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# Inclusion of different blends of poultry processed proteins in practical diets for African catfish (*Clarias gariepinus*) reared in RAS: Effects on nutrient digestibility, growth performance and fillet quality

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# **1. Introduction**

Global seafood consumption is expected to increase by 15 % and reach 21.4 kg per capita by 2030 ([Love et al., 2021\)](#page-9-0) to almost double by 2050 ([Naylor et al., 2021\)](#page-9-0), which calls for the diversification of farmed fish species to sustain aquaculture productions [\(Chan et al., 2024\)](#page-8-0). The global production of African catfish (*Clarias gariepinus*, Burchel, 1822) reached 231 million tons (674 million USD) in 2021 [\(FAO, 2022](#page-8-0)), with Nigeria leading the production followed by other African and Asian countries ([Dauda et al., 2018](#page-8-0)). Farming systems are based on earthy and clay pounds [\(FAO, 2022\)](#page-8-0) which are cost-effective and scalable, providing natural feed sources and ecological benefits, but challenging as for water management and disease control ([Onyia et al., 2021](#page-9-0)). In Europe, the farming of *Siluriformes* species is less widespread, with significant commercial production (6.8 million tons in 2020) ([FAO, 2022\)](#page-8-0) of African catfish limited to the Netherlands, Germany and Belgium,

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mainly reared in land-based systems [\(Rosa et al., 2007\)](#page-9-0). The lack of catfish production is mainly due to cultural habits, taste perceptions, poor marketing, concerns about farming practices, and competition with more established fish alternatives, which collectively reduce consumers' demand for catfish and diminish farmers' interest in this practice ([Varadi, 2001\)](#page-10-0). Nevertheless, catfish species would be promising for European land-based aquaculture because of their fast growth (specific growth rate up to 2.28 % day $^{-1}$ ) and efficient feed conversion ratio (0.87–0.97) [\(Ali and Jauncey, 2005; Hargreaves and Tucker, 2003;](#page-8-0)  [Hecht et al., 1996\)](#page-8-0). Specifically, African catfish would be suitable for intensive recirculating aquaculture systems (RAS) due to its high tolerance to a broad range of environmental and water quality conditions (e. g. dissolved oxygen <4.0 mg L<sup>−1</sup>, ammonia >0.34 mg L<sup>−1</sup>, presence of water re-use substances) (Prokešová [et al., 2021; Schram et al., 2014\)](#page-9-0) and high stocking densities (up to 500 kg m $^{-3}$ ) (Van de Nieuwegiessen [et al., 2009\)](#page-10-0).

Nowadays, fillets of RAS-reared African catfish are already marketed in leading production countries ([Rosa et al., 2007](#page-9-0)), where consumers appreciate its tasty meat with a high nutritive value, i.e. high-crude protein (17–18 % of which 42.5 % essential amino acids), relatively low-fat (4–8 %, primarily oleic, linoleic, and palmitic fatty acids), and valuable vitamins and micro-elements ([Abdel-Mobdy et al., 2021](#page-8-0)). Additionally, African catfish meat is suitable for different preparations and recipes, such as frying, grilling, smoking and soup ([Aderolu et al.,](#page-8-0)  [2010; Rosa et al., 2007\)](#page-8-0).

Despite advancements in aquafeed formulation, commercial diets for African catfish still use fishmeal as a crucial protein source. Current recommendations claim for an 8–10 % inclusion of fishmeal in the diet for optimal growth and health of this fish [\(Phonekhampheng et al.,](#page-9-0)  [2009\)](#page-9-0). This trend jeopardises the spread of its farming in Europe due to high feeding cost and environmental concerns [\(Naylor et al., 2021](#page-9-0)). Among the fishmeal-alternative ingredients tested in freshwater species, processed animal proteins (PAPs) are safe, low-cost, and protein-rich ingredients ([Kumar and Engle, 2017; Zhu et al., 2011](#page-9-0)); their use in aquafeeds has been allowed in EU countries (EU Commission Regulation No. 56/2013) since 2013. From this year onwards, different blends of by-products from terrestrial animal supply chains (i.e. meat, bone, feathers and blood meals) were tested in practical diets for all the major aquaculture species with inclusion levels ranging from 35 g  $kg^{-1}$  to 850 g kg<sup>-1</sup> (see [Woodgate et al., 2022](#page-10-0) for a review). Furthermore, PAPs contribute to satisfying the fish nutritional requirements of calcium, phosphorus, and vitamin  $B_{12}$  [\(Liland et al., 2015\)](#page-9-0). Among PAPs, poultry by-product meal (PBM) shows great potential as a fishmeal substitute because of its increasing availability (+8 % annual growth expected from 2024 to 2029), cost-effectiveness (380 US dollars per tons in 2023) ([Tridge, 2024](#page-10-0)), and high protein content (750–900 g  $\text{kg}^{-1}$ ) ([Galkanda-Arachchige et al., 2020; Heuz](#page-8-0)é et al., 2015). Then, hydrolysed feather meal has also a high protein content (800–850 g  $\text{kg}^{-1}$ ) (Heuzé et al., 2015), worldwide availability (+9 % annual growth expected from 2024 to 2029), and relatively low prices (178 US dollars per tons in 2023) ([Tridge, 2024](#page-10-0)), followed by poultry dry-blood meal (900–950 g kg<sup>-1</sup> of proteins), available in large quantities (+3 % annual growth expected from 2024 to 2029), and at competitive prices (288 US dollars per tons in 2023) [\(Tridge, 2024](#page-10-0)) in the aquafeed market ([Hertrampf and Piedad-Pascual, 2000; Heuz](#page-9-0)é and Tran, 2016).

The inclusion of PAPs has the potential to minimise the amount of fishmeal in aquafeeds, preserving production efficiency and potentially increasing the economic and environmental sustainability of fish farming, as demonstrated by previous studies in fish species such as Nile tilapia (*Oreochromis niloticus*; [Ibrahim et al., 2022](#page-9-0)), gibel carp (*Carassius auratus*; [Yang et al., 2004](#page-10-0)), and sea bream (*Sparus aurata*; [Martí](#page-9-0)[nez-Llorens et al., 2008](#page-9-0)). However, few information is available about the potential benefits of PAPs inclusion in diets for African catfish ([Abdel-Warith et al., 2001; Elesho et al., 2021\)](#page-8-0). In details, experimental diets including from 270 g kg<sup>-1</sup> to 290 g kg<sup>-1</sup> animal protein sources (including 80–120 g kg<sup>-1</sup> fishmeal), balanced with vegetable protein

sources (480–608 g  $kg^{-1}$ ), have resulted in promising dietary formulation for African catfish reared in RAS ([Shaw et al., 2024](#page-9-0)). While the maximum inclusion of PAPs in diet for catfish species is actually considered to be approximately 300 g kg<sup>-1</sup> [\(Kumar et al., 2017\)](#page-9-0), further research is warranted to assess the effects of high inclusion levels of PAPs in commercial diets in view of a higher environmental and economic sustainability of diet formulations.

Therefore, this study aimed at evaluating the effects of decreasing levels of fishmeal (90–10 g  $kg^{-1}$ ) and increasing levels of blends of poultry PAPs (227–392 g  $kg^{-1}$ ) in four commercially available, marketconsistent, and price-stable practical diets, on digestibility, growth performance and fillet traits of African catfish (*Clarias gariepinus*) reared in controlled RAS conditions.

#### **2. Materials and methods**

## *2.1. Ethics statement*

The experimental procedures were conformed to the European Community Directive (No. 2010/63/EU) ([EC, 2010\)](#page-8-0), and authorized by the Czech Ministry of Education Youth and Sports (No. MSMT-6744/2018–2), regarding the protection of animals used for experimental and other scientific purposes. Research staff involved in animal handling were animal specialists and veterinary practitioners.

# *2.2. Raw materials and diets*

Fishmeal (660 g kg $^{-1}$  crude protein − CP, 89 g kg $^{-1}$ ether extract − EE, and 170 g kg<sup>-1</sup> ash), poultry by-product meal (640 g kg<sup>-1</sup> CP; 99 g kg<sup>-1</sup> EE; 127 g kg<sup>-1</sup> ash), hydrolysed feather meal (850 g kg<sup>-1</sup> CP; 61 g kg<sup>-1</sup> EE; 21 g kg<sup>-1</sup> ash), and poultry dry-blood meal (900 g kg<sup>-1</sup> CP; 5 g kg<sup>-1</sup> EE; 20 g kg<sup>-1</sup> ash), commercially available products obtained according to standard practices ([De Blas et al., 2019](#page-8-0)), were used for the formulation and production of the tested practical diets. In details, these ingredients were included at different rates in four isonitrogenous (CP: 436 g kg<sup>-1</sup> DM), isolipidic (EE: 118 g kg<sup>-1</sup> DM), and isoenergetic (gross energy: 16.5 MJ  $kg^{-1}$ ) practical diets formulated to have a low and decreasing fishmeal content (from 91 to 10 g  $kg^{-1}$ ) and increasing levels of poultry PAPs (227–392 g kg<sup>-1</sup>) ([Table 1\)](#page-2-0). Specifically, we defined "low," "medium," and "high" based on the inclusion levels of fishmeal and PAPs of the four commercial diets. These diets were formulated according to the specifications and requirements of the aquafeed company NaturAlleva (VRM s.r.l., Cologna Veneta, Verona, Italy), ensuring they meet industry standards for nutritional balance, ingredient quality, and regulatory compliance. This approach ensures that the diets are representative of the practical and market-ready formulations used in commercial aquaculture. In detail, in diet HF-LP (i.e. high fishmeal and low PAPs), fishmeal was included at 91.3 g  $kg^{-1}$ , poultry by-product meal at 45.2 g kg<sup>-1</sup>; hydrolysed feather meal at 90.3 g kg<sup>-1</sup> and poultry dry-blood meal at 91.2 g  $kg^{-1}$  (total PAPs: 227 g  $kg^{-1}$ ). In diet MF-MP (i. e. medium fishmeal and medium PAPs) fishmeal was reduced at 46.1 g kg<sup>-1</sup> and balanced with 99.3 g kg<sup>-1</sup> of poultry by-product meal, 90.3 g kg<sup>-1</sup> of hydrolysed feather meal, and 91.2 g kg<sup>-1</sup> of poultry dry-blood meal (total PAPs: 281 g  $kg^{-1}$ ). In diet HF-HP (i.e. high fishmeal and high PAPs), fishmeal was included at 92.0 g  $kg^{-1}$ , poultry by-product meal was increased at 141.5  $g kg^{-1}$ , hydrolysed feather meal was increased at 127.4 g  $kg^{-1}$ , and poultry dry-blood meal was reduced at 45.5 g  $kg^{-1}$  (total PAPs: 314 g  $kg^{-1}$ ). Finally, in diet LF-HP (i.e. low fishmeal and high PAPs), fishmeal was further reduced at 10.1 g  $kg^{-1}$ , poultry by-product meal was included at 164.7 g kg<sup>-1</sup>, hydrolysed feather meal at 136.1 g kg<sup>-1</sup> and poultry dry-blood meal at 91.6 g kg<sup>-1</sup> (total PAPs: 392 g  $\text{kg}^{-1}$ ) [\(Table 1\)](#page-2-0). Other protein sources were included in all diets to reach the targeted protein level and to adjust nutrient and energy contents: soybean meal  $(0-144.5 \text{ g kg}^{-1})$ , rapeseed meal  $(135.5-181.4 \text{ g kg}^{-1})$ , wheat meal  $(162.5-226.8 \text{ g kg}^{-1})$ , and other minor sources (corn gluten meal, pea meal, whey protein concentrate,

#### <span id="page-2-0"></span>**Table 1**

Ingredients (g kg<sup>-1</sup> as fed) and proximate composition (% DM) of the tested practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs).

	<b>Diets</b>					
	HF-LP	MF-MP	$HF-HP$	LF-HP		
Ingredients, g $kg^{-1}$ as fed						
Fishmeal from by-products <sup>a</sup> (CP	91.3	46.1	92.0	10.1		
66 % DM)						
Poultry by-product meal <sup>b</sup> (CP 64 %	45.2	99.3	141.5	164.7		
DM)						
Hydrolysed feather meal <sup>c</sup> (CP 85 %	90.3	90.3	127.4	136.1		
DM)						
Poultry dry blood meal <sup>d</sup> (CP 90 %	91.2	91.2	45.5	91.6		
DM)						
Total $PAPS^{(2+3+4)}$	226.7	280.8	314.4	392.4		
Soybean meal	144.5	115.5	73.5	0.0		
Rapeseed meal	135.5	135.5	136.5	181.4		
Wheat meal	162.5	180.6	191.1	226.8		
Corn gluten meal	41.7	43.6	0.0	0.0		
Pea meal	90.3	90.3	91.0	84.9		
Total vegetable protein meals	574.5	565.5	492.1	493.1		
Whey protein concentrate	0.2	0.2	0.2	0.2		
Hydrolysed fish protein	12.3	12.3	12.3	12.3		
Rapeseed vegetable oil	75.6	75.6	69.3	72.0		
Fish oil	7.1	7.1	6.5	6.8		
Dl-methionine	4.5	4.5	5.5	5.4		
Emulsifier (E484)	1.3	1.3	1.2	1.2		
Vitamin and mineral premix <sup>e</sup>	5.9	5.9	5.9	5.9		
Vitamin <sub>C</sub>	0.7	0.7	0.7	0.7		
Proximate composition						
Dry matter, %	90.8	90.8	90.8	90.8		
Crude protein, % DM	43.5	43.5	43.6	43.6		
Ether extract, % DM	11.7	11.8	11.8	11.9		
Ash, % DM	5.11	4.79	5.87	4.57		
Crude fibre, % DM	3.48	3.43	3.36	3.83		
Gross energy <sup>f</sup> , MJ $kg^{-1}$	18.5	18.7	19.2	19.7		
Digestible energy <sup>f</sup> , MJ $kg^{-1}$	16.1	16.3	16.6	17.0		
Digestible protein / digestible	22.9	22.7	22.1	21.6		
energy <sup>f</sup> , g $MJ^{-1}$						

CP: Crude protein; DM: Dry matter; Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs.

<sup>a</sup> Fishmeal: ether extract 8.9 %, ash 17.0 %;

 $^{\rm b}$  Poultry-by product meal: ether extract 9.9 %, ash 12.7 %.

 $c$  Hydrolysed feather meal: ether extract 6.1 %, ash 2.1 %;

<sup>d</sup> Poultry dry-blood meal: ether extract 0.5 %, ash 2.0 %;

<sup>e</sup> Vitamin and mineral premix (quantities in 1 kg of mix): Vitamin A, 4000,000 IU; Vitamin D3, 800,000 IU; Vitamin C, 25,000 mg; Vitamin E, 15,000 mg; Inositol, 15,000 mg; Niacin, 12,000 mg; Choline chloride, 6000 mg; Calcium Pantothenate, 3000 mg; Vitamin B1, 2000 mg; Vitamin B3, 2000 mg; Vitamin B6, 1800 mg; Biotin, 100 mg; Manganese, 9000 mg; Zinc, 8000 mg; Iron, 7000 mg; Copper, 1400 mg; Cobalt, 160 mg; Iodine 120 mg; Anticaking and Antioxidant + carrier, making up to 1000 g. Antioxidant premix 0.50 g  $\text{kg}^{-1}.$ 

<sup>f</sup> Calculated based on data of raw materials available at NaturAlleva (VRM s.r. l., Cologna Veneta, Verona, Italy).

and hydrolysed fish protein). Amino acids profile of the four diets ([Table S1](#page-8-0)) was calculated based on data of amino acids composition of each raw material available at NaturAlleva production plant gate (VRM s.r.l., Cologna Veneta, Verona, Italy). Lipids were mainly provided by rapeseed oil (73.1 g  $\text{kg}^{-1}$ ) followed by fish oil (6.9 g  $\text{kg}^{-1}$ ). All diets met the nutritional requirements for African catfish set out by the National Research Council ([NRC, 2011](#page-9-0)).

The four diets were produced by NaturAlleva (VRM s.r.l., Cologna Veneta, Verona, Italy) as a sinking extruded pellet with a 3.0-mm diameter.

# *2.3. Rearing conditions and fish*

The trial run at the experimental facility of the Institute of Aquaculture and Protection of Waters (IAPW, Faculty of Fisheries and

Protection of Waters), University of South Bohemia in Ceske Budejovice, Czech Republic. The experimental RAS consisted of 12 rectangular rearing tanks (volume 300 L per tank), four sump tanks (volume 3500 L per tank), and four submerged biofilter tanks (volume 3500 L per tank) filled with bio-elements (BT 10, Ratz Aqua & Polymer Technik GmbH, Remscheid, Germany). The RAS used a water-gas mixer (Type 250, Ratz Aqua & Polymer Technik GmbH), a filter foam (Bioakvacit PPI 10, Jezirka Banat, s.r.o., Hnevotin, Czech Republic), and a drum filter (AEM ECO15, DVS-FilterTechniek, Kerkrade, Netherlands). Fish tanks were supplied with a water flow of 4–5 L min<sup>-1</sup> through pumps (DM-20,000 Vario, AquaForte, Veghel, Netherlands). The photoperiod was 8L:16D with a light period between 8:00–16:00. Light intensity over the tanks with fish, measured using a light metre (DT-8809, Cem, Shenzhen, China), was 500 lx. Water temperature, pH, and dissolved oxygen were daily measured using a multi-parameter probe (HQ40d multi, Hach Lange s.r.o., Prague, Czech Republic);  $NH<sub>4</sub>$ , NH<sub>3</sub>, and NO<sub>2</sub> contents were measured three times a week using LCK cuvette tests with barcode and spectrophotometer (DR 2800, Hach Lange, Loveland, Colorado, USA). During the trial, water was heated to  $27 \pm 0.5$  °C using air-conditioning in the room (Inverter, LG, Seoul, South Korea) and oxygen saturation was maintained at  $60 \pm 12$ % (air pump Airmac DBMX80, Air Mac, Inc., Dallas, Texas); water pH was 7.1  $\pm$  0.3; NH<sub>4</sub><sup> $+$ </sup> 0.83  $\pm$  0.45 mg L<sup>-1</sup>; NH<sub>3</sub>  $0.001 \pm 0.002$  mg L<sup>-1</sup>; NO<sub>2</sub>  $0.32 \pm 0.11$  mg L<sup>-1</sup>.

A total of 600 African catfish (initial live weight  $116 \pm 16$  g) were purchased from a commercial farm (AGRO Fish Farm Ltd., Handlová, Slovakia) and transported after one day of fasting to the IAPW facility. On arrival, fish were randomly distributed into the 12 tanks (50 fish per tank) with an initial stocking density of 19.2  $\pm$  3.3 kg m<sup>-3</sup>. Fish were acclimated to the system for one week before the beginning of the experiment. The trial lasted 84 days. Mortality was daily checked; fish were fed twice a day to visual apparent satiation (9:00 and 15:00).

#### *2.4. Fish performance and in vivo recordings*

At the beginning of the trial, live weight, total length, and standard length of catfish were individually measured. These measurements were repeated on days 21, 42, 63, and 84 of the trial (data not reported) to track the fish growth. For all these recordings, fish were removed from their tank, placed in a separate one with an oxygen supply, and anaesthetised with clove oil containing 87 % eugenol (0.03 mL  $L^{-1}$  of water). Then, fish were individually weighed on a scale (accuracy 0.01 g) and photographed (DSC-HX60, Sony, Tokyo, Japan), and their biometry was measured by a millimetre ruler (ImageJ programme; SKX222, Ohaus, Nänikon, Switzerland).

Finally, Fulton's condition factor  $(K_c)$  was calculated on an individual base, whereas the specific growth rate (SGR) and the feed conversion ratio (FCR) were calculated on a tank basis as follows:

Fulton's condition factor (K<sub>c</sub>) =  $100 \times$  live weight of the fish (g) / [total length of the fish (cm)] ^3.

Specific growth rate (SGR, % day<sup>-1</sup>) = [ln (average final weight (g) – ln (average initial weight (g)]  $\times$  100 / days of trial.

Feed conversion ratio (FCR) = weight of dry feed distributed (g)  $/$ weight gain of fish (g).

## *2.5. Faeces collection and digestibility assay*

The rearing tanks had a sedimentation cone with a valve located at the tank bottom ([Cho et al., 1982](#page-8-0)). To prevent contamination of faeces by feed, the removable chambers were cleaned 30 min after each feeding session. Faecal samples were daily and systematically collected at 9:30 and 15:30 throughout the 84-day trial period. To extract faeces, a siphoning process via cones was adopted. The collected material was left to settle before undergoing centrifugation (Heraeus Megafuge 16 R, Thermo Electron LED GmbH, Langenselbold, Germany) at 2000 rpm for 5 min. Subsequently, the faecal samples were freeze-dried using a lyophilisator (Home Pro Freeze Dryer, Harvest Right, LLC, North Salt Lake,

Utah, USA). Freeze-dried faeces collected during the trial were merged per tank; specifically, one pool per each of the 3 tanks (replicates) per each dietary treatment was sampled, resulting in a total of 12 samples analysed. Apparent digestibility coefficients (ADC, %) of diets were calculated for crude protein and crude fat, using crude fibre as indicator ([Krontveit et al., 2014](#page-9-0)), according to the following formula ([Cho et al.,](#page-8-0)  [1982\)](#page-8-0):

ADC (%) = 100 – [100  $\times$  (% crude fibre in feed / % crude fibre in faeces)  $\times$  (% nutrient in faeces / % nutrient in feed)].

# *2.6. Chemical analysis of diets and faeces*

The chemical composition of diets and faeces was measured in triplicates for dry matter (#930.15), crude protein (#984.13), crude fat (#920.39), and ash (#942.05) using AOAC methods ([AOAC, 2019](#page-8-0)). The determination of fibre content followed the procedure described in Reg. CE 152/2009 [\(EC, 2009\)](#page-8-0).

# *2.7. Recordings at fish slaughtering*

At the end of the trial (84 d), fish were anaesthetized through a eugenol bath in the same tank of rearing, followed by euthanasia via eugenol overdose. Subsequently, 12 fish per treatment (4 per tank) were individually weighed, eviscerated, and dissected and the weights of the carcasses and selected tissues (intraperitoneal fat, gonads, liver, spleen, and viscera) were measured to the nearest 0.0001 g (Adventurer Pro AV264C, Ohaus, Nänikon, Switzerland). Thereafter, intraperitoneal fat (%), gonadosomatic (%), hepatosomatic (%), spleen somatic (%), and viscerosomatic (%) indexes, along with carcass yield (%), head incidence (%), fillet with skin yield (%), and fillet without skin yield (%) were calculated as shown below:

Intraperitoneal fat index (IPF,  $\%$ ) = intraperitoneal fat weight (g) / fish weight (g)  $\times$  100.

Gonadosomatic index (GSI, %) = gonads weight (g) / fish weight (g)  $\times$  100.

Hepatosomatic index (HSI, %) = liver weight (g) / fish weight (g)  $\times$ 100.

Spleen somatic index (SSI, %) = spleen weight (g) / fish weight (g)  $\times$ 100.

Viscerosomatic index (VSI, %) = viscera weight (g) / fish weight (g)  $\times$  100.

Carcass yield (%) = carcass weight (g) / fish weight (g)  $\times$  100.

Head incidence (%) = head weight (g) / fish weight (g)  $\times$  100.

Fillet with skin yield (%) = fillet with skin weight (g) / fish weight (g)  $\times$  100.

Fillet without skin yield (%) = fillet without skin weight (g) / fish weight (g)  $\times$  100.

# *2.8. Fillet colour and pH*

On 12 fillets per dietary treatment (4 fillets per tank), 60 h after dissection, colour was measured in duplicate at three locations along the dorsal part (frontal, middle, and caudal) and at two locations along the ventral part (frontal and caudal) of each fillet using a spectrophotometer (CM-600d, Konica Minolta Inc., Japan) and expressed according to the CIELab system [\(CIElab, 1976\)](#page-8-0) in terms of lightness  $(L^*)$ , redness  $(a^*)$ , and yellowness (b\*) index. The pH was measured using a digital pH meter (Testo 206, Testo s.r.o. Prague, Czech Republic) by penetrating the pH probe at 2 cm into the dorsal muscle of the right fillet always at the same position.

#### *2.9. Dripping, thawing and cooking losses*

To determine dripping, thawing, and cooking losses, three pieces of 10 g flesh from the dorsal part of post-rigor fillets (12 fillets per dietary treatment; 60 h after filleting) were separated, weighed, and individually packed under vacuum in polyvinyl chloride bags. Samples for dripping losses were stored at  $2.0 \pm 0.5$  °C in a refrigerator for 24 h and 48 h. Packed samples for thawing losses determination were frozen at − 20 ◦C for 24 h and thawed to 4◦C in a refrigerator. Packed samples for cooking losses were cooked in a water bath at 75 ◦C for 20 min and cooled down in a refrigerator for 3 h. After 3, 24, or 48 h, samples were unpacked, exudate was wiped, and samples were weighed. Then, losses were calculated according to the following formulas [\(Varga et al., 2010](#page-10-0)):

Dripping losses (%) = [(weight of flesh sample at 0 h (g) – weight of flesh sample at 24 or 48 h (g)) / weight of flesh sample at 0 h (g)]  $\times$  100.

Thawing losses (%) =  $[$  (weight of flesh sample before freezing (g) – weight of flesh sample after thawing (g)) / weight of flesh sample before freezing  $(g)$ ]  $\times$  100.

Cooking losses  $(\%)$  = [(weight of uncooked flesh sample  $(g)$  – weight of cooked flesh sample (g)) / weight of uncooked flesh sample (g)]  $\times$ 100.

# *2.10. Sensory analysis of fillets and cooked meat*

A sensory panel consisting of 9 evaluators (six men and three women; 28–70 age) with expertise in evaluating fish products conducted the sensory analysis following ISO 8589:2007 guidelines ([ISO, 2007](#page-9-0)). Three pieces of fresh fillets ( $2 \times 2$  cm squares) from six fish per dietary treatment were placed into glass jars labelled with unique codes and then cooked in an electric oven (150 ◦C for 15 min). The evaluators assessed the freshly cooked fish samples individually (4 samples per dietary treatment; 4 samples per time for two rounds) in designated chambers equipped with utensils, water glass, bread pieces, evaluation forms, pencils, and paper towels. Each evaluator assessed sensory attributes (odour, texture, flavour, and aftertaste) of all samples in duplicate rounds. Evaluation scores ranged from 0 (very bad/negligible) to 100 points (very good/very strong), with provision for verbal notes on the evaluation forms.

#### *2.11. Economic analysis*

Feed production costs were calculated by cost data of each raw material available at NaturAlleva production plant gate (VRM s.r.l., Cologna Veneta, Verona, Italy). Costs of labour, packaging, and transport were not included. Then, feed cost to produce 1 kg of fish ([Fanizza](#page-8-0)  [et al., 2023](#page-8-0)), economic conversion ratio and economic profit index ([Martínez-Llorens et al., 2007](#page-9-0)) were calculated according to the following formulas:

Feed cost to produce 1 kg of fish (€ kg fish<sup>-1</sup>) = feed production cost  $(€ kg<sup>-1</sup>) × feed conversion ratio.$ 

Economic Conversion Ratio (ECR,  $\epsilon$  kg fish<sup>-1</sup>) = [feed conversion ratio  $\times$  feed production cost ( $\in$  kg<sup>-1</sup>)] / weight gain (kg).

Economic Profit Index (EPI,  $\epsilon$  fish<sup>-1</sup>) = [final weight (kg fish<sup>-1</sup>) × fish sale price ( $\epsilon$  kg<sup>-1</sup>)] – [ECR ( $\epsilon$  kg fish<sup>-1</sup>)  $\times$  weight gain (kg)].

where the fish sale price was set at 2.74  $\epsilon$  kg<sup>-1</sup> according to Scientific, Technical and Economic Committee for Fisheries [\(STECF Scientific](#page-9-0)  [Technical Economic Committee for Fisheries, 2023\)](#page-9-0).

#### *2.12. Statistical analysis*

All data were checked for normality through a Shapiro-Wilk test. Data related to individual catfish biometry and tank growth performance were submitted to ANOVA using the PROC MIXED of [SAS \(2013\)](#page-9-0), considering diet and time on trial as fixed effect, with interactions, and tank as a random effect (for individual data). Fish survival was analysed using PROC CATMOD with diet, time, and their interaction as fixed effects. Feed conversion ratio and specific growth rate, diet digestibility, slaughter results, and fillet traits were submitted to ANOVA and analysed using the PROC GLM, considering the effect of the diet. The Bonferroni test was used to compare least square means. Differences among least square means with *p<*0.05 were assumed to be statistically

significant.

# **3. Results**

# *3.1. Apparent digestibility coefficients of nutrients in the diets*

The ADC of protein was higher in fish fed the diet HF-LP (85.5 %) respect to those fed the diet LF-HP (82.3 %; +3.9 %; *p<*0.05), whereas fish fed the other diets showed intermediate values (83.8 %, on average) (Fig. 1a). The ADC of lipid was lower in fish fed the diet LF-HP (92.1 %) compared to those fed the other diets (94.2 %; –2.2 %; *p<*0.01) without differences among these latter (Fig. 1b).

# *3.2. Fish survival rate, growth performance, and somatic indexes*

Survival rate was higher in fish fed diet MF-MP than in fish fed the other diets  $(+2.4 \%; p<0.001)$  (Table 2). Final body weight was significantly higher in fish fed diets HF-LP and MF-MP than in fish fed diet HF-HP (+11 %; *p<*0.001), and in those fed diet HF-HP than in those fed diet LF-HP  $(+12 \%)$ ;  $p < 0.001$ ). Feed intake was higher in fish fed the diets HF-LP and MF-MP than in those fed diet HF-HP (+13 %; *p<*0.001) and in fish fed diet HF-HP than in those fed diet LF-HP (+10 %; *p<*0.001). The specific growth rate was higher in fish fed diets HF-LP compared to fish fed diet MF-MP and HF-HP (+6.5 %; *p<*0.001) and in fish fed diets MF-MP and HF-HP compared to those fed diet LF-HP (+10 %; *p<*0.001). Feed conversion ratio was higher in tanks belonging to LF-HP treatment respect to the other treatments  $(+17\%; p<0.001)$  (Table 2).

At slaughter (84 d of trial), total length and standard length were significantly higher in fish fed diets HF-LP and MF-MP compared to fish fed diet HF-HP ( $+2.8$  % and  $+3.1$  % respectively;  $p < 0.001$ ), and in fish fed diet HF-HP than in those fed diet LF-HP  $(+5.0 %$  and  $+5.3 %$ ; *p<*0.001). The same trend was recorded for the condition factor, being higher in fish fed diets HF-LP and MF-MP than in fish fed diet HF-HP (+3.4 %;  $p$ <0.001) and in fish fed diet HF-HP than in those fed diet LF-HP (+1.2 %; *p<*0.001) (Table 3).

Fish intraperitoneal fat (3.31 %, on average), gonadosomatic  $(0.46 \%)$ , hepatosomatic  $(1.33 \%)$ , spleen somatic  $(0.05 \%)$  and viscerosomatic (2.76 %) indexes did not differ among the four diets (*p>*0.05) (Table 3).

#### *3.3. Slaughter yields and fillet traits*

Carcass yield (90.8 %, on average), head incidence (27.0 %) and fillet yield without skin (39.0 %) were not different in fish fed the different diets. The fillet yield was higher in fish fed diet MF-MP respect to those fed diet LF-HP  $(+5.1 \%)$ ,  $p<0.05$ ), whereas the other groups showed intermediate values ([Table 4](#page-5-0)).

Muscle pH was higher in fish fed diets MF-MP and HF-HP than in those fed diet LF-HP ( $+2.7$  %;  $p < 0.001$ ). The lightness (L\*) was higher in fish fed diet LF-HP than in those fed diets HF-HP and MF-MP (+6.8 %;

**Table 2** 

Fish growth performance and survival in African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and processed animal proteins (PAPs).

	<b>Diets</b>				<i>p</i> -value	<b>RMSE</b>
	HF- LP	MF- MP	HF- HP	LF- HP		
Fish, n. Final weight, g Tanks, n.	142 704 <sup>c</sup> 3	145 690 <sup>c</sup> 3	141 624 <sup>b</sup> 3	142 551 <sup>a</sup> 3	< 0.001	196
Feed intake, g $d^{-1}$	317 <sup>c</sup>	$313^{\circ}$	$274^{\rm b}$	247 <sup>a</sup>	< 0.001	74.2
Specific growth rate, % $d^{-1}$	$3.23^{\circ}$	$3.18^{bc}$	3.00 <sup>b</sup>	2.69 <sup>a</sup>	< 0.001	0.82
Feed conversion ratio	$1.14^{a}$	1.06 <sup>a</sup>	$1.14^{a}$	1.34 <sup>b</sup>	< 0.001	0.15
Survival, %	$94.5^{\circ}$	$96.8^{b}$	$94.3^{\rm a}$	$94.6^{\circ}$	${<}0.001$	2.18

Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs. RMSE: root mean square error. a,b,c Different superscript letters within the same effect represent significant differences between means (*p <*0.05).

#### **Table 3**

Biometric measures and somatic indices of African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs) at slaughtering (84 days of trial).

	<b>Diets</b>				<i>p</i> -value	<b>RMSE</b>
	HF- LP	MF- MP	HF- HP	LF- HP		
Fish, n. Total length, mm Standard length, mm	142 431 <sup>c</sup> $390^{\circ}$	145 $427$ <sup>bc</sup> $382^{bc}$	141 417 <sup>b</sup> 374 <sup>b</sup>	142 396 <sup>a</sup> 354 <sup>a</sup>	${<}0.001$ < 0.001	41.3 39.0
Fulton's condition factor	0.87 <sup>b</sup>	0.86 <sup>b</sup>	$0.84^{ab}$	$0.83^{a}$	< 0.001	0.09
Fish, n.	12	12	12	12		
IPF, %	3.04	3.30	3.17	3.74	0.17	0.80
GSI, %	0.33	0.44	0.60	0.46	0.17	0.29
<b>HSI, %</b>	1.34	1.23	1.30	1.45	0.10	0.21
SSI, %	0.05	0.04	0.05	0.04	0.43	0.01
VSI, %	2.77	2.78	2.70	2.80	0.92	0.36

Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs. IPF: intraperitoneal fat index; GSI: gonadosomatic index; HSI: hepatosomatic index; SSI: spleen somatic index; VSI: viscerosomatic index. RMSE: root mean square error. a,b,c Different superscript letters within the same effect represent significant differences between means (*p <*0.05).



**Fig. 1.** Apparent digestibility coefficients of protein (a) and fat (b) in African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs); *n*=48 measurements. Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs. Data are represented as means  $\pm$  standard error of the mean. <sup>a,b,c</sup> Different letters above bars represent significant differences between means (*p<*0.05).

#### <span id="page-5-0"></span>**Table 4**

Slaughter results and fillet traits of African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and processed animal proteins (PAPs) at the end of the feeding period (84 d of trial).

	<b>Diets</b>				<i>p</i> -value	<b>RMSE</b>
	HF-LP	MF- MP	HF- HP	LF- HP		
Fish, n.	12	12	12	12		
Carcass vield, %	91.4	90.3	90.8	90.7	0.30	1.37
Head incidence, %	26.3	27.2	26.8	27.7	0.21	1.60
Fillet yield (with	$47.8^{b}$	$46.3^{ab}$	46.9 <sup>ab</sup>	45.5 <sup>a</sup>	0.03	1.87
skin), %						
Fillet yield (without	39.8	38.8	39.4	38.0	0.11	1.84
skin), %						
pН	6.09 <sup>b</sup>	$6.10^{b}$	$6.02^{ab}$	5.94 <sup>a</sup>	< 0.001	0.07
$L^*$	$42.5^{\circ}$	$42.3^a$	44.3 <sup>ab</sup>	$45.3^{b}$	0.00	1.73
a*	3.11	3.89	4.11	3.52	0.33	1.41
$h^*$	$9.35^{ab}$	8.92 <sup>a</sup>	10.2 <sup>b</sup>	$10.2^{b}$	0.01	1.02

Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs. RMSE: root mean square error. a,b,c Different superscript letters within the same effect represent significant differences between means (*p<*0.05).

*p<*0.05). The yellowness (b\*) was higher in fish fed diets HF-LP and LF-HP than in those fed diet HF-HP (+14.4 %; *p<*0.05). The redness (a\*) was not affected by the diets (Table 4).

As showed in Fig. 2, fillets of fish fed diet MF-MP showed the lowest thawing and dripping losses after 24 h respect to those fed diet HF-LP  $(-31.4 %$  and  $-29.1 %$ , respectively;  $p<0.01$ ), whereas the other fish performed in between (Fig. 2a and c). Dripping losses after 48 h (4.34 %, on average) and cooking losses (17.0 %) were not affected by the diets (Fig. 2b and d).

Regards sensory analysis of fish fillets and cooked meat [\(Fig. 3](#page-6-0)), no differences were found among the fish fed the four diets (*p>*0.05) in terms of pleasantness of appearance (81.1, average score), pleasantness of aroma (77.9), intensity of aroma (55.9), overall pleasantness of taste (80.4), intensity of fish flavour (35.8), greasiness (12.8), juiciness (66.0), pleasantness of consistency (84.3), meat firmness (55.1), meat solidity (78.1), and intensity of aftertaste (24.3).

### *3.4. Economic profile of the practical diets*

As showed in [Table 5](#page-6-0), the feed production cost was the highest for

the diet HF-LP (0.66 € kg<sup>-1</sup>) and the lowest for the diet LF-HP (0.58 €  $kg^{-1}$ ), whereas the highest feed cost to produce 1 kg of fish was reported for diet LF-HP (0.78 € kg fish<sup>-1</sup>) and the lowest for diet HF-HP (0.57 € kg  $fish^{-1}$ ). The economic conversion ratio of diet LF-HP was the highest (1.78 € kg fish<sup>-1</sup>), whereas that of diet MF-MP was the lowest (1.18 € kg  $fish^{-1}$ ). The highest economic profit index was obtained with fish fed the diet HF-LP (1.45 € fish<sup>-1</sup>), the lowest with fish fed the diet LF-HP (1.18 €  $fish^{-1}$ ).

#### **4. Discussion**

This study aimed to reduce the dependence on marine resources in practical aquafeeds for African catfish by partially replacing fishmeal with a blend of poultry PAPs, including poultry by-product meal, hydrolysed feather meal, and poultry dry-blood meal. The specific blends of the abovementioned PAPs were selected following industry standards, including considerations of market competitiveness, and considering the scarce literature available on optimal levels of PAPs for Siluriformes.

Previous research has highlighted the wide variability in nutrient digestibility and fish growth performance associated with PAPs inclusion in diet for different farming fish, such as omnivorous species ([Abdel-Warith et al., 2001\)](#page-8-0), freshwater carnivorous species [\(Gouveia,](#page-8-0)  [1992\)](#page-8-0) and marine carnivorous species ([Martínez-Llorens et al., 2008](#page-9-0)). The observed variability in nutritional profiles can also be attributed to disparities in the source and quality of different PAPs [\(Hoerterer et al.,](#page-9-0)  2022; Tomás-Vidal et al., 2019). These intrinsic differences need cautious interpretation when extrapolating results across studies, emphasising the importance of considering source-specific characteristics in comparative analyses. Nevertheless, there is the need for comprehensive evaluations of PAPs tailored to commercial feed formulations. Thus, to the best of our knowledge, this study represents the first evaluation of commercial formulations assessing digestibility and growth performance, fillet quality traits, and economic profitability of practical diets for African catfish containing different blends of PAPs.

The findings of this study on practical diets for African catfish show that is possible to partially replace fishmeal with poultry PAPs, maintaining a minimum fishmeal inclusion of 45 g  $kg^{-1}$ . Fishmeal replacement can be achieved by including poultry by-product meal and hydrolysed feather meal up to 100 g  $kg^{-1}$  and 90 g  $kg^{-1}$ , respectively. Through this formulation, while maintaining good growth performance and diet digestibility, it has been achieved a noteworthy improvement of



**Fig. 2.** Dripping losses after 24 h (a) and 48 h (b), thawing losses (c), and cooking losses (d) in fillets of African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs); *n*=48 measurements. Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs. Data are represented as means  $\pm$  standard error of the mean. a,b,c Different letters above bars represent significant differences between means (*p<*0.05).

<span id="page-6-0"></span>

-HF-LP -MF-MP -HF-HP -LF-HP

**Fig. 3.** Sensory analysis of the fillet and cooked meat of African catfish (*C. gariepinus*) fed practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs); *n*=72 measurements. Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs.

#### **Table 5**

Production costs and economic indexes of practical diets including different levels of fishmeal and poultry processed animal proteins (PAPs) fed to African catfish (*C. gariepinus*).



Diet HF-LP: high fishmeal and low PAPs; diet MF-MP: medium fishmeal and medium PAPs; diet HF-HP: high fishmeal and high PAPs; Diet LF-HP: low fishmeal and high PAPs.

<sup>a</sup> Feed production cost at production plant gate: costs of labour, packaging and transport are not included.

<sup>b</sup> African catfish sale price is calculated at 2.74  $\epsilon$  kg<sup>-1</sup>.

feed economic competitiveness compared to diets with a high inclusion of fishmeal and a low inclusion of poultry PAPs. Similar results were obtained by other authors ([Mustapha and Adeniyi, 2022\)](#page-9-0) in African catfish diets including low fishmeal (50 g  $kg^{-1}$ ) and poultry offal meal (100 g kg<sup>-1</sup>) ([Hag et al., 2017](#page-9-0)) as well as in diets with an inclusion of 95 g  $\text{kg}^{-1}$  of fishmeal and feather meal, respectively.

On the other hand, the diet characterized by the lowest inclusion of fishmeal and the highest inclusion of PAPs resulted in the lowest protein and fat digestibility compared with the other diets. In literature, different ADC values for protein (56–83 %) and fat (92–97 %) have been documented in practical diets (61 g kg<sup>-1</sup> of fishmeal and 300 g kg<sup>-1</sup> of poultry meal) for African catfish ([Orisasona et al., 2021\)](#page-9-0). This variability in ADCs may be attributed to potential fluctuations in the inherent content of feathers, connective tissue, and skin in poultry offal meals, which have a low digestibility [\(Hasan et al., 1997; Sevgili et al., 2019](#page-9-0)). Additionally, the processing method (e.g. autoclaving or oven-drying) and the long exposure of poultry by-product meal to high temperatures (over 200 ◦C for 10 h) [\(Orisasona et al., 2016](#page-9-0)) can result in lysine and cystine+cysteine losses, thereby further reducing protein and amino acid digestibility [\(McCallum and Higgs, 1989; Opstvedt et al., 1984;](#page-9-0)  [Sevgili et al., 2019\)](#page-9-0).

The reduced digestibility observed in diet LF-HP could be attributed also to its high content of rapeseed meal (181 g  $\text{kg}^{-1}$ ) and the absence of soybean meal in its formulation. Previous studies have shown that diets

containing more than 112 g  $kg^{-1}$  of rapeseed meal can depress the activity of digestive enzymes (i.e. pepsin, intestinal trypsin, lipase, and amylase) in Asian red-tailed catfish (*Hemibagrus wyckioides*; [Zhang et al.,](#page-10-0)  [2020\)](#page-10-0). Similar impairments in fish performance and feed utilisation have been reported in other catfish species fed diets high in rapeseed meal (>200 g kg<sup>-1</sup>), such as ussuri catfish (*Pseudobagrus ussuriensis*; Bu [et al., 2018](#page-8-0)), yellow catfish (*Tachysurus fulvidraco*; [Wang et al., 2017](#page-10-0)), wels catfish (*Silurus glanis*; [Slawski et al., 2011](#page-9-0)), and channel catfish (*Ictalurus punctatus*; [Lim et al., 1998](#page-9-0)). Rapeseed meal also contains antinutritional factors, particularly sinapine and sinapic acid, which could reduce feed palatability due to their bitter taste [\(Ye et al., 2016](#page-10-0)), thereby reducing feed intake, in line with the findings of this study. Conversely, soybean meal is highly regarded in fish feed due to its favourable chemical composition, wide availability, and stable price ([Pervin et al., 2020\)](#page-9-0). Therefore, in African catfish commercial diets, it is recommended to carefully incorporate rapeseed meal together with other vegetable protein sources like soybean meal, ensuring a balanced nutritional profile, minimising the impact of antinutritional factors, and enhancing overall diet palatability and acceptance by the fish.

In African catfish, other authors ([Elesho et al., 2021\)](#page-8-0) found that the ADCs of protein and fat of poultry meal (89 % and 99 %, respectively) and hydrolysed feather meal (92 % and 97 %) as raw materials (300 g kg<sup>-1</sup> inclusion level) were lower compared to fishmeal (95 % and 100 %). On the other hand, to the best of our knowledge, no information is available about the digestibility of diets containing different blends of animal proteins in catfish, tilapia and carp species. In rainbow trout, a diet including 150 g  $\text{kg}^{-1}$  of feather meal, 40 g  $\text{kg}^{-1}$  of blood meal and 110 g kg<sup>-1</sup> of poultry by-product meal (300 g kg<sup>-1</sup> total PAPs) showed a significant reduction (-5 %) in ADC of protein compared with diets without PAPs, whereas no difference was found in ADC of fat [\(Vale](#page-10-0)  [Pereira et al., 2023](#page-10-0)). In this study, the diet LF-HP, with the lowest inclusion of fishmeal (10 g  $kg^{-1}$ ) and the highest inclusion of poultry-by product meal (165 g kg<sup>-1</sup>) and hydrolysed feather meal (135 g kg<sup>-1</sup>), did not adequately support productive performance, showing the lowest nutrient digestibility and resulting in the worst fish growth rates. This latter result could be also related to potential deficiencies in essential amino acids and micronutrients in PAPs [\(Chaklader et al., 2020\)](#page-8-0). In details, while poultry by-product meal has acceptable levels of methionine, lysine, and arginine, ensuring adequate growth of African catfish ([Ali and Jauncey, 2005; Brown et al., 1985](#page-8-0)), its suboptimal levels of histidine, isoleucine, and phenylalanine compared to fishmeal could

potentially exert adverse effects on the growth performance of this species [\(Elesho et al., 2021\)](#page-8-0). These effects have been already observed in other warm water fish such as spotted rose snapper (*Lutjanus guttatus*; Hern´ [andez et al., 2014\)](#page-9-0), silver seabream (*Rhabdosargus sarba*; [El-Sayed,](#page-8-0)  [1994\)](#page-8-0), red drum (*Sciaenops ocellatus*; [Kureshy et al., 2000\)](#page-9-0), and cobia (*Rachycentron canadum*; [Zhou et al., 2011\)](#page-10-0). Nevertheless, all the diets tested in this study had a similar amino acid profile, as reported in [Table S1](#page-8-0) (calculated values). Therefore, the difference in fish growth performance could be related to a lower palatability of diets with high inclusion of poultry by-product meal and feather meal [\(Abdel-Warith](#page-8-0)  [et al., 2001; Glencross et al., 2016; Chaklader et al., 2020](#page-8-0)), confirmed by the notable reduction of feed intake (− 22 %) found in this study when comparing diet HF-LP with diet LF-HP. Overall, the findings of this study align with prior research on African catfish [\(Abdel-Warith et al., 2001](#page-8-0)), where performance indices (i.e., final weight, SGR and FCR) worsened with increasing inclusion levels of poultry by-product meal (from 90 to 455 g  $kg^{-1}$ ) especially when associated with low fishmeal contents (<24 g kg<sup>-1</sup>). In fact, an impairment of growth performance was observed when poultry by-product meal inclusions were higher than 140 g  $kg^{-1}$  and fishmeal inclusion was about 10 g  $kg^{-1}$ , as already observed in other warm-water species such as mirror carp (*Cyprinus carpio*; [Emre et al., 2003\)](#page-8-0), barramundi (*Lates calcarifer*; [Chaklader et al.,](#page-8-0)  [2020\)](#page-8-0), and spotted rose snapper (*Lutjanus guttatus*; Hernández et al., [2014\)](#page-9-0). The reduction observed in this study in feed intake and growth performance in fish fed diet LF-HP can be also attributed to the low inclusion of fishmeal, known for its high palatability and attractiveness in African catfish diets [\(Elesho et al., 2022\)](#page-8-0). As for the PAPs other than the poultry by-product meal, high inclusion levels of hydrolysed feather meal (>100 g kg<sup>-1</sup>) have shown to potentially reduce growth performance of African catfish due to a deficiency in essential amino acids associated with a low efficiency in the keratin hydrolysis process ([Papadopoulos et al., 1986\)](#page-9-0). In line with the results of the present study, African catfish fed diets high in feather meal (>100 g kg<sup>-1</sup>) showed reduced growth and feed conversion ratio as well as a significant reduction in feed intake ([Chor et al., 2013; Mustapha and Adeniyi,](#page-8-0)  [2022\)](#page-8-0). Consistently, reduced growth performance with high feather meal inclusion (>100 g kg<sup>−1</sup>) were found also in Indian major carp (*Labeo rohita*) ([Hasan et al., 1997](#page-9-0)).

Overall, based on the results of this study and previous observations as discussed above, a low inclusion rate of poultry by-product meal ( $\leq$ 100 g kg<sup>-1</sup>) and hydrolysed feather meal ( $\leq$  90 g kg<sup>-1</sup>) in African catfish diets, along with a medium-high inclusion rate of fishmeal (45–90 g kg<sup>-1</sup>) and poultry dry-blood meal (90 g kg<sup>-1</sup>), could ensure good digestibility and fish growth performance.

Then, PAPs are low-cost, protein-rich sources that could contribute to formulate market-competitive diets [\(Kumar et al., 2017](#page-9-0)). In this sense, a formulation containing 90 g  $kg^{-1}$  of fishmeal and replacing poultry dry-blood meal (from an inclusion of 90–45 g  $\text{kg}^{-1}$ ) with poultry by-product and hydrolysed feather meal resulted in reduced cost for feed production and reduced cost of the feed necessary to produce 1 kg of fish compared to a diet high in fishmeal and low in poultry PAPs. However, this reduction in dry-blood meal with other poultry PAPs worsened the economic conversion ratio and the economic profit index. On the other hand, the replacement of half of the dry-blood meal with poultry by-product and feather meal produced an impairment of growth performance and digestibility.

Alternatively, the replacement of half of the fishmeal (maintaining 45 g kg<sup>-1</sup>) with poultry dry-blood meal (up to 90 g kg<sup>-1</sup>) could work without impairment of fish growth performance and diet digestibility ([Subhadra et al., 2006](#page-9-0)) compared to the inclusion of poultry by-product and feather meals. In fact, high dietary inclusions (up to 330 g  $\text{kg}^{-1}$ ) of dry-blood did not compromise growth performance of African catfish ([Agbebi et al., 2009; Duwal et al., 2019\)](#page-8-0). In addition, dry-blood meals have other beneficial features, such as a high palatability and lysine content (approximately 70 g  $kg^{-1}$ ) [\(Sauvant et al., 2004](#page-9-0)) as well as a binding ability ([Kirimi et al., 2016](#page-9-0)) that could yield further advantages

in feed pellets for RAS. Then, haemoglobin oxidation in dry blood meals cause a dark colouring of the RAS water ([Duwal et al., 2019](#page-8-0)), which is beneficial for muddy-water species like (e.g., *Clariidae*, *Ictaluridae*, *Siluridae*) ([Eding and Kamstra, 2001; Shaw et al., 2022\)](#page-8-0). Nevertheless, processing factors (i.e. temperature) are crucial to prevent amino acid deterioration in dry-blood meals ([Cho et al., 1982](#page-8-0)) which could decrease diet digestibility ([Njieassam, 2016; Ogello et al., 2014](#page-9-0)).

In this study, it has been tested commercial diet formulations using different animal and vegetable raw materials to ensure costeffectiveness, consistency, and scalability for large-scale aquaculture. Diets were formulated to test different blends of poultry PAPs and vegetable protein meals were included to balance dietary nutrients, which also affected fish performance. In fact, based on the results of this study, performance was comparable between fish fed diets with similar inclusions levels of both protein and vegetable protein meals. In details, performance was favourable in fish fed diet HF-LP and those fed diets MF-MP (animal ingredients: 318 g  $kg^{-1}$  and 327 g  $kg^{-1}$ , respectively; vegetable protein meals: 575 g  $\text{kg}^{-1}$  and 566 g  $\text{kg}^{-1}$ ). Digestibility and growth performance impaired in fish fed diet HF-HP and in those fed diet LF-HP (animal ingredients: 406 g kg<sup>-1</sup> and 403 g kg<sup>-1</sup>, respectively; vegetable protein meals:  $492 g kg^{-1}$  and  $493 g kg^{-1}$ ). Similar results were observed in a recent study in African catfish reared in RAS, testing combinations of animal (from 138 g kg<sup>-1</sup> to 548 g kg<sup>-1</sup>) and vegetable (from 736 g  $kg^{-1}$  to 366 g  $kg^{-1}$ ) protein meals (Shaw et al., [2024\)](#page-9-0). In a nutshell, the diet MF-MP, with moderate fishmeal inclusion (45 g kg<sup>-1</sup>) and a medium inclusion of a PAPs blend (280 g kg<sup>-1</sup>), resulted in high survival rates and favourable growth performance, without compromising fillet quality and organoleptic characteristics, i. e., it was technically and economically viable.

In fact, under the specified conditions, slaughter results and fillet traits were slightly impaired by low-fishmeal and high-PAPs diets with poultry by-product meal (>140 g kg<sup>-1</sup>) and hydrolysed feather meal (>120 g kg<sup>-1</sup>). To the best of our knowledge, these are first results on the quality traits and organoleptic properties of fillets of catfish fed PAPs-based diets. In this study, the low muscle pH found in fish fed the lowest inclusion of fishmeal and the highest of PAPs could be related to a stressful condition resulting from a low feed intake and thus a poor body condition ([Wilkinson et al., 2008\)](#page-10-0). The reduction of pH in fish muscle has been also associated with colour changes, muscle tearing, blood spots, changes in flesh texture and shelf life [\(Jones and Carton, 2015;](#page-9-0)  [Wilkinson et al., 2008](#page-9-0)). Thus, fillet of fish fed high-PAPs diets showed the brightest and yellowish colours, which could lead to a negative consumer response [\(Alfnes et al., 2006](#page-8-0)). Similarly, fillets of barramundi fed diets high in PAPs (500 g kg<sup>−</sup> <sup>1</sup> ) and *Hermetia illucens* meal (350 g kg<sup>-1</sup>) [\(Chaklader et al., 2023](#page-8-0)) were brighter than fish fed a high fishmeal (720 g  $kg^{-1}$ ) diet. On the other hand, the increased yellowness could be related to a high presence of certain yellow pigments (e.g. carotenoids) in poultry PAPs [\(Marounek and Pebriansyah, 2018](#page-9-0)), which may influence skin and flesh colours ([Pulcini et al., 2020\)](#page-9-0). In this study, the highest thawing and dripping losses after 24 h in fillets of catfish fed PAPs-rich diets are consistent with previous finding on fillets of barramundi fed PAPs-rich diets ([Chaklader et al., 2022, 2023\)](#page-8-0).

Losses resulting from dripping, thawing, and cooking could significantly impact on the juiciness, appearance, and colour of fillets, thereby influencing their attractiveness to consumers. Nevertheless, under the present scenario, an increasing inclusion levels of poultry PAPs in diets for African catfish had no positive or negative effects on the visual appearance of the fresh fillets, nor on the organoleptic characteristics of the cooked meat.

#### **5. Conclusion**

Under the present conditions, a low dietary inclusion of fishmeal (10 g  $kg^{-1}$ ) and a high inclusion of poultry by-product meal and hydrolysed feather meal (total PAPs content of 392  $g kg^{-1}$ ) did not support adequately performance of African catfish reared in a RAS

<span id="page-8-0"></span>system which would increase the time necessary to reach a target final weight. On the other hand, a diet with a medium inclusion of fishmeal (45 g kg<sup>-1</sup>) and medium inclusion of a blend of PAPs (280 g kg<sup>-1</sup>) resulted technically and economically profitable, assuring high fish survival rate and good growth performance without affecting product quality. Future studies should further explore different proportions of poultry by-product meals, hydrolysed feather meals, and dry-blood meals in commercial dietary formulations for African catfish to refine their balance in view of enhancing the overall cost-effectiveness and scalability of aquafeed tailored for closed aquaculture systems.

## **CRediT authorship contribution statement**

**Angela Trocino:** Writing – review & editing, Formal analysis. **Francesco Bordignon:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Cecilia Fanizza:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Hung Quang Tran:** Writing – review & editing, Investigation, Formal analysis. **Mahyar Zare:** Writing – review & editing, Investigation, Formal analysis. **Gerolamo Xiccato:** Writing – review & editing, Formal analysis. **Fabio Brambilla:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Marketa** ´ **Proke**ˇ**sova:** ´ Writing – review & editing, Investigation, Formal analysis. **Vlastimil Stejskal:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Data curation, Conceptualization.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### **Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.aqrep.2024.102447.](https://doi.org/10.1016/j.aqrep.2024.102447)

#### **Data availability**

Data will be made available on request.

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