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Chemical composition of Berseem (*Trifolium alexandrinum*) leaf meal and leaf protein concentrate

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Abstract
Trifolium alexandrinum is commonly called as Berseem. It has been used to prepare berseem leaf meal (BLM) and leaf protein concentrate (BLPC). Proximate analysis revealed that the crude protein level in BLM and BLPC were 23.98% and 41.67% respectively. Level of anti-nutritional factor especially saponin content in BLM and BLPC were found to be 0.91% and 0.71% respectively. The alkaloids were higher in BLPC (5.55%) and BLM (3.46%). It has been recorded that saponin content reduced to 0.40% in BLM on 24 hours of water soaking. Evidence from the present study suggests that BLM and BLPC can be used as a good protein source in aquafeed.

Keywords: Antinutritional factor, berseem leaf meal, berseem leaf protein concentrate

1. Introduction
The aquaculture has shifted from traditional non-fed to feed based practices. It has led to increasing demand for feed ingredients. Feed contribute about 70% of the total operational costs in aquaculture. Cost of formulation is determined mostly by primary protein source such as fish meal (Average inclusion level of 24% in the diets). The raised protein based ingredient price is increasing day by day and feed industry is facing problem due to increased cost, shortages and conflict of demand with human consumption. Therefore, industry is searching for new ingredients and for exploration of the use of unconventional ingredients in fish feeds. However, recent price trends indicated that the price of rice bran, oil cakes, and soybean meal are increasing day by day. Hence, the need to find out an alternative source of rice bran, oil cakes, and soybean meal for making the aquaculture sector economically viable. Plants leaf meal is emerging as the cheapest sources of protein and energy. It is considered as a potential alternative in future and will reduce the cost of fish feed. Many studies have been conducted on leaf meals such as Cassava leaf meal [18], Alfalfa leaf meal [24], Alfalfa leaf protein concentrate [7], Alfalfa meal [4], *Leucaena leucocephala* leaf meal [6], Peanut leaf meal [10], Sweet potato leaf meal [2] etc. in the diet of various herbivores and carnivores fishes. Berseem (*Trifolium alexandrinum*) leaf meal is a new potential ingredient in fish feeds, and it contains a high amount of crude protein. Berseem is one of the oldest cultivated forage, domesticated in Egypt and later introduced into many other parts of the world [13]. It is now widespread in irrigation regions of the west and south Asia. Among the berseem growing countries, India has the highest cultivated area of around 2 million hectors, followed by Egypt (1.1 million hector) and Pakistan (0.71 million hectors) [17]. Berseem is a high-quality forage characterized by a high concentration of nutrients, protein (15-25%), minerals (11-19%) and carotene [8, 22] and the dry matter digestibility in ruminants is 70% [17]. Author [23] found that the nutritive value of Berseem leaves (% DM basis) is crude protein- 23.36%, ether extract-3.16%, crude fibre-17.04% and total ash- 19.19%. The inclusion of this leaf in the diets of animals such cattle [14], pigs [21] and poultry [3] since a long time. Recently in Tilapia, berseem has been proved that @ 5-10% inclusion in fish feed diet had shown good growth with high protein efficiency ratio (PER) as compared to control [12]. Author [20] substituted a fish meal with *Amaranthus hybridus* leaf protein concentrate (ALPC) in the diet of *O. niloticus* and found that ALPC replaced up to 80% of fishmeal without adverse effect on fish growth. Considering the above facts, the present study was aimed to analyse the chemical composition of Berseem

(*Trifolium alexandrinum*) leaf meal and leaf protein concentrate for future study in aquafeed.

2. Material and Methods

2.1 Berseem leaf meal preparation

Leaves of berseem were harvested in fresh condition from the agriculture field of Sukki dihtol village of Madhubani district, Bihar, India. The collected leaves were washed and kept for sun-dried. Dried leaves were milled to pass a 100 mesh sieve and then stored at room temperature in plastic containers until needed.

2.2 Berseem leaf protein concentrate

Leaf protein concentrate was prepared by following the method of [9]. Leaves of berseem were harvested in fresh condition and weighed, washed and chopped. Then, used for pulping in a grinder. This process was followed by pressing. The separated leaf juice was heated at 85-90 ^{0}C for 10 min to coagulate the leaf protein. The protein coagulum was separated by filtering through cloth filter followed by pressing. The BLPC was then washed with distilled water and repressed. Collected BLPC was spread for sun-drying. The dried BLPC was then grinded using a grinder machine and kept in an airtight container.

2.3 Proximate composition of BLM and BLPC

Proximate analysis of BLM and BLPC were done by the standard method [5].

2.3.1 Moisture

The moisture content of the sample was determined by taking a known weight of the sample in the Petri dish and allowing to dry it in a hot air oven at 100-105 ^{0}C till achieved a constant weight. The moisture content of the sample was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight of sample} - \text{Dried weight of sample}}{\text{Initial weight of sample}} \times 100$$

2.3.2 Crude protein (CP)

The nitrogen content of dried samples was estimated quantitatively by Kjeltec semi-automated method (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden) using titration as the means for determining the nitrogen percentage. The crude protein percentage was calculated by multiplying the nitrogen percentage by a factor of 6.25.

Crude protein (%) = N (%) × 6.25

2.3.3 Ether extract (EE)

Ether extract of dried samples were estimated by Soxhlet apparatus using petroleum ether (Boiling point 40-60^{0}C) as the solvent.

The calculation was made as follows.

$$\text{Ether extract (\%)} = \frac{\text{Initial weight of sample} - \text{Weight of extracted sample}}{\text{Initial weight of the sample}} \times 100$$

2.3.4 Total ash (TA)

Total ash content of the samples was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 600 ^{0}C for 5-6 hours.

The calculation was done using the following formula:

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.3.5 Crude fibre (CF)

Crude fibre content of the sample (fat-free samples) was determined by acid (1.25% HCL) and alkali digestion (1.25% NaOH) using Fibro TRON (Tulin equipment, India) followed by drying (100 ºC±2) and incineration (in a muffle furnace at 550 ^{0}C for 4hrs) of the samples.

The calculation was made as follows.

$$\text{CF (\%)} = \frac{\text{Weight of dried sample} - \text{Weight of ash}}{\text{Initial weight of the sample}} \times 100$$

2.4 Antinutritional factors

2.4.1 Saponin

For the estimation of total saponin contents in BLM and BLPC use a method recommended by [14].

2.4.2 Alkaloid

For the estimation of total alkaloid content in BLM and BLPC use method recommended by [12].

3. Statistical analysis

All data were analyzed in triplicate by using SPSS-22, and the results were expressed as mean value ± standard error.

4. Results and Discussion

4.1 Proximate composition

The result of the proximate composition of berseem leaf meal and berseem leaf protein concentrate is given in table 1. The berseem leaf meal has 23.98% crude protein and protein leaf protein concentrate has higher level of crude protein 41.67. Other nutrients such as the ether extract, total ash, crude fibre and nitrogen-free extract all had lower level in protein concentrate of berseem leaf compared to berseem leaf meal. The berseem leaf meal had 2.34% ether extract, 15.33% total ash, 13.94% crude fibre, 44.41 nitrogen-free extracts, and 396.83 (Kcal/100 g), gross energy. The berseem leaf meal concentrate had 5.98% ether extract, 8.75% total ash, 1.20% crude fibre, 42.40 nitrogen-free extracts and 470.70 (Kcal/100 g).

Proximate composition of the BLM and BLPC (Table 1) is supported by Singhal *et al.* (2008) found that the nutritive value of Berseem leaves (% DM basis) is crude protein-23.36%, ether extract-3.16%, crude fibre-17.04% and total ash-19.19%. Berseem is a high-quality forage characterized by a high concentration of nutrients, protein (15-25%), minerals (11-19%) and carotene [8, 22] and the dry matter digestibility in ruminants is 70% [17].

Table 1: Proximate composition of the BLM and BLPC (% DM basis)

Ingredients	M (%)	CP (%)	EE (%)	TA (%)	CF (%)	NFE (%)	GE (Kcal/100g)
BLM	7.35±0.06	23.98±0.22	2.34±0.05	15.33±0.07	13.94±0.34	44.41±0.22	396.83±0.80
BLPC	5.35±0.08	41.67±0.13	5.98±0.25	8.75±0.11	1.20±0.10	42.40±0.29	470.70±2.00

Abbreviations: M-Moisture, CP-Crude protein, EE-Ether extract, TA-total ash, CF-Crude fibre, NFE-Nitrogen free energy, GE-Gross energy, BLM-Berseem leaf meal, BLPC-Berseem leaf protein concentrate.

4.2 Anti-nutritional factor

The anti-nutritional factors such as the saponin and alkaloids were tested in both the ingredients such as berseem leaf meal and berseem leaf protein concentrate and it is presented in table 2. The berseem leaf meal had 0.91 (g/100g) and 3.46% alkaloids. The berseem leaf protein concentrate (BLPC) had 0.71 (g/100g) and 5.5% alkaloids. The protein concentrate making of leaf protein has resulted in a reduction in saponin level and increase in alkaloids level. The soaking of the leaf meal resulted more reduction in saponin content to 0.40% from 0.91. Anti-nutritional factor especially saponin and alkaloids contents of the BLM and BLPC (Table 2) is justified by [16] found that total saponin content (%DM basis) is 1.01%. But after water soaking of BLM for 24 hours saponin content was 0.40%.

Table 2: Saponin content of the BLM and BLPC (on% DM basis)

Ingredients	BLM	BLPC	SBLM
Saponin(g/100g)	0.91±0.02	0.71±0.04	0.40±0.06
Alkaloid (%)	3.46±0.07	5.55±0.09	-

Abbreviations: BLM-Berseem leaf meal, BLPC-Berseem leaf protein concentrate and BSLM- Soaked berseem leaf meal.

The anti-nutritional factors present in berseem leaf were of interest, and some anti-nutritional factors such as saponin and alkaloids were investigated. The major nutrient mostly considered for cost and quality is protein. As per the analysis, crude protein content in berseem leaf meal (BLM) and berseem leaf protein concentrate (BLPC) were found to be at good level of 23.98% and 41.67% respectively. Similarly, both the ingredients had good level of lipid. The ether extract was found to be significantly higher in BLPC. These levels indicate the partial use of the ingredients in the formulation.

Plant leaf has potential to become one of the cheapest sources of proteins and energy. Moreover, leaf protein concentrate contains a higher amount of protein. However, incorporation of plant edible materials in aquafeed has limitations due to presence of anti-nutritional factors such as alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haemagglutinin, saponin, mimosine, cyanoglycosides, etc. and imbalance in some essential amino acids, fatty acids and micronutrients [1]. The present study revealed that the berseem leaf meal and leaf protein concentrate have good potential as a protein source for the aqua-feed industry.

5. Conclusion

The present study serves as a basis to encourage the local fish farmers to exploit the nutritive potentials of the berseem fodder as fish feed ingredients. It may replace some conventional feed ingredients and contributes to overcoming the price of fish feed. The BLM and BLPC are rich in nutrients as evidenced by proximate composition. Though BLM contains certain anti-nutritional factors, mainly saponin (Others are under the acceptable limit) and after water soaking of BLM for 24 hours saponin content was reduced to 0.40%. Form the overall study, it can be suggested that BLM and BLPC are the potential raw material for aqua-feed industry.

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