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Effect of partial or total substitution of fish meal by poultry by-product meal in diets of juvenile fish *Dormitator latifrons* (Richardson, 1844)

Aguirre-García, Zinnia E.[1,2]; **Méndez-Soltero, Leticia**[2]; **Barreto-Curiel, Fernando**[4*]; **Ponce-Palomera, Kevin O.**[2]; **Vega-Villasante, Fernando**[2]; **López, Lus M.**[4]; **Badillo-Zapata, Daniel**[2,3*]

[1] Universidad de Guadalajara, Centro Universitario de la Costa, Programa de Doctorado en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas (BEMARENA). Av. Universidad 203, delegación Ixtapa, C. P. 48280, Puerto Vallarta, Jalisco, México.

[2] Laboratorio de Calidad de Agua y Acuicultura Experimental, Departamento de Ciencias Biológicas Centro Universitario de la Costa, Universidad de Guadalajara. Av. Universidad 203, delegación Ixtapa, C. P. 48280, Puerto Vallarta, Jalisco, México.

[3] Investigadoras e Investigadores por México. Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). Av. Insurgentes Sur 1582, Col. Crédito Constructor, Demarcación Territorial Benito Juárez. C. P. 03940, Ciudad de México.

[4] Universidad Autónoma de Baja California (UABC), Facultad de Ciencias Marinas. Carretera Transpeninsular Ensenada - Tijuana No. 3917. Colonia Playitas C. P. 22860. Ensenada, Baja California, México.

* Correspondence: danielbad00@hotmail.com, fbarreto8@uabc.edu.mx)

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ABSTRACT

Objective: The present study evaluated the impact of replacing fishmeal (FM) with Poultry by-product meal (PBM) in balanced diets for *Dormitator latifrons*, testing four replacement treatments (0, 33, 67 and 100%).

Design/methodology/approach: The experiment was conducted using 180 specimens with an initial weight of 33.80±0.08 g distributed in 600 L tanks fed at 2% of their total biomass, for 80 days.

Results: The results indicated that the inclusion of up to 33% of PBM did not significantly affect biological parameters compared to the control diet. However, the 100% PBM diet negatively affected blood parameters (such as glucose and protein) compromising the nutritional status of the fish. Although the proximate composition of muscle tissue was similar between treatments, the fatty acid profile showed deficiencies of Fatty acid composition (EPA) and docosahexaenoic acid (DHA, C22:6n3) in the 100PBM diet, essential for optimal growth and development.

Limitations of the study/implications: Poultry by-products represent a viable and sustainable alternative in the formulation of fish diets, with significant economic and environmental benefits. However, their use must be optimized through research that guarantees an adequate nutritional balance.

Findings/conclusions: In conclusion, the use of PBM up to 33% as a substitute for FM in the diet of *D. latifrons* is feasible, maintaining an adequate performance without compromising the flesh quality. Higher substitutions can impact negatively on both growth and physiological health of fish species, but also the nutritional quality of flesh, highlighting the need to adjust essential nutrient profiles in PBM-based diets. This approach combines sustainability and performance in the aquaculture of this species.

Keywords: Aquaculture; nutrition; by-products; animal welfare; native fish

INTRODUCTION

By-products of the food industry, defined as whole bodies or animal parts derived from the processing, manufacture or extraction of a primary product, mainly food intended for human consumption, represent a significant source of waste [1]. It is estimated that more than 1.3 billion ton of food waste, including by-products, are produced annually, equivalent to 13.8% of total world production [2]. This alarming figure has significant environmental, economic, and social implications [3]. In response to the growing demand for sustainable and environmentally friendly practices, increased emphasis has been placed on finding alternative applications for these waste materials. One promising strategy is the use of agro-industrial by-products as ingredients in diets formulated for aquaculture. Aquaculture has experienced accelerated growth in recent decades, consolidating its position as the most rapidly expanding food production system.

In Mexico, research on species with aquaculture potential has been predominantly directed towards exotic species such as tilapia (*Oreochromis niloticus*), however, there are native species with high culture potential that have outstanding nutritional characteristics and could be exploited in aquaculture. *D. latifrons*, known in different regions as Pacific fat sleeper, chopopo, chame, puyeque or popoyote, is a native species distributed along the Pacific coast, from California (USA) to Peru [4]. It inhabits freshwater environments such as riverbanks, marshes, coastal lagoons, and estuaries, and shows a preference for waters with temperatures between 21 °C and 30 °C. It has the ability to tolerate brackish water and low oxygen levels of up to 0.4 mg L^{-1} [5].

Aquaculture faces the challenge of producing high nutritional quality feeds that reduce costs, minimize environmental impact and achieve an optimal feed conversion factor (biomass gained per kilogram of feed provided) [6]. A crucial aspect of this challenge is to design diets that promote growth, decrease dependence on fishmeal (FM) as the main protein source and increase sustainability [7]. Poultry by-product meal (PBM) is a by-product of poultry processing and its quality depends on factors such as raw material composition, heating process, water and fat extraction, as well as cooking time [8]. This meal contains 30% to 65% protein and it has been shown that its inclusion in diets for aquatic organisms can significantly reduce the use of fishmeal without compromising growth [9]. Therefore, the main objective of this study was to evaluate the partial and total replacement of fishmeal with poultry by-product meal in diets formulated for juvenile *D. latifrons*, measuring growth and animal welfare indices.

EXPERIMENTAL DESIGN AND METHODOLOGY

Preparation of diets

The study was carried out at the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) of the Centro Universitario de la Costa, in Puerto Vallarta, Jalisco, Mexico. Four balanced isoproteic (30% total protein) and isolipidic (8% total lipids) diets were formulated following the methodology and nutritional requirements established by Badillo-Zapata *et al.* [10] in order to replace 0, 33, 67 and 100% of the fish meal with poultry by-product meal. The composition of ingredients is detailed in Table 1.

Table 1. Ingredients (g kg^{-1}) and proximal composition (g kg^{-1} dry weight), of the control diet (0PBM) and three experimental diets (33PBM, 67PBM and 100PBM) containing different levels of fishmeal substitution by poultry by-product meal. "Pet food grade" (PBM) used to feed *D. latifrons* juveniles, for 80 days.

	Experimental Treatments			
	0PBM	**33PBM**	**67PBM**	**100PBM**
Poultry by-product meal[a]	0	129	225	369
Fish meal[a]	368	235	130	0
Fish Oil	53	43	35	20
Corn flour	426	440	457	458
Corn starch	55	55	55	55
Gelatin	60	60	60	60
Vitamins and Minerals[b]	30	30	30	30
Vitamin C[b]	5	5	5	5
Sodium Benzoate	2	2	2	2
Choline Chloride	1	1	1	1
Alpha Tocopherol	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000
	Proximal composition (g/kg)			
Protein	330.3	320.9	340.1	350.1
Lipids	81.8	78.7	82.3	79.9
Ash	103.5	93.5	83.9	77.8
Carbohydrates (ELN)	484.4	506.9	493.7	492.2

NFE (g/kg)=100−(g/kg crude protein + g/kg total lipid + g/kg ash). NFE includes fiber.

[a] Poultry by-product meal (65.5% CP; 12.0% LC) and fish meal (68% CP; 8.0% LC) were obtained from Proteínas Marinas y Agropecuarias S.A. de C.V. in Guadalajara Jalisco, Mexico. [b] DSM Nutritional Products Mexico SA de CV. Rovimix®; Vitamin and mineral mix (g/kg): ρ-aminobenzoic acid 1.45; biotin 0.02; myo-inositol 14.5; nicotinic acid 2.9; Capantothenate 1.0; pyridoxine-HCl 0.17; riboflavin 0.73; thiamine-HCl 0.22; menadione 0.17; α-tocopherol 1.45; cyanocobalamine 0.0003; calciferol 0.03; L-ascorbyl-2-phosphate-Mg 0.25; folic acid 0.05; choline chloride 29.65; retinol 0.015; NaCl 1.838; $MgSO_4$-$7H_2O$ 6.85; NaH_2PO_4-$2H_2O$ 4.36; KH_2PO_4 11.99; $Ca(H_2PO_4)_2$-$2H_2O$ 6.79; Fe-citrate 1.48; Ca-lactate 16.35; $AlCl_3$-$6H_2O$ 0.009; $ZnSO_4$-$7H_2O$ 0.17; $CuCl_2$ 0.0005; $MnSO_4$-$4H_2O$ 0.04; KI 0.008; $CoCl_2$ 0.05 and Stay-C (Vitamin C) donated by DSM, Nutritional Products Guadalajara, Mexico.

Fish breeding, feeding and biometrics

A total of 180 juveniles with an average weight of 33.80±0.08 g and a length of 16.7±0.6 cm were selected from the LACUIC laboratory stock and randomly distributed in 12 tanks of 600 L, with 15 organisms per tank. Each experimental unit was connected to a canister filter (EF-05 SERIES) with constant aeration and a 30% weekly freshwater replacement. Water temperature was maintained at 23.0±1.0 °C using a heater (Thermal Pro 200W Lomas), and the dissolved oxygen level was maintained at 5.5±1.0 mg L^{-1}, measured with a Hanna oximeter® (HI9146-04). Water pH was recorded at 8.1±0.3, determined using a pH meter (HI8314), and the photoperiod was maintained naturally at 12 hours of light and 12 h of darkness. Feed was supplied once a day (at 16:00 h), and the amount administered in each tank corresponded to 2% of the total biomass of each experimental unit. An initial biometry was performed using a digital balance (OHAUS® PR2201) with an accuracy of ±0.01 g. At the end of the experiment, after 80 days, final biometry was performed to determine growth, fish feed efficiency and other performance indices, which were calculated using the following formulas:

Specific growth rate:

$$SGR = \frac{\left(\ln \text{ final weight} - \ln \text{ initial weight}\right)}{\text{time } \left(\text{days}\right)} \times 100$$

Weight increment:

$$\% \text{ Weight gain} = \left[\frac{\left(\text{final weight} - \text{initial weight}\right)}{\text{initial weight}}\right] \times 100$$

Food Conversion Ratio (FCR):

$$FCR = \frac{\text{dry weight feed consumed } \left(g\right)}{\text{wet weight gain } \left(g\right)}$$

Blood parameters

15 fish were randomly selected from each experimental treatment (five specimens per pond) for been anesthetized. Anesthesia was performed according to Aréchiga-Palomera *et al.* [11]. Once the fish showed erratic swimming, blood samples were collected under aseptic conditions by caudal vein puncture, using a 1 mL insulin syringe with 30G×13 mm needle, following the protocol described by Stoskopf [12].

Hematological profile

For the hematological profile, basic parameters were determined: hemato-crit (HCT), respiratory burst (NBT), erythrocyte count (RBC), and white blood cell count (WBC). HCT was determined using the microhematocrit technique [13]. NBT was evaluated following the methodology of Ibrahem *et al.* [14]. For erythrocyte and leukocyte counts, a 20 μL sample of EDTA-K2 blood was placed in 4 mL of Natt-Herrick's solution. In a Neubauer chamber with 1/400 mm^2 and 1/10 mm depth, it was filled with 5 μL of the dilution. The analysis was performed by observation under a Quasar Qm20 2500x Binocular Professional Microscope. Erythrocytes were counted in the central grid, from which five squares of 0.0025 mm^2 each were selected, and leukocytes in four large squares, from each corner, with an area of 1 mm^2 each.

Fatty acid composition

Samples were taken from the four diets and from the muscle tissue of the fish from each experimental unit (n=5). Extraction of fatty acids and transmethylation was performed using the technique described by Parrish *et al.* [15]. To separate and quantify fatty acids, an AGILENT gas chromatograph (GC 7820) was used, equipped with a Split/Splitless injector, a flame ionization detector (FID) and an AGILENT capillary column (122-2361 DB-23) 60 m × 0.25 mm with an internal diameter of 15 μm. Calculations were performed using the GC Chemstation Data Analysis software. The initial injection temperature was 120 °C for 1 min, then raised to 190 °C at a rate of 25 °C/min, then increased to 230 °C at a rate of 6 °C/min and nitrogen (N_2) was used as carrier gas at 1.0mL/min. Fatty acids were identified by comparison with the retention times of the standard mixture of 37 components FAME, PUFA1 and PUFA3 (Supelco/Sigma-Aldrich®).

Proximal analysis

Poultry by-product meal, experimental diets and muscle tissue were subjected to proximate analysis: quantification of crude protein (method 960.52), crude lipids (method 920.97) and ash (method 942.05) following the standard methods of the Association of Official Analytical Chemists [16].

Statistical analysis

All data are presented as mean and standard deviation (X±SD). Normality (Shapiro Wilk) and homoscedasticity (Bartlet) tests were performed. When the statistical assumptions were met for the data of biological indices, blood parameters, fatty acids and proximate analyses, a one-way analysis of variance (ANOVA) with an $\alpha = 0.05$, was applied among the four treatments. To identify differences between treatments, a Tukey's posterior test ($P < 0.05$) was used. All analyses were performed with Minitab® 19.1.1 software.

RESULTS

Biological indexes

The results obtained show that there were no significant differences in the initial weight between the treatments (Table 2). However, the final weight showed a significant reduction ($p < 0.05$) in the 100PBM group (64.8±9.4 g) compared to the other treatments, while the 0PBM group obtained the highest final weight (88.9±16.7 g). The specific growth rate (SGR) also decreased significantly in the 100PBM treatment (1.3±0.1%) compared to 0PBM and 33PBM. Similarly, the weight gain (%) was lower in 100PBM (49.3±74.5%), while 0PBM obtained the highest value (139.2±23.1%). Regarding the feed conversion ratio (FCA), a tendency to decrease with increasing PBM in the diet was observed, being significantly lower in 100PBM (0.4±0.2), suggesting lower feed efficiency. Finally, the survival rate was lower in the 100PBM treatment (77.7±6.6%) compared to the other groups, which maintained values higher than 95%. These results suggest that a high inclusion of PBM in the diet negatively affects the growth and survival of the fish.

Blood parameters

The results show that hematocrit (HCT) was significantly reduced ($p < 0.05$) in the 100PBM treatment (26.5±1.1%) compared to the other treatments, while there were no significant differences between the 0PBM, 33PBM and 67PBM groups (Table 3). Regarding the erythrocyte (RBC) count, a progressive decrease was observed as the

Table 2. Biological indices in juvenile poplar (*D. latifrons*) after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Biological indexes	**Experimental treatment**			
	0PBM	**33PBM**	**67PBM**	**100PBM**
Initial weight (g)	33.9±0.1	33.7±0.1	33.8±0.0	33.8±0.0
Final weight (g)	88.9±16.7^{a}	72.2±15.2^{b}	78.2±19.4^{ab}	64.8±9.4^{c}
Specific growth rate (%)^{1}	1.5±0.2^{a}	1.4±0.2^{b}	1.4±0.2^{ab}	1.3±0.1^{c}
Weight gain (%)^{2}	139.2±23.1^{a}	104.8±16.4^{b}	126.2±7.9^{a}	49.3±74.5^{c}
FCA^{3}	0.8±0.1	0.6±0.1	0.7±0.05	0.4±0.2
Survival (%)	97.7± 3.8	95.5± 7.7	97.7± 3.8	77.7± 6.6

Values are means ± standard deviation, different superscripts mean significant differences ($P<0.05$). ^{1}TEC=(ln final weight−ln initial weight)/time (days)×100. ^{2}Weight gain (%)=[(final weight−initial weight)/initial weight]×100; ^{3}FCA=feed dry weight consumed (g)/wet weight gain (g).

Table 3. Blood parameters of juvenile poplar (*D. latifrons*) after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Parameters blood	**Experimental treatments**			
	0PBM	**33PBM**	**67PBM**	**100PBM**
HCT (%) **	33.3±2.0a	32.3±1.5a	33.2±1.7a	26.5±1.1b
NBT (abs)*	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
RBC(×10^{6}/μL)	1.9±0.0a	2.0±0.1a	1.0±0.0b	0.8±0.0c
WBC(×10^{3}/μL)	14.3±1.2b	12.0±1.0b	13.5±1.0b	18.1±1.0a
Protein (g/dL	6.5±1.0a	6.4±1.2a	5.5±1.0b	4.3±1.0c
Albumin (g/dL)	1.5±0.2b	1.6±0.1a	1.5±0.2b	1.1±0.2c
Globulin (g/dL)	5.0±1.0a	4.7±1.2a	3.9±1.0a	3.1±1.0b
A/G Ratio	0.3±0.0	0.3±0.1	0.4±0.1	0.3±0.1
Glucose (mg/dL)	110.2±4.3b	129.7±7.6a	76.3±6.8c	66.7±3.7d
Cholesterol (mg/dL)	175.7±3.0d	225.2±4.2b	246.7±2.7a	188.9±1.4c
Triacylglycerides (mg/dL^{-1})	661.6±3.2a	444.0±5.1d	509.2±4.3c	643.4±7.5b

Values are means ± standard deviation, different superscripts mean significant differences ($P<0.05$). Hematological profile: hematocrit (HCT), respiratory burst (NBT), erythrocytes (RBC) and leukocytes (WBC). Biochemical parameters: protein, albumin, globulin, A/G ratio, glucose, cholesterol and triacylglycerides of blood and plasma under laboratory conditions for 80 days.

inclusion of PBM in the diet increased, being significantly lower in 67PBM and 100PBM compared to 0PBM and 33PBM. On the other hand, the leukocyte (WBC) count was significantly higher in the 100PBM group (18.1±1.0×10^{3}/μL) compared to the other treatments. The levels of total proteins, albumin and globulin decreased significantly in the 100PBM treatment compared to the other groups. Regarding metabolic parameters, glucose showed a significant decrease in 67PBM and 100PBM compared to 0PBM and 33PBM groups. Likewise, cholesterol levels were higher in 33PBM and 67PBM treatments, while triglycerides had their highest value in 0PBM group and the lowest in 33PBM. These results indicate that a higher inclusion of PBM in the diet negatively affects several hematological and biochemical parameters in fish.

Biochemical composition of the tissue

The table presents the results of the proximate composition and fatty acids in proximal muscle under different experimental treatments (0PBM, 33PBM, 67PBM, 100PBM) (Table 4 y 5). Regarding saturated fatty acids (SFA), no significant differences were observed between treatments, with values ranging from 27.8±0.6 to 32.1±1.7. Monounsaturated fatty acids (MUFA) showed a significant increase in the 100PBM treatment (34.3±0.3) compared to the other treatments. Polyunsaturated fatty acids (PUFA) presented their highest concentration in the 67PBM treatment (35.2±0.5), highlighting a high content of C18:2n6 (30.9±2.5). Regarding n3 fatty acids, a progressive decrease was observed from 22.8 in 0PBM to 1.0 in 100PBM.

The proximate muscle composition showed consistent values of protein (840.5±0.2 to 840.3±0.2 g/kg), lipids (50.7±0.2 to 50.9±0.4 g/kg) and ash (60.1±0.8 to 60.3±0.7 g/kg) across all treatments, indicating that these did not significantly affect the basic muscle composition.

Table 4. Fatty acid (FA) composition of the control diet (0PBM) and three experimental diets (33PBM, 67PBM and 100PBM) containing different levels of fishmeal substitution for "Pet food grade" poultry by-product meal (PBM) used to feed *D. latifrons* juveniles, for 80 days.

Experimental treatments				
AG	**0PBM**	**33PBM**	**67PBM**	**100PBM**
C14:0	5.6	4.7	3.7	3
C15:0	0.8	0.7	0.6	Nd
C16:0	21.3	22.1	22.1	28.2
C18:0	5.2	5.8	6.1	7.9
C24:0	0.3	Nd	Nd	Nd
∑SFA[1]	33.2	33.3	32.5	39.1
C16:1n-7	6.1	6.0	5.9	6.2
C18:1n-7	2.8	2.8	2.4	2.2
C18:1n-9	15.6	18.4	22	29.7
∑MUFAS[2]	24.5	27.2	30.3	38.1
C18:2n-6	8.9	11.4	13.6	10.2
C18:3n-3	1.9	1.7	1.5	0.5
C20:3n-3	1.8	1.9	1.8	0.8
C20:5n-3	6.4	5.2	3.8	Nd
C22:5n-3	1.0	0.8	0.6	0.5
C22:6n-3	9.1	6.9	4.8	0.5
∑PUFAS[3]	33.4	36.0	38.6	46.5
∑n3	44.7	43.7	42.8	40.4
∑n6	8.9	11.4	13.6	10.2
EPA/DHA	0.7	0.8	0.8	0

Fatty acid composition values are the mean (±SD) of two replicates. Nd not detected.

[1] Total saturated fatty acids included C13:0, C14:0, C15:0, C16:0, C18:0 and C24:0.

[2] Total monounsaturated fatty acids included C16:1n-7, C18:1n-9 and C18:1n-7.

[3] Total n-3 and n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C20:3n-3 C20:5n-3, C22:5n-3 and C22:6n-3.

Table 5. Fatty acid composition and proximate analysis of poplar (*D. latifrons*) muscle tissue after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Experimental treatments				
AG	**0PBM**	**33PBM**	**67PBM**	**100PBM**
C14:0	2.4±0.4a	1.7±0.2b	1.0±0.4c	0.8±0.0c
C15:0	0.6±0.4a	0.6±0.1a	Nd	0.3±0.0a
C16:0	18.1±0.8c	20.5±0.7b	19.4±1.0bc	24.6±0.9a
C18:0	8.6±0.3a	6.7±0.3b	6.8±0.7b	6.2±0.5b
C24:0	0.8±0.0a	0.9±0.0a	0.4±0.0b	Nd
∑SFA[1]	30.7±0.9	30.5±0.5	27.8±0.6	32.1±1.7
C14:1	0.7±1.7a	0.5±0.9a	1.8±2.8a	Nd
C15:1	0.5±0.0a	Nd	0.3±0.2b	Nd
C16:1	2.6±0.2a	2.9±2.3a	2.5±0.2a	4.8±0.4a
C18:1n9	15.5±0.7d	21.3±1.2b	18.1±1.0c	25.1±0.3a
C18:1n7	3.8±0.1a	3.8±0.2a	4.0±0.5a	4.3±0.2a
C20:1	0.3±0.1b	0.4±0.0b	0.8±0.4a	Nd
C24:1	0.8±0.1a	0.4±0.2b	Nd	Nd
∑MUFAS[2]	23.2±1.1b	29.0±0.5b	25.6±0.4b	34.3±0.3a
C18:2n6	11.2±0.4c	14.7±0.8b	30.9±2.5a	16.1±0.5b
C20:3n3b	0.4±0.0b	0.2±0.2b	0.5±0.0b	1.0±0.3a
C20:4n6	0.3±0.0a	0.2±0.0b	Nd	Nd
C20:4n3	4.9±0.3a	3.7±0.3b	1.8±0.1c	Nd
C20:5n3	4.4±0.2a	2.1±0.1b	0.5±0.1c	Nd
C22:5n3	3.9±0.1a	3.5±0.2a	0.9±0.0b	Nd
C22:6n3	8.9±0.4a	5.7±0.3b	0.6±0.2c	Nd
∑PUFAS[3]	20.5±1.4b	20.7±1.1b	35.2±0.5a	16.1±0.0c
∑n3	22.8	15.4	4.5	1.0
∑n6	11.5	15.0	30.9	16.1
EPA/DHA	0.5	0.4	0.9	0
Proximal muscle composition (g/kg dry basis)				
Protein	840.5±0.2	840.4±0.7	840.4±0.4	840.3±0.2
Lipids	50.7±0.2	50.9±0.4	50.8±0.6	50.8±0.3
Ash	60.1± 0.8	60.3±0.7	60.1±0.8	60.2±0.7
Carbohydrates (ELN)	48.7±1.1	48.4±1.1	48.7±0.6	48.6±1.0

Fatty acid composition values are the mean (±SD) of two replicates. Nd not detected.

[1] Total saturated fatty acids included C13:0, C14:0, C15:0, C16:0, C18:0 and C24:0.

[2] Total monounsaturated fatty acids included C16:1n-7, C18:1n-9 and C18:1n-7.

[3] Total n-3/n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C20:3n-3 and C20:5n-3, C22:5n-3 and C22:6n-3.

Values are means ± standard deviation of 100% of methylated fatty acids. Different superscripts mean significant differences ($P<0.05$).

DISCUSSION

The present study provides valuable information on the replacement of fishmeal with a significant amount of PBM in balanced diets for *D. latifrons* on biological indices, blood parameters and fatty acid composition of fish flesh. The result obtained in this experiment shows that the inclusion of PBM up to 33% had no adverse effects on biological indices (specific growth rate, weight gain, feed conversion factor and survival). A meta-analysis exploring the effects of replacing fishmeal with PBM found no significant differences in growth performance for most species studied [9]. This is likely due to the high crude protein content, favorable amino acid profile and lack of anti-nutritional factors usually associated with plant-based proteins [17]. However, like other animal-based proteins, variation in the quality of nutritional composition is common, largely due to alteration in raw material structure, quality and processing specifications, such as cooking process temperature, which is critical in determining the quality of the final product [18]. This variation results in deficiencies in certain essential amino acids, higher ash content and variability in digestibility. In this context, it has been shown that PBM can be used within diet formulations for aquatic organisms such as Nile tilapia (*Oreochromis niloticus*), catla (*Catla catla*), rohu (*Labeo rohita*), striped bass (*Morone saxatilis*), tench (*Tinca tinca*), trout (*Oncorhynchus mykiss*), grouper (*Epinephelus malabricus*), golden pompano (*Tachinotus ovatus*), juvenile gilthead sea bream (*Sparidentex hasta*) and juvenile totoaba (*Totoaba macdonaldi*) [7,19,20]. FM replacement percentages vary by species, however, replacement ranges from 15% to 67% have been observed that do not generate significant differences compared to organisms fed 100% FM [8,21-23]150 or 500 g kg^{-1} of fish meal protein was substituted by MBM (MBM15, MBM50. Particularly, it has been observed that proportions of 15, 25 and 35% PBM in juvenile gilthead sea bream (*Sparidentex hasta*) and 67% in totoaba (*Totoaba macdonaldi*), increase weight gain and improve feed conversion factor [24]. However, the importance of protein quality is highlighted, noting the lower availability of certain essential amino acids (AA) in PBM compared to fishmeal, which has the ideal AA profile for most aquaculture species [25]. In general, PBM protein is known to contain lower levels of methionine and lysine compared to FM, which is considered the limiting factor for growth in many species, such as Florida pompano (*Trachinotus carolinus*), African catfish (*Clarias gariepinus*), humpback grouper (*Chromileptes altivelis*), trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*), gibel carp (*Carassius auratus gibelio*) and sea bream (*Sparus aurata*) at higher dietary PBM inclusions [8,25-30]150 or 500 g kg^{-1} of fish meal protein was substituted by MBM (MBM15, MBM50.

Hematological profile and biochemical parameters, are fundamental tools for assessing fish welfare, health, immune system response, short- and long-term effects of "suboptimal" culture conditions, water quality, potential disease outbreak and nutritional status [31]. These parameters can be influenced by a wide variety of factors, including species type, season of the year, temperature, salinity, pH, presence of contaminants, nutrition, culture density, presence of diseases, farm conditions, and sampling method, among others [32].

Hematocrit (HCT) values are closely related to the activity and habitat of fish [33]. In this experiment, HCT ranged between 26.5% and 33.3%, these values, are similar to what was reported in the same species Todd [34] 39.1%. Ruiz-González *et al.* [35] 28%

and Santana-Piñeros *et al.* [36] 38%. El hematocrit remained stable in the 0PBM, 33PBM, and 67PBM treatments, but showed a significant reduction in 100PBM, suggesting a possible alteration in erythropoiesis or a deficiency of essential nutrients for red blood cell production [37]. This result is consistent with the decrease in RBC (red blood cell) count in the groups with higher inclusion of PBM, which could indicate an adverse effect on the oxygenation of the organism [38].

On the other hand, WBC values are similar to those of other demersal and sedentary species, such as the goldfish *Carassius auratus* ($10.1\text{-}14.7 \times 10^3$ cells μL^{-1}), the black goby *Gobius niger* ($8.0\text{-}10.8 \times 10^3$ cells μL^{-1}) and the African catfish *Clarias gariepinus* ($11\text{-}11.1 \times 10^3$ cells μL^{-1}) [33,39,40]. In *D. latifrons* our WBC values were very low, similar to the values of Santana-Piñeros *et al.* [36] ($8.9\text{-}10.3 \times 10^3$ cells μL^{-1}). WBC values decreased in 33PBM and 67PBM compared to 0PBM, which could reflect a lower immune activation. However, in 100PBM, a significant increase was observed, which could suggest an inflammatory response or a possible immune challenge associated with the high level of PBM in the diet. Despite these changes in leukocytes, neutrophil oxidative activity, measured by NBT, remained constant in all treatments, indicating that PBM did not significantly stimulate the production of reactive oxygen species in these cells [41].

Plasma protein levels also showed a decreasing trend as PBM in the diet increased. Both albumin and globulin decreased in treatments with higher inclusion of PBM, which could suggest a lower availability of proteins in the diet or an increase in their catabolism [42]. This could be related to a lower supply of essential amino acids in diets with high amounts of PBM or to a lower efficiency in nutrient absorption [43]. The A/G ratio remained stable, indicating that the reduction in plasma proteins proportionally affected both components.

Energy metabolism also showed important changes. The blood glucose levels differ considerably among fish species [44]. In the present study glucose levels were drastically reduced in 67PBM and 100PBM, which could indicate a lower availability of carbohydrates or an alteration in the regulation of energy metabolism [33]. The lipid profile presented variations, with an increase in cholesterol in 33PBM and 67PBM, followed by a decrease in 100PBM. This could be related to changes in lipid absorption or synthesis due to the composition of the diet.

The specific range of cholesterol and triacylglycerides levels in *D. latifrons* are not widely documented, however, it is known that fish triacylglyceride levels are usually relatively low, and variations may depend on factors such as diet, health status, time of year and environment [33]. Triglycerides, on the other hand, decreased with the partial inclusion of PBM, but in 100PBM they increased again, suggesting a possible alteration in lipid metabolism in the presence of high levels of PBM.

Taken together, these results indicate that moderate levels of PBM can be well tolerated without significantly affecting blood parameters, but a total inclusion of PBM in the diet can compromise red blood cell production, protein homeostasis and energy metabolism. It is recommended to evaluate micronutrient supplementation strategies to mitigate the adverse effects observed at high PBM inclusions and to conduct additional studies to better understand the physiological mechanisms involved.

The fatty acid (FA) composition of muscle tissue of most fish species is strongly influenced by the FA content of the diet [45]. Poultry by-product meal generally contains high levels of monounsaturated fatty acids (MUFAs) particularly oleic acid (OA, C18:1n9) and polyunsaturated fatty acids particularly linoleic acid (LA, C18:2n6), but contains low levels in essential fatty acids (EFAs) such as the long-chain polyunsaturated fatty acids LC-PUFAs of series n-3 (EPA and DHA) [46]. In the present study the inclusion of hydrolyzed feather meal (PBM) in the diet significantly altered the fatty acid profile of proximal muscle, increasing saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), while polyunsaturated fatty acids (PUFA), especially those of the n3 series, progressively decreased with increasing PBM [47]. The increase in palmitic acid (C16:0), present in vegetable oils such as palm and in animal fats, and stearic acid (C18:0), abundant in animal fats and butters, suggests a greater accumulation of SFA due to the lower availability of PUFA in PBM compared to other protein sources, such as fishmeal [7]. Likewise, the increase in oleic acid (C18:1n9), characteristic of vegetable oils such as olive and canola, indicates a possible metabolic adaptation to the lipid profile of the diet, although the reduction in myristoleic acid (C14:1), originating from dairy fats and fish oils, and pentadecenoic acid (C15:1), of bacterial origin and present in some dairy products, suggests a lower bioavailability of these fatty acids in PBM [48]. The drastic decrease in n3 PUFA, particularly eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), both present in fish oils and marine microalgae, in 100PBM is worrying, since these compounds play essential roles in fish health, including the regulation of anti-inflammatory processes and the development of the nervous system [49]. The reduction of these fatty acids may affect the nutritional quality of the muscle and its value for human consumption, suggesting the need to supplement these diets with sources rich in n3 PUFA, such as marine oils or microalgae, to mitigate the negative effects of the total replacement of fishmeal by PBM [50].

CONCLUSIONES

Replacing fish meal (FM) with hydrolysed feather meal (PBM) in fish diets resulted in significant changes in several physiological, biochemical and muscle composition parameters, highlighting both its advantages and limitations. In haematological terms, the reduction in haematocrit and red blood cells at the highest levels of PBM could suggest a lower efficiency in blood oxygenation, while the increase in leukocytes at 100PBM indicates a possible immune or inflammatory response. Biochemical parameters revealed a negative impact on plasma protein and albumin levels, which could be related to a lower bioavailability of certain essential amino acids in PBM. Furthermore, the reduction of polyunsaturated fatty acids (PUFA), especially those of the n3 series, together with the increase of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), suggests a negative effect on the nutritional quality of muscle, affecting its value both for fish health and for the final consumer. However, the stability of the proximal composition of muscle in terms of protein and lipids suggests that PBM may be a viable protein source, provided that supplementation strategies are implemented to mitigate its nutritional deficiencies, particularly in essential fatty acids and limiting amino acids. In conclusion, although PBM represents a promising alternative for the reduction of FM use in aquaculture, its inclusion should be carefully formulated to avoid adverse effects on the physiology, health and final quality of the product.

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