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IMPACT OF VARYING AMOUNTS OF PEANUT (Arachis hypogaea) MEAL ON RAINBOW TROUT (Oncorhynchus mykiss) DIETS INSTEAD OF SOYBEAN MEAL ON THE ELEMENTAL COMPOSITION AND BIOMAGNIFICATION FACTORS IN FILLETS

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ABSTRACT:

In this study, the effects of using different ratios of peanut meal (PNM) instead of soybean meal in rainbow trout (*Oncorhynchus mykiss)* diet on elemental composition and biomagnification factor (BMF) were evaluated for 12 weeks. Three experimental diets containing 50% (PNM_{50}), 75% (PNM_{75}), and 100% (PNM_{100}) peanut meal were prepared. In the study, two control groups were fed a commercial feed (CF) and a diet containing 0% PNM (C), respectively. The CF had the greatest Ca/P ratio and the highest Se, Ca, and P levels, and these values were also significantly different from the other experimental diets. The PNM_{75} diet had significantly higher Mn and Cu values ($p<0.05$). Fe, Mg, and Na levels increased with the increasing level of PNM ratios. The PNM100 group had the highest values of these elements. At the end of the study, the CF group had high BMF of K, Mg, Mn, and Cu elements. Compared with the C group, rainbow trout fillets in the CF group had high BMFs of Ca, Fe, Na, and Se. The BMF of the P element was high in PNM75 group. In summary, the element values of rainbow trout fillets did not have an adverse effect when using PNM instead of soybean meal in their diets.

KEYWORDS: Biomagnification factor, element composition, *Oncorhynchus mykiss*, peanut meal.

1. INTRODUCTION

Feed expenses represent the largest operational cost in intensive aquaculture systems, accounting for over half of total production expenditures. Traditional reliance on fishmeal as the primary protein source faces economic challenges due to rising prices and competition from livestock feed industries. Plant-based ingredients have emerged as viable alternatives, offering cost advantages and greater availability (Cai *et al*., 2013; Gatlin *et al*., 2007; Van Vo *et al.,* 2020). However, their incorporation faces limitations, including amino acid deficiencies, antinutritional factors, and reduced feed appeal. Notably, many aquatic species demonstrate greater tolerance for plant-derived proteins compared to land animals (Van Vo *et al*., 2020).

Among plant-based protein sources, soybean meal has become the predominant choice for animal feed formulations owing to its optimal protein content, balanced amino acid composition, and reliable supply (Trosvik *et al*., 2012). In aquaculture feed formulations, SBM typically constitutes 20-60% of the total dietary composition, with the exact inclusion level varying according to species-specific nutritional requirements (Junior *et al.,* 2016; Koch *et al.,* 2016). However, its cost significantly raises feed prices because of sustainability and external dependency (Hossain *et al*., 2012). Therefore, it is necessary to determine alternative, available, and sustainable plant protein sources that do not negatively affect the growth performance of fish. Vegetable protein sources, such as canola, sesame, cottonseed, pea, wheat gluten, and peanut meals, have been used instead of soybean meal in several fish-feeding experiments with positive outcomes (Collins *et al*., 2013; Dernekbaşı *et al.,* 2021; Guo *et al*., 2011; Hosseini Shekarabi *et al.,* 2021; Li *et al.,* 2023; Yue & Zhou, 2008; Zhou & Yue, 2010; Kleemann *et al.,* 2011; Ustaoğlu Tırıl & Kerim, 2015; Tunca, 2019; Kumar *et al*., 2020).

The peanut, *Arachis hypogaea* L., is the fourth-largest oilseed crop in the world, with approximately 51.5 million tons produced worldwide (USDA, 2024). In addition to being high in fat and protein, peanut meal (PNM) is a good source of iron, calcium, selenium, salt, manganese, phosphorus, potassium, copper, and zinc (Ayoola *et al.,* 2012; Davis & Dean, 2016; Hasan *et al*., 2013; Jonnala *et al.,* 2006; Sales & Resurreccion, 2010; Zhang *et al.,* 2017). PNMs are becoming increasingly popular as a protein source to replace SBM in animal feeds because of their lower cost per unit of protein than SBM (Batal *et al*., 2005; Dernekbaşı *et al*., 2021; Goes *et al*., 2004;).

This research indicates that while studies have been conducted on the use of PNM in rainbow trout diets (Adelizi *et al*., 1998; Acar & Türker, 2018; Dernekbaşı *et al*., 2021), no studies have been conducted to assess the elemental values and biomagnification factor of the fish. In this regard, the study sought to ascertain the impact of PNM used at varying rates in rainbow diets on fish fillets' element and biomagnification values.

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2. MATERIAL AND METHOD

Experimental trials were carried out at Sinop University's Faculty of Fisheries Research and Application Center in Sinop (Türkiye). Juvenile rainbow trout (Oncorhynchus mykiss) specimens were sourced from Kuzey Su Ürünleri A.Ş., a commercial aquaculture operation located in Samsun, Turkey. Prior to experimentation, fish underwent a 10-day acclimatization period while being maintained on a commercial diet (Black Sea Feed). Following this adjustment phase, specimens were individually weighed and randomly allocated across 15 fiberglass rearing tanks (300 L capacity each), with stocking densities maintained at 20 fish per experimental unit. All tanks featured continuous flow-through water exchange and supplemental aeration systems.

The 12-week nutritional trial employed a completely randomized design with five dietary treatments; each replicated three times. Juvenile rainbow trout (initial mean weight = 89.73 ± 0.08 g) were stocked in experimental tanks at equal densities. Four isonitrogenous (45% crude protein) and isolipidic (17% crude fat) test diets were formulated with graded peanut meal (PNM) inclusion levels: 0% (control, C), 50% (PNM50), 75% (PNM75), and 100% (PNM100). A fifth treatment group received commercial feed (CF) as an industry reference. Both the CF and C groups served as experimental controls for comparative analysis. All formulated diets were nutritionally balanced to match the proximate composition of the commercial reference feed.

Throughout the 12-week experimental period, fish received hand-fed rations twice daily (09:00 and 15:30 hours) to apparent satiation. Water quality parameters were monitored twice per day using a Thermo Scientific Orion 4 Star multiparameter instrument, recording mean values of:

Temperature: 15.98 ± 0.02°C, Dissolved oxygen: 6.12 ± 0.04 mg/L, and pH: 8.15 ± 0.02. For biological sampling, specimens (n=5 per time point) were humanely euthanized at both the initiation and termination of the trial using clove oil anesthetic at concentrations sufficient to ensure rapid loss of consciousness. All collected tissue samples were immediately flash-frozen and maintained at -80°C in a WiseCryo WUF-D500 ultra-low temperature freezer until subsequent.

All elemental analyses were conducted at SUBITAM (Sinop University Scientific and Technological Research Center) following established protocols. Tissue sample preparation followed EPA Method 200.3 for spectrochemical determination of recoverable elements in biological matrices. Fillet samples underwent microwave-assisted acid digestion (Milestone Systems Start D 260) using Suprapur® grade nitric acid, following the manufacturer's HPR-FO-67 protocol for animal tissues. Quantification of elements was performed using an Agilent Technologies 7700X ICP-MS system operated by an accredited scanning electron microscopy laboratory. The analytical protocol incorporated: Three replicate measurements per sample, Certified reference material (Lobster TORT-2) for quality control, Multi-element calibration standards (Agilent 8500-6940 series), and Continuous internal standard monitoring (1 ppm Agilent 5188-6525). Method validation demonstrated: Analytical precision ≤ ±10% RSD, CRM recovery rates of 90-100%, and Results expressed as mean ± standard error.

The biomagnification factor (BMF) is defined as the ratio of the concentration of a given element in fish tissue to its concentration in the fish's diet. In this study, BMF was calculated using the following equation (Kelly *et al*., 2008; Majid *et al*., 2019)

$BMF = C_{fish}/C_{diet}$

where C_{fish} represents the concentration of an element in the fillets of rainbow trout exhibiting different reproductive characteristics, and C_{diet} denotes the concentration of the same elements in the administered diet

Data processing utilized IBM SPSS Statistics 21 software. Normal distribution was verified using Anderson-Darling tests, while group variance homogeneity was assessed with Levene's test. Intergroup differences were determined through one-way ANOVA with appropriate post-hoc testing.

3. RESULTS

Table 1 shows the live weight initially and at the end of the 12-week study. The control (C) group attained the best weight at the end of the trial. While no statistical difference was observed between the final weights of the groups administered peanut-containing diets (PNM50, PNM75, PNM100) (p>0.05), there was a significant difference between the live weights of the commercial feed (CF) and C groups (p<0.05).

Table 1: The live weight values of rainbow trout fed with experimental diets for 12 weeks

	CF	C	PNM_{50}	PNM_{75}	PNM_{100}
Initial (g)	89.7±0.15^{a}	89.5±0.12^{a}	89.7±0.12^{a}	89.7±0.25^{a}	89.8±0.24^{a}
Final (g)	261.42±20.57^{c}	274.90±9.52^{a}	265.10±16.83^{b}	266.74±8.04^{b}	268.37±21.03^{b}

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).

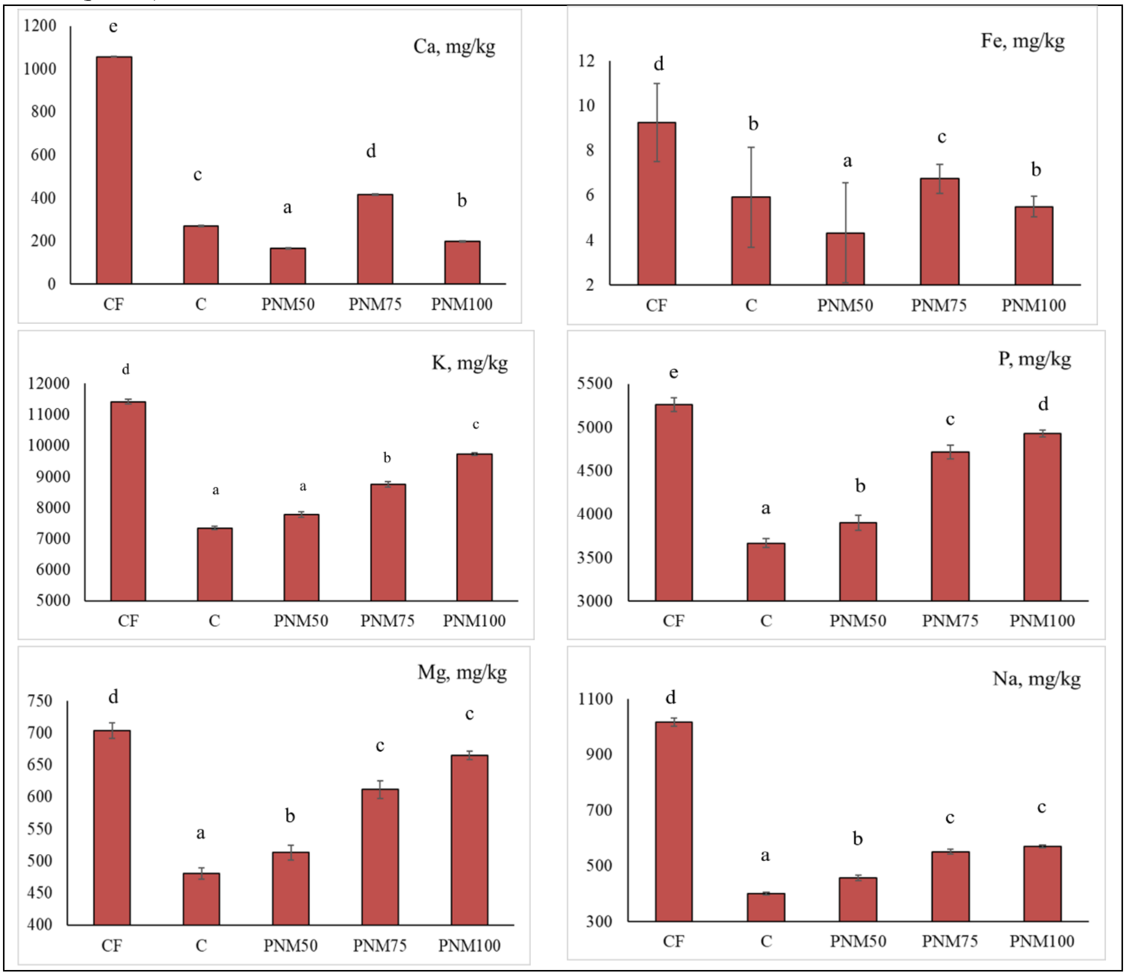
Table 2 displays the element values in the experimental diets. The study that CF had the highest Se, Ca, and P values, as well as the Ca/P ratio. The differences among Se, Ca, and P values and Ca/P ratio in the experimental diets were also statistically significant (p <0.05). The CF had a high K value (p<0.05), while the PNM_{75} diet had high Mn and Cu values (p<0.05). As the amount of peanut meals in the diet increased, so did the values for Fe, Mg, and Na; the PNM_{100} group had the highest values of these elements (p<0.05).

Figure 1 shows the selected elements in the rainbow trout fillets of the groups at the end of the experiment. The levels of Ca, Fe, P, K, Mg, Na, Mn, and Se were higher in rainbow trout fillets fed commercial diets than in those fed experimental diets. As the diet's peanut meal ratio increased, so did the elements K, P, Mg, and Na in rainbow trout fillets given experimental diets. Fe, Zn, Mn, and Cu levels in rainbow trout fillets fed experimental diets were found to be highest in the group fed feed containing 75% peanut meal. Significant statistical differences were found between the selected element values in the fillets (p<0.05).

Table 2: The selected element values in the experimental diets (mg/kg)

Diets (mg/kg)	**Experimental groups**				
	CF	**C**	**PNM$_{50}$**	**PNM$_{75}$**	**PNM$_{100}$**
Calcium (Ca)	63847.27±1018.21^{d}	14638.72±39.86^{a}	19634.98±152.64^{b}	18367.75±186.89^{b}	24930.56±127.94^{c}
Iron (Fe)	585.21±4.48^{b}	208.36±1.36^{a}	880.39±0.52^{c}	1187.43±7.49^{d}	1460.21±12.45^{e}
Potassium (K)	26043.13±337.70^{a}	30958.28±121.36^{c}	29237.77±196.84^{c}	27452.13±197.10^{b}	27943.45±147.26^{b}
Magnesium (Mg)	6033.03±123.46^{b}	5781.58±35.76^{a}	6430.50±34.54^{c}	6850.06±60.39^{d}	7150.61±36.19^{e}
Sodium (Na)	13974.22±224.56^{c}	4474.52±16.77^{a}	9944.33±68.67^{b}	10494.38±113.74^{b}	17935.96±118.42^{d}
Phosphorus (P)	41249.02±727.44^{d}	19392.83±2.10^{a}	20917.33±176.87^{b}	19784.59±224.29^{a}	23406.57±99.17^{c}
Manganese (Mn)	103.67±0.56^{b}	93.50±0.84^{a}	102.89±0.65^{b}	114.47±0.30^{d}	107.44±0.20^{c}
Zinc (Zn)	265.16±2.12^{a}	329.14±2.58^{c}	303.37±0.51^{b}	333.11±0.90^{d}	372.74±1.27^{e}
Selenium (Se)	2.31±0.08^{d}	1.93±0.05^{b}	1.76±0.06^{a}	1.79±0.05^{a}	2.14±0.12^{c}
Copper (Cu)	24.02±0.17^{a}	29.25±0.14^{b}	32.81±0.10^{c}	38.12±0.18^{e}	35.32±0.13^{d}
Ca/P	1.55	0.75	0.85	0.66	0.96

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



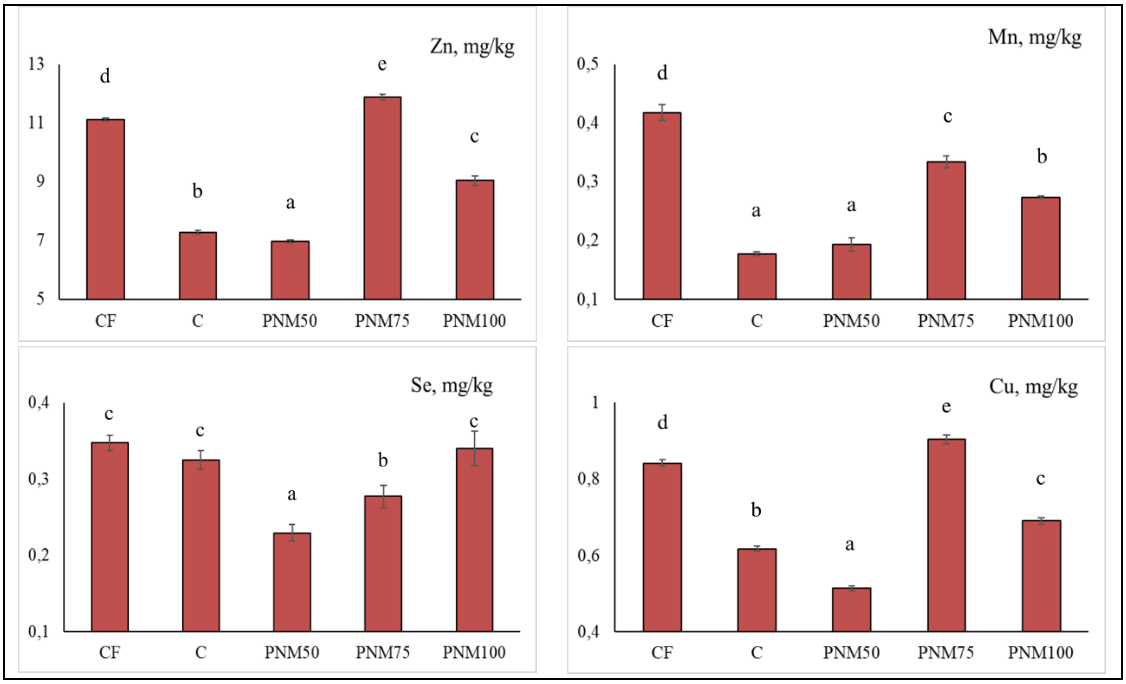


Figure 1: Selected element values determined in the fillets of the experimental groups (mg/kg)

Figure 2 depicts the biomagnification factor (BMF) of selected elements in the fillets of rainbow trout after the 12-week experimental period. The BMF of K, Mg, Mn, and Cu elements was to be high in rainbow trout fillets given commercial feed (CF group) at the end of the study. The BMF of Se, Fe, Ca, and Na were high in rainbow trout fillets given to the C group. The fillets in the group-fed feed containing 75% peanut meal (PNM_{75}) had a high BMF of the P element.

4. DISCUSSION

This study determined selected element values and biomagnification factors in fillets of rainbow trout fed with feeds containing different amounts of peanut meal (50, 75, and 100%) instead of soybean meal. Rainbow trout with initial weights of 89.68±0.05 g reached the highest value with 274.90±9.52g in the control group (C) after 90 days, followed by the PNM_{100} group with 268.37±21.03g. The final weight of the group fed with commercial feed (CF) was 261.42±20.57g. In the study, using peanut meal instead of soybean meal did not have an adverse effect on fish weights. Since the subject of the study was fillet element values and biomagnification values, no discussion was made on growth performances.

Table 2 lists the selected element values of the diets used in the research. The results showed that the element contents in the diets used were different. These differences can be attributed to the differences in the raw materials in both the commercial and experimental feeds. Ca, P, and Se were more dominant in the commercial diet mixtures studied. The progressive incorporation of peanut meal (PNM) in formulated feeds demonstrated a corresponding elevation in calcium (Ca), iron (Fe), magnesium (Mg), and sodium (Na) concentrations. Current findings align with established nutritional benchmarks, where Watanabe (1988) and Goddard (1995) identified optimal dietary mineral requirements for rainbow trout as 30 mg/kg Fe, 3 mg/kg Cu, 13 mg/kg Mn, and 30 mg/kg Zn. However, commercial aquaculture practices often exceed these recommendations, as evidenced by Tacon and De Silva's (1983) documentation of European salmonid diets containing 80-540 mg/kg Fe, 35-100 mg/kg Mn, 5-40 mg/kg Cu, and 50-260 mg/kg Zn - representing 2-11 fold increases above standard requirements. More recent analyses by Sissener *et al.* (2013) further confirm this variability, reporting typical dietary ranges of 65-493 mg/kg Fe, 2.5-21 mg/kg Cu, 36-330 mg/kg Zn, 4.4-226 mg/kg Mn, and 0.39-4.1 mg/kg Se. These micronutrients play indispensable roles in biological systems, supporting critical physiological processes including growth performance, reproductive success, and metabolic regulation across aquatic species (Fallah *et al.*, 2011; Roy & Lall, 2006; Yıldız *et al.*, 2008;). Analytical evaluation confirms that the mineral profiles of the experimental diets in this investigation satisfy the essential nutritional requirements for rainbow trout cultivation.

Compared to proteins, lipids, and carbohydrates, research on elemental nutrition in fish remains limited, primarily due to the complex interactions between dietary intake and environmental uptake (Antony Jesu Prabhu *et al*., 2016). Like all organisms, fish require essential elements for critical physiological functions, but unlike terrestrial animals, they can absorb minerals directly from water—except for elements like phosphorus and iodine, which are often present in insufficient concentrations (Kaushik & Lall, 2021). For rainbow trout, established dietary requirements include: Macroelements: Phosphorus (0.07%), magnesium (0.05%). Trace elements: Copper (5 mg/kg), manganese (10 mg/kg), selenium (0.15 mg/kg), zinc (37 mg/kg) (NRC, 2012). Even when elements are present in culture water, studies suggest that dietary supplementation remains crucial for certain minerals, such as calcium, to prevent deficiencies (Vielma *et al.*, 1998). Additionally, metabolic demands may necessitate higher selenium intake than growth requirements alone, as this element supports enzyme function (Fontagné-Dicharry *et al.*, 2015). Traditionally, fishmeal-rich diets provided sufficient minerals, reducing the need for supplementation (Kamalam *et al.*, 2020). However, as fishmeal inclusion decreases in modern aquafeeds, ensuring adequate dietary supply of both macro- and trace elements—with consideration for bioavailability—has become increasingly important (Antony Jesu Prabhu *et al*., 2016). This research examined the concentrations of Fe, Mn, Ca, Cu, Mg, P, Zn, K, Na, and Se in rainbow trout fillets to assess: Elemental utilization efficiency from diets with varying peanut meal inclusion and Tissue retention patterns to determine storage and metabolic allocation.

Finally, the highest element values, except Cu and Zn, in rainbow trout fillets were obtained from the group fed with commercial feed (CF) (Figure 1). The peanut meal in the diet was increased, and the Na, P, Mg, and K values of fish fillets increased. The Ca, Fe, Mg, Zn, and Cu values of the fillets of the experimental groups were obtained from the groups fed with feed containing 75% peanut meal. The number of studies on fillet element values using different amounts of peanut meal in fish diets is limited. For example, it was reported that using different amounts of peanut flour in feeds affects the elemental values of tilapia fillets (Yılmaz, 2022). Apart from this, Antony Jesu Prabhu *et al*. (2018) reported that if completely plant-based meals are to be used in rainbow trout diets, mineral supplements should be made to meet the needs of the fish. The current study added the same amount of mineral mix to all experimental diets except the commercial feed (CF). Apart from all these evaluations, the element values of rainbow trout fillets in this study portion weight (250g) were within the ranges reported by USDA (2019). Selected elements' biomagnification factor values were less than 1 after the 12-week study. When all fillets were evaluated in general, the element with the highest BMF was determined to be K in all groups. Except for the group fed with CF, the BMF value increased as the peanut meal ratio in the trial feeds increased. While no such increase is observed in the K values of the diet (Table 2), it has been determined that there is a parallelism between the K element values in fish fillets (Figure 1) and the BMF of K. Table 2 and Figure 1 demonstrate that the element levels in each of the study experimental diets were higher than those found in fish fillets. Analogous to the current study, Jiang *et al*. (2016) found that all the elements in the diets they utilized were two to three times higher than in the fillets and that the diets significantly impacted the biomagnification factor measured in fish. According to Varol *et al*. (2017), variations in the concentrations of certain elements in wild and farmed fish may also be directly related to variations in the amounts of those elements in typical and commercial fish diets. Several studies have shown that the changes in elements used in diet formulation and the addition of varied percentages of mineral premix to diet mixes result in differences in the amounts of elements in commercial feeds (Dadar *et al*., 2016; Kaya Öztürk, 2022; Kaya Öztürk, 2023 ; Majlesi *et al*., 2019). The finding that rainbow trout fed varying quantities of PNM had Biomagnification factor values <1 is significant when analyzed in terms of selected elements.

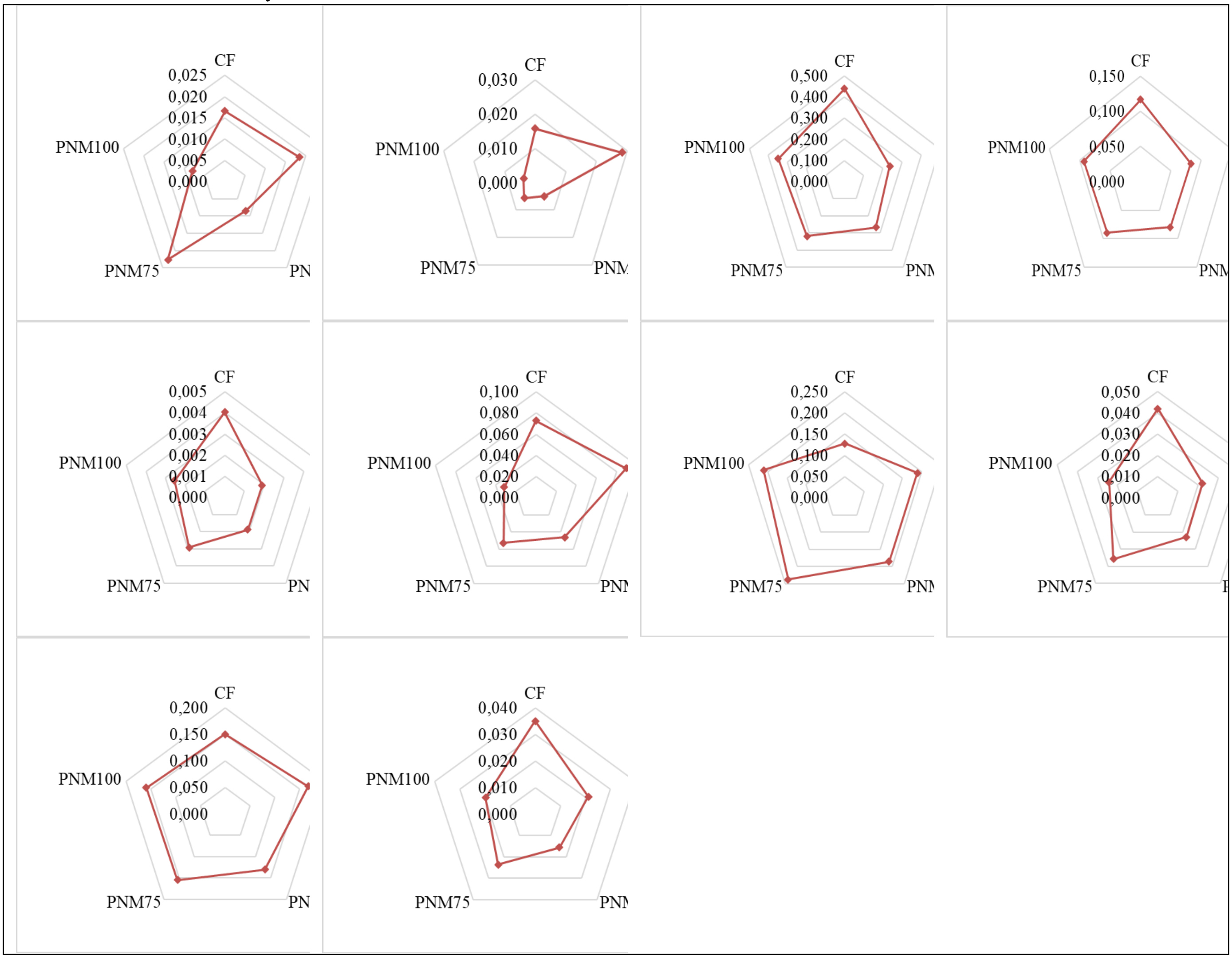


Figure 2: Biomagnification factor of experimental groups

CONCLUSION

This study is the first to evaluate the elemental composition and biomagnification factors in the fillets of rainbow trout fed diets containing peanut meal (PNM) as a replacement for soybean meal. The results showed a parallel relationship between the dietary inclusion level of PNM and the biomagnification values of various elements. In conclusion, substituting soybean meal with PNM in rainbow trout diets did not significantly affect the elemental concentrations in the fillets.

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Ethical statement:

This study was conducted in compliance with the rules for animal experiments for scientific purposes, and permission was given by the Sinop University, Animal Experiments Local Ethics Committee with permission No. 2020/02 on February 17th, 2020.

Author Contributions:

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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