.063	0.052	0.100

Table 9 Whole-body amino acid composition (percentage wet weight) in juvenile (initial mean weight, 2.66 g) Nile tilapia fed practical diets containing graded levels of distiller's dried grains with solubles (DDGS), poultry by-product meal (PBM) and soybean meal (SBM), with (+) amino acid (AA) and without (−) enzyme (Enz) supplementation, as total replacement of menhaden fish meal (MFM). Values are means of $N = 3$ replicate tanks per treatment. Linear or quadratic effects of DDGS level were considered significant at $P \leq 0.05$

Diet	DDGS	AA	Enz	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Tau	Thr	Tyr	Val
3	45	+	−	1.04	0.046	0.44	0.599	1.24	1.23	0.43	0.73	0.26	0.78	0.53	0.76
7	30	+	−	1.18	0.052	0.46	0.670	1.38	1.40	0.48	0.79	0.25	0.86	0.57	0.84
6	15	+	−	1.16	0.052	0.47	0.673	1.37	1.35	0.48	0.80	0.34	0.85	0.57	0.85
Pooled SEM				0.04	0.001	0.01	0.007	0.01	0.02	0.01	0.02	0.05	0.02	0.01	0.01
ANOVA contrast, Pr > F															
Linear				0.086	0.007	0.071	<0.001	0.001	0.012	0.032	0.062	0.313	0.028	0.089	0.002
Quadratic				0.232	0.078	0.821	0.015	0.015	0.017	0.163	0.509	0.539	0.133	0.499	0.059

Discussion

As small juvenile fish require higher protein levels than more mature fish, FM is typically added to diets to increase protein and amino acid levels for rapidly growing fry and juveniles. However, given the high price of FM, it is vital that aquaculture diets reduce or eliminate its use from diets for all life stages of fish. Growth responses of small (2.7 g initial average weight) tilapia fed DDGS diets 2–5 were slightly reduced with respect to fish fed the fishmeal control diet, but the magnitude of differences was modest. For example, juveniles fed the DDGS test diets (51–53 g average final weight) were 9–10 g lighter on average, with an average 0.4 increase in feed conversion and 0.5 reduction in protein efficiency ratio, when compared to their FM diet fed counterparts. Nevertheless, average weight gains in all dietary treatments were high, ranging from 1533% to over 2000% of initial weight. These data suggest that all test diets were well utilized and that refinements in DDGS amino acid availability data for tilapia with appropriate adjustments to amino acid supplementation levels may yield narrower margins in performance between DDGS-based, as opposed to fishmeal-based, diets for juvenile tilapia. Indeed, when DDGS was reduced from 45% to 30% with a concomitant increase in SBM (from about 3% to 12%) with supplemental Lys and Met (Diet 7), the differences in final average weight (57 g vs. 62 g), weight gain (1875% vs. 2040%), SGR (7.1 vs. 7.3), FCR (1.3 vs. 1.1) and PER (2.2 vs. 2.6) with respect to the FM control diet were narrower.

The present study is the first to report that small (<3 g) Nile tilapia can be fed a diet containing appreciable concentrations (up to 45%) of DDGS without dramatic adverse effects on growth, feed conversion, body composition or

whole-body amino acid composition compared to fish fed a diet with 20% menhaden fish meal. While there have been several previous reports of DDGS use in tilapia diets (Coyle *et al*. 2004; Lim *et al*. 2007, 2009; Shelby *et al*. 2008), the diets fed in those studies contained some FM. Additionally, the use of all-plant protein ingredients in tilapia diets has resulted in reduced growth (Coyle *et al*. 2004). The addition of PBM to our diet formula, however, appears to allow higher inclusion of DDGS with minimal reductions in fish growth and no discernable differences in whole-body proximate or amino acid composition. If black soldier fly larvae production increases, and the larvae are processed into a meal for use in animal diets, any excess substrate will need to be utilized, either within the BSFL production facility, or by utilizing the substrate in another industry (bio-ag reutilization). While substrates from agricultural products could be used as fertilizer, use of the by-product in aquaculture diets may hold more economic advantage. It appears that DDGS used as substrate for BSFL has the same nutrient composition as commodity DDGS and is suitable for use in diets for Nile tilapia with minimal negative impacts on performance.

One readily available and renewable ingredient is poultry by-product meal (PBM), which is a by-product of the poultry processing industry that is high in protein (550 g kg^{-1}) and contains a favourable profile of indispensable amino acids (IAA) for fish production (Gaylord & Rawles 2005). Thompson *et al*. (2012) reported that PBM is high in protein (570 g kg^{-1}), contains a favourable profile of essential amino acids with high availability, and its lower price makes it an ideal candidate for replacing FM in aquafeeds. When combined with alternative protein sources, PBM has been shown to completely replace FM in juvenile tilapia diets without adverse effects on growth. Thompson *et al*. (2012) reported that Nile tilapia fed a diet containing 20% soy protein concentrate (SPC) and 20% feed-grade PBM had similar growth as fish fed a diet containing 20% FM. However, the quality of PBM varies considerably, which may cause a deficiency in one or more essential amino acids (Rawles *et al*. 2006).

Among plant protein ingredients, soybean meal (SBM) has received the most attention and is the most widely used plant protein ingredient in aquaculture diets due to its wide availability, nutritional consistency, balanced amino acid profile and high digestibility (Lovell 1988). Although SBM has several positive attributes that make it a good overall candidate for FM replacement, it also has negative attributes that may limit its use at high percentages or as the sole protein ingredient in commercial aquaculture diets. When compared to FM, SBM has lower essential amino acid (EAA) concentrations and there are deficiencies in the EAA methionine, lysine and threonine, as well as a lack of essential n-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (NRC 2011). Soybean meal is known to contain antinutritional factors such as trypsin inhibitors, lectins, phytic acid, saponins, antivitamins and high levels of non-starch polysaccharides and oligosaccharides that may affect nutrient digestibility and/or availability (Francis *et al*. 2001; Gatlin *et al*. 2007). Additionally, global demand for SBM is expected to increase along with its price on world trade markets. Soybean meal prices have risen by 118% since 1998 from USD $197 to $431 per metric ton. In the light of the rising cost of SBM, it has become imperative to evaluate suitable alternative ingredients that can be used to formulate fish diets that support adequate growth rates necessary for commercial production.

Although growth responses were not appreciably affected by amino acid or enzyme supplementation, or the level of DDGS in the diet, several performance metrics, for example feed intake (ADI), FCR, PER and survival of fish, were similar or superior to other reports (Lim *et al*. 2007, 2009; Thompson *et al*. 2012). Interestingly, a negative interaction between dietary amino acid and enzyme supplementation was clearly indicated ($P = 0.05$–0.1) in almost all the whole-body amino acid results, while the negative interaction between histidine and enzyme supplementation was highly significant ($P = 0.03$). Hence, amino acid or enzyme addition alone tended to improve whole-body amino acid profiles, but the combined addition of enzyme mix with amino acids reduced essential amino acid content in the body. It is important to point out, however, that the magnitude of differences in amino acid content due to either enzyme or amino acid diet supplementation was small, in the order of 0.1–0.2 percentage points. Hence, our results are somewhat contrary to Lin *et al*. (2007) who reported that a commercial enzyme product containing xylanase and beta-glucanase did improve growth, protein retention and intestinal enzyme activities in hybrid tilapia. However, Lin *et al*. (2007) did not supplement limiting amino acids in their study. Still, an explanation for our observed negative interaction between enzyme and amino acid supplementation is elusive. It is tempting to suggest that improved protein digestibility due to enzyme supplementation may have increased free amino acid concentrations from intact protein causing a competitive inhibition of dietary crystalline amino acid absorption (Ambardekar *et al*. 2009). The enzyme product used in the present study, however, did not include a protease, whereas Lin *et al*. (2007) used an

enzyme mixture that included a natural protease and improved protein digestibility of their diet. Similarly, the use of pancreatic enzymes has beneficial effects on growth and protein utilization in Atlantic salmon, *Salmo salar* (Carter *et al*. 1994), but Drew *et al*. (2005) did not find any effects on growth of rainbow trout, *Oncorhynchus mykiss*, when fed diets containing various plant protein meals with protease addition.

The enzyme product used in the present study also contained phytase; however, the diets contained supplemental phosphorus to meet the requirement of Nile tilapia so that the effectiveness of the enzyme may have been attenuated. Furthermore, phytate-bound phosphorus in DDGS is released during the fermentation process, which makes it available to monogastric animals like fish. Several studies have reported that phosphorus retention and absorption increase in rainbow trout when DDGS, or high protein distiller's dried grains, is added to the diet (Cheng & Hardy 2004; Overland *et al*. 2013; Prachom *et al*. 2013). Thus, it appears that addition of phytase to diets containing DDGS for Nile tilapia may not be required.

Supplementation of the test diets with lysine and methionine did not affect growth performance or proximate body composition in Nile tilapia in the present study. This is in agreement with other studies (Nguyen *et al*. 2009; Thompson *et al*. 2012). Nguyen *et al*. (2009) stated that addition of 0.5% crystalline methionine had no effect on growth, survival and FCR when the total sulphur amino acid (TSAA) levels of a diet already met or exceeded requirement. Furuya *et al*. (2004) found that growth was improved, however, by addition of supplemental essential amino acids to a SBM-based diet, but they also added dicalcium phosphate, which may have improved growth if the diets did not meet the phosphorus requirement of tilapia. In the present study, all diets were formulated to meet the known requirements of essential amino acids and phosphorus. Hence, the lack of response to supplemental amino acids in growth metrics and body proximate composition may not be surprising, particularly as our diets were formulated on a digestible protein basis.

A potential candidate ingredient for replacement of FM and SBM in tilapia diets is DDGS due to its nutrient content, relative low cost per unit protein basis, lack of antinutritional factors found in other plant-based ingredients and increasing availability. There have been several studies to evaluate inclusion of DDGS in diets for different fish species including tilapia (Coyle *et al*. 2004; Lim *et al*. 2007), hybrid striped bass (Thompson *et al*. 2008; Metts *et al*. 2011), rainbow trout (Cheng & Hardy 2004) and channel catfish (Webster *et al*. 1991, 1992; Robinson & Li 2008; Li *et al*. 2010, 2011). Growth in the present study was similar to other reports. Thompson *et al*. (2012) found that tilapia juveniles fed a diet containing 200 g kg^{-1} FM had higher growth rates than fish fed a diet containing 100 g kg^{-1} FM. Therefore, the best growth in juvenile Nile tilapia occurs when fish are fed a diet containing 200 g kg^{-1} FM. In the present study, weight gain of fish fed a diet containing 450 g kg^{-1} DDGS was slightly less than that of fish fed a diet containing 200 g kg^{-1} FM (control). Lim *et al*. (2007) reported that Nile tilapia fed a diet containing 200 g kg^{-1} DDGS and 80 g kg^{-1} FM had similar weight gains, feed conversion, protein efficiency ratios, body composition, blood and immune parameters as fish fed a control diet formulated with 80 g kg^{-1} FM and 540 g kg^{-1} SBM. However, in the same study, feeding fish a diet containing 400 g kg^{-1} DDGS and 80 g kg^{-1} FM resulted in reduced growth. Although lysine supplementation of the 400 g kg^{-1} DDGS diet improved growth and PER in Lim *et al*. (2007), it did not match the performance of their FM control diet. Again, the diets used in that study were formulated on an equal crude protein basis, as opposed to digestible protein basis in the case of our study, which may account for the lack of an appreciable amino acid supplementation effect on our measured fish responses. In contrast, Shelby *et al*. (2008) reported that Nile tilapia fed a diet containing 600 g kg^{-1} DDGS and 80 g kg^{-1} FM, plus 9 g kg^{-1} supplemental lysine, had similar final weight, weight gain and body composition compared to fish fed a control diet (80 g kg^{-1} FM and 450 g kg^{-1} SBM), but that fish fed a diet containing 600 g kg^{-1} DDGS without supplemental lysine had significantly reduced growth. Coyle *et al*. (2004) stated that hybrid tilapia, *Oreochromis niloticus* X *Oreochromis aureus*, fed a diet containing 300 g kg^{-1} DDGS and 260 g kg^{-1} meat-and-bone meal had weight gain, PER and FCR similar to fish fed a control diet containing 120 g kg^{-1} FM and 410 g kg^{-1} SBM, but fish fed a diet containing 300 g kg^{-1} DDGS and 460 g kg^{-1} SBM had reduced growth and increased FCR.

Whole-body proximate composition of Nile tilapia in the present study did not exhibit any differences among treatments. While this is not surprising for moisture, protein and ash, the similar lipid content of fish among treatments is in contrast to some reports where fish were fed diets containing DDGS. Rahman *et al*. (2013) concluded, for example, that differences in lipid deposition in olive flounder, *Paralichthys olivaceus*, were due to increased diet intake when fish were fed diets containing increasing percentages of DDGS. Likewise, increased body lipid has been correlated

with higher lipid levels in DDGS, or increased energy digestibility of DDGS, compared to other ingredients, in channel catfish diets (Webster *et al*. 1991; Robinson & Li 2008; Li *et al*. 2010, 2011). However, the increase in lipid reported in channel catfish may not occur in Nile tilapia (Coyle *et al*. 2004; Lim *et al*. 2007) or rainbow trout, *Oncorhynchus mykiss* (Prachom *et al*. 2013). All diets in the present study were formulated to contain similar energy and lipid levels that may have contributed to similar body lipid levels observed among our dietary treatments. Further, when dietary phosphorus levels are too low, lipid accumulation can occur in fish, but all test diets in the current study were formulated to meet phosphorus requirement of tilapia.

It is interesting to note that whole-body amino acid content of fish fed the amino acid balanced diets with graded levels of DDGS (diets 3, 7 and 6) slightly increased in a linear or quadratic fashion with decreasing diet DDGS level. However, the magnitude of increases, although statistically discernible, was minimal and ranged from 0.03 to 0.17 percentage points. Given that SBM was increased as DDGS level decreased, while all other intact protein ingredients were held constant in diets 3, 7, and 6, the resulting increase in whole-body AA content may be due to slight differences between *in vitro* SBM and DDGS digestibilities as opposed to coefficients taken from the literature and used for formulating the current test diets.

In conclusion, results of the present study suggest that the former limit of DDGS inclusion in tilapia diets (200 g kg^{-1}; Lim *et al*. 2007) may be increased when diets are formulated on a digestible protein basis and DDGS is combined with a highly digestible animal (PBM) and plant protein (SBM). While the current data does not convincingly support the addition of Rovabio® Max AP-10 as a digestibility enhancer to such diets, developing a database of amino acid availabilities from common and novel ingredients for tilapia and then balancing FM replacement diets with limiting amino acids may be an appropriate strategy for eliminating the performance gap between FM- and DDGS-based diets for tilapia.

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