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A Preliminary Study on the Molecular Weight Profile of Soluble Protein in Niger (*Guizotia abyssinica* (L.f.) Cass)

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Abstract: In present study, ash, protein, and crude fiber contents of niger meal were higher than that of niger seed. However, niger seed had higher lipid levels than that of niger meal ($p<0.05$). The ash, lipid, protein and crude fiber values of niger meal were found as 7.31 %, 7.16 %, 28.80 % and 20.02 %, respectively. The ash, lipid, protein and crude fiber values of niger seed were determined as 4.89 %, 20.61%, 19.63% and 18.96 %, respectively. The saturated and unsaturated FAs were determined as 20.52% and 45.23% in niger meal, 18.78% and 53.1% in seed, 25.35% and 52.05% in oil, respectively. The linoleic acid levels of niger meal, seed and oil were 37.49%, 41.76% and 41.61%, as highest FAs respectively. Amongst the saturated FAs, palmitic and stearic acids are found at higher levels with palmitic ranging from 9.85% (niger seed) to 15.76% (oil), and stearic ranging from 3.5% (oil) to 5.48% (seed). α-linolenic acid (ALA) levels of niger seed and niger oil were found as 0.85% and 1.2%, respectively. The MWPs of niger meal and seed were found as 48.74 % and 42.92 % in 2532 Da ≥ group by followed 34.46 % and 38.72 % in 67000 Da ≤ group, 14.18 % and 15.76 % in 13700-67000 Da group, 2.72 % and 2.62 % in 2532-13700 Da group, respectively. Considering taking into account protein, FA and MWPs, except for the high levels of crude fiber it could be suggested to use at moderate levels of niger meal remaining after the extraction of niger seed and seed oil in aquaculture feeds.

Keywords: Niger seed, niger meal, niger oil, biochemical compositions, molecular weight profiles

Nijerin (*Guizotia abyssinica* (L.f.) Cass) Çözülebilir Proteininin Moleküler Ağırlık Profili Üzerine Bir Ön Çalışma

Özet: Mevcut çalışmada, soğuk ekstrakte nijer ununun kül, protein ve ham selüloz içeriklerinin, Nijer tohumundan daha yüksek olduğu, buna karşılık, nijer tohumunun, ununda daha yüksek lipit seviyelerine sahip olduğu tespit edilmiştir ($p<0.05$). Nijer ununun % olarak kül, lipit, protein ve ham selüloz değerleri sırasıyla 7.31, 7.16, 28.80 ve 20.02, tohumunun ise 4.89, 20.61, 19.63 ve 18.96 olarak belirlenmiştir. Doymuş ve doymamış yağ asitleri sırasıyla nijer unu % 20.52 ve % 45.23, tohumunda % 18.78 ve % 53.1, yağında % 25.35 ve % 52.05 olarak belirlenmiştir. Nijer unu, tohumu ve yağının linoleik asit seviyeleri sırasıyla % 37.49, % 41.76 ve % 41.61 olarak en yüksek yağ asidi içeriği olduğu bulunmuştur. Doymuş yağ asitleri arasında, % 9.85 (tohum) ile % 15.76 (yağı) arasında değişen palmitik ve % 3.5 (yağı) ile % 5.48 (tohumu) arasında değişen stearik ile daha yüksek seviyelerde bulunmuştur. Nijer unu ve tohumunun moleküler ağırlık dağılımları 2532 Da ≥ grubunda % 48.74 ve % 42,92 olarak bulunurken, bunu sırasıyla 67000 Da≤ grubunda % 34.46 ve % 38.72, 13700-67000 Da grubunda % 14.18 ve % 15.76, 2532-13700 Da grubunda % 2.72 ve % 2.62 değerleri izlemiştir. Sonuç olarak, yüksek ham selüloz değerleri dışında protein, yağ asidi ve moleküler ağırlık profilleri dikkate alındığında su ürünleri yemlerinde soğuk eksrakte nijer ununun ve tohum yağının orta seviyelerde kullanılması önerilebilir.

Anahtar Kelimeler: Nijer tohumu, nijer unu, nijer yağı, biyokimyasal bileşimler, moleküler ağırlık profilleri

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1. Introduction

Nowadays, together with the increase in aquaculture production, the demand for feeds is rising. Fish feed includes the principal operating cost in aquaculture sector and the main protein source of fish feed has been fishmeal. However, fishmeal is expensive due to high demand and limited global supply. Therefore, replacing fishmeal with protein rich animal or plant sources is a necessary. Lovell (1998) revealed that feed ingredients containing 20% level or more crude protein may use as protein sources. In this concept, oil seed meals have been found to have considerable sustainable and economic potential (Ng and Romano, 2013; El-Sayed, 2004; Hardy, 2010; Tacon, 1997). In addition, substitution of fish oil in fish feeds has become inevitable due to the limited availability of fish oil (Dalbir et al., 2015; Naylor et al., 2009; Turchini, 2009). Vegetable oils commonly used fish feeds consisted of an important part of the research on fish nutrition (Menoy et al., 2007; Martins et al., 2009). Vegetable oils are rich in FAs with 18 carbons such as linoleic or linolenic acids, essential for fish species (Sener and Yıldız, 2003; Olurin et al., 2004; Fontagne et al., 1999).

Digestive processes, protein digestion and particularly the protein forms provided by feeds have been the biggest limitations to the use of feeds by fish larvae. Some authors revealed that the protein forms used in feeds has an important role on the development of the larval digestive system (Cahu and Zambonino Infante, 1995a,b; Zambonino Infante et al., 1997). Therefore, the identification of proteins forms as feed ingredient may contribute towards the formulation of more appropriate feeds for different larval stages (Ronnestad et al., 1999; Holt, 2000).

Niger (*Guizota abyssinica* (L.f.) Cass.) is an oilseed crop cultivated in Ethiopia and India. Ethiopian and Indian oilseed production were 50% and 3%, respectively. The process of niger seeds are inexpensive, and the cake remaining after oil extraction is used as a protein source in diets. In general, niger meal remaining after the extraction of oil includes 30% protein and 23% crude fibre (Getinet and Sharma, 1996). The oil content of niger was reported as 29-39% (Dutta et al., 1994), 30-35% (Kandel and Porter, 2002), 42-44% (Dagne and Jonson 1997). Studies on the FA composition of niger, linoleic acid is the dominat fatty acid followed by palmitic, oleic and stearic acids (Dutta et al., 1994; Ramadan and Morsel, 2003; Dagne and Jonson 1997). The oil of niger seeds appears to be nutritionally valuable, as the high content of linoleic acid known to prevent cardiovascular diseases (Vles and Gottenbos, 1989), and also, antioxidant activity mainly due to sterols and tocopherols (Dutta et al., 1994).

The biochemical characterization and functional properties of Niger (*Guizotia abyssinica* Cass) have been described by some researchers (Syume and Chandravanshi, 2015; Akewake et al., 2015; Tesfaye et al 2017). However, the information about MWP of soluble protein in Niger is not available. The study aimed to reveal the MWP of Niger protein considering as a sustainable protein source. Also, biochemical compositions of niger seed, niger meal and niger oil were determined.

2. Materials and Methods

Niger seed was purchased from regional spice shop. The oil from the niger seeds was obtained by the method of cold-press extraction in machine (Ozdemir Reducer FN063-B09 Serial No:220916011668). The cake remaining after the mechanical oil extraction process was used as niger meal. In the current study, the biochemical compositions and molecular weight profiles of niger seed, niger meal and niger oil were determined.

2.1. Biochemical compositions

The different forms such as niger meal and niger seed as alternative feed ingredient were tested. Biochemical compositions such as the protein, lipid, crude fiber (Anonymous (2018) and ash of two niger forms were done according to the methods described by the AOAC (2000) and lipid analyses were determined according to the method described by Bligh and Dyer (1959).

2.2. Fatty acid compositions

FAs of the niger meal, niger seed and niger oil were analyzed according to the method described by Garces and Mancha (1993). FAs were determined by comparisons with standard mixture.

2.3. Molecular weight profiles

MWPs of niger meal and niger seed were performed according to Boza et al. (1994). Standards were ranged from bovine albumine (67000 Da) to p-aminobenzoic acid (137 Da).

2.4. Statistical methods

The analyses were performed in triplicates. All data were analyzed to one-way (ANOVA) and mean±standard error (SE) differences by using SPSS 15.0 statistical package (SPSS, 2006).

3. Results

The biochemical compositions of the niger meal and niger seed were given in Table 1. The ash, protein, and crude fiber contents of niger meal were higher than that of niger seed. However, niger seed had higher lipid levels than that of niger meal.

The relative levels of individual FAs found are given in Table 2. The saturated and unsaturated FAs were determined as 20.52% and 45.23% in niger meal, 18.78% and 53.1% in niger seed, 25.35% and 52.05% in niger oil, respectively. The highest FA of niger meal, niger seed and niger oil were linoleic acid. The linoleic acid levels of niger meal, niger seed and niger oil were 37.49%, 41.76% and 41.61%, respectively. Amongst the saturated FAs, palmitic and stearic acids are found at higher levels with palmitic ranging from 9.85% (niger seed) to 15.76% (niger oil), and stearic ranging from 3.5% (niger oil) to 5.48% (niger seed). α-linolenic acid (ALA) levels of niger seed and niger oil were found as 0.85% and 1.2%, respectively.

Figure 1 reveals the MWPs of soluble proteins in niger meal and niger seed. A remarkable similarity in the MWPs of soluble protein was observed among these two types of niger such as niger seed and niger meal but the differences were statistically significant except for 2532-13700 Da group ($p<0.05$). The MWPs of niger meal and niger seed were found as 48.74±0.5% and 42.92±0.1% in 2532 Da ≥ group by followed 34.46±0.4% and 38.72±0.08% in 67000 Da≤ group, 14.18±0.01 and 15.76±0.05 in 13700-67000 Da group, 2.72±0.03% and 2.62±0.05% in 2532-13700 Da group, respectively. The lowest and highest values were determined in 2532-13700 Da and 2532 Da ≥ group, respectively.

4. Discussion

In present study, the MWPs of soluble protein in niger seed and niger meal (*Guizotia abyssinica* Cass) were determined by Gel Filtration Chromatography. Also, we determined the ash and FA, protein, crude fiber, lipid contents of Niger seed, niger meal and niger oil. Until now, the knowledge about the MWP of Niger (*Guizotia abyssinica* Cass) is not available.

The present results showed that ash, protein, and crude fiber contents of niger meal were higher than that of niger seed. The earlier studies indicated that oil extracted niger meal contained approximately 30% protein and 23% crude fibre (Getinet and Sharma, 1996). The ash, protein, and crude fiber levels of niger meal remaining after the extraction of oil are within the range of reported levels. However, lipid levels of niger seed had higher than that of niger meal remaining after the extraction of oil in niger seed. In earlier studies, the niger meal oil content is variously reported as 29-39% (Dutta et al., 1994), 30-35% (Kandel and Porter, 2002), 42-44% (Dagne and Jonson, 1997). Thus, results of the present study were lower range of data reported earlier.

The data indicated that the highest FA of niger meal, niger seed and niger oil were linoleic acid. The dominant levels of linoleic acid are generally in agreement with those found in current study whereas oleic acid and α-linolenic (ALA) acid levels are generally lower. In the study, ALA levels of niger seed and niger oil were reported as 0.85% and 1.2%, respectively. Bhatnagar et al. (2014) showed that niger oil extracted by mechanical methods had a concentration of 0.08% α- linolenic acid. The present findings were higher than that of the reported by Bhatnagar et al. (2014).

Table 1. The proximate compositions of niger meal and niger seed (mean±S.E.)

	Ash	Lipid	Protein	Crude fiber
Niger meal	7.31±0.08[a]	7.16±0.19[b]	28.80±0.24[a]	20.02±0.22[a]
Niger seed	4.89±0.58[b]	20.61±1.97[a]	19.63±0.08[b]	18.96 ±0.4[b]

Table 2. Fatty acid levels of niger meal, niger seed and niger oil

	Niger Meal (cold extracte)	Niger Seed	Niger Oil
14:0	0.08	0.08	0.13
15:0	0.04	0.04	0.05
16:0	10.07	9.85	15.76
16:1	0.08	0.12	nd
18:0	5.44	5.48	3.5
18:1n-9	7.66	9.82	7
18:2n-6	37.49	41.76	41.61
18:3n-3	nd	0.85	1.2
20:0	1.55	1.85	1.44
20:1n-9	nd	nd	0.58
20:2n-6	nd	nd	0.48
20:3n-3	nd	0.55	1.18
22:0	2.02	nd	2.89
24:0	1.32	1.48	1.58
Saturated	20.52	18.78	25.35
Unsaturated	45.23	53.1	52.05

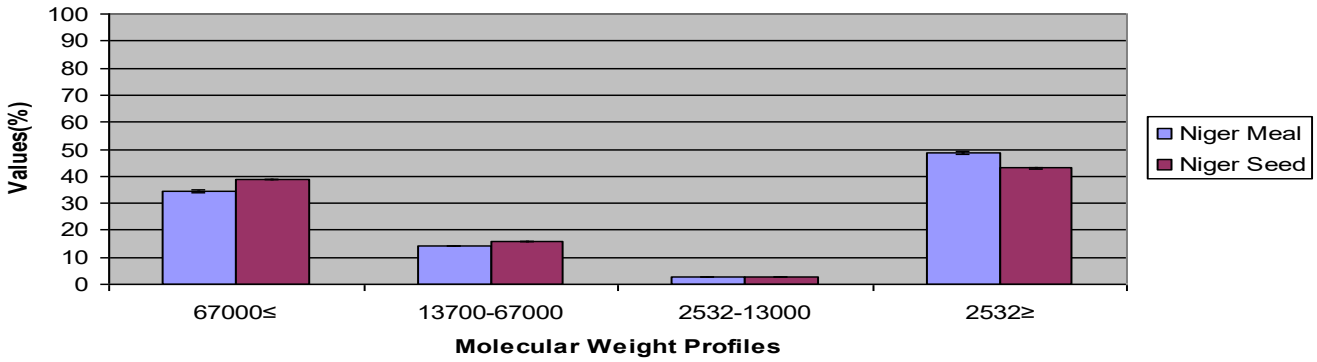

Figure 1. Molecular weight profiles of soluble protein in the niger meal and niger seed

Linoleic acid known as a member of the w-6 acids is less susceptible to oxidation than the essential w-3 acids. Therefore, high linoleic acid contents of niger forms are advantageous due to it has a longer shelf life. Kiralan et al. (2010) indicated that ALA found as the dominate FA in linseed oil is more susceptible to oxidation. Therefore, there are need the combinations between oilseed crops cultivated to provide an optimal balance and to produce an oil with a higher shelf life that is more suitable as a food supplement.

A remarkable similarity in the MWPs of soluble protein in niger meal and niger seed was observed. The lowest and highest values were observed in 2532-13700 Da and 2532 Da ≥ group, respectively. Peptide profiles of niger meal and niger seed have a balance in 67000 Da ≤ and 2532 Da ≥ groups. Free amino acids (FAAs) appear to regulate the larval growth when supplied at moderate levels to feeds, but not excessive of FAAs (Szlaminska et al., 1993; Carvalho et al., 1997; Cahu et al., 1999; Cahu and Zambonino Infante 1995a; Lopez-Alvarado and Kanazawa, 1995). The faster absorption of FAAs compared to protein bound amino acids (AAs) may lead to AA irregulars in larval intestine and followed a decrease on protein utilization (Ronnestad et al., 2000). Zambonino Infante et al. (1997) indicated that the partial substitution of whole protein by di-and tripeptides improved larval growth. Carvalho et al. (2003) revealed that protein macromolecules are digested into peptides and AAs in the larval intestine and followed, di-and tripeptides are rapidly converted into AAs for absorption. Also, Carvalho et al. (2003) indicated that a balance among the peptide groups was important to optimize the protein utilization. Carvalho et al. (2003) advised that the supplementation of moderate levels of protein hydrolysates to feeds must be considered in order to reach the mentioned purpose. In addition, it is recommended that feeds used for fish larvae should have a nitrogen solubility and MWP similar to that found in live food.

In conclusion, the niger meal is a good source of protein and if suitably processed has a good potential for aquaculture feeds. In addition, the high linoleic acid content makes the oil of Niger (*Guizotia abyssinica* Cass) seed nutritionally valuable. Also, a balance exists in peptide profiles containing 67000 Da ≤ and 2532 Da ≥ groups of niger meal and niger seed. Considering taking into account protein, fatty acid and molecular weight profiles, except for the high levels of crude fiber it could be suggested to use at moderate levels of niger meal remaining after the extraction of niger seed and niger seed oil in aquaculture feeds.

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