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Fisheries and Aquaculture Journal

Research Article

Effects of Amino Acid Supplements in Plant-based Yellow Perch Diets

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ABSTRACT

High protein distillers dried grains (HP-DDG) were tested in combination with fermented soybean meal (FSBM) or soy protein concentrate (SPC), with and without an essential amino acid complex, to assess utility of these plant protein alternatives in Yellow Perch *Perca flavescens* diets in a 63-d feeding trial. Four experimental diets were formulated to contain equal amounts of HP-DDG and FSBM or SPC, each with and without essential amino acids and compared to a fish meal/HP-DDG reference diet. Fish that received diets containing SPC displayed the greatest weight gain, feed conversion, and apparent protein digestibility. Weight gain was significantly reduced and feed conversion significantly increased in the diet containing FSBM without essential amino acids. No mortalities or health assessment differences were observed during the trial and all treatment fish readily accepted the experimental diets.

Keywords: Yellow perch; Aquaculture; Alternative proteins; Amino acids

INTRODUCTION

The demand for fishmeal (FM) as a feedstuff for aquaculture and other livestock feed production will likely exceed sustainable supplies if feed demand for the aquaculture industry continues to increase at its current rate [1]. Aquaculture production increased at a rate of 10% annually during the 1990s and has averaged nearly 6% annually since 2000 [2]. As FM demand increases, prices have followed a similar trend leading to increasingly expensive aquaculture feeds. As such, fish feed is often the most expensive aspect in aquaculture production and can account for over 50% of operational costs [3,4]. Most carnivorous and omnivorous commercial fish feeds contain FM due to the fact that it is the most cost-effective complete protein source available; however, prices rose sharply since 2008 have and fluctuated around $1,550 per metric ton on average from 2010-2020 (Commodity Prices, Fish Meal, Index Mundi.com, 2020). Increased FM price and volatility has led to interest in developing more economical and sustainable alternatives to FM protein. In an effort to reduce dependence on fish meal and cost of fish feeds, plant-based proteins have been tested and continue to be a high priority area of research [3,5,6].

Cereal grain and oilseed production have experienced an increase during the last two decades as a result of increased plantings, higher yields, more efficient use of fertilizer, plant breeding, and policies that have driven biofuel (e.g., ethanol and biodiesel) production [1]. Generally, soybean meal has been the most commonly tested soybean feedstuff, however several studies have tested FSBM in fish diets [7-13] and SPC supplementation in fish diets [14-21]. Soybean protein concentrates (SPC) and fermented soybean meals (FSBM) may be incorporated at higher levels in feeds than defatted meal because further processing reduces anti-nutritional factors and increases the protein concentration [22,23], ideally concentrations that approximate that of fish meal.

Because of these increased production factors, co-product meals have experienced a large increase and are more broadly available. An increase in corn ethanol production has increased the volume of co-products, such as distillers dried grains with (DDGS) or without solubles (DDG). Conventional wet processes used in the ethanol industry yields DDGS with a protein concentration range of 27 to 31% [24-27] and a lipid range of 8 to 12% [26,28], however a higher protein DDG (HP-DDG) (~40% crude protein) is now available. Several studies have tested fuel or beverage-based DDGS in aquaculture feeds

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Received: October 18, 2019; **Accepted:** June 22, 2020; **Published:** June 29, 2020

Citation: Von Eschen AJ, Brown ML, Rosentrater K (2020) Effects of Amino Acid Supplements in Plant-based Yellow Perch Diets. Fish Aqua J 11:276. doi: 10.35248/2150-3508.20.11.276

[21,29-36], however few studies have reported on the use of HP-DDG in aquaculture diets [37,38].

Aquaculture of Yellow Perch *Perca flavescens* has increased in response to the declining commercial supply provided by the Great Lakes [39,40]. Limited published studies are known to have evaluated plant-based proteins as FM replacements in Yellow Perch diets. Schaeffer et al. [34] and Von Eschen et al. [21] investigated the use of DDGS in the diets of Yellow Perch. Likewise, few studies [21,34,40] have reported on the use of soybean feedstuffs in the diets of Yellow Perch. The objective of this study was to use HP-DDG in combination with SPC or FSBM as a FM replacement and determine nutrient utilization and performance of Yellow Perch fed these alternative proteins, with and without crystalline amino acid supplements.

METHODS AND MATERIALS

Experimental diets

Four experimental feeds were formulated to test plant meal combinations of HP-DDG (40% crude protein, 5% crude lipid) and FSBM (55% crude protein, 1% crude lipid) or SPC (73% crude protein, 0% crude lipid) (Table 1) for comparison to a FM/HP-DDG reference diet (50% FM/50% HP-DDG) (Table 2). Experimental diets were formulated to contain equal amounts of HP-DDG and FSBM or SPC, each with (4g/100g) or without an essential amino acid (EAA) complex (arginine, lysine, isoleucine, histidine, glycine, methionine, and a sulfonic acid-taurine). To avoid deficiencies in experimental diets, amino acids were determined a priori from feedstuff analyses (Table 1) and the EAA complex was formulated to meet or exceed recommended concentrations for Yellow Perch by Hart et al. [41] (Table 3). Test ingredients were included in equal amounts (30g/100g) and diets contained an average of 43.0 ± 1.5% (mean ± SE) crude protein, 7.9 ± 0.34% crude lipid, and 13.3 ± 0.31 MJ/kg gross energy (GE) (Table 3). Gross energy values were estimated by multiplying 17.2, 23.6, or 39.5 MJ/kg for carbohydrates, proteins, or lipids, respectively, times the analyzed composition values of each ingredient (Table 3) [4].

Table 1: Comparative values for essential amino acid (EAA) concentrations and proximate composition of fermented soybean meal (FSBM), soy protein concentrate (SPC), high protein distiller's dried grain (HP-DDG), and menhaden fish meal (FM) ingredients (g/100g, dry matter basis).

	Protein Source			
EAA	**FSBM**	**SPC**	**HP-DDGS**	**FM**
Arginine	3.6	5.3	1.5	6.3
Histidine	1.4	2	1.1	2.3
Isoleucine	2.6	3.5	1.7	4.4
Leucine	4.3	5.7	5.4	7.2
Lysine	3.1	4.7	1.2	7.7
Methionine	0.8	1.1	0.8	2.9
Phenylalanine	2.7	3.7	2.1	3.8
Threonine	2.1	2.7	1.5	4
Tryptophan	0.8	1	0.3	1.1
Valine	2.8	3.7	2.1	6
EAA sum	24.2	33.4	17.7	45.7
Proximate values				
Crude protein	54.9	73.2	40.3	70
Crude lipid	1.3	0	4.8	9.7
Crude fiber	2.8	0.2	8.6	0
Ash	7.7	6	2.4	20.3

Table 2: Diet formulations (dry matter basis, g/100g) containing menhaden fish meal (FM), fermented soybean meal (FSBM), soy protein concentrate (SPC), and high protein distiller's dried grain (HP-DDG).

	Diets				
Ingredients (%)	1	2	3	4	5
Menhaden FM[a]	30	0	0	0	0
HP-DDG[b]	30	30	30	30	30
FSBM[c]	0	30	30	0	0
SPC[d]	0	0	0	30	30
Whole wheat flour[e]	15	15	15	15	15
Corn gluten meal[f]	10	10	10	10	10
Menhaden oil[a]	3	5.3	5.3	5.65	5.65
Celufil[g]	9	0.5	4.5	0	4
Vitamin premix[h]	0.5	0.5	0.5	0.5	0.5
Ascorbic acid (Stay-C)[f]	0.05	0.05	0.05	0.05	0.05
Choline chloride[f]	0.2	0.2	0.2	0.2	0.2
Mineral premix[i]	0.1	0.1	0.1	0.1	0.1
Sodium chloride[f]	1	1	1	1	1
Potassium chloride[f]	0.8	0.8	0.8	0.8	0.8
Magnesium oxide[f]	0.05	0.05	0.05	0.05	0.05
Calcium phosphate[f]	0.2	2.6	2.6	2.6	2.6

Phytase[j]	0.037	0.037	0.037	0.037	0.037
Amino acid complex[kl]	0	4	0	4	0
Arg	~	0.5	~	0.5	~
His	~	0.3	~	0.3	~
Ile	~	0.2	~	0.2	~
Lysine	~	1.5	~	1.5	~
Met	~	0.5	~	0.5	~
Glx	~	0.5	~	0.5	~
Tau (sulfonic acid)	~	0.5	~	0.5	~

[a]Omega Protein, Inc., Houston, TX, USA; [b]Poet Ethanol, Chancellor, SD, USA; [c]PepSoyGen Nutraferma, North Sioux City, SD, USA; [d]The Solae Company, St. Louis, MO, USA; [e]Bob's Red Mill Natural Foods, Inc., Milwaukie, OR, USA; [f]Consumers Supply Distributing Company, Sioux City, IA, USA; [g]USB Corporation, Cleveland, OH, USA; [h]ARS 720 Vitamin Premix, USDA Agricultural Research Service, USA; [i]ARS 640 Trace Mineral Premix, USDA Agricultural Research Service, USA; [j]Ronozyme P, DSM Nutritional Products, Ames, IA, USA; [k]Arg, DL-Arginine; His, DL-Lysine; Ile, DL-Isoleucine; His, DL-Histidine; Glx, DL-Glutamine; Met, DL-Methionine; Tau, DL-Taurine; [l]Pure Bulk, Inc., Roseburg, OR, USA.

Table 3: Diet composition analyses (g/100g, db). Gross energy (GE) was estimated as crude protein × 23.6 MJ/kg + crude fat × 39.5 MJ/kg (NRC 2011). Analysis was conducted on post-extrusion pellets. Amino acid requirements are estimated (EST) values from Hart et al.

	Diets					
Component (%)	**1**	**2**	**3**	**4**	**5**	**EST**
Crude protein	42.9	42.11	38.4	47.45	44.15	
Arginine	1.81	2.17	1.8	2.62	2.23	2
Histidine	0.86	1.07	0.89	1.23	1.03	0.9
Isoleucine	1.6	1.79	1.64	2.03	1.84	1.4
Leucine	3.99	4.1	4.1	4.41	4.41	2.3
Lysine	1.92	2.58	1.46	3.01	1.83	2.6
Methionine	0.84	0.97	0.6	1	0.62	0.8
Phenylalanine	1.86	1.98	1.92	2.21	2.22	1.4
Threonine	1.4	1.35	1.33	1.46	1.44	1.4
Tryptophan	0.45	0.52	0.47	0.55	0.51	0.3
Valine	1.86	1.8	1.86	2.06	2.09	1.6
EAA sum	16.59	18.33	16.07	20.58	18.22	
Crude lipid	8.14	8.82	8.13	7.64	6.75	
Crude fiber	2.51	4.04	3.09	2.76	2.81	
GE (MJ/kg dry matter)	13.34	13.42	12.27	14.22	13.09	

Feeds were processed using a single-screw autogenous extruder (Bepex International LLC, Minneapolis, MN, USA), with a barrel length of 600 mm and a barrel length to diameter ratio of four, and barrel speed of 228 rpm. Feeds were extruded into 2-mm diameter pellets, dried at room temperature, coated with fish oil, crumbled and sieved to achieve uniform pellet size, and stored at -20°C. Diets were analyzed for crude protein (AOAC [42], method 990.03), crude fat (AOAC [42], method 990.03), crude fiber (AOAC [42], method 978.10), moisture (AOAC [42], method 934.01), ash (AOAC [42], method 942.05), and amino acids [AOAC [42], method 982.30 E (a,b,c)].

Fish and culture system

Age-0 Yellow Perch were held in a 340-L recirculating aquaculture system (RAS) and feed trained to accept a commercial pelleted diet (BioDiet Grower, Bio-Oregon, Warrenton, OR) for a period of 63 days. Following the feed training interval, 360 fish (8.6 ± 0.4g) were randomly selected and stocked into 20, 110-L circular tanks to provide four replicate tanks per diet with 18 fish per experimental unit.

The stocking rate was determined by the predicted loading density of the RAS estimated for the end of trial. Total tank weights (±0.5 g) were measured and a random subsample of 20 fish was selected for individual length (±1 mm) and weight (±0.1 g) measurements. Fish were fed the reference diet (1) (Table 2) during a two-week acclimation period then diet treatments were randomly assigned to replicate tanks.

The feeding trial was conducted in a closed loop recirculation system consisting of a solids separation tank, bio-reactor, and 100-µm bag, charcoal and UV irradiation filtration. System flow provided 25 exchanges per 24-hour period. Water temperature was held constant at 23°C with an 1800 watt, single-phase bayonet heater (Process Technology, Mentor, OH). Culture tanks were wrapped with double-backed foil insulation to help maintain water temperatures. Fecal and uneaten feed solids were removed daily with a siphon.

Nitrite (Hach [43], method 8153), nitrate (Hach [43], method 8039), and total and free ammonia (Hach [43], method 8038) nitrogens were monitored weekly using a Hach DREL 2000 spectrophotometer (Hach Company, Loveland, CO). Water pH was measured weekly using an Oakton multi-parameter PCS Testr 35 (Eutech Instruments, Vernon Hills, IL).

Dissolved oxygen (DO) was measured twice weekly using an YSI Model 55 DO meter (Yellow Springs Instrument Corp., Yellow Springs, OH). Water quality remained favorable throughout the duration of the trial. Temperature was held constant at 23°C and pH ranged from 7.4 to 8.0. Nitrate-nitrogen ranged from

4.2 to 8.4mg/L. Nitrite-nitrogen ranged from 0.052 to 0.229 mg/L throughout the duration of the feeding trail. Unionized free ammonia was calculated from total ammonia nitrogen and ranged from 0.002-0.008 mg/L.

Values for nitrite and ammonia nitrogen remained well below the tolerance levels (0.29 mg/L and 0.77 mg/L) reported for Yellow Perch [43]. The DO ranged from 7.0 to7.5 mg/L. Photoperiod was maintained at 15h light: 9h dark for the duration of the feeding trial.

Fish were hand-fed fixed rations of 2 to 3% of tank biomass, split into two feedings per day. Consumption was monitored to estimate feed intake, and to minimize waste in the RAS. Total tank weights were measured every 21 days and feed rations were adjusted according to tank weight and observed consumption. Upon completion of the feeding trial, total tank weights were measured as well as individual lengths and weights.

Whole body, liver, viscera, and fillet weights were measured to determine organosomatic indices, condition, and muscle ratio (MR). Muscle tissues were collected from euthanized fish for proximate composition and amino acid analyses.

Fish analyses

Performance indices were used to determine responses to treatments. Percent weight gain was calculated as WG=100 × (final weight (g)-initial weight (g))/initial weight (g)) [4]. Feed conversion ratio was estimated as FCR=(weight of diet fed (g)/ total wet weight gain (g)) [4]. Consumption was estimated as the total amount of feed fed minus the unconsumed portion. Protein efficiency ratio was estimated as PER=(weight gain (g)/ protein fed (g)) [4].

Following completion of the trial, five fish from each experimental unit were euthanized (150 mg/L tricaine methanesulfate) after a 24-hr fasting period. General health and condition indices were determined from necropsy data including: viscerosomatic index [VSI=(visceral weight (g)/body weight (g)) × 100], hepatosomatic index [HSI=(liver weight (g)/ body weight (g)) × 100], and muscle ratio [MR=((fillet weight (g) × 2)/body weight (g)) × 100] [4]. Fulton-type condition factor was calculated [$K=(W(g)/L(cm)^3) \times 100{,}000$] using individual lengths and weights.

Statistical analysis

All response variables were analyzed using one-way analysis of variance (ANOVA). Significant ANOVA results ($p \leq 0.05$) were further analyzed with Tukey's range tests to determine mean differences [44,45]. Systat (version 11) software (SPSS Inc. Chicago, IL) and Microsoft Excel (Microsoft, Redmond, WA) were used to perform all statistical analyses.

RESULTS

No mortalities were observed during the trial and all fish fed actively on the experimental diets. During the trial, no visible sign of nutritional deficiencies or sickness were observed. When EAAs were compared among the FM and plant ingredients, FM contained the highest total concentrations of EAAs, followed by SPC, FSBM, and HP-DDG, respectively (Table 1). SPC and FSBM met or exceeded the estimated amino acid requirement for Yellow Perch suggested by Hart et al. [41]. HP-DDG failed to meet the estimated requirement for arginine and lysine, suggesting the need for EAA supplements. SPC had a similar crude protein content compared to FM (Table 1).

The only diet to exceed the suggested lysine requirement by Hart et al. [41] was HP-DDG+SPC with EAA supplements (Table 3). Treatment diets containing FSBM failed to meet the estimated requirement of threonine (Table 3). Diets without FM or EAA supplements did not meet the estimated requirement of methionine (Table 3).

Muscle ratio was not significantly different (F=2.56, df 4, 15, P=0.08) among treatments, however supplemented EAAs appeared to have a positive effect on fillet weight as the diets that contained EAAs had the higher MR values (Table 4). Fillet amino acid composition only differed for histidine in fish fed the HP-DDG+FSBM diet, (Table 5). Fillet protein percentages ranged from a low of 89% for fish fed HP-DDG+FSBM to 93% for fish fed the reference diet.

Consumption was significantly different (F=32.52, df 4, 15, P<0.01), but only for fish fed HP-DDG+FSBM. Fish fed diets containing SPC, regardless of EAA supplementation, consumed the most feed however consumption was highest overall for fish fed HP-DDG+SPC+EAA. Diet composition also significantly affected PER (F=55.3, df 4, 15, P<0.01) and FCR (F=28.3, df 4, 15, P<0.01); diets supplemented with amino acids had more favorable PER and FCR values (Table 4). Protein efficiency ratios were more favourable in diets containing EAAs when compared to their counterparts; however, SPC diets had higher PERs than all other diets. Feed conversion ratios were improved with EAAs additions to FSBM and SPC blends. FSBM had the highest FCR values followed by the reference diet; both diets containing SPC had the lowest FCR's.

Growth performance was significantly influenced by diet composition (F=131, df 4, 15, P<0.01) (Table 4). Overall weight gain was highest in fish fed diet HP-DDG+SPC+EAA. FSBM with EAAs produced similar weight gain to the FM+HP-DDG reference diet. FSBM without EAAs produced the least weight gain of any experimental diet.

Condition factor was significantly affected by diet composition (F=2.56, df 4, 15, P<0.01) (Table 4). HSI only differed between the reference diet (lowest) and the HP-DDG+FSBM (highest) treatments. VSI was significantly different (F=3.51, df 4, 15, P=0.03). No correlation was observed in VSI with SPC or FSBM based diets, nor EAA supplements.

Table 4: Mean weight gain (WG), food conversion ratio (FCR), protein efficiency ratio (PER), viscerosomatic index (VSI), hepatosomatic index (HSI), Fulton-type condition factor (K), muscle ratio (MR) of experimental diets containing varying levels of feed grade soy protein concentrate (SPC) and with or without amino acid (EAA) supplements. Values are treatment means (±SE) for experimental diets. Values not significantly different (P>0.05) have the same letter within a given column.

Diet	EAA	WG (%)	FCR	PER	VSI (%)	HSI (%)	K	MR (%)
1 (HP-DDG+FM)	No	63.7 ± 6.1 z	3.40 ± 0.27 z	0.81 ± 0.07 z	8.5 ± 0.43 z	1.54 ± 0.08 z	1.14 ± 0.07 z	28.3 ± 1.30 z
2 (HP-DDG+FSBM)	Yes	63.8 ± 7.4 z	3.10 ± 0.33 z	0.90 ± 0.90 z	10.5 ± 0.41 y	1.80 ± 0.11 zy	1.14 ± 0.01 z	31.0 ± 0.81 z
3 (HP-DDG+FSBM)	No	19.1 ± 2.8 y	10.5 ± 1.44 y	0.34 ± 0.60 y	10.7 ± 0.48 y	1.97 ± 0.15 y	1.02 ± 0.02 y	28.4 ± 1.18 z
4 (HP-DDG+SPC)	Yes	178.3 ± 3.6 x	1.50 ± 0.02 z	1.58 ± 0.02 x	10.3 ± 0.68 y	1.64 ± 0.12 zy	1.30 ± 0.02 x	32.5 ± 1.04 z
5 (HP-DDG+SPC)	No	80.0 ± 4.1 w	2.71 ± 0.10 z	0.97 ± 0.04 z	10.0 ± 0.21 y	1.71 ± 0.10 zy	1.20 ± 0.02 z	30.8 ± 1.30 z

Table 5: Resulting amino acid (EAA) and proximate compositions (g/100g db) of Yellow Perch fillets. Values not significantly different (P>0.05) have the same letter within a given row.

	Diet (FM/(FSBM or SPC))				
Amino Acid	**1**	**2**	**3**	**4**	**5**
Arginine	5.50 ± 0.08 z	5.34 ± 0.07 z	5.19 ± 0.11 z	5.47 ± 0.04 z	5.36 ± 0.05 z
Histidine	2.73 ± 0.03 z	2.82 ± 0.05 z	2.64 ± 0.04 x	2.84 ± 0.02 z	2.80 ± 0.02 z
Isoleucine	4.43 ± 0.04 z	4.27 ± 0.09 z	4.13 ± 0.10 z	4.38 ± 0.06 z	4.26 ± 0.07 z
Leucine	7.59 ± 0.10 z	7.27 ± 0.10 z	7.16 ± 0.12 z	7.44 ± 0.05 z	7.35 ± 0.06 z
Lysine	8.83 ± 0.12 z	8.51 ± 0.10 z	8.26 ± 0.14 z	8.68 ± 0.08 z	8.52 ± 0.05 z
Methionine	2.82 ± 0.03 z	2.74 ± 0.04 z	2.66 ± 0.06 z	2.80 ± 0.02 z	2.75 ± 0.03 z
Phenylalanine	4.07 ± 0.06 z	3.86 ± 0.07 z	3.86 ± 0.07 z	3.97 ± 0.02 z	3.92 ± 0.03 z
Threonine	3.92 ± 0.07 z	3.76 ± 0.07 z	3.74 ± 0.05 z	3.83 ± 0.04 z	3.85 ± 0.08 z
Tryptophan	1.14 ± 0.03 z	1.14 ± 0.06 z	1.07 ± 0.03 z	1.18 ± 0.02 z	1.15 ± 0.01 z
Valine	4.64 ± 0.05 z	4.56 ± 0.06 z	4.35 ± 0.09 z	4.58 ± 0.06 z	4.55 ± 0.11 z
	Proximate Composition (%)				
Crude Protein	93.4	90.13	89.86	92.02	91.43
Crude Fat	3.83	5.77	4.86	4.77	5.27
Crude Fiber	0.16	0.07	0.23	0.06	0.07
Ash	8.74	8.34	9.15	8.46	8.09

DISCUSSION

No mortalities occurred during this study. Similar results were found by Cheng et al. [46] who reported high survival rates of Rainbow Trout *Oncorhynchus mykiss* fed diets containing DDGS +SBM in which only one of the diet combinations test resulted in reduced survival. Kasper et al. [37] noted that higher numbers of mortalities were observed in Yellow Perch as SBM increased in the diet. Schaeffer et al. [34] observed nearly 100% survival (two mortalities) when feeding DDGS+SBM to Yellow Perch. No mortalities were observed for Yellow Perch fed FSBM as a replacement for FM at various levels up to 100% [13]. Von Eschen et al. [21] reported 100% survival in Yellow Perch fed varying levels of conventional DDGS+SPC with and without EAA supplements. The results from these studies indicate Yellow Perch can utilize combinations of corn derived protein and soy protein without negatively impacting survival.

Measurements for growth, PER, MR, and FCR indicated that diets containing SPC showed the most favorable results (Table 4). Supplemented and unsupplemented diets containing SPC displayed better performance (i.e. WG, FCR, PER, and K) than all other diets. Similar results were observed by Von Eschen et al. [20] who fed Yellow Perch combinations of DDGS+SPC, who observed increased performance of Yellow Perch when EAA were added to the diets. MR and FCR were also higher than the reference diet however, neither was significantly different. Results from this study demonstrate that ingredient blends such has HP-DDG+SPC can be used to replace FM providing that EAA supplements are included in the diet of Yellow Perch. HP-DDG+FSBM without EAA supplements do not appear to be a suitable direct-replacement for FM. Fish fed HP-DDG+FSBM did not achieve any appreciable weight gain during the first 42 days of the trial, and failed to reach a 20% growth increase by the end of the trial. Fish fed diets containing HP-DDG+FSBM performed the poorest in nearly every metric measured. Von Eschen et al. [13] found that as FSBM directly replaced FM in the diets of Yellow Perch, performance decreased in WG and FCR. However, in this study when EAA supplements were incorporated with a HP-DDG+FSBM diet blend, performance did improve and was similar to the reference diet. Schaeffer et al. [34] tested conventional DDGS (27.9% CP, 11.5% CL) in combination with (defatted, toasted) soybean meal and reported that Yellow Perch were able to utilize combinations of fuel-based DDGS and soybean feedstuffs. However, the authors did not determine if amino acid supplements increased Yellow Perch performance. Von Eschen et al. [21] determined when EAA were added to combinations of conventional DDGS+SPC performance increased. Other studies have determined the effectiveness of amino acid supplements in plant-based fish feeds. Cheng et al. [46] noted success using DDGS+SBM with lysine and methionine supplements in Rainbow Trout diets, in that weight gain did not differ from a FM-based reference diet. Davis and Morris [47] found that while Rainbow Trout growth was limited when soy-based proteins were added to the diet, inclusion of EAAs increased fish performance.

With EAA supplementation, HP-DDG+SPC+EAA met and or exceeded estimated EAA requirements suggested by Hart et al. [41] for Yellow Perch (Table 3). The reference diet and un-supplemented experimental diet compositions failed the meet the minimum estimated requirement for lysine and methionine, and only experimental diets without EAA supplements failed to reach the requirement for methionine. The EAA concentrations determined in HP-DDG+SPC+EAA provide an explanation as to why this diet performed the best in nearly all measured performance responses. Additionally, HP-DDG+SPC failed to reach the minimum requirement for lysine and methionine yet still produced greater growth performance than the reference diet, which was found to be deficient in arginine, histidine, and lysine. The results are further evidence that fish are better able to utilize plant proteins with EAA supplements [46-48].

The amino acid complex improved Yellow Perch FCR values (Table 4). Cheng et al. [46] noted similar findings, observing that FCR values improved with lysine and methionine supplements until methionine reached 2.2% in diets fed to Rainbow Trout. The FCR values for un-supplemented diets in this study are similar to those reported by Schaeffer et al. [34] who fed DDGS+SBM to Yellow Perch found that the diet containing 40% DDGS+9.5% SBM had the lowest FCR values. Lowest values in this study, of diets without EAA supplements, were observed for HP-DDG+SPC. Takagi et al. [48] found feed efficiency in juvenile Red Seabream *Pragus major* improved when methionine or a combination of methionine and lysine were added to diets containing SPC, however feed efficiency did not meet that of the FM diet. Those authors also determined that methionine and lysine had a more pronounced positive effect on growth in juvenile Red Seabream compared to yearling Red Sea Bream.

The presence of EAA supplements improved PER values over diets not containing EAA supplements. Von Eschen et al. [21] noted improved PER when EAA were added to plant protein diets fed to Yellow Perch. Lim et al. [49] noted that PER increased when lysine was added to a DDGS+SBM diet fed to Channel Catfish *Ictalurus punctatus*. Takagi et al. [48] found that PER increased in Red Seabream fed SPC when methionine, or methionine+lysine, was supplemented in the diets, however when only lysine was added to SPC diets it failed to provide the same PER as SPC without supplements. The authors also found that EAA supplements had a greater impact on PER in juvenile fish when compared to yearling fish. Zhou et al. [36] noted that hybrid catfish (Channel Catfish *Ictalurus punctatus* × Blue Catfish *I. furcatus*) fed 20% and 30% DDGS with lysine in combination with SBM had higher PER values than the same DDGS concentrations without additional lysine supplements.

HSI values were lower but not significantly different for fish fed diets with EAA supplements but were lowest for fish fed HP-DDG+FM. Von Eschen et al. [21] observed no significant difference in HSI values when Yellow Perch were fed 20 or 40% DDGS+SPC with and without EAA supplements. Schaeffer et al. [34] found increasing HSI values with decreasing SBM and increasing DDGS in the diets of Yellow Perch. However, the authors noted that 40% DDGS+9.5% SBM had the highest HSI values, this diet also produced the highest growth. Takagi et al. [48] found increasing HSI values when feeding SPC with EAA supplements to juvenile Red Seabream. The authors noted that EAA supplements increased HSI values in juvenile fish, with the highest values derived from fish fed a FM reference diet.

Condition (K values) were significantly higher in Yellow Perch fed diets with HP-DDG+SPC+EAA, however both diets containing SPC had the highest K values indicating a higher relative robustness. Conversely, fish fed the HP-DDG+FSBM had significantly lower K values. Schaeffer et al. [34] found no difference in K values until DDGS inclusion reached 50%. Von Eschen et al. [21] observed a difference in K values in only two of their study diets fed to Yellow Perch.

Significant VSI differences were observed in fish fed HP-DDG +FM versus fish fed diets containing 100% plant-proteins. Plant protein supplements had a significant impact on Yellow Perch VSI, producing higher VSI values than the diet without, indicating higher lipid storage. Different results were observed by Schaeffer et al. [33] who fed DDGS+SBM to Yellow Perch; they found no difference in VSI's regardless of varying amounts of DDGS+SBM. The results from that study and the current study indicate that Yellow Perch can utilize corn- and soybean-based feedstuffs without affecting VSI. Overall, no differences were detected in MR, but the reference diet had the lowest MR while HP-DDG+SPC+EAA had the highest MR (Table 4). Similarly Schaeffer et al. [34] found no difference in MR when feeding Yellow Perch varying amounts of DDGS+SBM.

CONCLUSION

In conclusion, when SPC combined with HP-DDG was supplemented with EAA the feedstuff provided promising results. Additionally when not supplemented with EAA the SPC diet performed better than the reference diet for most performance metrics. It appears that SPC offers a favourable alternative when compared to fishmeal in combination of HP-DDG. The primary drawback is that soy protein concentrate ($1,000-$2,000 per metric ton) will not offer a financial benefit over FM ($1,300-1,500 per metric ton) until processing costs decrease. This should not discourage future research of this protein source based on the performance of the product when used as a FM substitute with EAA supplements. FSBM does offer a financial benefit to culturists and researchers though ($900 per metric ton), but without EAA supplements this product appears to be an unsuitable direct replacement for FM when accompanied with HP-DDG. When FSBM is supplemented with EAA and combined with HP-DDG it achieves similar performance to FM and in that case offers a comparable alternative to FM.

ACKNOWLEDGMENTS

The authors thank the South Dakota Soybean Research and Promotion Council, Hatch funds, grant no. SD00H404-11/ project accession no. 227032 from the USDA National Institute of Food and Agriculture, South Dakota Agricultural Experiment Station, Department of Natural Resource Management at South Dakota State University, and the South Dakota Department of Game, Fish and Parks for funding, facilities, equipment, and supplies. Furthermore, the assistance S. Nichols, K. Mjoun and numerous technicians was greatly appreciated.

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