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Replacement of fishmeal using poultry-based protein sources in feeds for pikeperch (*Sander lucioperca*, Linnaeus, 1758) during grow out phase

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Abstract
A 61-day growth experiment was carried out to evaluate the potential of a poultry-based protein (PBP) comprising of feather meal (FeM) and poultry meat and bone meal (PMBM), as a fishmeal (FM) substitute in diets of juvenile pikeperch (*Sander lucioperca*, L.). Pikeperch (initial body weight 113.12 g) were randomly distributed in groups of 250 fish in twelve 1.8 m^3 circular recirculating aquaculture system (RAS) tanks, and fed four isonitrogenous (52% crude protein), isolipidic (17% crude lipid), and isocaloric (21.80 KJ/g energy) experimental diets. The feeds contained FM as the primary protein source (PBP0) or PBP replacing 20 (PBP20), 40 (PBP40), or 60% (PBP60) of the FM. Based on appetite and calculated uneaten feed, the feeding rate was initially set at 1.5% and subsequently reduced to 1% of the total tank biomass towards the end of the study. At the end of the experiment, the average feed intake ranged from 1.93 to 2.00 g/fish/day ($p > 0.05$). No adverse effects on growth and feed efficiency were observed in fish fed diets PBP0, PBP20, and PBP40. Particularly, the final body weight (FBW) (248.73, 240.11 g), feed conversion ratio (FCR) (0.86, 0.91), specific growth rate (SGR) (1.29, 1.35%/day), and protein efficiency ratio (PER) (2.20, 2.12) of fish fed PBP20 and PBP40 were not significantly different from the control PBP0 whose values ranged from 248.11 g, 0.89, 1.27%/day, and 2.18, respectively ($p > 0.05$). In all treatments, the condition factor (k) (1.31 to 1.33), hepatosomatic index (HSI) (2.35 to 2.70%), visceral somatic index (VSI) (6.87 to 7.19%), and whole-body composition of crude protein (57.77–58.28%) and crude lipid (23.85–26.85%) were not significantly influenced by the dietary PBP inclusion level ($p > 0.05$). However, whole-body ash content was significantly higher in PBP60 (14.66%) compared to the other treatments (12.57–14.43%) ($p < 0.05$). Based on the results from this study, up to 40% of FM can be replaced by PBP in diets for pikeperch juveniles without compromising growth performance and feed utilization.

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Introduction

Global aquaculture production has significantly increased to meet the supply gap created by the decline in capture fisheries (FAO 2020). Consequently, this has translated into higher aquaculture feed production to meet the nutritional requirements of farmed fish. However, the reliance on marine feed ingredients, i.e., fishmeal (FM), and the rising cost of aquaculture feeds resulting from the high cost of FM threatens the sustainability of the sector (Boyd 2015; Oliva-Teles et al. 2015; Cottrell et al. 2020). While FM is an ideal protein source for aquaculture feeds because of its optimal amino acid profile, high digestibility, and high contents of essential minerals and fatty acids (Woodgate et al. 2022), its use as the primary source of protein in aquaculture feeds needs to be lessened (Boyd 2015; Oliva-Teles et al. 2015).

Considerable progress has been achieved in that regard using protein-rich plant-based feedstuffs majorly from oil seeds, legumes, and cereal grains (Gatlin et al. 2007). Over the last two decades, studies have investigated the potential of complete or partial FM replacement with these plant-based derivatives (Daniel 2018). Findings from these studies showed that significant reductions of FM can be achieved without negatively impacting the growth of fish (Daniel 2018). Even though the use of plant-based alternatives of FM is extensive and projected to increase in the future, their inclusion is limited by unbalanced amino acid profiles, reduced palatability, and the existence of anti-nutritional factors which negatively affect growth and welfare of the fish (Bandara 2018). Furthermore, the high prices and competition for plant ingredients among the livestock sector, aquaculture sector, biodiesel production, bioethanol production, and directly for human consumption present additional limitations for their incorporation in aquaculture feeds (Karapanagiotidis 2014). Moreover, with the expansion and intensification of terrestrial crop production, there is increased competition on the limited resources like arable land and freshwater for plant production, and negative impacts of climate change (FAO 2017, 2022). Therefore, there is need to further investigate more nutritionally suitable, widely available, competitively priced, and sustainably sourced alternative protein sources for inclusion in aquaculture feeds.

Processed animal proteins (PAPs) present an opportunity as a more pragmatic, environmentally friendly, and economical alternative to FM than plant ingredients. PAPs are underutilized feed ingredients produced from the rendering of animal by-products (Karapanagiotidis 2014). Compared to plant ingredients, PAPs contain high level of protein with good amino acid profiles, lack anti-nutritional factors, and are widely accessible at a competitive price (Bureau 2006; Naylor et al. 2009; Karapanagiotidis 2014; Moutinho et al. 2017). PAPs are also rich in phosphorus, which is limiting mineral in many feedstuffs (Karapanagiotidis 2014). The use of PAPs in aquaculture feeds is highly variable depending on the region (Moutinho et al. 2017). In the European Union (EU), the use of PAPs was prohibited between 1990 and 2000 due to the threat of transmission of bovine spongiform encephalopathy (BSE) (European Commission 2013; Woodgate et al. 2022). However following some amendments, category-three non-ruminant PAPs, i.e., carcasses and scraps of non-ruminant animals suitable for human food but not intended for that purpose (EU 2002), were re-approved for use (Woodgate et al. 2022), thus providing an opportunity for

utilization of waste generated from non-ruminant animal by-products as feed ingredients (Jędrejek et al. 2016). .

Hydrolyzed feather meal (FeM) and poultry meat and bone meal (PMBM) are two of the PAPs approved for use in aquaculture feeds in the EU. FeM is produced by the hydrolysis of poultry feathers (Psofakis et al. 2020) and has a high protein level (55–80%) and palatability (Hasni et al. 2014). It is estimated that the annual production of feather meal in the EU is around 175,000 tonnes (Adler et al. 2014). Several amino acids, including cystine, arginine, and aspartic acid, are more abundant in FeM compared to FM (Psofakis et al. 2020). However, FeM is poorer in methionine, histidine and lysine (Psofakis et al. 2020). FeM is also a good source of biological resources like proteases and antioxidant peptides (Lasekan et al. 2013). The use of FeM as a FM replacement was shown to be possible up to 76% in European seabass (*Dicentrarchus labrax*) (Campos et al. 2017), 20–100% in Nile tilapia (*Oreochromis niloticus*) (Arunlertaree and Moolthongnoi 2008; Yong and Mohammad 2018), 30% in rainbow trout (*Oncorhynchus mykiss*) (Bureau et al. 2000), 25% in Malabar grouper (*Epinephelus malabaricus*) (Li et al. 2009), and 25% in African catfish (*Clarias gariepinus*) (Absalom et al. 2017).

On the other hand, PMBM is a product of the poultry carcass rendering process (Bureau et al. 1999; Plazzotta and Manzocco 2019). PMBM is a widely available ingredient, and the EU is reported to produce more than 3.5 million tonnes of it annually (Coutand et al. 2008). Similar to FeM, PMBM has a high protein content, an amino acid profile that is well balanced, and is abundant in minerals like calcium and phosphorus (Moutinho et al. 2017). The use of PMBM as FM replacement was shown to be possible up to 25–75% in Pacific white shrimp (*Litopenaeus vannamei*) (Forster et al. 2003; Tan et al. 2005; Wang et al. 2007), 34% in Ussuri catfish (*Pseudobagrus ussuriensis*) (Tang et al. 2018), 50% in gibel carp (*Carassius auratus gibelio*) (Yu et al. 2004), 20% in snakehead (*Ophiocephalus argus*) (Yu et al. 2015), and 50% in gilthead seabream (*Sparus aurata*) (Moutinho et al. 2017).

It is clear from earlier studies that the degree to which FM is replace by either FeM or PMBM considerably varies between species. This variation can be due to differences in fish species, feeding behaviors, and/or the nutritive quality of the ingredients (Moutinho et al. 2017). The freshness, quality, and/or processing method of raw materials have a significant impact on their nutritional value of the PMBM and FeM produced (Campos et al. 2017; Moutinho et al. 2017; Yong and Mohammad 2018). Compared to FM, one major drawback of FeM as a feed ingredient is the reduced digestibility due to indigestible keratin protein content despite significant improvements due to the hydrolyzation processing (Bureau et al. 1999). In the case of PMBM, it is the high ash content owing to the presence of inorganic matter such as bone that significantly affects digestibility (Bureau et al. 1999). Despite this, there is great potential for poultry by-products like FeM and PMBM to be incorporated in fish feeds and to reduce the long-term reliance on FM. Therefore, accurately characterizing their nutritional content for specific fish species is crucial to maximizing their use in aquaculture feeds.

Due to its appealing market price and widespread consumer acceptance, the European pikeperch (*Sander lucioperca*, (Linnaeus, 1758)) is seen as a promising species for intensive culture in recirculating aquaculture systems (RAS) (Nguinkal et al. 2019; Rapp et al. 2019). Since pikeperch is a carnivorous fish, it requires a high level of protein in its diets (> 43%) (Nyina-Wamwiza et al. 2005; Geay and Kestemont 2015) traditionally met by FM. A limited number of studies have examined FM substitution in pikeperch feeds. However, recently, Schafberg et al. (2018) and Schafberg et al. (2021) investigated the possibility of substituting FM and fish oil using a microbial blend of cyanobacteria (*Arthrospira* sp.),

dinoflagellate microalgae (*Crypthecodinium cohnii*), and yeast (*Rhodotorula glutinis*) in feeds for pikeperch juveniles. Tran et al. (2021) also examined how defatted black soldier fly (*Hermetia illucens*) larvae meal in diets of pikeperch juveniles affected the gut microbiota, histomorphology, and antioxidant biomarkers. To our knowledge, no information has been published on the use of FeM or PMBM (either alone or in combination) as FM substitutes in pikeperch diets. The current study offers the first scientific evaluation of the potential of a combination of a poultry-based protein comprising of FeM and PMBM as a FM substitute in practical diets for pikeperch. The growth performance of the fish, feed utilization, and final body composition were used to assess the impact of this substitution.

Materials and methods

Experimental diets

The experiment was performed out at the INAGRO aquaculture research facility (Rumbeke, Belgium). The protocols for the handling of animals and experimental methods were carried out with the approval of the institutional internal research and review board of INAGRO. The protocols used in the experiment followed the guidelines of the directive 2010/63/EU for animal experiments.

Feed ingredients and the chemical composition of the four test diets are presented in Table 1. The experimental diets were formulated to be isonitrogenous (approximately 50% crude lipid), isolipidic (approximately 17% crude lipid), and isocaloric (approximately 21.80 KJ/g energy) with varying inclusion levels of a poultry-based protein (PBP). The PBP comprised of FeM (EM'PAQ®) and PMBM (EMMEAT®) produced by Empro Europe NV (Dendermonde, Belgium). FM was the primary protein source in the control feed (PBP0), while the three additional feeds had 20 (PBP20), 40 (PBP40), or 60% (PBP60) of the FM replaced by PBP. The diets (3-mm floating pellets) were produced by RDS BV (Utrecht, Netherlands) using a twin-screw extruder. Diets were stored in air-tight containers at room temperature for the duration of the trial.

Crude lipid in the experimental diets was determined using the method described by Folch et al. (1957). Phosphorus was determined using a phosphate photometric kit (Merck KGaA, Darmstadt, Germany, Spectroquant®). All other chemical analyses followed standard AOAC methods (AOAC 1990). Dry matter in the feeds was determined by drying in a drying oven (103 °C for 4 h); ash was determined by combustion in a muffle furnace (500 °C for 6 h) and protein (N × 6.25) using a tecator digester (Foss, Hillerød, Denmark, model 1015) and distillation apparatus (Gerhardt, Konigswinter, Germany, vapodest®). A bomb calorimeter (Parr Instrument company, Illinois, USA, model 1261) was used to determine gross energy. Analysis of crude protein, crude lipid, gross energy, and phosphorus was done in duplicate while that for dry weight and ash was done in triplicate.

Experimental facilities and conditions

Rearing facility and conditions

The growth experiment was performed in three recirculating aquaculture systems (RAS) for a total of 61 days. Each RAS consisted of four identical circular black tanks (1.8 m^3/tank), one drum filter (Faivre, Baume-les-Dames, France, model 4–80) with 36 µm screen

Table 1 Ingredients and proximate composition of the feeds

Ingredients (%)	Treatments			
	PBP0	PBP20	PBP40	PBP60
Fishmeal	55.00	44.00	33.00	22.00
EM'PAQ	0.00	5.56	11.11	16.67
EM'MEAT	0.00	4.84	9.68	14.53
Wheat gluten	11.02	11.19	11.35	11.52
Wheat flour	18.58	17.14	15.71	14.28
Fish oil	10.47	10.21	9.95	9.68
Lysine	0.00	0.39	0.78	1.17
Methionine	0.00	0.17	0.35	0.52
Threonine	0.00	0.04	0.08	0.12
$CaHPO_4$	0.56	1.80	3.04	4.28
Vitamin mix	1.85	1.85	1.85	1.85
Feed pellet binder	0.50	0.50	0.50	0.50
NaCl	0.00	0.29	0.58	0.87
Vitamin C	0.02	0.02	0.02	0.02
Digestibility marker	2.00	2.00	2.00	2.00
Proximate composition				
Dry matter (%)	96.34 ± 0.01	96.20 ± 0.01	95.17 ± 0.12	96.24 ± 0.08
Crude protein (%)	51.93 ± 0.08	52.67 ± 0.55	51.98 ± 0.12	51.61 ± 0.09
Crude lipid (%)	17.44 ± 0.10	17.48 ± 0.16	17.16 ± 0.17	15.94 ± 0.10
Gross energy (KJ/g)	23.09 ± 0.82	21.74 ± 0.27	21.60 ± 0.22	20.78 ± 0.29
Ash (%)	11.99 ± 0.68	12.39 ± 0.04	13.39 ± 0.09	15.26 ± 0.05
Phosphorus (mg/g)	70.39 ± 1.56	88.26 ± 3.11	92.96 ± 1.83	103.23 ± 2.81

PBP0, 0% poultry-based protein core; *PBP20*, 20% poultry-based protein core; *PBP40*, 40% poultry-based protein core; *PBP60*, 60% poultry-based protein core (in diets PBP20, PBP40, and PBP60 the sum of EM'PAQ, EMMEAT, lysine, methionine, and threonine is equal to the percentage of FM replaced

mesh size, a 5.4 m^3 moving bed filter, UV filter (SIBO Fluidra, Veghel, Netherlands, model bio-UV), and an oxygen cone (Multivis Waterbehandling, B.V, Nietap, The Netherlands). Water temperature, pH, conductivity, and dissolved oxygen (DO) were measured daily in each RAS sump tank using a portable multimeter probe (Hach, Colorado, USA, model HQ40D). The TAN, NO_2^-, and NO_3^- levels in each RAS were analyzed weekly using test and tube reagent kits (Hach, Colorado, USA, model AmVer™ low range NH_3 reagent set 26045-45, NitriVer® 3 reagent set 26093-45 and NitriVer® X reagent set 26053-45). Readings were made using a colorimeter (Hach, Colorado, USA, model DR/890).

Fish and feeding procedures

Pikeperch juveniles used for the growth study were provided by INAGRO. The fish were obtained from the 2018 out-of-season reproduction of pikeperch carried out at INAGRO and were 6 months post-hatch. The fish used for the experiment were part of a batch already being reared in the experimental tanks under the experimental conditions for more than one month. During this pre-experimental period, fish were fed 3 mm Skretting R-3 Europa Salmon feed (55% crude protein, 16% crude lipid). Fish were transferred from the experimental tanks into holding tanks supplied with oxygen to begin the experiment. Fish were individually weighed, and those ranging 90–120 g were randomly re-distributed into the 12 circular tanks, ten fish at a time. At the end of stocking, each tank had 250 fish. Following stocking, the experimental diets were randomly allocated to triplicate tanks.

Fish (mean live weight 113.12 g) were fed equal rations five times a day at 8:10 am, 10:00 am, 12:00 pm, 2:00 pm, and 4:00 pm following a 12 L:12D photoperiod. Over the course of seven days, the fish were gradually acclimated to the experimental feeds by adding 15% more experimental feed to the total feed ration daily. Feed was dispensed using an automatic feeder (Multivis Waterbehandling, B.V, Nietap, The Netherlands) and monitored at every feeding time. Any uneaten feed from the tanks was collected 20 min after the automatic feeder stopped dispensing feed using a sieve. To determine the precise feed intake, it was then dried in an oven and weighed. Similarly, any feed that was left in the feeders at the end of the day, was taken out, weighed, and recorded. Based on appetite and calculated uneaten feed, the feeding rate was initially set at 1.5% and subsequently reduced to 1% of the total tank biomass towards the end of the study. A theoretical specific growth rate (SGR) of 1.5%/day was used during the first 2 weeks of the experiment according to data supplied by technical staff at INAGRO. Fish were sampled bi-weekly by taking individual wet weight measurements of 30 fish in the tank to adjust the feeding rate. All the fish in the tank were also bulk weighed bi-weekly to determine the total tank biomass. Fish were monitored weekly for the presence of *Trichodina* or *Costia* parasites in the system by taking mucus samples.

Fish sampling

During stocking, ten fish within the weight range were randomly selected and euthanized by pithing. Five fish were frozen at −20 °C for subsequent whole-body proximate analysis. Samples of liver and viscera from five fish were weighed to determine visceral somatic index (VSI) and hepatosomatic index (HSI). At the end of the experiment, fifteen fish per tank were euthanized as earlier described. Ten fish were used to determine VSI and HSI. Five fish were stored in a freezer at −20 °C to determine whole-body composition. To determine whole-body proximate analysis, the fish were ground, spread on aluminum foil trays, and dried in an oven

at 103 °C for 4 h. Thereafter, the fish was re-ground and sieved, and crude lipid, protein, and ash were determined using methods described in the "Experimental diets" section. At the end of the experiment, the fish were individually weighed to determine the final tank biomass. At the start and end of the experiment, length measurements of 30 weighed fish per tank were taken to determine the condition factor (k). Fish were not fed 16 h prior to sampling.

Growth performance indices were calculated as follows:

$$Feed\ intake\ (g/fish/day) = \frac{(Amount\ of\ dry\ feed\ eaten\ in\ the\ period)}{(Number\ of\ fish * Number\ of\ days)}$$

$$Survival\ (\%) = \frac{No\ of\ fish\ alive\ after\ culture\ period}{No\ of\ fish\ stocked} * 100$$

$$Specific\ growth\ rate\ (SGR)\ (\%/day) = \frac{\ln(final\ body\ weight) - \ln(initial\ body\ weight)}{Time\ in\ between\ weighings(days)} * 100$$

$$Initial/Final\ body\ weight\ (IBW/FBW)(g) = \frac{Sum\ of\ individual\ weights\ of\ fish\ in\ tank}{Number\ of\ fish}$$

$$Weight\ gain(g/fish/day) = \frac{Final\ Body\ Weight - Initial\ Body\ Weight}{Time\ in\ between\ weighings}$$

$$Average\ body\ length(ABL)\ (cm) = \frac{Sum\ of\ body\ lengths\ of\ fish\ sample}{Number\ of\ fish\ in\ the\ sample}$$

$$Condition\ factor\ (k) = \frac{Mean\ weight}{Mean\ length^3} * 100$$

$$Coefficient\ of\ variation\ (COV)\ (\%) = \frac{Standard\ deviation\ of\ weight\ in\ tank}{Average\ body\ weight\ in\ tank} * 100$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Dry\ weight\ of\ feed\ eaten}{Live\ weight\ gain\ by\ fish}$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{Live\ weight\ gain}{Protein\ Intake}$$

$$Visceral\ somatic\ index\ (VSI)\ (\%) = \frac{Weight\ of\ Viscera}{Wet\ weight\ of\ fish} * 100$$

$$Hepatosomatic\ index\ (HSI)(\%) = \frac{Weight\ of\ liver}{Wet\ weight\ of\ fish} * 100$$

Statistical analysis

Results are expressed as means ± standard deviation (SD). Levene's test was used to assess equality of variances, while normality was assessed using the Shapiro-Wilk test. All percentage data were arcsine square-root transformed prior to statistical analysis. Results were analyzed using one-way ANOVA and Tukey's multiple range tests were carried out to compare means among treatments. Differences were considered statistically significant at $p < 0.05$. All analyses were performed using the statistical software RStudio version 1.2.5042 (RStudio, Boston, USA).

Results

Water quality parameters

Water temperature (23.95 ± 0.23 °C), dissolved oxygen concentration (8.42 ± 0.22 mg/L), pH (7.81 ± 0.21), conductivity (2.47 ± 0.66 ms/cm), TAN concentration (0.08 ± 0.11 mg/L), NO_2^- concentration (0.16 ± 0.13 mg/L), and NO_3^- concentration (228.52 ± 81.10 mg/L) were within the limits for pikeperch culture.

Feed analysis and feed intake

Proximate analysis of the experimental feeds (Table 1) showed that percentage crude protein and dry matter were similar. Gross energy was highest in feed PBP0 and decreased as the FM replacement increased. Feed PBP60 had the lowest percentage of crude lipid; however, it had the highest percentage ash content. Overall daily feed intake which ranged from 1.93 ± 0.03 to 2.00 ± 0.15 g/fish/day across treatments was not significantly influenced by the dietary PBP inclusion level ($p > 0.05$).

Fish growth and survival rate

After 61 days, the survival rate was high ranging from 97.1 ± 2.34 to 98.7 ± 0.61% amongst treatments ($p > 0.05$). Fish fed experimental diets showed an increase in body weight (approximately doubling the initial body weight) (Table 2). No adverse effects on growth and feed efficiency were observed in fish fed diets PBP0, PBP20, and PBP40. However, the SGR in treatment PBP60 (1.13 ± 0.03%/day) was significantly lower compared to the SGR in PBP0 and PBP20 (between 1.27 and 1.29%/day) ($p < 0.05$), with PBP40 showing an intermediate value. The FBW in treatment PBP60 was also 10% lower than PBP0 and PBP20 and 8% lower than PBP40 ($p < 0.05$).

Feed utilization

Feed utilization was lowest in fish in treatment PBP60 (Table 3). The FCR in treatment PBP60 (1.03 ± 0.02) was significantly higher than that of all the other treatments (0.86 ± 0.04–0.91 ± 0.04), while the protein efficiency ratio was

Table 2 Growth parameters of pikeperch juveniles fed experimental diets

Parameter	Treatments			
	PBP0	PBP20	PBP40	PBP60
SGR (%/day)	1.27 ± 0.04^{a}	1.29 ± 0.04^{a}	1.25 ± 0.04^{ab}	1.13 ± 0.03^{b}
IBW (g)	114.16 ± 1.53^{a}	113.34 ± 0.30^{a}	112.32 ± 0.90^{a}	112.64 ± 0.59^{a}
FBW (g)	248.11 ± 2.86^{a}	248.73 ± 5.64^{a}	240.11 ± 4.27^{a}	224.99 ± 4.64^{b}
Weight gain (g/fish/day)	2.20 ± 0.07^{a}	2.22 ± 0.10^{a}	2.09 ± 0.08^{ab}	1.84 ± 0.07^{b}
IBL (cm)	20.78 ± 0.25^{a}	20.83 ± 0.00^{a}	20.56 ± 0.19^{a}	20.38 ± 0.68^{a}
FBL (cm)	25.9 ± 0.48^{a}	26.21 ± 0.25^{a}	26.15 ± 0.29^{a}	25.55 ± 0.16^{a}
Initial k	1.30 ± 0.04^{b}	1.30 ± 0.00^{b}	1.27 ± 0.02^{b}	1.38 ± 0.07^{a}
Final k	1.33 ± 0.07^{a}	1.33 ± 0.01^{a}	1.31 ± 0.02^{a}	1.31 ± 0.04^{a}
Initial COV (%)	7.73 ± 0.23^{a}	7.81 ± 0.11^{a}	7.68 ± 0.05^{a}	8.32 ± 1.00^{a}
Final COV (%)	14.97 ± 1.11^{b}	15.08 ± 0.63^{b}	12.97 ± 0.15^{a}	14.03 ± 0.25^{ab}

SGR, specific growth rate; *IBW*, initial body weight; *FBW*, final body weight; *IBL*, initial body length; *FBW*, final body weight; *k*, condition factor; *COV*, coefficient of variation

Values are mean ± SD (n = 3). Different superscript letters on the same row denote significant differences ($p < 0.05$)

significantly lower in treatment PBP60 (1.89 ± 0.03) compared to the other treatments (2.12 ± 0.09–2.20 ± 0.10) ($p < 0.05$).

Hepatosomatic index and visceral somatic index

There were no significant differences ($p > 0.05$) observed in VSI and HSI among treatments (Table 4).

Whole-body analysis

No significant differences among treatments were observed ($p > 0.05$) in the crude protein and lipid levels in the fish carcasses after proximate analysis (Table 5). However, the ash content of the carcass of fish in treatment PBP60 ($14.66 \pm 0.10\%$) was significantly higher than that of the control ($12.57 \pm 0.13\%$) ($p < 0.05$).

Table 3 Feed conversion ratio (FCR) and protein efficiency ratio (PER) of pikeperch juveniles fed experimental diets

Parameter	Treatments			
	PBP0	PBP20	PBP40	PBP60
FCR	0.89 ± 0.06^{a}	0.86 ± 0.04^{a}	0.91 ± 0.04^{a}	1.03 ± 0.02^{b}
PER	2.18 ± 0.15^{a}	2.20 ± 0.10^{a}	2.12 ± 0.09^{a}	1.89 ± 0.03^{b}

Values are mean ± SD ($n = 3$). Different superscript letters on the same row denote significant differences ($p < 0.05$)

Table 4 Hepatosomatic index (HSI) (%) and visceral somatic index (VSI) (%) of pikeperch fed experimental diets

Parameter (%)	Treatments			
	PBP0	PBP20	PBP40	PBP60
HSI	2.70 ± 0.06^{a}	2.61 ± 0.27^{a}	2.35 ± 0.09^{a}	2.37 ± 0.17^{a}
VSI	7.19 ± 0.36^{a}	6.99 ± 0.27^{a}	6.87 ± 0.25^{a}	7.00 ± 0.78^{a}

Values are mean ± SD ($n = 3$). Different superscript letters on the same row denote significant differences ($p < 0.05$)

Table 5 Proximate analysis (% of dry weight) of whole body of pikeperch fed experimental diets

Parameter (%)	Initial	Treatments			
	Initial	PBP0	PBP20	PBP40	PBP60
Crude protein	57.80 ± 0.11^{a}	58.06 ± 1.42^{a}	58.28 ± 0.95^{a}	58.75 ± 1.71^{a}	57.77 ± 0.56^{a}
Crude lipid	26.85 ± 0.59^{a}	26.12 ± 1.25^{a}	24.89 ± 1.89^{a}	23.85 ± 2.18^{a}	25.56 ± 0.87^{a}
Ash	12.57 ± 0.13^{a}	13.97 ± 0.76^{ab}	13.44 ± 0.43^{ab}	14.43 ± 0.71^{ab}	14.66 ± 0.10^{b}

Values are mean ± SD ($n = 3$). Different superscript letters on the same row denote significant differences ($p < 0.05$)

Discussion

The results of the current study showed that a mix of poultry-based protein (PBP) consisting of FeM and PMBM can be used in pikeperch diets as a partial FM substitute during the grow out phase. While the weight of the fish used in the current study doubled by the end of the experiment, the SGR, FBW, and overall daily weight gain were significantly lower in fish fed diets with 60% replacement of FM (Table 2). The feed utilization parameters, i.e., FCR and PER, followed a similar trend (Table 3), suggesting that the feed PBP60 was poorly assimilated by the fish. This could be attributed to the higher levels of PMBM, and FeM included in the experimental diet. PMBM is associated with higher ash content owing to the presence of inorganic matter like bone which has been reported to reduce digestibility (Robaina et al. 1997), and subsequently, feed efficiency and growth performance (Goda et al. 2007; Xavier et al. 2014). It is also likely that the reduced feed utilization at PBP60 resulted from reduced digestibility of FeM because of the indigestible keratin despite improvements in processing methods (Bureau et al. 1999; Psofakis et al. 2020). The lower gross energy levels in feed with 60% and 40% FM replacement level seem not to have affected overall feed intake ($p > 0.05$), although it might have had an impact on growth performance. Lower energy levels in feeds are associated with increased feed intake for fish to meet their required metabolic energy demand (De Silva and Anderson 1995), which was not the case. However, it is likely that increased feed intake was not observed in fish fed PBP40 and PBP60 because of reduced palatability resulting from either higher ash content in the feeds due to increasing levels of PMBM (Moutinho et al. 2017; Tang et al. 2018) or higher levels of FeM (Yu 2008).

The use of blended poultry by-product and FeM was shown to be possible up to 40% in juvenile giant croaker (*Nibea japonica*) (Wu et al. 2017), 25% in juvenile Siberian sturgeon (*Acipenser baerii*) (Zhu et al. 2011) and Malabar grouper when using a blend of FeM, PMBM, and other poultry by-products (Wang et al. 2008), and 75–100% in gilthead

seabream when using a blend of FeM and PMBM (Nengas et al. 1999). Similarly, when used as individual ingredients, FeM and PMBM inclusion levels are highly variable. FeM incorporation levels have reached 76% in seabass (Campos et al. 2017), 20–100% in Nile tilapia (Arunlertaree and Moolthongnoi 2008; Yong and Mohammad 2018), and 25% in African catfish (Absalom et al. 2017), while PMBM inclusion levels have reached 40% in Pacific white shrimp (Wang et al. 2007), 34% in Ussuri catfish (Tang et al. 2018), 50% in gibel carp (Yu et al. 2004), and 50% in gilthead seabream (Moutinho et al. 2017) without negatively affecting fish performance.

Besides the high ash content, reduced feed utilization beyond 40% replacement in the present study could have resulted from the deficiency of one or several essential amino acids (EAA) in either FeM or PMBM (Yu 2008; Xavier et al. 2014). It is a limitation that our study did not determine the EAA profile of the experimental feeds as EAA deficiencies are of concern when FM is replaced in aquaculture feeds. Methionine has been reported as one of the limiting EAA in PMBM (Klemesrud et al. 1997). Evidently, higher level of FM replacement from 75 to 100% using PMBM resulted in reduced performance of African catfish due to deficiency in methionine, lysine, and isoleucine compared to the FM control (Goda et al. 2007). Hydrolyzed FeM has also been reported to be deficient in lysine, methionine, and histidine (Baker et al. 1981; Klemesrud et al. 2000; Psofakis et al. 2020). Furthermore, the freshness, quality, and/or processing method of raw materials have a significant impact on their nutritional value of the PMBM and FeM produced (Bureau et al. 1999; Campos et al. 2017; Moutinho et al. 2017; Yong and Mohammad 2018). The heat treatment during the cooking and drying processes could damage nutrients such as amino acids thus reducing their nutritive value (Bureau et al. 1999), and subsequently lowering feed utilization efficiency.

The assessment of nutrient availability or digestibility is thought to be the initial stage in evaluating the potential of feed ingredients in aquaculture (Allan et al. 2002). This is because it provides preliminary evidence of the availability of nutrients thus providing a good foundation for diet formulation to maximize fish growth and limit fish waste production (Bureau et al. 1999). In some fish species, the use of FeM and PMBM in feeds for some fish species has been limited due to their poor digestibility and quality variability (Bureau et al. 2000). While we hoped to carry out digestibility measurements of the fish subjected to the different PBP feed treatments at the end of the growth trial, we faced grave challenges related to the quality and quantity of fecal sample collected. Firstly, it was observed that pikeperch feces were not compact, i.e., did not have a solid consistency; hence, the fecal particles were suspended in the water once released from the anus. Since our experimental tanks were not fitted with fecal collectors at the time, fecal collection by this method was not a possibility. Fecal collection by stripping (Windell et al. 1978; Fernandez et al. 1996) was equally futile as only few drops of feces were produced at a go. Stripping would have to be done repetitively at the cost of stressing the fish and possibly causing physical tissue damage, and yet the amount of sample collected would still be insufficient. The most suitable option seemed to be dissection (Windell et al. 1978) but even then, 30 fish per treatment had to be sacrificed to produce 30 g (wet weight) of fecal sample. To keep the animal sacrifices to a minimum, we opted not to collect any fecal sample further. The digestibility measurements would have been valuable supplemental information to assess the potential of using PBP in pikeperch feeds.

There were no discernible differences in the condition factor (k), hepatosomatic index (HSI), or visceral somatic index (VSI) in the current investigation (Table 4). These performance indices provide an indication of the health status of fish (Sogbesan et al. 2017). Both initial and final k were higher than 1, indicating that the fish were in above-average

condition throughout the experiment, thus in good health, and showing good growth (Datta et al. 2013; Wu et al. 2017) reported similar outcomes for giant croaker (*Larimichthys crocea*) however, Siberian sturgeon juveniles were found to have a condition factor lower than one (Zhu et al. 2011). In both studies, fish were fed diets that substituted FM with a mixture of poultry by-product meal and FeM. Regarding HSI and VSI, Zhu et al. (2011) and Wu et al. (2017) observed similar outcomes to the present study in Siberian sturgeon juveniles and giant croaker fingerlings when FM was substituted with a mix of poultry by-product and FeM. Similar results were also reported in sobaity sea bream (*Sparidentex hasta*) (Hekmatpour et al. 2018) and humpback grouper (*Cromileptes altivelis*) fingerlings (Shapawi et al. 2007) while using only poultry by-product meal.

Based on the body composition results from this study, all parameters appeared to be unaffected by the substitution of dietary FM with PBP except ash content (Table 5). In experiments where dietary FM was replaced with rendered poultry protein ingredients, similar outcomes were observed in Malabar grouper (Wang et al. 2008), Siberian sturgeon (Zhu et al. 2011), and giant croaker (Wu et al. 2017). But just like in the current study, Yigit et al. (2006) reported higher levels of ash with increasing levels of poultry by-product in the diets.

Conclusion

This study describes the first use of a blend of FeM and PMBM as an alternative to FM in feeds for pikeperch juveniles in RAS. The results suggest that a significant amount of FM, i.e., up to 40%, can potentially be replaced using a combination of FeM and PMBM in the diet of pikeperch without adverse effects on growth performance, feed utilization, and whole-body composition. As a carnivorous fish, pikeperch have a high protein requirement; therefore, the findings of the present study must be viewed as a crucial step for the inclusion of alternative protein sources. Poultry by-products are readily available and a considerably cheaper source of protein in comparison to FM and as such have the potential to reduce cost of pikeperch feeds. To identify a suitable combination that will optimize inclusion level while guaranteeing good growth performance and nutritional quality of the fish, more research is required on this topic (and products). Furthermore, the analyses carried out in the present study do not reveal the full potential of the feed and/or how the fish utilize them. Comprehensive analyses of apparent digestibility, nutrient retention efficiencies, and amino acid profile of the experimental ingredients should be carried out, as these would provide a better understanding of how the feed is utilized and how diets can be improved.

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Author contribution All authors contributed to the study's conception and design. Data collection and analysis were done by Sandra Langi. The first draft of the manuscript was written by Sandra Langi, and all authors commented on subsequent versions of the manuscript. All authors read and approved the manuscript before submission.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval The protocols for the handling of animals and experimental methods were carried out with the approval of the institutional internal research and review board of INAGRO. All the procedures were based on the directive 2010/63/EU for animal experiments.

Conflict of interest The authors declare no competing interests.

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