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Growth and physiological effects of replacing fishmeal with dry-extruded seafood processing waste blended with plant protein feedstuffs in diets for red drum (*Sciaenops ocellatus* L.)

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Highlights

- The present study proposed an alternative method to recycle the by-products of fish processing plants by mixing with plant protein feedstuffs through dry extrusion.
- The resulting products were nutrient-enriched ingredients that could possibly reduce processing wastes and diminish aquaculture's reliance on marine ingredients derived from wild-caught animals.
- The replacement of fishmeal by the processed blends in the red drum diets enhanced production performance and did not negatively affect most of the physiological markers.

ABSTRACT

The utilization of seafood processing waste (SPW) is a potential means of reducing aquaculture's reliance on marine forage fish. Therefore, in an effort to recycle valuable nutrients such as high-quality proteins and polyunsaturated fatty acids from potentially wasted seafood processing, a novel approach was evaluated to enrich plant-derived feedstuffs. Four thermally-processed blends were manufactured by dry-extruding a mixture of either soybean meal (SBM) or distillers dried grains with solubles (DDGS) with two different ratios of SPW (60:40, and 40:60 of SPW: plant-derived feedstuffs on a wet-weight basis). Five diets were formulated to contain 36% of crude protein and 12% of lipid, and each of the four blends comprising treatments (SBM 60:40, SBM 40:60, DDGS 60:40, DDGS 40:60) which contributed 30% of the dietary crude protein, with SBM providing 45% and FM providing the remaining 25%. The reference diet had its protein provided solely by FM. Groups of 30 fish (~98.8 g/fish) were distributed into 15 fiberglass tanks (1200 L), and fed the experimental diets in triplicate to apparent satiation twice a day for 8 weeks. At the end of the trial, four fish per tank had their intestine samples collected and flash frozen to measure digestive enzymes activities. The remaining fish were pooled per treatment, re-distributed into two tanks per treatment, and fed the experimental diets for an additional week. A transport-induced stress challenge was then performed, and fish were transported in a hauling tank for 2 h. Blood hematocrit, and plasma cortisol, lactate and osmolality, were measured from four fish per treatment at five sampling points: prior to and 30 min after transportation, and at 24, 36 and 48 h after the transport-induced stress challenge. Weight gain was significantly affected by the dietary treatments, with fish fed all blends but the SBM 40:60 outperforming those fed the reference diet. Fish fed all treatments also were significantly different from those fed the reference diet for hepatosomatic index. Dietary treatments also significantly impacted, relative to the reference diet, the activity of trypsin, alkaline phosphatase, and amylase. A lower percentage of red blood cells were observed for fish fed SBM 60:40 when compared to those fed the reference diet, but only before the transport-induced stress challenge. Based on the results of this study, the inclusion of both SPW blends had a favorable influence on production performance of red drum while reducing the fishmeal and fish oil in the diet formulation.

Keywords: protein replacement, digestive enzymes, dry-extrusion, fishmeal, protein ingredient, transport stress challenge

Abbreviations: SBM: Soybean meal; DDGS: Distiller's dried grains with solubles; PSE: Pooled standard error; BW: Body weight; HSI: Hepatosomatic index; IPF: Intraperitoneal fat; PCE: Protein conversion efficiency

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Author contribution

Delbert M. Gatlin III: study conception and design, funding acquisition, assisted the sampling procedures, draft the manuscript; critical revision

Fernando Y. Yamamoto: conducted the experiment; assisted the sampling procedures; conducted laboratorial analysis; data curation; statistical analysis and interpretation of the data; drafting the original manuscript

Kequan Chen: conducted the experiment; assisted the sampling procedures; draft the manuscript

Sergio Castillo: assisted the sampling procedures; conducted laboratorial analysis; revising the manuscript

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Joseph P. Tomasso: study conception and design, funding acquisition, sampling procedures assistance; conducted laboratorial analysis; critical revision

1. INTRODUCTION

The rapid growth of aquaculture over the last several decades increased the demand for marine ingredients in the feed industry worldwide, escalating the prices of fishmeal and fish oil, both of which are often produced from highly regulated captured forage fisheries (Froehlich et al., 2018). Even with the refinement and development of novel alternative ingredients (*e.g.*, insect meals, microbial and microalgae meal, etc.), their volume, price, and availability restrict their inclusion in fish feeds (Hua et al., 2019; FAO, 2020). On the other hand, fishmeal is still considered a "golden reference" for aquafeed ingredients by having a well-balanced essential amino acid profile and long-chain polyunsaturated fatty acids, while being highly digestible and palatable for farmed fish and crustaceans.

The predominant use of this resource by aquaculture has raised concerns and labeled its practices as unsustainable in the eyes of the general public when some press outlets reported that fish farming is strictly converting low-value wild fish to highly-priced farmed carnivorous fish. In response to those accusations, reduction and/or elimination of wild-caught marine ingredients has become a challenging priority which also relates to increasing the economic viability of fish and shellfish production while limiting dependence on marine resources. But additional steps still can be taken towards more environment-friendly aquaculture, like recycling nutrients from fisheries by-products wasted by the seafood processing industry and converting them into nutritious feed ingredients. Such approach holds promise for not only making more efficient use of harvested marine resources, but also decreasing the reliance of aquafeeds on forage fish products (Yan and Chen, 2015; Mo et al., 2018).

Historically, fish by-products were disposed of as waste; fed directly as "trash-fish" for in aquaculture, processed to feed livestock, pets, or other animals, made into silage or plant fertilizers, or simply dumped in the sea or landfills (Olsen et al., 2014; FAO, 2020). Roughly 35% of the global captured and farmed fish is wasted throughout the food supply chain (FAO, 2020). In fish processing plants alone, approximately 11.7 million tonnes of fish by-products are not collected yearly but could be available for the production of marine ingredients (Jackson and Newton, 2016). The last figure could represent an increment of 65% of marine products to the 18 million tons of forage fish captured on average each year that are reduced into fishmeal and fish oil (FAO, 2020). Manufacturing fishmeal and fish oil from seafood processing waste (SPW) has been gaining increased attention as prices or marine-derived ingredients increase, and it can represent up to a quarter of global fishmeal and fish oil production (FAO, 2020). European countries, as leading examples, have been able to reutilize their processing waste and halve the use of whole-fish for fishmeal production (Jackson and Newton, 2016). But producing fishmeal from SPW often yields an ingredient with lower protein and higher mineral contents than traditional forage fishmeal, (Naylor et al., 2009). In addition, fishmeal manufacturing can be a costly and energy-demanding multistep process that requires large volumes and a constant supply of raw materials to be economically viable, which can be a constrain when producing a less nutritious ingredient (Naylor et al., 2009). Moreover, transporting SPW from the processing plant to the fishmeal manufacturer can be logistically challenging if not stored and transported in appropriate conditions, because this commodity is highly perishable not only due to its high microbial and endogenous enzyme load but also for the high content of long-chain polyunsaturated fatty acids that are prone to oxidation (Khawli et al., 2019). Therefore, a cost-effective approach to recycle the valuable nutrients from SPW may be attained at the processing plant by applying dry-extrusion technology, in which ground SPW is mixed with plant protein ingredients, then partially dried and sterilized with brief exposure to high temperature and pressure provided by the sheer of a single-screw extruder.

The dry extruder was initially developed to process soybeans and other grains on farm, in order to reduce anti-nutritional factors and increase lipid digestibility of processed ingredients to livestock and poultry (Kearns, 2018). Using friction as the only source of heat, the single-screw, relatively low-cost extruder, is able to cook, sterilize and dehydrate products in a high-pressure environment in a relatively short time (~30 seconds) (Nelson et al., 1987; Said, 2000). The dry-extrusion processing method also has proven to be a practical approach to reduce the pathogenic bacterial load of raw ingredients (Said, 1996; Kelley and Walker, 1999;) and adequately recycle rendered products like poultry by-products (Samocha et al., 2004; Bandegan et al., 2010), as well as fish and shellfish by-products for animal feeds (Carver et al., 1988; Hernández et al., 2004). Bringing this to light, dry extrusion can be a relatively inexpensive alternative processing method to reduce wastes from seafood processing plants and recycle the invaluable nutrients such as high-quality proteins, essential amino acids, highly unsaturated fatty acids and essential trace minerals, from their by-products.

Red drum (*Sciaenops ocellatus*) is a well-established species for aquaculture that naturally occurs from Tuxpan (Mexico) in the Gulf of Mexico to Massachusetts (USA) in the Atlantic Ocean (Matlock, 1987). In the early 1980's, the aggressive capture of the wild stocks by commercial fisheries led to restricted management regulations in the United States, including closure of the commercial fishery in the Gulf of Mexico and federally protecting it as a game species in 2007 (Watson et al., 2014). Fortunately, the declining wild stocks of red drum prompted the development of technology for aquacultural purposes, such as optimizing spawning and fingerling production in captivity for enhancement of natural stocks as well as for fish farming. In its natural habitat, this species has a high-trophic-level feeding behavior, preying mainly, arthropods, mollusks, and other fish (Matlock, 1987). However, due to extensive research to determine various nutrient requirements of this species including all indispensable amino acids (Gatlin, 2002; NRC, 2011) and development of practical diets, in captivity, this strict marine carnivore can consume manufactured diets containing relatively low levels of fishmeal and/or high levels of plant-derived feedstuff (PDFs)

(Rossi et al., 2013, 2017b; Denson et al., 2018; Watson et al., 2019). Another desirable characteristic for culturing red drum is its adaption to wide variations in water quality. This euryhaline species can thrive in a wide range of water salinity (Watson et al., 2014), tolerate moderate levels of ammonia and nitrite (Wise et al., 1989; Wise and Tomasso, 1989), and survive the seasonal temperature fluctuations as observed in their natural habitat (Neill, 1987).

The objectives of this study were two-fold. First, SPW co-products at two different ratios (40:60 and 60:40) were blended with two separate plant protein ingredients, soybean meal (SBM) and distillers dried grains with solubles (DDGS) then subjected to dry extrusion. Those co-products were evaluated in a comparative feeding trial to evaluate their impacts on growth performance and digestive enzyme activity of red drum. Second, at the end of the feeding trial, representative fish fed the various co-products were subjected to transport stress and their physiological responses were assessed.

2. MATERIALS AND METHODS

2.1 Manufacturing of Seafood Processing Waste (SPW) Blends

The raw materials used to manufacture the SPW blends consisted of viscera and skeletal remains from filleted black drum (*Pogonias cromis*), provided by Austin Seafood Products (Austin, TX), and SBM or DDGS, both provided by the Producers Cooperative Association (Bryan, TX). During transportation from Austin to the Texas A&M Process Engineering Research & Development Center (College Station, TX), the processing wastes were held in chest coolers filled with cube ice and then stored frozen at -20°C until further processed. The SPW was ground using an industrial meat grinder, mixed in a horizontal ribbon mixer for 15 min, with either SBM or DDGS at two different ratios (60:40 and 40:60; of feedstuff on a wet-weight basis). The resulting mixtures were dry-extruded by screw-pressing through an extruding barrel (Insta-Pro 600JR, Insta-Pro

International, Grimes, IA). The machine was primed with soybean grains to reach constant temperature (135˚C), and a fraction of each mixture initially exiting the barrel was discarded to ensure that the extruded products consisted exclusively of the specific blends. The processed blends were dried overnight using a forced airflow oven at 50˚C, ground using a hammer mill (LM6, Kelly Duplex, CO), and stored frozen (-20˚C) before incorporation into experimental diets. The raw SPW material, plant protein ingredients, and the manufactured blends were analyzed for proximate composition (AOAC, 2005) (Table 1), and gross energy was determined by combustion of the samples using a bomb calorimeter (Parr 6200; Parr Instrument Company, Moline, IL). The pH of the ingredients also was determined as described by Castillo et al. (2014), and the amino acid composition was determined using high-pressure liquid chromatography (UPLC-Acquity system, Waters, Milford, MA) according to procedures as described by Castillo et al. (2015).

2.2 Experimental diets and fish

All experimental diets were formulated to be isonitrogenous, isolipidic, and isoenergetic, containing 36% crude protein (CP), 12% crude lipid, and approximately 12 MJ kg^{-1} of digestible energy. Diets were formulated to meet the protein and amino acid requirements previously established for this species (Castillo et al., 2015; Castillo and Gatlin, 2018; Daniels and Robinson, 1986; Gatlin, 2002; Peachey et al., 2018). Each blend comprised a dietary treatment and was evaluated independently, contributing 30% of total CP in each diet while soybean meal contributed 45% of CP, and the remaining 25% was provided by menhaden fishmeal (Table 2). A diet based exclusively on menhaden fishmeal for its protein was formulated to serve as a reference. Each diet's ingredients were mixed using a V-mixer (Blend master, Buflovak, NY) for 30 min, and oil and water were gradually incorporated into the mixture using an industrial mixer (A-200 Hobart meat grinder, Hobart Corporation, OH). The resultant dough was cold-pelleted through a 5-mm die plate and dried at room temperature for 48 h. The dried pellets were ground manually using a corn mill grinder to appropriate size, sieved, and the resulting fines were sampled for proximate analysis, gross energy, and pH, using the methods referenced above.

Red drum fingerlings were provided by Texas Parks and Wildlife Department (Lake Jackson, TX) and transported to the Aquacultural Research and Teaching Facility of the Texas A&M University System. Fish were acclimated to local conditions and fed a commercial diet (Rangen Inc., Angleton, TX, 40% crude protein, 12% crude fat) until the desired average weight was attained (~100 g). This study was carried out with the compliance of the Institutional Animal Care and Use Committee at Texas A&M University (IACUC 2016-0368).

2.3 Comparative feeding trial

Prior to starting the feeding trial, ten fish were euthanized with an overdose (300 mg L^{-1}) of tricaine methanesulfonate (MS-222, Tricaine-S, Western Chemical Inc., Ferndale, WA) (Topic Popovic et al., 2012) for analysis of initial body composition. Groups of 30 red drum (~98.8 g) were stocked into 15, 1200-L circular fiberglass tanks operating as three independent recirculating systems. Each system consisted of five circular tanks which were each randomly assigned one of the experimental diets, resulting in a randomized complete block design with three replicates per diet. Water was recirculated to each tank, with exiting water flowing by gravity to a settling chamber and a biological filter. Before returning to the tanks, water was forcedly pumped through a sand filter and an ultraviolet chamber for mechanical filtration and reduction of the microbial load, respectively. Aeration was supplied to each tank through three air stones connected to a central regenerative blower system. The water temperature was kept steady throughout the trial by conditioning ambient air. A 12:12 h light: dark photoperiod was maintained using fluorescent lighting controlled by timers.

Fish were fed to apparent satiation twice a day for 56 days, during which they were individually counted and biomass quantified at days 0, 28, and 56. Partial water exchange was performed daily to maintain suitable water quality for red drum culture (Neill, 1987), and synthetic marine salt was added periodically to keep salinity within 3 ppt (Red Sea Salt, Red Sea U.S.A., Houston, TX). Water quality parameters for each system were measured thrice a week (Table 3). Dissolved oxygen and temperature were recorded using an optic dissolved oxygen meter (ProOdo, YSI, OH), pH was measured using a portable pH meter (Pocket Pro pH tester, Hach Company, Loveland, CO), salinity was measured using a portable salinity meter (EC170, Extech, Boston, MA) and total ammonia-, and nitrite-nitrogen dissolved in the culture water were measured photometrically (Hach DR 2000 spectrophotometer and test reagents, Hach Company). Fish were harvested 15 h after the last ration, where they were individually counted, group weighed, and seven fish per tank were euthanized with an overdose of MS-222 (~300 mg L^{-1}) for tissue collection, and measurement of condition indices including fillet yield, intraperitoneal fat (IPF) ratio and hepatosomatic index (HSI) (4 fish), as well as whole-body proximate composition (3 fish). One side of each fish was dissected and skinned to obtain the muscle yield values. Proximate composition of the whole-body was measured following the same procedures as used for the ingredients and prepared diets (AOAC, 2005). Production parameters and condition indexes were computed as follows:

$$\text{Weight gain (WG)(\% of initial)} = 100 \times [(\text{Final weight-initial weight})/\text{initial weight}]$$

Protein retention efficiency (PCE) (%)=

$$\{[(\text{Final body weight (g)} \times \text{final body protein (\%)}) - (\text{initial weight (g)} \times \text{initial body protein (\%)})]/\text{protein intake}\} \times 100$$

$$\text{Feed efficiency (FE)} = \text{weight gain}/\text{dry feed intake}$$

Feeding rate (% BW/day)

$$= \left[\text{dry feed intake (g)} \div \left(\sqrt{\text{initial body weight} \times \text{final body weight}}\right) \div \text{days on feed}\right] \times 100$$

$$\text{Muscle yield (\%)} = [\text{fillet muscle weight (g)} \times 2\ /\text{body weight (g)}] \times 100$$

$$\text{Viscerosomatic indices (HSI or IPF ratio)(\%)} = [\text{liver or IPF (g)}/\text{body weight (g)}] \times 100$$

$$\text{Survival (\%)} = 100 \times (\text{number of surviving fish}/\text{initial number of fish})$$

2.4 Digestive enzymes

Intestines from 4 fish per tank were aseptically dissected, stored in 5-ml centrifuge tubes, flash-frozen in liquid nitrogen, and stored at -80°C prior to tissue homogenization. The dissected intestines were segmented into three parts (anterior, medium, and posterior) to evaluate if fishmeal replacement by the manufactured SPW blends affected the activity of various digestive enzymes. The frozen segments were weighed and placed in a 2-ml microtube with cold Tris-HCl buffer (50 mM, 20 mM $CaCl_2$, pH 7), maintaining a ratio of 50 mg of wet tissue per mL^{-1} of buffer. Tissues were homogenized in ice with a handheld homogenizer (PT1200 E, Kinematica AG, Luzern, Switzerland), and centrifuged at 15,000 × *g* for 10 min. The supernatant of the homogenized samples were carefully pipetted out and aliquoted into individual 500-μL microtubes for each digestive enzyme analysis and stored frozen at -80°C. The activity of the following enzymes: trypsin, aminopeptidase, alkaline, and acid phosphatase, lipase, and amylase were determined as described by Anguiano et al. (2013) and Castillo et al. (2014).

2.5 Transport stress challenge and blood sampling

After sampling on the eighth week, the remaining fish were combined by treatment, and each treatment was re-distributed into two 1200-L circular fiberglass tanks each in separate recirculating systems. After the redistribution, fish were fed their assigned experimental diets once a day to apparent satiation for 1 week to fully recover from handling and sampling at the end of the feeding trial, and prior the transport stress challenge. Fish were fasted for 24 h prior to the transportation stress challenge, and then water from the culture system was pumped to a fiberglass transport tank mounted to a truck with five separate compartments (700 L each), and one tank of fish per dietary treatment was randomly assigned to each compartment. Pure oxygen was pumped through air stones to keep dissolved oxygen of the transport water above saturation levels. Four fish from each tank were netted, and blood was drawn immediately using a 21-G needle equipped with a heparinized sterile vacuum blood collection tube (6 mL), to establish basal levels of stress parameters. The remaining fish per tank were netted, placed in a hauling compartment and transported for 2 h, after which they were brought back to the Aquacultural Research and Teaching Facility. Upon arrival, fish were stocked back to their respective tanks. Then blood from a set of four fish from each dietary treatment was collected at 0.5, 24, 36, and 48 h after the transport challenge. Fish that had their blood sampled were placed in a separate tank in a third recirculating system to ensure that the same fish would not be sampled twice and any other biological or environmental conditions would not stress the remaining experimental fish. The transport stress challenge was performed twice, on two different days, to acquire data for two replicate groups of fish per dietary treatment. This procedure was performed in two different days due to the limited number of hauling compartments of the truck.

An aliquot of the whole blood was sampled to measure the ratio of red blood cells:plasma using glass hematocrit tubes. Briefly, blood samples were collected from the tube by capillarity in duplicate per sample and centrifuged at 10,000 × *g* for 10 min. The remaining blood was centrifuged at 3000 × *g* for 10 min, and the supernatant was aliquoted into separate vials using sterile 1-mL tips. Plasma cortisol was measured by an enzyme immunoassay (Cortisol ELISA, cat# EIA-1887, DRG International, Mountainside, NJ) following the manufacturer’s instructions. Frozen aliquots of plasma samples were shipped overnight in dry ice to Auburn University (Auburn, AL) where plasma glucose, osmolality, and lactate were measured. Plasma glucose was measured photometrically using a commercial kit by the glucose oxidase method (Glucose oxidase reagent cat# G7519-500, Pointe Scientific, Canton, MI). Osmolality was measured by a vapor pressure osmometer (Vapro 5520, Wescor Inc., Logan, UT). Plasma lactate was measured photometrically using a commercial kit (Lactate oxidase cat# L7596-50, Pointe Scientific) by oxidizing lactate to pyruvate and hydrogen peroxide.

2.5 Statistical analysis

Each experimental diet was assigned once to three independent recirculating systems, in a fashion that each system would be considered a statistical block, to reduce the variance provided by the environmental conditions and different water quality parameters. Data were thus analyzed using JMP PRO software (SAS Institute Inc., Cary, NC) by one-way ANOVA as a completely randomized block design (CRBC), having the independent recirculating systems as the statistical blocks. When significant differences were identified ($P<0.05$), test diets were compared to the fishmeal-based diet (reference) using Dunnett's multiple comparison procedure.

3. RESULTS

Most of the blends presented an enrichment pattern for protein, lipid, ash, and energy when compared to the raw plant protein ingredients (Table 1). As the inclusion ratio of SPW increased, the pH of the ingredient DDGS followed the same increasing trend. On the other hand, a downward trend was observed for the dehulled SBM ingredient when compared to the SBM blends. The concentration of all essential amino acids and taurine in the blends numerically increased as the ratio of SPW increased compared individually to the dehulled SBM and DDGS.

Final weight, weight gain, feeding rate, HSI, and fillet yield of red drum were significantly affected by the inclusion of the SPW blends (Table 4). Fish fed the treatments SBM 60:40, DDGS 60:40, and DDGS 40:60 had a higher final weight and weight gain which was significantly different from the reference group; whereas, fish fed SBM 40:60 had similar performance to that of the reference. Fish fed all blend treatments had a higher feeding rate and lower HSI value when compared to the reference. Fish fed the SBM 60:40 diet had a higher fillet yield than those fed the reference diet. No statistical differences were detected for IPF ratio, feed efficiency, or survival (Table 4). No differences were observed for protein conversion efficiency, or moisture, protein, lipid, and mineral composition of whole-body tissues (Table 5). Nevertheless, it is worth mentioning that whole-body lipid was marginally significant ($P=0.15$), with fish fed the reference and DDGS 60:40 diets having higher lipid content.

Higher trypsin activity was observed in the anterior part of the intestine for fish fed SBM 60:40, SBM 40:60, and DDGS 40:60 compared to fish fed the fishmeal-based reference diet (Table 6). Interestingly, significant differences among diets were observed for alkaline phosphatase in the three segments of the intestine. Fish fed SBM 60:40 and SBM 40:60 had a lower alkaline phosphatase activity in the anterior part of the intestine than fish fed the reference diet (Table 6). The alkaline phosphatase activity of the middle and posterior parts of the intestine for all fish fed the blends was lower when compared to fish fed the fishmeal-based reference diet. Amylase activity was significantly different for the posterior intestine with only the fish fed the SBM 60:40 diet having a higher activity when compared to those fed the reference (Table 6). No differences were observed for aminopeptidase, acid phosphatase, or lipase for the three intestinal segments regardless of diet (Table 6).

Red drum fed the SBM 60:40 diets had significantly lower blood hematocrit values than fish fed the reference diet prior to the transport stress challenge (Table 7). Plasma glucose and cortisol were significantly lower for fish fed all the dietary blends when compared to that of fish fed the reference diet 24 hours after the transport stress challenge. No differences were observed for plasma osmolality and lactate among the dietary treatments for the different time points after the transportation stress. All fish subjected to the transport challenge survived after the 48 h observation period.

4. DISCUSSION

A public misconception branded aquaculture as the main driver for the increased capture of forage fish to manufacture fish meal and fish oil (Froehlich et al., 2018). In reality, the rather stable reduction fisheries have long been used to produce feed ingredients, and only shifted in terms of consumers, when terrestrial livestock (*e.g.*, swine and poultry) producers sought other alternatives when the prices of fishmeal began to escalate (Froehlich et al., 2018). However, the necessity to meet the demand for quality ingredients in aquaculture feeds is paramount to ensure food security as the global demand for seafood continues to increase. Therefore, recycling SPW with the dry-extrusion technology can bring valuable but underutilized nutrients back into the food chain and possibly reduce feed cost formulation by naturally enriching plant protein ingredients with proteins and energy. This processing method also aligns with the target 12.3 of the 17 Sustainable Development Goals established in 2015 by the United Nations (UN), aiming to halve the food waste by 2030 (UN, 2015). Furthermore, uncertainties caused by global trade turmoil in previous years resulted in sudden scarcity/abundance of soybean meal and other plant protein products in the commodity markets (Fuchs et al., 2019; He et al., 2019). Thus, co-extruding these ingredients with SPW appears to be a desirable opportunity for processing plants and feed millers to reutilize raw materials and possibly reduce costs with a more nutritious ingredient.

By blending SPW with plant protein ingredients, a clear enrichment pattern for most nutrients could be numerically observed when compared to the plant protein ingredients *per se*. The most limiting amino acids for animals (*e.g.*, lysine and methionine), and the conditionally essential organic acid, taurine, were substantially enriched in the blends as the ratio of SPW increased. The same trend was observed for crude protein and lipids, minerals, and energy. These results corroborate findings from previous studies from our laboratory with SPW blends manufactured in a similar fashion (Yamamoto et al., 2020). During that investigation, however, we were able to manufacture blends with up to 70:30 ratio of SPW. As in the current investigation, we were unsuccessful in reproducing the same ratio levels of SPW to plant protein blends using DDGS. The composition of the SPW used in the current investigation varied somewhat from the earlier study (Yamamoto et al., 2020). Several other variables can greatly affect the nutrient composition of fish carcasses, such as life stage, season of collection, and storage conditions after processing. In addition to the proximate composition of the collected carcasses, perhaps the amount of fiber in this specific batch of DDGS used in current trial, could have also vary greatly depending on fermentation conditions (USGC, 2018), which may have hindered the dry-extrusion process. For this treatment, not only the minimum temperature for extrusion was not achieved, but also the fluidity of the mixture inside the extruder barrel was erratic, and the dye of the extruder was constantly plugging, presumably by the fibrous mixture. From this experience, the authors strongly suggest that for future studies or practical implementation, the SPW and plant protein ingredients should not surpass a 60:40 ratio. In addition, the nutrient composition of the SPW and the DDGS can vary, and when comparing the manufactured blends with the previous study conducted by our research group (Yamamoto et al., 2020), the resulting lipid enrichment of the plant protein ingredients were numerically higher, and the mineral enrichment was numerically lower. These variables that ultimately affect the nutritional composition of the blends may require them to be individually analyzed for proximate composition and perhaps mineral profile (*e.g.*, calcium and phosphorus) to ensure a precise feed formulation, if this method is to be translated to practical conditions.

In contrast to the findings of our previous study (Yamamoto et al., 2020), the advanced red drum juveniles fed all experimental treatments, except SBM 40:60, presented superior growth performance when compared to those fed the reference diet, in which the protein was solely provided by menhaden fishmeal. In that study, however, a lower digestibility of the protein from the menhaden fishmeal was observed when compared to the blends with DDGS of advanced red drum juveniles, which could be related to the growth results from the present study. It is intriguing that an ideal ingredient such as forage fishmeal would be outperformed by mixtures of SFW mixed with plant protein ingredients, nevertheless this was consistent with digestibility of other seafood processing waste materials and marine by-products (Li et al, 2004). Less favorable results with the highest inclusion of SBM may have been due to the lower levels of indispensable amino acids, despite the enrichment with seafood waste and the addition of crystalline amino acids to the diet formulation. The weight gain of red drum was likely limited by the reduced levels of methionine (requirement previously reported as 0.88-1.00 g/100 g of diet) (Moon and Gatlin, 1991) as the ratio of SBM increased.

Discussing the results from the present study is cumbersome because few studies have been published evaluating seafood waste by-products dry-extruded with plant protein feedstuffs. However, findings from this study could be compared to previous investigations reporting the effects of fishmeal replacement by plant-protein feedstuffs in the diet of red drum and other marine sciaenids, like yellow croaker (*Larimichthys crocea*), totoaba (*Totoaba macdonaldi*), and shortfin corvina (*Cynoscion parvipinnis*). In such studies, similar improved production performance was observed for fish fed diets partially replacing fishmeal with plant protein feedstuffs (Rossi et al., 2013, 2017b; López et al., 2015; Trejo-Escamilla et al., 2017; Wang et al., 2017). Other carnivorous marine species like the cobia (*Rachycentrum canadum*), Florida pompano (*Trachinotus carolinus*), and red sea bream (*Pagrus major*) also showed enhanced growth performance with intermediate levels of soybean meal replacing fishmeal (Zhou et al., 2005; Riche and Williams, 2011; Kader et al., 2012;). Conversely, many other studies have reported opposite findings, where incremental replacement of fishmeal by plant protein ingredients suppressed growth performance and feed efficiency of yellow croaker, red drum, and totoaba (Davis et al., 1995; Fuentes-Quesada et al., 2018; Rossi et al., 2017a; Villanueva-Gutiérrez et al., 2020; Wang et al., 2019; Watson et al., 2019). Fishmeal is an ingredient of superior nutrient availability and quality with a balanced amino acid profile, combined with its high protein and energy digestibility for red drum (Gaylord and Gatlin, 1996; Mcgoogan and Gatlin, 1997). The extrusion of processed blends evaluated in the present study could have potentially denatured protease inhibitors and reduced levels of other anti-nutritional factors from the plant protein feedstuffs to enhance the overall nutritional quality of the SPW blends Previous authors hypothesize that the partial inclusion of plant protein ingredients would provide dispensable amino acids that could be used as a metabolic substrate for energy production, and thereby promote growth (Riche and Williams, 2011). Another hypothesis to be considered in regard to the present findings is that the substantial lipid accretion to the blends originating from the SPW, increased the levels of monounsaturated fatty acids, palmitic and oleic acid (Arvanitoyannis and Kassaveti, 2008), which are preferred metabolic substrate for energy generation via β-oxidation (NRC, 2011).

Survival, whole-body proximate composition, and intraperitoneal fat of red drum were unaffected by the replacement of fishmeal with the SPW blends. However, the hepatosomatic index presented lower values, and the feeding rate and the fillet yield (SBM 60:40) were higher for fish fed the SPW blends when compared to the fish fed the reference diet. A reduced feed intake was also reported for red sea bream and yellow croaker fed fishmeal-based diets when compared to the treatments where soybean products replaced fishmeal (Kader et al., 2012; Wang et al., 2019). Those authors reported that enhanced feed intake of the diets could be attributed to a better palatability of the diets by the increasing levels of essential amino acids other marine supplements, which could be similar to the findings of the present study. It is speculated that a higher concentration of free amino acids (FAA) from the SPW could also have improved the organoleptic properties of the SPW blends and thus increased feeding rate. Hernandez et al. (2014) also reported similar findings of an enhanced feed intake of spotted rose snapper (*Lutjanus guttatus*) fed diets containing tuna by-products partially replacing sardine fishmeal, which the authors also hypothesized to be related to the FAA provided by the by-product. A comparable relationship between hepatosomatic index (HSI) and fishmeal replacement with soybean products has been observed in red drum and totoaba, presenting higher percentages for fish fed fishmeal-based diets (Minjarez-Osorio et al., 2016; Rossi et al., 2017b; Fuentes-Quesada et al., 2018). In a separate study with totoaba, hepatic composition and key enzymes of amino acid catabolism and gluconeogenesis were significantly affected when fish were fed diets containing soy protein concentrate (Bañuelos-Vargas et al., 2014). Although there were no significant differences in HSI of fish in the latter study, those fed fishmeal-based diets had livers containing more water and protein when compared to fish fed the other diets. Unfortunately, in the present study, the dissected livers were not preserved or chemically analyzed.

The modulation of intestinal enzymes upon dietary change has been described in teleost fish, which sometimes can adapt the secretion of enzymes to counteract protease inhibitors found in plant ingredients (Francis et al., 2001; NRC, 2011). The increased trypsin activity in the anterior intestine of fish fed the SPW blends corroborates this statement, along with previous studies investigating fishmeal replacement by soybean products with sciaenids and salmonids (Krogdahl et al., 2003; Refstie et al., 2006a; Wang et al., 2017; Villanueva-Gutiérrez et al., 2020). A reduced pH of the diets resulting from the addition of SPW to plant protein ingredients could have indirectly acidified the intestine and positively influenced the activity of trypsin in the anterior segment of the red drum intestine, especially for fish fed diets containing the DDGS-based blends. On the other hand, recent studies reported decreased activity of trypsin in rainbow trout (*Oncorhynchus mykiss*) (Kumar et al., 2020), and trypsin and chymotrypsin activities were negatively affected in red drum and totoaba (Rossi et al., 2017b; Fuentes-Quesada et al., 2018) when soybean products were included in the diet at the expense of fishmeal. These contrasting results were apparently related to lower feed intake and perhaps the disruption of normal digestive capacity caused by the anti-nutritional factors contributed by the soybean products. It is noteworthy to mention that unlike the previous reports, the present study used advanced juveniles, which could be less sensitive to the detrimental effects of soybean products as compared to juvenile fish, similar to what has been observed in salmonids studies (Storebakken et al., 2000; Kaushik, 2008).

The activity of enteric alkaline phosphatase (ALKP) has been proposed to be an indicator of the intestinal health and enterocyte maturation of teleost fish (Lallès, 2020). This enzyme is produced by the enterocytes, and it helps maintain intestinal homeostasis by reducing inflammation, detoxifying microbial endotoxins, improving the expression of tight junction proteins, and regulating intestinal microbiota (Bates et al., 2007; Rombout Jan et al., 2011; Lallès, 2019, 2020). In the present study, the replacement of fishmeal by the SPW blends significantly reduced the ALKP activity of red drum for all dietary treatments and intestinal segments, with the exception of DDGS 60:40 and 40:60 in the anterior part. A fraction of the DDGS ingredient consists of fermented yeast, which can be a source of functional nutrients like nucleotides, vitamins, and prebiotics (Shurson, 2018), and possibly diminished the potentially deleterious effects of fishmeal replacement, but only for the anterior segment of the intestine of red drum. Suppressed intestinal ALKP activity also was observed in totoaba and Atlantic cod (*Gadus morhua*) when fishmeal was gradually replaced by soybean products (Refstie et al., 2006b; Trejo-Escamilla et al., 2017; Fuentes-Quesada et al., 2018). Even though the growth performance of red drum was enhanced by the replacement of fishmeal with most SPW blends, the reduction in activity of this enzyme over almost all SPW treatments and intestinal segments may be of concern. The activity of ALKP is just one indicator of several that can reflect intestinal health of fish; nevertheless, this reduced activity deserves to be further explored in upcoming studies addressing the dietary inclusion of dry-extruded SPW blends. Increased intestinal amylase activity in the anterior part of the intestine was observed for the fish fed SBM 60:40, when compared to the intestine of the fish fed the reference diet. This finding is in agreement when fishmeal was replaced by soybean products in diets for Atlantic salmon (*Salmo salar*) and cod (Refstie et al., 2006a, 2006b).

Farmed fish are constantly being exposed to physical and environmental disturbances, such as handling, crowding, and chemical variation in water quality. These disturbances often disrupt the fish's normal physiological state, which can adversely affect its well-being (Green and Haukenes, 2015). During confinement and transport, all these stressors can be imposed on the fish in a short time-frame (Harmon, 2009). Dietary formulations and regimes can directly affect the fish's health and its compensatory mechanisms to cope with stressors, to restore homeostasis, and ultimately to combat pathogens (Trichet, 2010; Oliva-Teles, 2012). Prior to the transport-stress challenge, a reduced hematocrit was observed for fish fed the SBM 60:40 treatment compared to those fed the reference diet, which could be an indication that SBM 60:40 compromised erythropoiesis of those fish. Decreased hematocrit values also were observed for rainbow trout and amberjack (*Seriola dumerili*) fed diets containing high inclusion of soybean meal (Haghbayan and Mehrgan, 2015; Hossain et al., 2018). Nevertheless, no differences in hematocrit were subsequently detected for all treatments throughout the collection points in the transportation-stress challenge. Overall, no detrimental effects were detected with regard to hematological and physiological responses with the inclusion of the SPW blends. An aberrant spike of glucose and cortisol was detected for fish fed the reference diet at 24 h post transport, which is a curious response because both parameters have been reported to be restored to basal levels after a 24h resting period for red drum (Robertson et al., 1987). It is hypothesized that the high individual variability allied with the limited number of replicates available for this rather elaborate stress challenge may have limited to statistical differences in lactate and osmolality, as well as for the other stress-related responses at the different sampling points.

In conclusion, the present study demonstrated the feasibility of manufacturing feed ingredients with superior nutritional quality compared to traditional plant-protein feedstuffs by relying on relatively low-cost machinery and SPW, which is underutilized and often discarded by seafood processing plants. The manufactured ingredients succeeded in replacing fishmeal in red drum diets without compromising growth performance or critically affecting other physiological responses. More studies are encouraged to explore further the nutritional quality of dry-extruded seafood by-products, and to appraise the economic viability of implementing the dry extrusion technology in seafood processing plants or feed mills.

Conflict of interest

The authors declare none.

Table 1: Proximate composition and amino acid profile of the plant protein ingredients and extruded seafood waste blends.

	DDGS	DDGS 40:60	DDGS 60:40	Dehulled SBM	SBM 40:60	SBM 60:40	SPW
Dry matter	894.7	925.2	950.5	903.9	955.7	969.1	316.8
Protein	319.9	356.8	378.4	520.9	538.7	515.8	453.4
Lipid	115.9	176.5	217.3	34.4	96.3	151.8	163.6
Ash	50.5	96.7	112.1	68.4	102.6	128.5	294.3
pH	4.53	5.22	5.48	6.96	6.69	6.50	5.90
Energy (MJ kg^{-1})	19.7	21.8	21.9	19.0	19.7	20.5	22.9
Analyzed amino acid composition							
Arg	10.6	19.2	21.6	27.5	37.7	36.3	37.1
His	5.3	12.2	11.7	8.1	18.1	14.4	18.8
Ile	10.8	14.4	15	19.1	25.4	25	30.8
Leu	34.8	39.9	37	34.4	44.2	44.9	48.1
Lys	8.8	17.1	21.1	22.4	38.1	41.3	49.0
Met	3.6	6.5	7.0	2.9	5.4	7.5	16.9
Phe	14.1	17.6	17.2	23.5	27.8	26.1	37.1
Tau	0.2	1.8	2.8	0.0	1.7	3.3	6.9
Thr	11.3	16.6	17.2	16.6	24.3	25.5	30.5
Val	13.6	18.9	19.4	19.0	27.1	27.5	33.4

Values expressed as g kg^{-1} on a dry-matter basis unless otherwise stated

Abbreviations: DDGS: Distiller's dried grains with solubles; SBM: Soybean meal; SPW: Seafood processing waste

Table 2: Formulation and analyzed proximate composition of the experimental diets

Ingredients	SBM 60:40	SBM 40:60	DDGS 60:40	DDGS 40:60	Control
Menhaden Fishmeal[1]	136.0	136.0	136.0	136.0	567.0
SBM 60:40	224.0	0.0	0.0	0.0	0.0
SBM 40:60	0.0	214.0	0.0	0.0	0.0
DDGS 60:40	0.0	0.0	305.0	0.0	0.0
DDGS 40:60	0.0	0.0	0.0	324.0	0.0
Soybean meal[2]	330.0	330.0	330.0	330.0	0.0
Menhaden Oil[1]	58.0	71.0	25.0	32.0	58.0
Dextrinized Starch[3]	50.0	50.0	50.0	50.0	150.0
Vitamin Premix[4]	30.0	30.0	30.0	30.0	30.0
Mineral Premix[4]	40.0	40.0	40.0	40.0	40.0
Dicalcium Phosphate[5]	10.0	10.0	10.0	10.0	0.0
Lysine[6]	5.0	5.0	5.0	5.0	0.0
Methionine[7]	5.0	5.0	5.0	5.0	0.0
Taurine[3]	5.0	5.0	5.0	5.0	0.0
CMC[3]	20.0	20.0	20.0	20.0	20.0
Celufil[3]	86.0	82.0	38.0	12.0	134.0
Analyzed proximate composition[*]					
Dry matter	926.5	927.1	923.8	920.8	925.5
Protein	382.8	382	382.3	384.3	387.5
Lipid	112.7	114.2	116.5	120.5	118.5
Ash	121.7	114	125.2	123	150.8
pH	6.35	6.33	5.93	6.15	6.97
Energy (MJ kg^{-1})	20.21	19.8	19.59	19.81	19.46
Analyzed amino acid composition					
Arg	34.5	24.3	30.7	21.8	33.6
His	16.7	10.1	13.4	10.6	15.1
Ile	22.9	17.6	18.5	13.9	23.2
Leu	43.7	31.3	32.6	23.8	42.2
Lys	49.1	37.9	32.1	25	45.9
Met	11	8.1	10.4	7.1	13.8
Phe	23.4	17.6	21.6	15.6	22.7
Tau	10.7	9.4	11.7	7.6	8.7
Thr	23.4	17.2	21.4	14.2	27.4
Val	26.1	19.2	20.1	15	27.8

[*] Values expressed as g kg^{-1} on a dry matter basis otherwise stated

[1] Omega Protein Corporation, Abbeville, LA

[2] Producers cooperative association, Bryan, TX

[3] MP Biomedicals, Solon, OH

[4] Same as in Moon and Gatlin III (1991)

[5] Fisher Scientific, Pittsburg, PA

[6] ADM Animal Nutrition, Quincy, IL

[7] Ajinomoto North America Inc., Itasca, IL

Abbreviations: CMC: Carboxymethyl cellulose; DDGS: Distiller's dried grains with solubles; SBM: Soybean meal

Table 3: Average values of water quality parameters sampled in each recirculating system throughout the trial.

	Systems			
	1	2	3	PSE
Total ammonia nitrogen (mg L^{-1})	0.13	0.11	0.13	0.01
Total nitrite nitrogen (mg L^{-1})	0.035	0.066	0.073	0.08
pH	8.05	7.87	7.96	0.03
Salinity (g L^{-1})	3.16	3.01	3.23	0.20
Temperature (°C)	24.7	25.1	25.2	0.37
Dissolved oxygen (mg L^{-1})	7.30	7.28	7.37	0.11

Table 4: Growth performance, feeding rate, feed efficiency, condition indexes, fillet yield and survival of red drum after 8 weeks feeding the experimental diets

Dietary treatments	Initial weight (g)	Final weight (g)	Weight gain (%)	Feeding rate (%BW day^{-1})	HSI (%)	IPF (%)	Fillet yield (%)	Feed efficiency	Survival (%)
SBM 60:40	99.2	291.1*	193.6*	2.30*	1.39*	1.37	30.2*	0.83	95.5
SBM 40:60	98.4	266.2	170.5	2.39*	1.34*	0.98	24.8	0.76	98.8
DDGS 60:40	99.2	292.5*	195.0*	2.37*	1.50*	2.22	27.5	0.84	98.8
DDGS 40:60	98.4	284.6*	185.5*	2.36*	1.67*	1.94	26.1	0.80	96.6
Control	99.0	244.6	146.1	2.17	2.01	2.20	26.7	0.78	100.0
PSE	0.3	7.8	7.5	0.02	0.04	0.37	0.7	0.22	0.3
One-way ANOVA		0.01	0.008	0.001	<0.0001	0.16	0.01	0.28	0.69
Block P value		0.78	0.72	0.43	0.18	0.45	0.17	0.02	2.12

* Asterisks represent treatments significantly different than the positive control identified by the Dunnet test. Abbreviations: SBM: Soybean meal; DDGS: Distiller's dried grains with solubles; PSE: Pooled standard error; BW: Body weight; HSI: Hepatosomatic index; IPF: Intraperitoneal fat.

Table 5: Proximate composition of the whole body of red drum and protein conversion efficiency (PCE) after 8 weeks feeding the experimental diets. Values are expressed on a fresh-wet basis, unless otherwise stated.

		Proximate composition of the whole-body			
Dietary treatments	PCE (%)	Moisture (g kg^{-1})	Protein (g kg^{-1})	Lipid (g kg^{-1})	Ash (g kg^{-1})
SBM 60:40	39.1	733.4	180.2	48.4	39.8
SBM 40:60	35.5	734.8	173.6	42.7	42.1
DDGS 60:40	38.3	720.3	183.5	59.7	41.3
DDGS 40:60	35.5	733.7	180.1	46.9	41.9
Control	34.8	725.8	188.4	58.9	39.2
PSE	1.9	5.67	4.64	5.05	1.31
One-way ANOVA	0.45	0.37	0.32	0.15	0.45
Block P value	0.53	0.63	0.46	0.68	0.31

Abbreviations: SBM: Soybean meal; DDGS; Distiller's dried grains with solubles; PSE: Pooled standard error; PCE: Protein conversion efficiency.

Table 6: Digestive enzymes activities of different intestine sections of red drum fed experimental diets. Activity expressed unit per mg of tissue.

Enzyme	Trypsin			Aminopeptidase			Alkaline phosphatase			Acid phosphatase			Amylase			Lipase		
Segment	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P
SBM 60:40	3.39*	2.42	1.36	7.91	9.28	7.79	26.92*	20.16*	24.18*	225.8	215.1	146.7	2.47	1.16	3.10*	11.5	7.4	9.7
SBM 40:60	3.01*	2.67	1.02	5.59	8.85	7.58	27.9*	21.34*	28.75*	220.6	235.3	162.6	4.24	1.1	1.11	14.5	6.6	6.6
DDGS 60:40	2.01	2.37	0.93	6.66	8.85	9.02	35.54	23.49*	28.21*	225.8	225.6	198.4	1.39	0.94	0.63	11.3	9.0	5.9
DDGS 40:60	3.05*	2.64	0.92	6.59	9.61	8.46	34.97	26.07*	27.25*	230.0	219.9	185.6	2.38	1.29	1.97	11.0	6.9	5.8
Control	2.5	2.89	1.01	7.94	9.57	10.48	37.12	39.67	48.91	215.2	271.3	196.2	2.78	0.93	0.56	13.6	9.6	6.5
PSE	0.29	0.22	0.11	0.81	1.06	0.71	1.66	3.27	3.23	17.3	24.1	24.1	1.29	0.63	0.68	1.1	1.2	1.4
One-way ANOVA	0.01	0.48	0.06	0.29	0.97	0.11	<0.001	<0.01	0.004	0.97	0.52	0.52	0.65	0.99	<0.001	0.85	0.25	0.33
Block P value	0.13	0.03	0.28	0.13	0.65	0.01	0.03	0.38	0.19	0.68	0.57	0.08	0.51	0.81	0.36	0.02	0.1	0.54

* Asterisks represent treatments significantly different than the positive control identified by Dunnet's test. Abbreviations: A: Anterior; M: Medium; P: Posterior; SBM: Soybean meal; DDGS: Distiller's dried grains with solubles; PSE: Pooled standard error.

Table 7: Hematologic and stress-related parameters of red drum after 8 weeks of feeding the experimental diets and being subjected to a transport-stress challenge.

	Hours	SBM 60:40	SBM 40:60	DDGS 60:40	DDGS 40:60	Control	*PSE*	*One-way ANOVA*	*Block P value*
Hematocrit (%)	0	24.3*	24.6	26.2	29.1	27.5	0.90	0.003	0.28
	0.5	25.7	25.1	23.3	25.4	26.6	1.04	0.29	0.30
	24	24.4	25.9	24.8	24.4	26.4	0.91	0.41	0.003
	36	25.2	25.9	26.5	27.1	27.3	0.68	0.18	0.42
	48	25.5	26.6	26.2	25.8	27.8	0.79	0.38	0.01
Osmolality (mOsm kg^{-1})	0	316.5	328.5	321	328	342	5.80	0.21	0.13
	0.5	336.5	345.0	332.0	327	339.5	13.21	0.71	0.34
	24	333.5	349.5	338.0	333.5	342	8.19	0.68	0.35
	36	339.5	351.0	332.0	331.5	337	6.67	0.92	0.23
	48	333.0	334.5	328.5	333	341	3.47	0.82	0.51
Glucose (mg dL^{-1} plasma)	0	51.6	39.6	52.6	36.4	38.7	6.37	0.36	0.53
	0.5	61.1	60.7	62.2	60.7	69.4	9.3	0.87	0.18
	24	39.8*	37.7*	39.1*	39.3*	49.2	1.32	0.01	0.04
	36	50.4	43.2	41.3	43.9	62.4	6.8	0.33	0.68
	48	45.5	48.9	51.9	43.5	63.4	3.5	0.07	0.20
Lactate (mg dL^{-1} of plasma)	0	5.8	7.15	7.45	6.75	7.5	0.43	0.18	0.05
	0.5	6.35	8.45	9.5	8.25	10.95	2.19	0.68	0.31
	24	6.9	7.65	7.25	6.85	7.1	1.10	0.98	0.01
	36	7.9	7.8	7.2	7.45	8.4	0.46	0.51	0.26
	48	8.4	8.15	8.15	7.55	9.95	0.58	0.2	0.06
Cortisol (ng mL^{-1} of plasma)	0	9.4	29.9	9.1	32.6	38.6	16.2	0.63	0.98
	0.5	35.9	35.2	17.9	30.9	18.5	17.3	0.88	0.27
	24	35.6*	38.8*	47.9*	46.9*	84.7	6.7	0.03	0.01
	36	16.9	9.2	17.5	11.1	15.3	10.5	0.96	0.24
	48	11.7	8.0	6.1	5.0	24.5	13.6	0.84	0.59

Abbreviations: SBM: Soybean meal; DDGS; Distiller’s dried grains with solubles; PSE: Pooled standard error

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