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## A Comparative Approach to Sustainable Fish Meal: Prussian Carp Meal (*Carassius gibelio* Bloch, 1782)

### Sürdürülebilir Balık Ununa Karşılaştırmalı Bir Yaklaşım: İsrail Sazanı Unu (*Carassius gibelio* Bloch, 1782)

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**Abstract:** In the present study, the usability of Prussian carp (*Carassius gibelio*) meal (PCM) as an alternative animal protein source in fish feeds was investigated by comparing it with anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) meals in terms of their biochemical, fatty acid, amino acid and element compositions. Prussian carp were obtained by fishing and made into a meal (PCM). Anchovy (AM), and sprat (SM) meals were purchased from a commercial company. The amino acid analysis results show that PCM's total, essential, and non-essential amino acid values were lower than that of AM and SM ( $P < 0.05$ ). The polyunsaturated fatty acids (PUFA) and Omega-6 values of PCM were higher than AM and SM; and lower than saturated fatty acids (SFA), Omega-3, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), atherogenicity index (AI) values ( $P < 0.05$ ). According to the element analysis, the P and Ca values of the PCM were higher than the AM and SM ( $P < 0.05$ ), and the Na, K, Fe, Cu, Zn and Se values were lower. According to the results of the present study, PCM can be utilized in the feed industry to boost the sustainability of fish meals used in feed production, which in turn will reduce the foreign dependency on vegetable and fish meals, and lower feed costs.

#### Keywords

- Prussian carp meal
- Anchovy meal
- Sprat meal
- Fatty acid composition
- Amino acid composition

**Özet:** Çalışmada İsrail sazanı (*Carassius gibelio*) ununun hamsi (*Engraulis encrasicolus*) ve çaça (*Sprattus sprattus*) unlarının biyokimyasal, yağ asidi, amino asit ve element kompozisyonları ile karşılaştırılarak balık yemlerinde alternatif protein kaynağı olarak kullanılabilirliği araştırılmıştır. İsrail sazanı avcılık yoluyla elde edilip un haline getirilmiş (PCM); hamsi (AM) ve çaça (SM) unları ise ticari bir firmadan alınmıştır. Çalışma sonunda PCM'nin toplam esansiyel ve esansiyel olmayan amino asit değerleri AM ve SM'den düşük olduğu belirlenmiştir ( $P < 0.05$ ). PCM'nin çoklu doymamış yağ asitleri (PUFA) ve Omega-6 değerleri AM ve SM'den yüksek; doymuş yağ asitleri (SFA), Omega-3, eikosapentaenoik asit (EPA), dokosaheksaenoik asit (DHA) ve aterosite indeksi (AI) değerleri düşük bulunmuştur ( $p < 0.05$ ). Element analizine göre PCM'nin P ve Ca değerleri AM ve SM'den yüksek ( $p < 0.05$ ), Na, K, Fe, Cu, Zn ve Se değerleri ise düşük olduğu belirlenmiştir. Çalışma sonunda, yem üretiminde kullanılan balık unlarının sürdürülebilirliğinin artırılması, bitkisel ve balık unlarında dışa bağımlılığın azaltılması ve yem maliyetlerinin düşürülmesi amacıyla PCM'nin yem sektöründe kullanılabileceği belirlenmiştir.

#### Anahtar kelimeler

- İsrail sazanı unu
- Hamsi unu
- Çaça unu
- Yağ asitleri kompozisyonu
- Amino asit kompozisyonu

## 1. INTRODUCTION

Population increases, dwindling terrestrial and aquatic supplies, increased demand for products



made from animals, excessive dependence on fossil fuels, and a changing climate are all major pressures on the world food system (Neff et al., 2011; Gerile et al., 2017). The production of seafood has also undergone significant change in recent decades. Currently, aquaculture, or farmed fish, accounts for half of all seafood consumption worldwide and is growing more quickly than any other area of animal production (FAO, 2014). The dietary requirements of aquatic species vary, and they are grown in a variety of ways. Feed is needed for around two-thirds of the output of farmed aquatic animals. Some species, such as tilapia (*Oreochromis sp.*) and grass carp (*Ctenopharyngodon idella*) are herbivorous and can consume 100% vegetarian feed derived from crops, other foods, and agricultural waste. Carnivores species such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), and bream (*Sparus aurata*), always consume fish/animal protein and lipids as part of their diet. Fish meal is a key component of aquaculture diets and is commonly used in animal diets to increase feed palatability, which promotes feed efficiency and animal growth, as well as taking in nutrients, digestion, and absorption (Mile & Chapman, 2006). It is estimated that roughly 30% of the entire fish catch is converted into fish meal and fish oil for use in animal and fish feeds (Ogunji et al., 2006). Fish used in diets are generally obtained from short-lived, fast-growing fish (e.g., anchovy: *Engraulis encrasicolus*, sprat: *Sprattus sprattus*, herring: *Clupea harengus*, capelin: *Mallotus villosus*, etc.), that are not intended for human consumption or byproducts of seafood processing plants. However, fish populations are constantly changing due to the variability of stocks, fishing pressure, and environmental conditions. The development of sustainable aquaculture, on the other hand, is dependent on the availability of raw resources such as fish meals. Many studies were conducted with alternative and low-cost different vegetable protein sources (Kaushik et al., 2004; Naylor et al., 2009; Rana et al., 2009; Cabral et al., 2011, Siddik et al., 2021) and animal protein sources (Yang et al., 2004; Xiaoming et al., 2010; Tabinda & Butt, 2012; Da et al., 2012; van Huis & De Prins, 2013; Sevgili et al., 2015; Rahman et al., 2016; Zhang et al., 2016; Wang et al., 2017; Devic et al., 2018; Ye et al., 2019; Choi et al., 2020) to fish meal.

*Carassius gibelio*, which will be evaluated as meal in the current study, is a species found in streams, lakes, and ponds in Northern, Central, and Eastern Europe, the Balkans, and Asia (Hong et al., 2005). Because of its rapid distribution, it has gained interest in many nations, and surveillance studies have increased in recent years (Wheeler, 2000; Halačka et al., 2003; Paschos et al., 2004; Tsoumani et al., 2006 Gozacan & Becer, 2018). *C. gibelio* benefits from a considerable portion of the nutrients in its environment due to its omnivorous nature (benthic and planktonic invertebrates, insects and fish larvae, plants, and so on) (Balık et al., 2003; İnnal & Erkakan, 2006; Yılmaz et al., 2007). The *C. gibelio* was captured in its native habitat and its biochemical components were examined in several investigations (İzci, 2010; Süle, 2011; Dağtekin Gözü & Baştürk, 2014). Following these investigations, research was conducted to introduce the species into the economy by employing several processing processes to ensure customer preference (Baygar, 2012; Dağtekin Gözü, 2013; İzci & Bilgin, 2015). In this study, the possibilities of using the Prussian carp (*C. gibelio* Bloch, 1782), which is reported as invasive in Turkish waters (Özuluğ et al., 2004; Yılmaz et al., 2007), as fishmeal were investigated. In addition, a comparative analysis of Prussian carp meal with anchovy and sprat meals, which are currently used as fish meals, was conducted in terms of biochemical, fatty acid, amino acid, and element compositions, and their usability as an alternative raw material for aquaculture was evaluated.

## 2. MATERIAL and METHODS

Prussian carp (*C. gibelio*) samples were obtained from the Bafra Fish Lakes (Bafra, Samsun, Türkiye) by fishing monthly throughout the year (2019). The sampled fish were transferred to the Feed Technology Laboratory (Sinop University). The whole body was used in the production of fish meal and no sorting was done. The fish were placed on drying trays and dried in an oven at 105°C for 12 hours. After the drying process, the fish were allowed to cool down to room temperature (24°C), and they were ground into meal. Prussian carp meals (PCM) were kept in plastic bags in a deep freezer until they were analyzed. In the study, anchovy (*E. encrasicolus*) (AM) and sprat meals (*S. sprattus*), (SM) used in fish feeds were obtained from a commercial production company (Kardez Seafood Company -Samsun/Turkey). Biochemical analyses of Prussian carp, anchovy, and sprat meals were

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made according to AOAC (1995). Dry matter analysis was performed in an oven ( $105\pm 1^\circ\text{C}$ ), crude protein analysis was performed using the Kjeldahl method, crude oil analysis was performed using soxhlet extraction, and crude ash analysis was performed using a muffle furnace ( $550\pm 1^\circ\text{C}$ ). All biochemical analyses in meals were triplicated. Element, amino acid, and fatty acid analyzes of meals were made by Sinop University Scientific Research and Application Center (SUBITAM). The elemental measurements of fish meals were based on EPA Method 200.3 (Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements in Biological Tissues). Fish meals (up to 0.5g) (HPR-FO-67) were prepared using a microwave digestion system (Milestone SK10), concentrated grade  $\text{HNO}_3$  (suprapur 65%) and  $\text{H}_2\text{O}_2$  (suprapur 30%) according to temperature and pressure profile (7:1) was digested in teflon containers containing the mixture. After adding the acid, the teflon bombs were closed and heated at  $200^\circ\text{C}$  for 15 min. and kept at the same temperature for another 15 min. The digested solution was transferred to 50ml polypropylene falcon tubes and filled with ultrapure water to 50ml. Concentrations of elements were measured with an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS, Agilent 7700X). Quality assurance and control were performed using triplicate measurement and certified reference material (UME CRM 1201-UME CRM 1204). Multi-element standard solutions (27-element mixtures: 8500-6940 2A and 8500-6940 Hg) supplied by Agilent were used for calibration curves. Analytical precision was within  $\pm 10\%$ . In this study, a 1 ppm internal standard (Agilent 5188–6525) was analyzed continuously with samples. The accuracy of items in the CRM ranged from 90% to 100%. Amino acid analyses of fish meals were performed using the Jasem LC-MS/MS amino acid assay kit. The concentration of the target amino acids was determined using the electrospray ionization (ESI)-based multiple reaction monitoring (MRM) mode. 0.5g sample was taken into a glass vial with a screw cap, and 4ml of reagent-2 was added, and then, a hydrolysis reaction was performed at  $110^\circ\text{C}$  for 24 hr. The hydrolysate was centrifuged for 5 min at 4000 rpm when it reached room temperature. Then, 100 $\mu\text{l}$  of the supernatant was transferred to a vial and completed to 1 ml with distilled water. This dilution procedure was repeated one more time to yield 800-fold diluted hydrolysate of the sample. 50 $\mu\text{l}$  of the diluted hydrolysate was transferred to a sample vial and 50 $\mu\text{l}$  of internal standard mixture with isotope labelled and 700 $\mu\text{l}$  of reagent-1 was added, respectively, and then, the mixture was vortexed for 5s. All samples were prepared according to the above procedures and injected into the LC-MS/MS system where the amounts of amino acids were read. The following formulae were used to determine the quantity and quality of amino acids (Li et al., 2009).

Essential Amino Acids (EAA) = Histidine + Lysine + Phenylalanine + Methionine + Threonine + Leucine + Isoleucine + Valine + Arginine + Tryptophan

Semi-Essential Amino Acids (SEAA) = Histidine + Arginine

Non-Essential Amino Acids (NEAA) = Alanine + Aspartic acid + Glutamic acid + Tyrosine + Glycine + Serine + Proline

Branched-chain amino acid (BcAA) = Leucine + Isoleucine + Valine

Sulfur-containing amino acids (SAA) = Cystine + Methionine

Aromatic amino acids (ArAA) = Phenylalanine + Tyrosine

Basic (alkaline) amino acids (BAA) = Lysine + Arginine + Histidine

Acidic amino acids (AAA) = Aspartic acid + Glutamic acid

For fatty acid analysis, fish meals were transformed to methyl esters by derivatizing fat samples in a gas chromatography instrument (Thermo Scientific Trace 1310). For this purpose, 0.25g of the extracted oil was removed, and 4 ml of heptane and 0.4ml of 2N KOH were added. The mixture was stirred in a vortex for 2 min, and then centrifuged at 5000rpm for 5 min. After centrifugation, 1.5–2ml of the heptane phase was collected and transferred to glass tubes for GC/MS analysis. The injection of samples into the device was carried out with an automatic sampler (Autosampler AI 1310). Samples were analyzed by Thermo Scientific ISQ LT model GC/MS. For this analysis, Trace Gold TG-WaxMS capillary column (Thermo Scientific code: 26088-1540) with a film thickness of 0.25  $\mu\text{m}$  and 60 m length was used. The injection block temperature was set to  $240^\circ\text{C}$ , and the column temperature was increased from  $100^\circ\text{C}$  to  $240^\circ\text{C}$  in the temperature program. Helium gas (1 ml/min) was used as a carrier gas at constant flow, and 1:20 split ratio was applied. The MS unit (ISQ LT) was used in electron ionization mode. Fatty acids were defined by comparing the standard FAME mixture of 37

components based on the arrival times. Additionally, the quality of fatty acids (peroxidability index (PI), atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic/hypercholesterolemic ratio (HH)) was determined according to Arakawa & Sagai, (1987); Ulbricht & Southgate, (1991), and Santos-Silva et al., (2002).

Total saturated fatty acids ( $\Sigma$ SFA) = C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0

Total monounsaturated fatty acids ( $\Sigma$ MUFA) = C14:1 + C15:1 + C16:1 + C17:1 + C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9 + C24:1

Total polyunsaturated fatty acids ( $\Sigma$ PUFA) = C18:2n-6t + C18:2n-6c + C18:3n-3 + C18:3n-6 + C20:2 + C22:2 + C20:3n-6 + C20:5n-3 + C20:4n-6 + C22:6n-3

$\Sigma$ Omega-3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3;

$\Sigma$ Omega-6 = C18:2n-6t + C18:2n-6c + C18:3n-6 + C20:4n-6 + C20:3n-6

$\Sigma$ Omega-9 = C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9

Atherogenicity Index (AI) = [(C12:0 + (4 x C14:0) + C16:0)] / (MUFA + Omega-3 + Omega-6)

Thrombogenicity Index (TI) = (C14:0 + C16:0 + C18:0) / [(0.5 x MUFA) + (0.5 x Omega-6) + (3 x Omega-3) + (Omega-3/Omega-6)]

Hypocholesterolemic/Hypercholesterolemic ratio (HH) = (C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:6n-3) / (C14:0 + C16:0)

Peroxidisability index (PI) = (MUFA x 0.025) + (C18:2n-6 + C20:2) x 1 + [(C18:3n-6 + C18:3n-3) x 2] + [(C18:4n-3 + C20:4n-6 + C22:4n-6) x 4] + [(C20:5n-3 + C22:5n-3) x 6] + [(C22:6n-3) x 8]

The data were reported as average values with standard error (SE). Statistical analysis was performed using the IBM-SPSS21 statistical package program. The differences between the values were tested with a one-way analysis of variance (ANOVA), these differences were compared with the Tukey test, and the significance values were taken as  $P < 0.05$ .

### 3. RESULTS

The biochemical compositions of Prussian carp, anchovy, and sprat meals are given in Table 1. When the biochemical composition of PCM were examined, it was determined that the crude protein value was lower than AM and SM ( $P < 0.05$ ). The statistical difference between the crude fat value of fish meals was not significant ( $P > 0.05$ ). The crude ash values were in high amounts ( $28.09 \pm 0.37\%$ ) in PCM and the statistical difference was significant ( $P < 0.05$ ).

**Table 1.** The biochemical compositions of Prussian carp (PCM), anchovy (AM), and sprat (SM) meals (%).

	Crude protein	Crude Fat	Crude Ash	Dry Matter
PCM	58.43 $\pm$ 0.23 <sup>a</sup>	12.39 $\pm$ 1.17	28.09 $\pm$ 0.37 <sup>b</sup>	97.47 $\pm$ 0.04 <sup>c</sup>
AM	74.93 $\pm$ 0.50 <sup>b</sup>	10.18 $\pm$ 0.01	18.90 $\pm$ 0.63 <sup>a</sup>	94.08 $\pm$ 0.03 <sup>a</sup>
SM	73.02 $\pm$ 1.28 <sup>b</sup>	11.47 $\pm$ 0.01	18.93 $\pm$ 0.11 <sup>a</sup>	96.07 $\pm$ 0.23 <sup>b</sup>

Each value represents the mean  $\pm$  standard error. Values expressed with different exponential letters on the same column are statistically different from each other ( $P < 0.05$ ).

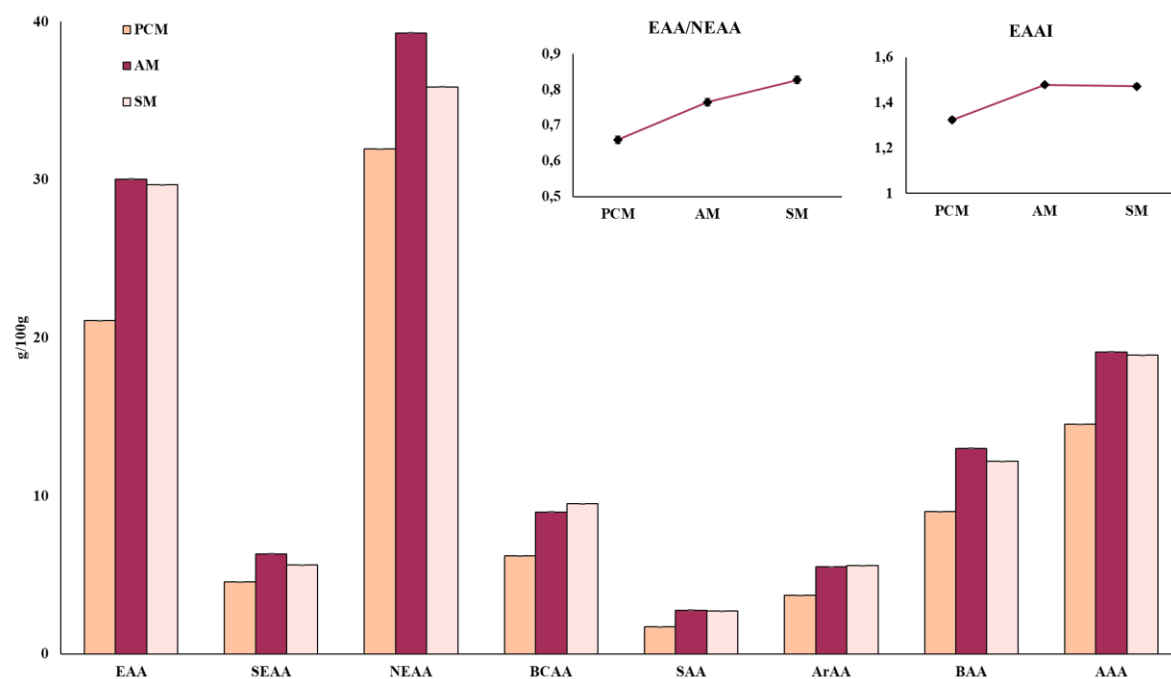
The amino acid compositions of Prussian carp (PCM), anchovy (AM) and sprat meals (SM) are given in Table 2. The highest total amino acid values (TAA) were determined in AM and the lowest in PCM ( $P < 0.05$ ). The glycine and proline values were high in PCM ( $P < 0.05$ ). The alanine, arginine, cystine, glutamic acid, histidine, leucine, lysine, phenylalanine and taurine were prominent in AM ( $P < 0.05$ ). The aspartic acid, isoleucine, methionine, serine, threonine, tyrosine and valine values peaked in SM ( $P < 0.05$ ).

**Table 2.** The amino acid compositions of Prussian carp (PCM), anchovy (AM), and sprat meals (SM) (g/100g).

Amino acid	PCM	AM	SM
<b>Essential Amino Acids</b>			
Arginine	3.43±0.01 <sup>a</sup>	4.12±0.01 <sup>b</sup>	4.11±0.01 <sup>b</sup>
Histidine	1.12±0.01 <sup>a</sup>	2.23±0.01 <sup>c</sup>	1.54±0.01 <sup>b</sup>
Isoleucine	1.00±0.01 <sup>a</sup>	1.49±0.01 <sup>b</sup>	1.78±0.01 <sup>c</sup>
Lysine	4.45±0.01 <sup>a</sup>	6.67±0.01 <sup>c</sup>	6.54±0.01 <sup>b</sup>
Leucine	3.75±0.01 <sup>a</sup>	5.27±0.01 <sup>c</sup>	5.13±0.02 <sup>b</sup>
Methionine	1.65±0.01 <sup>a</sup>	2.32±0.01 <sup>b</sup>	2.39±0.01 <sup>c</sup>
Phenylalanine	2.06±0.03 <sup>a</sup>	2.90±0.01 <sup>c</sup>	2.65±0.01 <sup>b</sup>
Tyrosine	1.67±0.01 <sup>a</sup>	2.62±0.01 <sup>b</sup>	2.93±0.01 <sup>c</sup>
Valine	1.48±0.01 <sup>a</sup>	2.24±0.01 <sup>b</sup>	2.59±0.01 <sup>c</sup>
Taurine	ND	0.46±0.01 <sup>b</sup>	0.12±0.01 <sup>a</sup>
<b>Non Essential Amino Acids</b>			
Alanine	4.16±0.01 <sup>a</sup>	5.60±0.01 <sup>c</sup>	4.30±0.01 <sup>b</sup>
Aspartic Acid	7.37±0.01 <sup>a</sup>	8.51±0.01 <sup>b</sup>	9.55±0.01 <sup>c</sup>
Cystine	0.06±0.01 <sup>a</sup>	0.45±0.01 <sup>c</sup>	0.32±0.01 <sup>b</sup>
Glutamic Acid	7.18±0.01 <sup>a</sup>	10.61±0.01 <sup>c</sup>	9.37±0.01 <sup>b</sup>
Glycine	5.27±0.01 <sup>b</sup>	5.24±0.05 <sup>b</sup>	3.61±0.01 <sup>a</sup>
Proline	3.50±0.01 <sup>c</sup>	3.35±0.01 <sup>b</sup>	2.76±0.01 <sup>a</sup>
Serine	2.83±0.01 <sup>a</sup>	3.36±0.01 <sup>b</sup>	3.37±0.01 <sup>b</sup>
Threonine	2.16±0.01 <sup>a</sup>	2.83±0.01 <sup>b</sup>	2.96±0.01 <sup>c</sup>
ΣTAA	53.09±0.01 <sup>a</sup>	70.25±0.01 <sup>c</sup>	65.99±0.01 <sup>b</sup>

Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ( $P < 0.05$ ). ND: not detected.

The amino acid quality of Prussian carp (PCM), anchovy (AM) and sprat meals (SM) (g/100g) are shown in Figure 1. The ΣEAA, ΣSEAA and ΣNEAA values were highest in AM and lowest in PCM, and the statistical difference was significant ( $P < 0.05$ ). The highest ΣEAA/ΣNEAA ratio was in SM ( $0.82±0.01$ ) and the lowest in PCM ( $0.66±0.01$ ) ( $P < 0.05$ ).

**Figure 1.** The amino acid quality of Prussian carp (PCM), anchovy (AM) and sprat meals (SM) (g/100g).

The ΣBCAA, ΣKAA, ΣBAA, and ΣAAA values were high in AM, and ΣArAA value was in SM. The lowest EAAI value was in PCM and the highest in AM, and the statistical difference between the

EAAI values of the meals is not significant ( $P > 0.05$ ).

According to the fatty acid composition analysis, the most determined fatty acids in meals were C16:0, C18:0, C18:1n-9c, C18:2n-6c, C18:3n-3, C20:4n-6, C20:5n-3, and C22:6n-3 (Table 3). The highest  $\sum$ SFA value was in AM, while the lowest was in PCM ( $P < 0.05$ ). While the  $\sum$ MUFA value was the highest in SM, the lowest was in AM and the statistical difference was significant ( $P < 0.05$ ).

**Table 3.** The fatty acid compositions of Prussian carp (PCM), anchovy (AM), and sprat meals (SM) (%).

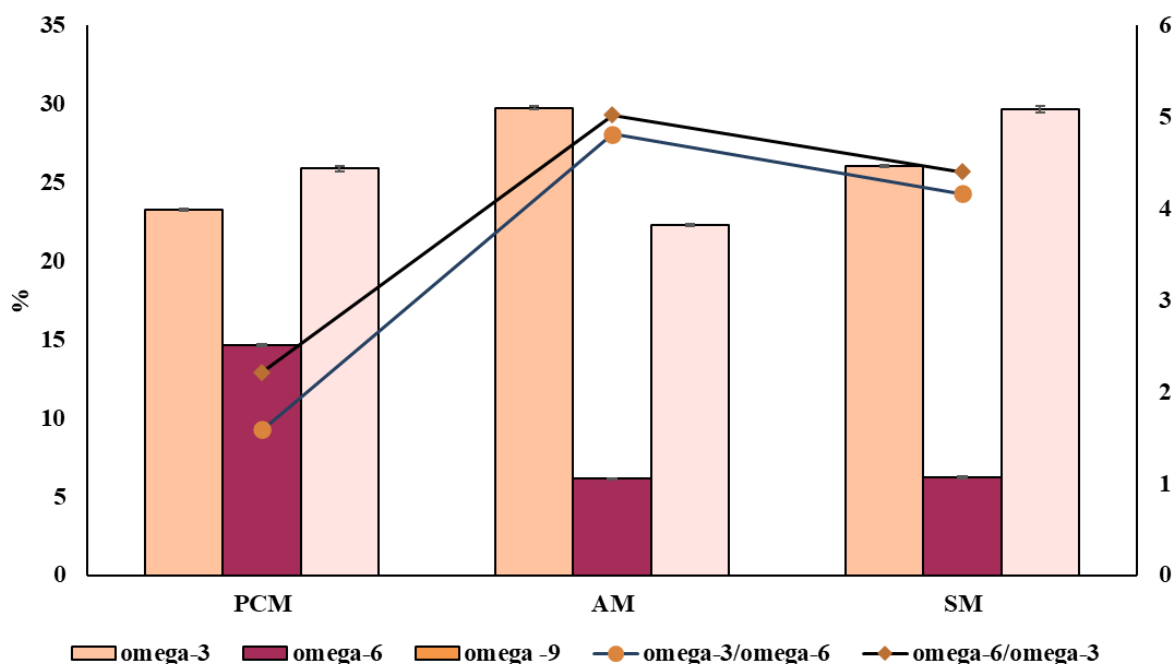
Fatty acids	PCM	AM	SM
C12:0	0.20±0.01 <sup>c</sup>	0.08±0.01 <sup>a</sup>	0.11±0.01 <sup>b</sup>
C13:0	0.12±0.01 <sup>c</sup>	0.08±0.01 <sup>a</sup>	0.10±0.01 <sup>b</sup>
C14:0	4.70±0.01 <sup>a</sup>	6.04±0.04 <sup>b</sup>	6.07±0.02 <sup>b</sup>
C15:0	1.32±0.01 <sup>a</sup>	1.55±0.01 <sup>b</sup>	1.61±0.01 <sup>c</sup>
C16:0	14.69±0.01 <sup>a</sup>	17.31±0.18 <sup>c</sup>	15.10±0.05 <sup>b</sup>
C17:0	1.13±0.01 <sup>b</sup>	1.60±0.01 <sup>c</sup>	0.97±0.01 <sup>a</sup>
C18:0	5.33±0.01 <sup>b</sup>	7.00±0.03 <sup>c</sup>	4.60±0.03 <sup>a</sup>
C20:0	0.47±0.01 <sup>a</sup>	1.46±0.01 <sup>c</sup>	1.24±0.01 <sup>b</sup>
C21:0	0.04±0.01	0.04±0.04	0.08±0.07
C22:0	0.20±0.03 <sup>a</sup>	0.82±0.01 <sup>c</sup>	0.70±0.04 <sup>b</sup>
C23:0	0.13±0.02 <sup>b</sup>	0.04±0.02 <sup>a</sup>	0.06±0.02 <sup>a</sup>
C24:0	0.12±0.01 <sup>b</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>
$\sum$ SFA	28.45±0.03 <sup>a</sup>	36.05±0.16 <sup>c</sup>	30.65±0.14 <sup>b</sup>
C14:1	1.33±0.01 <sup>c</sup>	0.46±0.01 <sup>a</sup>	0.73±0.01 <sup>b</sup>
C15:1	0.77±0.01 <sup>b</sup>	0.28±0.01 <sup>a</sup>	0.29±0.01 <sup>a</sup>
C16:1	0.85±0.01 <sup>a</sup>	1.14±0.01 <sup>b</sup>	1.19±0.01 <sup>c</sup>
C17:1	1.81±0.01 <sup>b</sup>	0.85±0.01 <sup>a</sup>	0.95±0.04 <sup>a</sup>
C18:1n-9c	14.59±0.05 <sup>b</sup>	14.20±0.06 <sup>a</sup>	13.98±0.08 <sup>a</sup>
C18:1n-9t	6.07±0.14 <sup>c</sup>	3.97±0.02 <sup>b</sup>	3.55±0.01 <sup>a</sup>
C20:1n-9c	3.05±0.02 <sup>b</sup>	2.71±0.01 <sup>a</sup>	5.86±0.08 <sup>c</sup>
C22:1n-9	2.17±0.02 <sup>b</sup>	1.43±0.03 <sup>a</sup>	6.26±0.06 <sup>c</sup>
C24:1	0.61±0.01 <sup>a</sup>	2.17±0.01 <sup>b</sup>	3.36±0.01 <sup>c</sup>
$\sum$ MUFA	31.24±0.09 <sup>b</sup>	27.20±0.08 <sup>a</sup>	36.16±0.18 <sup>c</sup>
C18:2n-6t	0.70±0.01 <sup>c</sup>	0.11±0.01 <sup>a</sup>	0.20±0.01 <sup>b</sup>
C18:2n-6c	9.18±0.01 <sup>c</sup>	3.78±0.01 <sup>a</sup>	4.05±0.01 <sup>b</sup>
C18:3n-3	7.42±0.02 <sup>c</sup>	1.99±0.01 <sup>a</sup>	2.53±0.01 <sup>b</sup>
C18:3n-6	0.10±0.01 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>
C20:2	2.30±0.01 <sup>b</sup>	0.76±0.01 <sup>a</sup>	0.77±0.01 <sup>a</sup>
C20:3n-3	1.64±0.01 <sup>c</sup>	0.45±0.01 <sup>b</sup>	0.39±0.01 <sup>a</sup>
C20:5n-3	6.37±0.04 <sup>a</sup>	8.58±0.03 <sup>c</sup>	6.74±0.03 <sup>b</sup>
C20:4n-6	4.00±0.01 <sup>c</sup>	1.97±0.01 <sup>b</sup>	1.45±0.06 <sup>a</sup>
C22:6n-3	7.83±0.02 <sup>a</sup>	18.73±0.11 <sup>c</sup>	16.39±0.06 <sup>b</sup>
C22:2	0.07±0.01 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>
C20:3n-6	0.66±0.03 <sup>c</sup>	0.31±0.02 <sup>a</sup>	0.55±0.01 <sup>b</sup>
$\sum$ PUFA	40.26±0.07 <sup>c</sup>	36.70±0.12 <sup>b</sup>	33.10±0.06 <sup>a</sup>

Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ( $P < 0.05$ ).

The highest  $\sum$ PUFA value was in the PCM value, the lowest was in SM and the statistical difference between the  $\sum$ PUFA values of the meals was significant ( $P < 0.05$ ). The C20:5n-3 (EPA) values of PCM, AM and SM were 6.37±0.04, 8.58±0.03 and 6.74±0.03%, respectively ( $P < 0.05$ ). The

DHA value of PCM was approximately half of the C22:6n-3 (DHA) values of SM and AM and the statistical difference between the DHA values of the meals was significant ( $P < 0.05$ ).

The  $\sum\omega$ -3,  $\sum\omega$ -6, and  $\sum\omega$ -9 values and  $\omega$ -3/ $\omega$ -6 and  $\omega$ -6/ $\omega$ -9 ratios of the meals used in the study are shown in Figure 2.

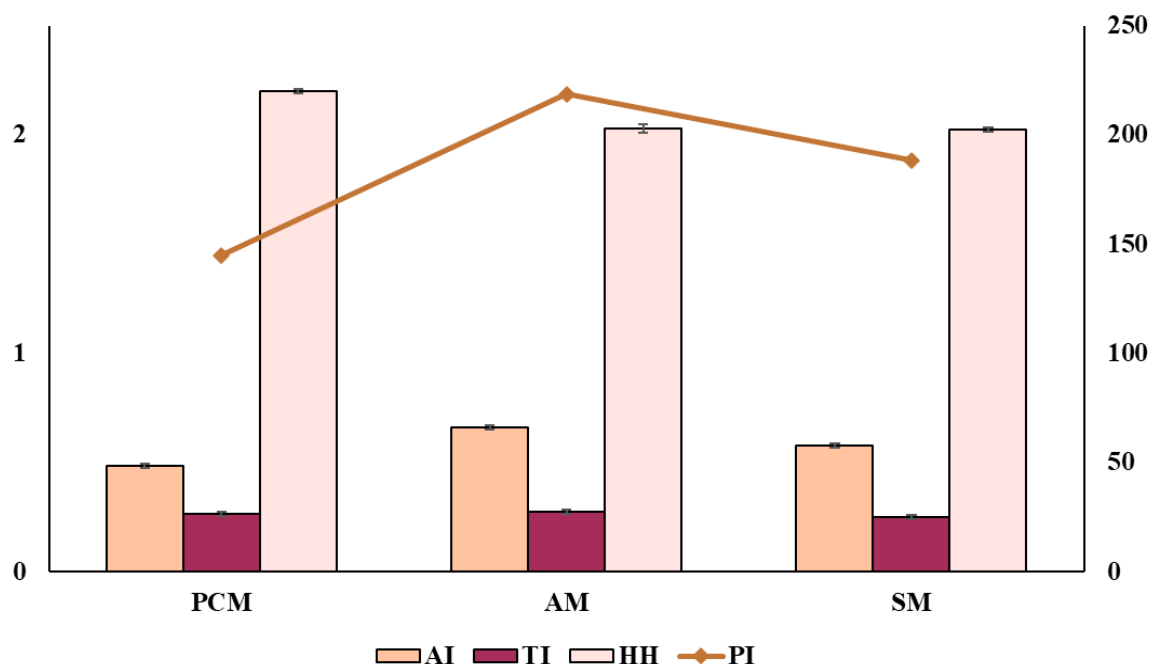


**Figure 2.** The  $\sum\omega$ -3,  $\sum\omega$ -6, and  $\sum\omega$ -9 values (%) and  $\omega$ -3/ $\omega$ -6 and  $\omega$ -6/ $\omega$ -9 ratios of Prussian carp (PCM), anchovy (AM) and sprat meals (SM).

In the current study, the  $\sum\omega$ -3 value was prominent in AM ( $P < 0.05$ ),  $\sum\omega$ -6 value in PCM ( $P < 0.05$ ) and  $\sum\omega$ -9 value in SM ( $P < 0.05$ ). The  $\omega$ -3/ $\omega$ -6 ratio was calculated as  $1.59 \pm 0.01$ ,  $4.81 \pm 0.02$ , and  $4.16 \pm 0.06$  for PCM, AM, and SM, respectively ( $P < 0.05$ ). The  $\omega$ -6/ $\omega$ -9 ratio was the highest in PCM and it was statistically different from the  $\omega$ -6/ $\omega$ -9 ratios of other meals ( $P < 0.05$ ).

The atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic ratio (HH), and peroxidisability index (PI) values of fish meals are shown in Figure 3. When lipid quality values were evaluated, the atherogenicity index (AI) values were highest in AM and lowest in PCM, and the statistical difference in AI values was significant ( $P < 0.05$ ). Thrombogenicity index (TI) values of fish meals were found to be similar ( $P < 0.05$ ). The hypocholesterolemic/hypercholesterolemic ratio (HH) value was high in PCM and statistically different from the HH values of other meals ( $P < 0.05$ ). Peroxidisability index (PI) values of fish meals were highest in AM and lowest in PCM, and it was determined that the statistical difference between PI values was significant ( $P < 0.05$ ).





**Figure 3.** The atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic ratio (HH), and peroxidisability index (PI) values of Prussian carp (PCM), anchovy (AM) and sprat meals (SM).

The element compositions of Prussian carp (PCM), anchovy (AM) and sprat meals (SM) are given in Table 4. The most abundant elements in anchovy meal were K, Ca, Fe, Cu, and Zn. Na, Mg, Mn, Se, Al, Cr, Co, and Ni were prominent in the sprat meal.

**Table 4.** The element compositions of Prussian carp (PCM), anchovy (AM), and sprat (SM) meals (mg/kg).

Elements	PCM	AM	SM
Na	5743.30±28.99 <sup>a</sup>	8425.03±34.81 <sup>b</sup>	9578.28±28.48 <sup>c</sup>
Mg	3360.96±22.41 <sup>a</sup>	3333.81±10.80 <sup>a</sup>	4343.22±21.47 <sup>b</sup>
P	66364.80±445.23 <sup>c</sup>	30240.92±142.43 <sup>a</sup>	31158.00±157.84 <sup>b</sup>
K	11144.59±34.15 <sup>a</sup>	11860.15±61.24 <sup>b</sup>	11714.80±56.94 <sup>b</sup>
Ca	128353.32±1021.10 <sup>c</sup>	45816.52±440.51 <sup>b</sup>	41678.76±215.40 <sup>a</sup>
Mn	33.14±0.28 <sup>b</sup>	23.73±0.11 <sup>a</sup>	36.28±0.21 <sup>c</sup>
Fe	241.30±0.84 <sup>a</sup>	514.95±3.12 <sup>b</sup>	524.21±1.70 <sup>b</sup>
Cu	2.94±0.02 <sup>a</sup>	4.43±0.02 <sup>c</sup>	3.92±0.04 <sup>b</sup>
Zn	136.65±0.38 <sup>a</sup>	176.42±1.02 <sup>b</sup>	171.62±0.63 <sup>b</sup>
Se	1.09±0.06 <sup>a</sup>	2.32±0.04 <sup>b</sup>	3.00±0.03 <sup>c</sup>
Al	190.99±1.40 <sup>b</sup>	40.73±0.37 <sup>a</sup>	333.55±2.25 <sup>c</sup>
Cr	0.45±0.01 <sup>a</sup>	0.55±0.01 <sup>b</sup>	2.39±0.02 <sup>c</sup>
Co	0.16±0.01 <sup>a</sup>	0.18±0.01 <sup>a</sup>	0.25±0.01 <sup>b</sup>
Ni	0.77±0.01 <sup>a</sup>	0.72±0.01 <sup>a</sup>	1.93±0.01 <sup>b</sup>

Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ( $P < 0.05$ ).

The most P was determined in Prussian carp meal. While the statistical difference between the Mg values of Prussian carp and anchovy meals was not significant ( $P > 0.05$ ), the Mg value of the sprat meal was significant ( $P < 0.05$ ). The Fe and Zn values of the Prussian carp meal were statistically different from the Fe and Zn values of the anchovy and sprat meals ( $P < 0.05$ ).

#### 4. DISCUSSION

In the current study, biochemical, fatty acid, amino acid, and element compositions of the Prussian carp, which was recorded as an invasive species in Turkey, were determined and compared to anchovy

and sprat flours, which are commonly utilized as the primary animal protein source in fish diets. In the current study, the CP values of PCM were lower than that of AM and SM ( $P < 0.05$ ), and the CF value was higher than the CF value in other meals ( $P < 0.05$ , Table 1). A report on the biochemical compositions of different fish meals (herring meal, sardine meal, Peruvian anchovy meal, haddock meal, sprat meal, tuna meal, capelin meal, mackerel meal, sand eel meal, salmon meal, cod meal) used in fish feeds disclosed that the CP values of the fish meal in question are between 53.24 and 76.6% (Windsor & Barlow, 1981; Widsor et al., 2001; Kop & Korkut, 2010; Suresh et al., 2011; Tabinda & Butt, 2012; Sevgili et al., 2015; Rahman et al., 2016; Zhang et al., 2016; Guo et al., 2019). The CP value of the different fish meals in the mentioned references was higher than the CP value of the PCM, and the CP values of the AM and SM were similar. When compared with alternative animal raw material sources that can be used as protein, except for fish meals, it is observed that the protein values of PCM were similar [meat-bone meals: 27.35-50.40% (NRC, 1994; Hardy, 1996); meat meals: 54% (NRC, 1994); chicken scraps meals: 46.6-66.88% (Firman, 2003; Ertürk & Çelik, 2004; Feedipedia, 2023); fishery by-products meals: 51.71% (Kop & Korkut, 2010); apple snail meals: 56.4% (Da et al., 2012); krill meals 58.8-59.4% (Suresh et al., 2011; Choi et al., 2020); yellow mealworm larvae meals 51.93% (Bovera et al., 2015)]. The CF values were the opposite of CP, and the CF values of fish meals in many studies (Windsor, 2001; Kop & Korkut, 2010; Suresh et al., 2011; Tabinda & Butt, 2012; Sevgili et al., 2015; Rahman et al., 2016; Zhang et al., 2016; Guo et al., 2019; Choi et al., 2020) were lower than the CF value of PCM in the current study. These differences in the biochemical compositions of different fish meals are thought to be due to the living environments (sea/inland water), feeding, and breeding conditions of the fish used as meals.

In the study, the AM was found higher  $\sum TAA$ ,  $\sum EAA$ ,  $\sum SEAA$ ,  $\sum NEAA$ ,  $\sum SAA$ ,  $\sum ArAA$ ,  $\sum AAA$ , and  $\sum BAA$ , the SM was higher of  $EAA/NEAA$  ratio and  $\sum BcAA$  (Figure 1). Since there is no literature on the evaluation of total amino acids of animal protein sources added to feeds such as fish meals, an evaluation could not be made. The total amino acid values show that the amino acid values of AM and SM were better than PCM. When essential amino acids are evaluated separately, the leucine value in different studies (Windsor & Barlow, 1981; NCR, 1994; Windsor, 2001; Sevgili et al., 2015; Choi et al., 2020; Feedipedia, 2023) was higher than the leucine values in all meals in the current study. Otherwise, lysine values, which is an essential amino acid determined in meals obtained from animal protein sources in different studies, were lower or similar to the lysine values in the current study (NRC, 1994; Da et al., 2012; Bovera et al., 2015; Rahman, 2016; Guo et al., 2019; Feedipedia, 2023). These differences in amino acid compositions are assumed to be effective in the life stage, nutritional conditions, and freshness of the animal protein source, as well as being of terrestrial or aquatic origin.

In the current study, the highest  $\sum SFA$  value was in AM, the highest  $\sum MUFA$  value in SM, and the highest  $\sum PUFA$  value in PCM, and the statistical difference was significant for each fatty acid ( $P < 0.05$ ) (Table 3). The  $\sum \omega\text{-}3$  value was high in AM and  $\sum \omega\text{-}6$  value in PCM ( $P < 0.05$ ) (Figure 2). In studies conducted with meals used in fish feeds and especially obtained from marine products (Sevgili et al., 2015; Rahman et al., 2016), the total fatty acids values in question differ from the values in the current study. It is thought that these differences are caused by the nutritional characteristics of the fish used in making the meals, the fishing season, the processing conditions and the stocking conditions.

The element values determined in fish meals in the current study are given in Table 4. Elemental values of fish meals to be used in fish feeds (Çaklı, 2007), especially Ca and P values are very important in fish feed ration preparation (Chavez-Sanchez et al., 2000). Ca and P values of PCM in the present study were approximately two times higher than AM and SM ( $P < 0.05$ ). The fact that both Ca and P values of PCM are higher than the other two meals can be explained by the fact that the bone and awn structure of Prussian carp is larger than that of sprat and anchovy. The reason why the Na value in AM and SM is higher than that of PCM can be explained by their living environments (marine). The element values of AM and SM were different from the values given in the literature (Windsor & Barlow, 1981; Guo et al., 2019; Anonymous, 2023). These differences are assumed to be caused by the periods in which the fish were caught, biological (age, species, size) and environmental (marine/inland water, salinity, etc.) factors, and the processing method used to make fish meal.

## 5. CONCLUSION

As a result, when all analyzes in the current study are evaluated, it does not seem possible to use PCM in fish feeds instead of 100% AM and SM. Although PCM contains around 60% protein and may be used as an animal protein source, its high raw ash values limit its utilization. In addition, it is known that the main problems of vegetable raw materials include an unbalanced profile in terms of essential amino acids and high antinutritional factors. By limiting the use of these vegetable raw materials in fish feed, terrestrial animal by-product meals appear as a suitable and practical alternative at the technical and economic level. However, there are strict regulations and restrictions on the use of these meals. Apart from these, insect meals, which are used as feed raw materials as an alternative, are rich in  $\Sigma$ omega-6 and poor in  $\Sigma$ omega-3. When all these factors are evaluated together, it is thought that PCM can be used instead of AM and SM in certain proportions. In conclusion, the comparison between Prussian carp, anchovy, and sprat meal reveals that Prussian carp meal has the potential to be used in fish feeds, but further experimental studies are needed to validate this. Our study highlights the need for continued research and analysis in this field to determine the best options for fish feed.

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## CONFLICT OF 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.

## AUTHOR CONTRIBUTIONS

Fiction: BB; Literature: BB, DKÖ; Methodology: BB, DKÖ, GUG; Performing the experiment: DKÖ, GUG; Data analysis: DKÖ; Manuscript writing: DKÖ; Supervision: BB; All authors approved the final draft.

## ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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