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Is the Nutritional Composition of Safflower Oilseed Meal Sufficient for Alternative or Complementary Aqua Feeds-raw Material?

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is grown in many countries, even in arid regions. Due to its important nutrients, safflower has the potential to be used as raw material for the nutrition of many animals and aquaculture species. For this reason, the objective of this study is to determine crude protein, crude oil, ash, and nitrogen-free extract (NFE) values, as well as amino acid, fatty acid, and mineral values in safflower seed meal. In addition, fish meal, soybean meal, wheat, and canola meal values are compared with safflower seed. On a dry matter basis, the crude protein, crude oil, crude ash, and NFE values were found at 19.42% ±0.32, 8.76% ±0.21, 2.82% ±0.1, and 62.68% ±0.88 respectively. Safflower meal contains significant arginine, histidine, and phenylalanine levels, with C18:2 n-6 being one of the most abundant fatty acids. According to the fatty acid values examined, the total saturated fatty acid values were to be 9.79%; the total monounsaturated fatty acid values are 27.58%; total n-6 PUFA values are 61.49%; total n-3 fatty acid values are 0.55% and total n-3 HUFA values are 0.22% in safflower oilseed meal. The potassium and magnesium content in safflower seed meal is similar to that of soybean meal and fishmeal. Safflower oilseed meal or oil can be used as complementary feedstuff in both marine and freshwater fish feeds. In future studies, observing the balance of essential amino acids and polyunsaturated fatty acids and conducting detailed studies will be effective in closing the gap in this area.

Keywords: Safflower meal and oil, Nutritional composition, Model for amino acids, Cultured fish nutrition, Complementary feedstuff

1. Introduction

The aquaculture industry ensures that cultured fish receive the food and nutrients they need, both in terms of quality and quantity (FAO 1996). However, in aquaculture, while the fish must be fed to survive, a nutritionally balanced level is required (Tecnovit 2014). Feed costs are the major portion (about 40-70%) of aquaculture expenditure. The aquaculture feed industry has expanded rapidly in recent years, and this trend is expected to continue. Fish meal is the preferred raw material in fish feeds due to its high protein and essential amino acid content. According to the FAO (2011), the reliance on fishmeal for aquaculture jeopardizes both marine biodiversity and human food security. Because aquaculture feeds' over-reliance on fishmeal may be unsustainable, the aquafeed industry's costs require the discovery of alternative ingredients (Kiron et al. 2016; Sarker et al. 2016). In 2020, the world produced 4.9 million tons of fish meal and 1.1 million tons of fish oil (OECD/FAO 2022). Fish meal and oil (FM and FO) are the most used raw materials in the aquafeed industry. However, despite the occasional difficulties in obtaining sufficient quantities of these raw materials, the search for alternative raw materials is continuous. For the most part, it is predicted that including different raw material sources in the ratios as a protein source would be more beneficial both economically and nutritionally for aquafeeds (Turchini et al. 2019).

Research continues on fish meal and oil, which is one of the most important raw materials in fish diets, and on marine and terrestrial raw materials that can be partially substituted for these two critical raw materials. On the other hand, Turchini et al. (2019) examined 7390 articles/documents in their research with the keywords "alternatives and aquafeeds". Raw materials must be consistent and economical, available in sufficient quantities, have essential nutrients, be free from contaminants and other undesirable factors, and be capable of withstanding a range of processing constraints. In addition, consideration should be given to how new raw materials may interact with others. Knowing the positive and negative effects of raw materials on each other during feed production creates very useful information.

Glencross (2020), recommends that seven steps (characterization, palatability, digestibility, utilization, immunological, processing effects, and product quality influence) should be considered when deciding whether or not a feed raw material will be used.

With its high protein percentage, balanced amino acid profile, low price, high digestibility, and easy accessibility, soybean meal is an important raw material that can be used in the feed of many aquaculture species (Storebakken et al. 2000). Because of supply issues and the environmental effects of fish meal, the use of soybean meal in aquafeed has increased in recent years, and it has emerged as a dietary protein source (Murashita et al. 2015). In 2019, the world's total soybean production was 333 million tons (FAOSTAT 2020).

Despite soybean meal being the most commonly used vegetable raw material in aquafeeds, the following studies have attempted to summarize the fact that many vegetables raw materials are used in various fish species.

In European seabass feeds, Tibaldi & Kaushik (2005), used corn gluten meal, wheat gluten, wheat meal, soybean meal, and linseed meal. It was determined that there were no negative effects on somatic growth or nitrogen absorption. Suárez et al. (2009), in their 2009 study, used a mixture of soybean and canola meal was used in white-leg shrimp feeds and found that growth was also not affected. Köprücü and Sertel (2012), fed grass carp linseed meal, sunflower meal, and corn meal and observed no negative effect on somatic growth or nitrogen utilization. Rubber meal was used in carp feeds by Suprayudi et al. (2015), who found in growth and feed evaluation no observable negative effects.

The safflower (*Carthamus tinctorius* L.) oilseed plant is a tall and yearly plant with a length can that can range from 0.3 to 1.5 meters and roots that can grow to be 2-3 meters long. The first root's contact with the soil occurs at a depth of 30 cm. (Oyen & Umali 2007; Ecoport 2010) When compared to soybean and sunflower, it requires less water when growing in arid conditions. It is a very sustainable plant because it is an oilseed and easy to extract (Gilbert 2008).

Safflower is grown in over 60 countries (Ekin 2005; Kinupp & Lorenzi 2014). It is grown in tropical regions (1400 m in Ethiopia, 1800 m in Kenya) between latitudes of 20 °S and 40 °N, from sea level to 900 m and above, due to its tolerance to cold and drought (Ecoport 2010).

It is known that there are 25 wild safflower species in the world, and some of these species C*arthamus lanatus, C. tinctorius* and *C. dentatus* are also found in Türkiye. Safflower plants have been grown around the world for many years, particularly in China, Japan, India, Egypt, and Iran. It is known that it was cultivated in the Medieval Era in Italy, France, and Spain and that after the discovery of America, it was first taken to Mexico by the Spanish, and then to Venezuela and Colombia. The safflower plant arrived in the United States in 1925 via the Mediterranean countries.

In Türkiye, the genus *Carthamus*, which has 25 taxons worldwide, is *C. oxyacantha* (2n=24), and *C. tinctorius* (2n=24) is cultivated (Meshram et al. 2011; Çulha Erdal et al. 2021). Between 1929 and 1930, the Eskişehir-Sazova breeding station began cultivating and breeding safflower in Türkiye (Babaoğlu 2017).

Although there are many companies producing safflower oilseed meal in Türkiye, the RIPSA company (Kayseri), safflower oilseed meal supplier, is one of the leading companies in this field and has been producing the "Balcı" seed, which is the most widely used seed type in Türkiye recently. Therefore, the "Balcı" seed used in this study was thought to reflect the safflower profile in Türkiye (RİPSA 2021).

Safflower (*Carthamus tinctorius* L.) is an oleaginous plant that was once widely known throughout the world (Mansouri et al. 2018). Safflower is a member of the Asteraceae family and is grown in many parts of the world due to its adaptability to a variety of conditions, including areas with low rainfall (Baümler et al. 2006). Currently, it is grown in nearly 60 countries around the world (FAO 2017), with Kazakhstan, India, Mexico, and the United States being the main producers of safflower.

In 2019, the total global safflower production was 627,653 tons. Kazakhstan (214,149), the United States (107,200), Mexico (58,675), India (55,000), and Türkiye (35,000) were the world's top manufacturers (FAO 2020). According to FAOSTAT (2017), the average global cycle of safflower cultivation for seed production varies from 200 to 230 days in the autumn and winter seasons and from 120 to 160 days in the spring and summer seasons, when including the emergence periods and harvest of seeds/grains.

Safflower whole seed is mainly composed of 33-60% coat and 40-67% food stores (Dajue & Mündel 1996; Pahlavani 2005). The oil content of the seed ranges from 15% to 45% depending on the variety and environment in which it grows (Emongor 2010).

Safflower oilseed plant meal is found in the feed of many farm animals and some cultured fish species. Studies have revealed that safflower meal can be used as a protein source up to 20% in rainbow trout and shrimp feeds (Galicia-González et al. 2010; Ustaoglu Tiril & Kerim 2015; Ustaoğlu Tiril et al. 2016; Çantaş & Yıldırım 2020).

Safflower is used in pharmaceuticals, infant formulas, cosmetics, and biodiesel production (GRDC 2010) and does not require much nitrogen fertilizer (which may be deleterious to livestock and oil quality). Due to its taproot, safflower uses NO_3 leachates left in groundwater and for this reason is considered environment friendly (Yau & Ryan 2010).

There are some deficiencies in the literature in studies evaluating nutrients as a whole (protein, lipid, NFE, amino acids, fatty acids, and minerals). As a result, in this study, we determined the nutritional properties of safflower oilseed meal, which is an alternative or complementary source of raw materials in terms of all nutrients, and we revealed which limits should be considered as a vegetable protein source in aquatic feeds.

Due to the rapid development of the aquaculture industry, the demand for fishmeal/fish oil in aquafeed has increased, but its availability and increase in price have also raised concerns. For this reason, it is vital to search for high-quality, economical, and environmentally friendly feed raw materials (Hekmatpour & Mozanzadeh 2021). Efforts are being made to reduce the use of fishmeal and oil in fish nutrition. In aquaculture, feeding studies have been conducted to reduce fishmeal oil to 10% or less in the diet without adversely affecting fish growth performance (Apper-Bossard et al. 2013). The purpose of this research is to evaluate safflower oilseed plant meal, its history, production conditions, and its current status in Türkiye and around the world. In addition, it is aimed to compare the nutrients of safflower oilseed meal with other raw materials (soybean, wheat, and canola meal) and to reveal to what extent it can meet the nutritional needs of species of importance in aquaculture (rainbow trout, carp, and Nile tilapia).

2. Material and Methods

The safflower oilseed meal used in this study was obtained from a private company (RİPSA) and analyzes were carried out in the laboratory with 3 replications. The safflower meal known as "Dinçer" is the oldest registered type of safflower. In recent years, the "Balcı" seed variety has been widely used in Türkiye and the safflower variety we examined in this study is this oilseed.

Crude protein, crude lipid, crude ash, and moisture of safflower meal were determined using standard methods (AOAC 1990; AOAC 1995; AOAC 2002; AOAC 2006).In this study, the cold-press extraction method was used to extract the safflower oilseed. The oil extraction process obtains two types of safflower meal (Knowles & Ashri 1995), indicating a significant variation in composition. One is the hull and labelled as a hulled safflower meal, while the other is de-hulled (totally or partially) and labelled as a de-hulled safflower meal. The safflower oilseed meal in which coat is not separated is commonly used in the safflower variety we analyzed in this study.

2.1. Amino acid content

The LCMS/MS system was used to measure amino acid concentrations to determine the amino acid profiles of the samples. The method in question is described further below. The Jasem LC-MS / MS amino acid analysis kit was used, which included a calibrator set containing standards at five different concentrations, a stable isotope-labeled internal standard mix, mobile phases, reagents, chromatographic separation and mass detection method parameters, and a modified sample preparation process that included acidic hydrolysis. The concentration of target amino acids was determined using the multiple reaction monitoring mode based on electrospray ionization (Bilgin et al. 2018).

2.2. Fatty acid content

Lipids were extracted using the method described by Folch et al. (1957). Following lipid extraction, fatty acid methyl esters (FAME) were prepared as described by Metcalfe and Schmitz (1961), and analyzed as previously described (Czesny & Dabrowski 1998) with some modifications. Briefly, the FAME obtained was separated using gas chromatography (Agilent 6820 A), equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d., and 0.25 μm). The injector temperature program was set to 190 °C for 35 minutes, and then increased at a rate of 30 °C per minute until it reached 220 °C, where it was held for 5 minutes. The carrier gas was hydrogen (2 mL min^{-1} with a split ratio of 30:1). Individual fatty acids were identified by comparing their retention times to a standard fatty acid mixture (Supelco 37 component FAME mix).

2.3. Mineral analysis

Analyses were performed on samples that had been dried at 70 °C. Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe) Potassium (K) with Total Nitrogen (N) Kjeldahl device, Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P) and Zinc (Zn) were determined by reading the solution obtained by wet burning with acid in ICP-OES by reading the solution obtained by wet burning with acid (USEPA 2007). All analyses were done in triplicate, and the mean values were used for data analysis.

2.4. Comparison of nutritional compositions of feed raw materials (simple comparison model for amino acids)

The data obtained in the analysis of the safflower seed meal were compared with the nutritional composition data of fish meal, soybean meal, wheat meal, and canola meal in the literature. The essential amino needs of three important cultured species were also compared with data from NRC (2011). In this study, fishmeal was used as a basic feed ingredient and compared with other ingredients. Furthermore, considering that fishmeal covers all essential amino acid needs, we have taken this value 100% and developed a simple and useful comparative model with other raw materials (safflower, soybean, wheat, rapeseed meal). This is a simple way to model the availability of other ingredients in feed compared to fishmeal.

3. Results

Analyses were performed to determine the amino acid content, fatty acid content, and mineral content, which were then compared in Tables 1-4.

3.1. Nutritional values of feed raw materials

The safflower oilseed meal contains relatively moderate levels of protein 19.42%; besides the crude lipid 8.76%; the ash 2.82% and the nitrogen-free extract 62.68%. The biochemical and essential amino acid values of fish meal, safflower oilseed meal, soy meal, wheat meal, and canola meal are given comparatively in Table 1.

Table 1- Nutritional values of feed raw materials

Proximate analysis	***Marine meals***	***Terrestrial plant meals***			
	Fish meal[1]	***Safflower oilseed meal[2*]***	***Soybean meal[3**]***	***Wheat meal[4]***	***Canola meal[5**]***
Dry matter (%)	91.80	96.24	88.00	88.30	92.10
Protein (%)	67.00	19.42	55.20	13.35	36.90
Lipid (%)	10.90	8.76	1.70	1.63	33.20
Fibre (%)	-	-	4.40	12.05	14.90
Ash (%)	13.90	2.82	7.60	1.66	3.70
NFE (%)	-	62.68	18.40	59.6	10.20
EAA (g $100g^{-1}$ DM)					
Arginine	3.90	1.68	3.43	0.72	2.28
Histidine	2.50	0.81	1.22	0.35	1.38
Isoleucine	3.00	0.51	2.19	0.55	1.21
Leucine	5.00	1.21	3.97	0.99	2.43
Lysine	5.30	0.81	2.67	0.41	2.02
Methionine	2.00	0.13	0.49	0.58	0.68
Phenylalanine	2.70	0.93	2.51	0.68	1.40
Threonine	2.50	0.64	2.41	0.43	1.62
Valine	3.70	0.94	2.50	0.72	1.66

[1]Data on proximate composition and amino acid contents of fishmeal are from Glencross (2020); [2]Amino acid contents of safflower meal obtained are from this study; [3]Data on proximate composition of soybean meal are from Heuzé et al. (2020) and Zheng et al. (2022); [4]Data on proximate composition and amino acid contents of wheat meal are from Thacker and Widyaratne (2012); [5]Data on proximate composition and EAA of canola meal are from Mejicanos et. al. (2016) & MacIntosh et al. (2021); [*]Cold press extraction process; [**]Solvent extraction process

3.2. Amino acid content

The amount of essential and non-essential amino acids is determined in safflower oil seed meal using standard methods. Arginine, leucine, valine, and phenylalanine were the most abundant amino acids in the safflower oilseed meal. The methionine level of the safflower oilseed meal was low and limited. The amino acid contents are shown in Table 2. The essential amino acid comparison between raw materials is shown in Figure 1.

Table 2- Safflower oilseed meal amino acid contents

Essential amino acid (g/100g)		***Non-essential amino acid (g/100g)***	
Arginine	1.68	Aspartic acid	1.96
Histidine	0.81	Glutamic acid	4.09
Isoleucine	0.51	Ornithine	0.19
Leucine	1.21	Proline	1.02
Lysine	0.81	Taurine	0.00
Methionine	0.13	Serine	1.11
Phenylalanine	0.93	Alanine	0.88
Threonine	0.64	Cystine	0.26
Valine	0.94	Glycine	1.13

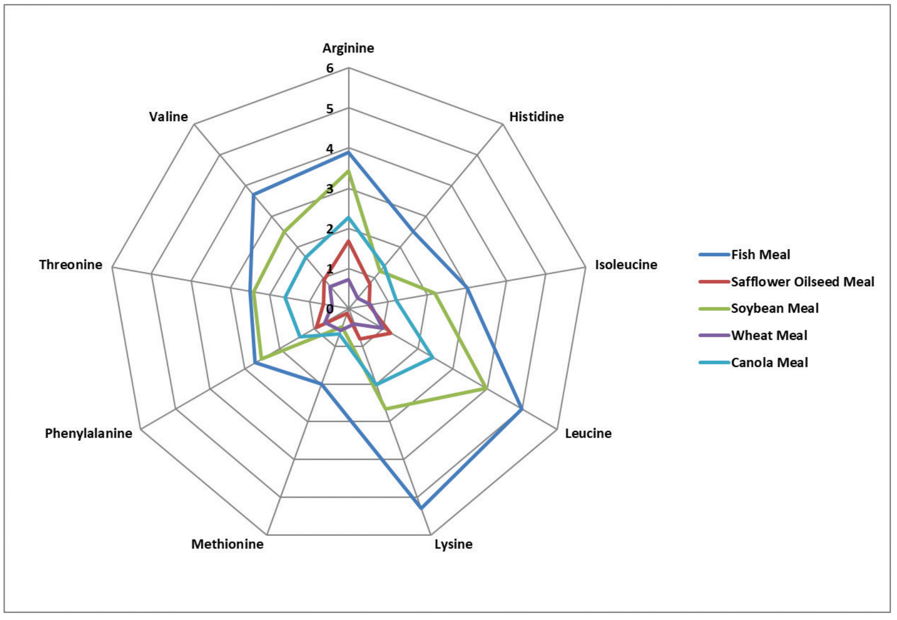

Figure 1- Comparison of essential amino acid content in raw materials

3.3. Fatty acid content of safflower oilseed meal

According to the fatty acid analysis, linoleic acid made up 60.57% while oleic acid made up 26.03%. The total saturated fatty acid values were 9.79; total monounsaturated fatty acid values were 27.58; total n-6 PUFA values were 61.49; total n-3 fatty acid values were 0.55 and total n-3 HUFA values were 0.22 in safflower oilseed plant meal. When compared with other raw materials, it is clear that they are present in significant quantities. A comparison of the fatty acid contents of fish meal, soybean meal, wheat meal, and canola meal with safflower meal is shown in Table 3.

Table 3- Fatty acid content of safflower oilseed meal

Fatty acid	*Marine meals (g/100g)*	*Terrestrial plant meals (g/100g)*			
	Fish meal[1]	*Safflower seed meal*[**2]	*Soybean meal*[**3]	*Wheat meal*[4]	*Canola meal*[**5]
C14:0	3.40	0.13	0.08	-	0.08
C15:0	0.30	0.02	0.01	-	-
C16:0	13.10	6.94	11.13	19.56	4.31
C18:0	18.90	2.69	4.08	1.37	2.21
C20:0	0.20	0.00	0.31	-	0.48
C22:0	0.10	-	0.36	0.26	0.19
C24:0	0.20	0.01	0.13	-	0.10
Total saturates	36.20	9.79	16.10	1.63	7.37
C16:1n-9	0.10	-	0.05	-	-
C16:1n-7 (PA)	3.80	0.04	0.13	-	0.27
C18:1n-9 (OA)	6.00	26.03	23.18	20.28	57.07
C18:1n-7	1.80	0.70	-	-	-
C20:1n-11	0.10	0.24	-	-	-
C20:1n-9	0.60	0.01	0.26	-	0.95
C20:1n-7	0.10	-	-	-	-
C22:1n-11	0.40	0.24	-	-	-
C22:1n-9	0.10	0.29	-	-	-
C24:1n-9	0.60	0.03	-	-	-
Total monounsaturated	13.40	27.58	23.57	20.28	58.29
C18:2n-6 (LA)	0.60	60.57	52.91	57.67	19.34
C18:3n-6	0.10	0.49	0.02	-	9.20
C20:2n-6	0.10	0.02	-	-	0.06
C20:3n-6	0.10	0.37	-	-	-
C20:4n-6 (ARA)	0.60	0.04	-	-	-
C22:4n-6	0.00	-		-	-
C22:5n-6	0.30	-	-	-	-
Total n-6 PUFA	1.80	61.49	52.93	57.67	28.60
C18:3n-3 (LNA)	0.50	0.22	5.92	-	11.18
C18:4n-3	1.40	-	-	-	0.03
C20:3n-3	0.10	0.07	-	-	0.03
C20:4n-3	0.50	-	-	-	0.07
C20:5n-3 (EPA)	9.50	0.07	-	-	0.05
C21:5n-3	0.40	-	-	-	-
C22:5n-3 (DPA)	1.40	0.04	-	-	0.07
C22:6n-3 (DHA)	14.60	0.15	-	-	0.15
Total n-3 PUFA	28.40	0.55	5.92	0.00	11.58
Total n-3 HUFA	24.10	0.22	0.00	0.00	0.20

[1]Data on proximate composition and fatty acid contents of fishmeal is from Glencross (2020); [2]Fatty acid contents of safflower oilseed meal obtained are from this study; [3]Fatty acid contents of soybean meal is from Oliveira et al. (2021); [4]Wheat meal fatty acid content is from Nikolić et al. (2008);
[5]Canola meal fatty acid content is from MacIntosh et al. (2021). [*]Cold press extraction process, [**]Solvent extraction process

3.4. Mineral composition of safflower meal

A comparison of the mineral content of a fish meal, soybean meal, wheat meal, and canola meal with the mineral content of a safflower meal is shown in Table 4. A Safflower oilseed meal is a better source of phosphorus, potassium, calcium, and iron than a wheat meal. As for magnesium, it is relatively low.

Table 4- Safflower oilseed meal, fish meal, soybean meal, wheat meal, and mineral contents

	Marine meals	*Terrestrial plant meals*			
Mineral name	*Fish meal*[1]	*Safflower meal*[*2]	*Soybean meal*[**3]	*Wheat meal*[3]	*Canola meal*[**3]
Phosphorus (P)	2.35%	0.39%	0.55%	0.34%	1.07%
Potassium (K)	1.36%	0.62%	1.9%	0.22%	1.36%
Calcium (Ca)	3.5%	0.26%	0.37%	0.04%	0.62%
Magnesium (Mg)	1.9%	0.25%	0.30%	0.27%	0.50%
Iron (Fe) (ppm)	27	55	36	33	373
Copper (Cu) (ppm)	6	7	41	5	6
Manganese (Mn) (ppm)	3	6	36	30	67.1
Zinc (Zn) (ppm)	47	18	67	20	66.2

[1]Data on proximate composition and mineral contents of fishmeal are from Glencross (2020); [2]Mineral contents of safflower oilseed meal are obtained from this study; [3]Data on proximate composition and mineral contents of soybean meal, wheat meal, and canola meal are from Hertrampf & Wiedad-Pascual (2000); [*]Cold press extraction process; [**]Solvent extraction process

4. Discussion

The average safflower seeds yield ha^{-1} is lower (0.72 tons) when compared with other oilseeds (Alizadeh et al. 2010), such as soybeans (2.34 tons), and rapeseeds (1.51 tons), peanuts (1.37 tons), and sunflowers (1.14 tons). Dubois et al. (2007) determined safflower oilseed meal contains a rich linoleic fatty acid.

The weight of a 1000-seed varies between 15-104 g with a coat percentage that varies between 18-59% of the total seed weight (Smith 1996; Bowles et al. 2010). It has been emphasized in several studies that these characteristics are influenced by genetic variation (Smith 1996; Amini et al. 2008; Ben Moumen et al. 2015). The oil extraction process, two of safflower product types are used (Knowles and Ashri 1995). One is the hull and labeled as a hulled safflower meal; the other is de-hulled (totally or partially) and labelled as a de-hulled safflower meal. Safflower meal in which the coat is not separated is commonly used in the safflower variety (Balcı) we discussed in our study.

Safflower seeds used for oil production may be either cold-pressed, expeller-pressed, or solvent-extracted (GRDC 2010). In this study, it was determined that the cold-press extraction method was used to extract safflower oil.

Safflower oilseeds are cold-pressed in a cold-press machine with fixed parameters of 10 m output mold, 40 rpm screw rotation speed, and a maximum 40 °C output temperature. After each cold pressing, the oil (liquid phase) and meal (solid phase-meal) are collected and weighed, while the oil fraction is immediately filtered through a 40 mm sieve to separate the suspended materials. The oil is separated in a centrifuge that has been chilled to a constant temperature of 10 °C (Aydeniz et al. 2014; Aksoylu Özbek & Günç Ergönül 2020). Safflower producers in Türkiye extract oil from the safflower oilseed plant by using the cold-press method (RIPSA 2021). In animal nutrition, safflower seed meal can be used as a protein source, although nutritional value of safflower oilseed meal may vary depending on the amount of coat and oil extracted (Heuzé et al. 2015).

According to the findings and results of this research, it was determined that the use of safflower meal in animal feeds, either alone or in combination with another raw material, had no negative effects on the growth performance, feed conversion, and physiological characteristics of aquaculture species.

4.1. Proximate analysis

According to crude protein content, safflower meal is similar to wheat meal, which has lower protein ratios than fish meal, soybean meal, and canola meal. When the results of our study's analysis are compared with other in the literature it can be seen that the oil content is higher than fish meal, soybean meal, wheat meal, and canola meal. In the studies in which safflower meal was used instead of fish meal and soybean meal in aquafeeds for protein sources up to 20%, no negative effects occurred in terms of growth performance and feed evaluation (Galicia-González et al. 2010; Ustaoglu Tiril & Kerim 2015; Çantaş & Yıldırım 2020). In addition, it can be used as a source of oil as well as a source of protein in fish feeds. In this case, it can benefit both in terms of sustainability and cost-effectiveness.

4.2. Comparing amino acids

The amino acid content of safflower oilseed meal is relatively low when compared to fish meal and soybean meal. It is remarkable that the content of arginine, histidine and phenylalanine percentages as vegetable protein sources in fish feeds are close to that of canola meal. Upon understanding the importance of the amount of amino acids in feeds, this should be taken into account in the feedstuff to be substituted for fishmeal. This is one of the factors that directly affect the growth and health in fish (Tibaldi & Kaushik 2005). Cysteine and methionine constitute the whole sulfur amino acid of fish. Methionine is an essential sulfur amino acid that plays an important functional role in initiating protein synthesis (Elesho et al. 2021). The nutritional value of the safflower oilseed meal is limited by sulfur amino acids. Rodehutscord (1997) determined that 2.77% lysine, 0.2% tryptophan. 0.13% leucine, and 1% isoleucine are required to achieve 95% maximum protein utilization. It has been determined that if the ratios are lower, the growth performance will be adversely affected. Tibaldi and Kaushik (2005) have determined that the optimum protein ratio for European sea bass and sea bream is Arg, 4.6-5.4; Lys 4.8-5.0; Phe 1.6-1.7; Ile 2.6; Leu 4.3-4.5; Val 2.9-3.0; Met+Cys. 2.3-2.4; Phe+Tyr. 2.6-2.9; His 1.3-1.7 respectively.

The levels of arginine, histidine, leucine, valine, and phenylalanine in rainbow trout diets are similar to those found in safflower oilseed meal according to the expected essential amino acid ratios. However, isoleucine, lysine, methionine, and threonine are not sufficient to meet the needs of fish in safflower oilseed meal. The arginine and histidine requirements of common carp and Nile tilapia are affordable with safflower oilseed meal alone. It seems unlikely that other essential amino acids can be supplemented with safflower oilseed meal alone and used together with other raw materials. Table 5 below shows the amino acid values of safflower oilseed meal and the essential amino acid levels required by rainbow trout, carp, and Nile tilapia.

Table 5- Essential amino acid requirements of some freshwater fish

Essential amino acids	***Safflower oilseed meal[1] (% of diet)***	***Rainbow trout[2] (% of diet)***	***Common carp[2] (% of diet)***	***Nile tilapia[2] (% of diet)***
Arginine	1.68	1.50	1.70	1.20
Histidine	0.81	0.80	0.90	1.00
Isoleucine	0.51	1.10	1.00	1.00
Leucine	1.21	1.50	1.40	1.90
Lysine	0.81	2.40	2.20	1.60
Methionine	0.13	0.70	0.70	0.70
Phenylalanine	0.93	0.90	1.30	1.10
Threonine	0.64	1.10	1.50	1.10
Valine	0.94	1.20	1.40	1.50

[1]Values based on this study; [2]Values based on dry matter (NRC 2011)

In Table 6, assuming that fish meal, which is the basic feed raw material, meets all the essential amino acid needs, a full score of 100 is obtained and it is shown at what rate other raw materials can be used in feeds. Safflower oilseed meal can be used directly in place of wheat meal in terms of all essential amino acids except methionine. In general, safflower oilseed meal can be used at a level of 20% instead of fish meal and soybean meal in terms of essential amino acids.

Table 6- Comparison of the essential amino acid content of raw materials

Essential amino acids	*Fish meal*[*]	*Safflower oilseed meal*[*]	*Soybean meal*[*]	*Wheat meal*[*]	*Canola meal*[*]
Arginine	100	43.07	87.94	18.46	58.46
Histidine	100	32.40	48.80	14.00	55.20
Isoleucine	100	17.00	73.00	18.33	40.33
Leucine	100	24.20	79.40	19.80	48.60
Lysine	100	15.28	50.37	7.73	38.11
Methionine	100	6.50	24.50	29.00	34.00
Phenylalanine	100	34.44	92.96	25.18	51.85
Threonine	100	25.60	96.40	17.20	64.80
Valine	100	25.40	67.56	19.45	44.86

[*]Assuming that fish meal, which is the basic feed raw material, meets all the essential amino acid needs, a full score of 100 is obtained and it is shown at what rate other raw materials can be used in feeds. Also, references to raw materials in Table 1 were used

In the studies of Ustaoglu Tiril & Kerim (2015), Çantaş & Yıldırım (2020) the essential amino acid and nutritional composition data of safflower meal are found to be similar to this study. Galicia-González et al. (2010), the quantity of amino acid in the safflower meal used in a study is relatively high compared to our study.The reasons for this can be listed as the type of safflower meal, the soil, environmental conditions, and maintenance differences. In safflower meal, the first limiting amino acid is methionine followed by isoleucine. These ratio changes are expected to be normal as a result of these differences. Diets relatively low in amino acids may have negatively affected the growth performance of the cultured species.

4.3. Comparing fatty acid

Compared to fish meal, soybean meal, wheat, and canola meal the analysis results show us that safflower oilseed is a good source of linoleic (61%) and oleic acid (26%). It is noteworthy that they are at similar levels compared to these raw materials (wheat meal and soybean meal). Lipids in the diet play an important role in fish nutrition as a source of body energy and essential fatty acids (Yıldız 2008). Essential fatty acids are important for osmoregulation in fish. In the absence of essential fatty acids, it is thought that digestion and absorption will be adversely affected and will have also negative effects on neural tissues over time (Glencross 2009). As a result, essential fatty acids are crucial in juvenile fish and crustacean feeds (Glencross 2009). In general, the requirements for n-3 HUFA in Mediterranean aquaculture species range from 0.64% to 2.2% in broodstocks diets and from 0.8% to 3.5% in juvenile feeds (Izquierdo 2005).

In rainbow trout and common carp, the requirement for 18:3n-3 varies between 0.7-1% and 0.5-1%, respectively. Furthermore, the requirement for n-3 PUFA in rainbow trout ranges between 0.4% and 0.5% (NRC 2011).

Galicia-González et al. (2010) studied three different safflower meals that had C18:1n-9 content as a fatty acid content. These contents were higher than the values in our study. In terms of C16:0, C18:3n-3 and C20:1n-11, it was determined that they had similar results to our study results. In our study, the C18:2n-6 (LA) value, which is one of the total PUFAs, was quite high. At the same time, the HUFA value was found to be 0.22

4.4. Comparison of mineral composition

It was discovered that safflower meal only meets the mineral substance levels required by the cultured species when combined with other raw materials. The essentiality macro minerals were calcium, phosphorus, magnesium, and potassium. Calcium and phosphorus play important roles in the development and maintenance skeletal system. The Ca to P ratio of fish bone may show some changes during the development stage of fish species, however, Calcium: Phosphorus reported in several fish species ranges from 1.6:1 to 2:1 (Lall & Kaushik 2021). In this study, the Ca:P ratio is close to 1.0. Magnesium is a modulator of ion channels, an important intracellular signaling molecule involved in nerve conduction, muscle contraction, and potassium transport, and a modulator of oxidative phosphorylation (Lall & Kaushik 2021). Safflower oilseed meal contains significant potassium, iron, and phosphorus from mineral elements. Safflower meal has a higher potassium (0.62%) and calcium level (0.26%) than wheat meal. Comparing the magnesium content, it is found to be less than fishmeal, similar to soybean meal and wheat meal.

4.5. If we summarize the studies of safflower meal over aqua feeds

In rainbow trout feeds, Ustaoglu Tiril & Kerim (2015) used safflower meal at three different rates (10, 20, and 30). Their results indicate that safflower meal is a promising feed ingredient and can be used up to a concentration of 20% in the rainbow trout diet with no adverse effects on growth performance, nutrient digestibility, or body composition. Çantaş & Yıldırım (2020) used safflower meal instead of soybean meal in two different amounts (10% and 20%) in rainbow trout feeds and supplemented the feeds with phytase enzyme. The group, which can be considered suitable for rainbow trout due to its sustainability and reduced environmental impact due to decrease phosphorus excretion, was the group containing 20% safflower meal and 2000 IU/kg phytase instead of soybean meal. Ali Assaf (2018) used 0%, 25%, 50%, 75%, and 100% safflower meal instead of fish meal in herbivore ratfish (*Siganus rivulatus*) diets. His study's findings noted that as the rate of safflower meal increased, the feed efficiency and protein utilization decreased. It is thought that a 25% or more usage of safflower oilseed meal instead of fish meal is not appropriate in herbivorous marine fish species such as ratfish because it indirectly reduces protein intake (Ali Assaf 2018). In addition, according to Ali Assaf's (2018) study, this type of safflower meal is not appropriate for use as a substitute for fish meal. This is because the palatability of the feed is not accepted by the fish. It was also understood that there was uneaten feed at the bottom of the tank. Galicia-González et al. (2010) investigated the digestibility of three different safflower meals used in white-legged shrimp feeds in their study. It was revealed that high-protein safflower oilseed plant meal feeds performed the best. Turchini et al. (2019) drew attention to the need to determine the positive or negative interaction of this raw material with other feed ingredients during commercial feed production. As Glencross (2020) points out, seven important steps must be taken into account in safflower oilseed meal/oil as well as in other raw materials.

5. Conclusions

The safflower oilseed plant meal is found in the feed of many animals and cultured fish. Researchers have revealed that up to 20% protein source can be used experimentally, especially in rainbow trout diets. Safflower oilseed meal is more advantageous in terms of potassium percentage (0.62%) when compared to wheat meal. While the amount of arginine, histidine, leucine, valine, and phenylalanine needed in the nutrition of rainbow trout can be met with safflower oilseed meal, it seems unlikely that isoleucine, lysine, methionine, and threonine can be satisfied with safflower oilseed meal alone. The arginine and histidine levels required by carp and Nile tilapia can be fulfilled with a safflower meal. The use of aquaculture as a protein source in their feed is important for cost and sustainability. It is also predicted that it can be evaluated as a protein and lipid source as well as a linoleic and oleic acid supplement. Studies are needed testing different proportions of oilseed safflower meal in rations for more species. Knowing which raw materials can be used in what amounts can make a difference in terms of cost and sustainability and focus on future research. Considering the balance of amino acids, fatty acids, and energy, research on using safflower oil seed meal and safflower oil as dietary supplements for fish fills the existing gaps.

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