GREEN ALGAE AS A PROTEIN SOURCE IN ANIMAL FEED

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Green alga predominantly *Chlorella spp.*, which grows wildly in waste alkaline water (pH 8.5 to 9.8) were harvested from different sites in Peshawar and Nowshera (NWFP) in both spring and winter seasons. Composite samples from each locations were dried and analysed for their protein, amino acids and mineral composition. It was found that dried algae contained 45 to 51% crude protein (Kjeldhal N x 6.25), which was rich in lysine and threonine, but deficient in methionine and cystine (sulphur) – containing amino acids). The ash content varied from 6.8 to 7.7% with a mean calcium, phosphorus and iron contents of 2.0, 5.2 and 1.0 g/kg. It was concluded that green alga is an adequate protein and mineral supplement in animal feed, and in poultry rations.

Key words: Algae, Protein, Animal feed.

INTRODUCTION

Protein food and feed is a matter of concern in most legumes are mainly used for livestock feed. The protein content of these foddars is low and therefore need protein supplement to increase feed efficiency. Oilseed meals are generally used for this purpose, which are usually expensive and affect the cost of animal production. Hence, the cheap protein-rich feed sources must be exploited to lower the cost of milk and meat production in the country. Efforts have been made in recent years to use the single cell protein (SCP) produced by unicellular green algae in feeding farm animals [1]. This may be due to their rapid growth and reproduction rate and to the fact that they can be produced on commercial scale from raw materials which are economical and easily available. It has been estimated that the yield per unit area of algae ranged from 7 to 43 g day matter/m²/day, corresponding to 4 to 25 g protein/m²/day [2]. (swald [3] reported that algae Chlorella can produce protein more than ten-fold the rate of soybean on per unit area basis. These estimates high light the biological efficiency of algae in protein production.

In Pakistan algae are found abundantly. They grow wild mostly in polluted and waste waters. The algae biomass from waste effluents in oxidation ponds can serve as a good protein supplement in animal feed [4]. In order to assess the nutritional quality of algal protein, this work was taken up to determine the amino acid composition of indigenous species of algae (predominantly *Chlorella* spp.) found in winter and spring seasons in NWFP.

MATERIALS AND METHODS

Green algae predominately Chlorella spp., which

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grow wildly in waste waters were harvested from different locations in Peshawar (Site I) and Nowshera (NWFP) (Site II) during winter and early spring seasons. The water from where samples were collected was alkaline (pH 8.5 to 9.8) and had a high salt content (15 to 21 g/1) mainly owing to sodium carbonate and bicarbonate (HCO₃, 7.0 to 9.5 g/1; CO₃⁻², 3.5 to 4.8 g/1). Composite samples from each place was first washed thoroughly with water and then screened to remove undesired materials. Each sample was then concentrated by centrifuge and dried in air by spreading the material in thin layers. Further drying was performed in an oven at 60 to 70°. The oven-dried material was crushed and mixed, and sub-samples were used for chemical analysis. Samples from each location within a site was considered as a replicate. At least three replicates from each site were analysed and the average result reported herein.

Proximate composition. The percent moisture, crude protein (N x 6.25), crude fat (ether extract), crude fibrer, and ash contents were determined by the standard methods of A.O.A.C. [5]. The nitrogen-free-extract which represent the digestible carbohydrates was calculated by difference.

Amino acid analysis. Protein hydrolysate for amino acid analysis was prepared by treating 150 mg of the dried material with 5 ml 6 N HC1 in evacuated pyrex tube at 105° for 24 hours. The hydrolysate was then filtered and dried on a rotary evaporator. It was repeatedly dissolved in water and dried to remove excess HC1. Finally the hydrolysate was diluted to known concentration with buffer solution (pH 2.2). The amino acid composition of the hydrolysate was determined by automatic amino acid analyser (LKB 4101) [6].

For tryptophan determination, the hydrolysate was prepared by the similar procedure but barium hydroxide was used instead of HC1, since acid hydrolysis destroys the tryptophan.

Mineral composition. Wet ashing of the dried material was performed by treating 0.5 g sample first with small volume of conc. HNO_3 : and then with HNO_3 : (1:1) mixture. The acid digest so prepared was diluted and used for mineral analysis. Calcium, sodium and potassium were determined by Flame photometer [7]. Phosphorus and iron were determined by Spectrophotometric method [7].

RESULTS AND DISCUSSION

The proximate composition of indigenous species of algae (mostly, *Chlorella*) collected from sewage is shown in Table 1. Variations in the composition of the material

Table 1. Proximate composition of dried algae.

Components	Site I (Peshawar)	Site II (Nowshera)	Mean* (± SE)	
Moisture	8.7	6.5	7.6 ± 0.15	
Crude Protein	45.2	50.8	48.0 ± 0.52	
Crude Fat	5.4	6.2	5.8 ± 0.21	
Crude Fiber		5.6	6.2 ± 0.33	
Ash	- 7.2	6.8	7.0 ± 0.51	
Nitrogen-Free-				
Extract**	26.7	24.1	25.4 ± 0.38	

** Nitrogen-free-extract representing digestible carbohydrates, was calculated by difference.

from two different sites are obvious. Differences among samples from various locations within each site were also observed. The protein content varied from 45.2 to 50.8%, the average being 48.0%. This indicates that algae contained approximately the same crude protein level as commonly used supplements, such as soybean or cotton seed meal. The crude fat content of algae was comparatively lower than crude fiber and ash contents. These results suggest that algae is a good protein and mineral supplement, which can alleviate the deficiencies of these nutrients in animal feed. Nutritional experiments with rats [8], chicks [9] and calves [2, 4] support this idea. The use of uniccllular algae (*Chlorella*) as food for space travel is also documented [10].

As found in this study, Hintz *et al* [40] also reported significant variations in the composition of different samples of algae (*Chlorella* spp.) grown on sewage and collected over a period of four years. Such differences may be due to the composition of the waste water on which the biomass grow. In agreement to the results of the present work, they also observed high protein and ash contents in the dry matter of algae.

The high protein content of algae is an attractive factor. However, the protein quality is also important from nutritional point of view. The amino acid composition determines the quality of the protein. A good quality protein contains all the essential amino acids in proper proportion. The amino acid composition of algae.

(Table 2) reveals that it contained considerable quantity of lysine, threonine and tryptophan. These amino acids are

Table 2. Amino acid composition of algae protein.

Amino Acid (g/100g protein		Site II (Nowshera)	Mean* (SE)	Reference** protein	Brioler*** require ment
Lysine	4.8	5.4	5.1 ± 0.21	4.2	5.3
Leucine	7.2	6.0	6.6 ± 0.22	4.8	7.3
Iso-leucine	4.2	3.6	3.9 ± 0.35	4.2	3.9
Threonine	5.0	4.2	4.6 ± 0.42	2.8	3.5
Histidine	1.2	1.8	1.5 ± 0.11	-	2.0
Methionine	1.6	1.2	1.4 ± 0.02	2.2	4.0
Cystine	0.4	0.6	0.5 ± 0.05	1000	-
∀aline	6.2	5.8	6.0 ± 0.26	4.2	4.3
Tryptophan	1.2	1.6	1.4 ± 0.03	1.4	1.0
Arginine	4.8	5.6	5.2 ± 0.32	-	5.7
Glycine	5.0	4.2	4.6 ± 0.30		5.0

S.E. (Standard error of 6 to 8 replicates, atleast 3 from each site.
Amino Acid Pattern recommended by FAO/WHO (1973) for adult man.

*** Amino Acid Pattern of protein required for brioler

present above the level required for adult man and broiler. The lysine content often reaches an order of magnitude similar to that in fish meal, skim milk powder and soya extraction grits. The lysine and threonine content of wheat and other cereals is quite low [11]. Hence algae can supplement cereal feeds and diets.

The amount of sulphur containing amino acids, such as methionine and cystine was low in algal protein. They are probably the limiting amino acids. In this respect algae are similar to food legumes which also contain insufficient methionine and cystine [12]. These results support the earlier work [2] which suggest that methionine supplementation of the order of magnitude of 0.2% of the dry matter caused considerable improvement in the biological value (BV) and net protein utilization (NPU) of algal protein. The deficiency of sulphur containing amino acid in algal protein can be balanced when algae is fed in combination with other food sources. cook *et al* [8] using a combination of algae, oats, wheat flour and dried skim milk, were able to get just as good protein efficiency ratio (PER) values as when using a protein composed exclusively of dried skim milk.

Algae proteins are digestible and have fairly good biological value. Kraut *et.al* [13] reported that dried algae protein was about 80% digestable by rats. Kofrany and Jekat [14] using the same material, found in man a biological value of 82% as related to whole egg protein equal to 100. Lubitz [15] observed 86% absorption of the protein from freeze dried algae (*Chlorella*). It should be noted that steaming decreased the net protein utilization (NPU) of al-

Mineral (g/kg)	ала	Site I (Peshawar)	Site II (Nowshera)	Mean* (± S.E.)
Calcium		2.3	1.7	2.0 ± 0.15
Phosphorus	5	4.4	6.0	5.2 ± 0.22
Potash		2.8	3.4	3.1 ± 0.05
Iron		0.8	1.2	1.0 ± 0.06
Magnesium	1	1.8	2.4	2.1 ± 0.08

Table 3. Mineral composition of dried algae.

* Standard error of 6 to 10 replicates, atleast 3 from each site.

Although the use of algae as human food may require to consider certain chemical and toxicological aspects, it appears that algae have considerable potential as a livestock feed, because of the high content of good quality protein plus significant amount of mineral matter.

REFERENCES

- 1. I.A. Khalil and H. Shah, Sarhad J. Agric., 4, 579 (1988).
- 2. E. Schulz and H.J. Oslage, Ani. Res. and Devel., 6, 1 (1977).
- 3. W.J. Oswald, Am. J. Pub. Health, 52, 235 (1962).
- H.F. Hintz, H. Heitman, W.C. Weir, D.T. Torell and J.H. Meyer, J. Ani. Sci., 25, 675 (1966).

enabled me to succeed can be stated what has been mentioned before [3]. On p. 172 I wrote "When a tissue inflected with a germ is teased out and exposed to air it acquires bactericidal propertites speciefic to that germ. I have come to imagine that the bacteria produce fatty acids and they give rise to specific peroxides which are highly bactericidal. The one trick I resort to in culturing symbiotic germs was to leave a bit of intestine as a centre where reduction is assured and peroxide formation avoided. The lac yearsts are embeded in fatty tissue so that the insect fat liself may be realized that the symbiotic germ does not grow a random within the host and is kept within control. What then is the mechanism by which the germ is kept in check by the host."

Realizing that a tissue can produce a metabolite which is antibiotic, along with a colleague. Dr. Bakshi 1 prepared liver extracts. What was water soluble enabled bacteria to grow well but the fraction which was also alcoholsoluble was antibiotic (Mahdihassan and Bakshi, [4]. Further it was found that Stein [5] published an illuminating article on antibacterial substances produced by tissues. And we have to assure that peroxides, acting upon tissue metabolites,

- A.O.A.C., Official Methods of Analysis, (A.O.A.C. Arlington, VA 22209, USA, 1980), 13th ed., pp. 211.
- 6. D.H. Spackman, W.H. Stein and S. Moore, Anal. Chem., **30**, 1190 (1058).
- 7. G.D. Christian, Analytical Chemistry (John Wiley, New York, 1986), 4th ed., pp. 126.
- 8. B.B. Cook, E.W. Lau and B.M. Bailey, J. Nutr., **81**, 22 (1963).
 - 9. S. Uesaka, World Rev. Ani. Prod., 1, 11 (1965).
 - 10. P.A. Lachance and J.E. Vanderveen, Fd. Technol., 23, (1963).
 - I.A. Khalil, T. Hussain and H. Shah, Pak J. Biochem., 19, 13 (1986).
 - 12. I.A. Khalil, S. Akbar and S. Khatoon, J. Sci. Tech., 66, 114 (1982).
 - 13. H. Kraut, F. Jekat and W. Pabst, Nutr. Dieta., 8, 130 (1966).
 - 14. E. Kofrany and F. Jekat, Z. Physiol. Chem., 348, 84 (1967).
 - 15. J.A. Lubitsz, J. Fd. Sci., 28, 229 (1963).
 - G. Clement, C. Giddey and R. Menzi, J. Sci. Fd. Agri., 18, 497 (1967).
 - 17. J. Okumura, K. Fusuya and I. Tasaki, Jap. Poultruy Sci., **10**, 157 (1973).
 - 18. Y. Marimura and T. Nabuko, Fd. Tech., 8, 179 (1954).

that termini degradation products of definite openits have been mistaken for real germs, while these were overfooked as components of normal tissues. Nevertheless in some insects, like species of Lecanium, which is a scale insect or coccid, the symbiote is an yeas-like microorganism. Even an auther of a handbook on that subject, could not isolate the symbiote of a Lecanium. I had the honour of knowing him personally and I can report what had been his actual atting symbiotic years. I tried to grow them but at first with negative results. Believing that the symbiotic gern would prefer an extract of lac insects, since it grew within their prefer as pecial media was prepared. Even with such an additive to the culture media attempts to grow the symbiotic gern would such the symbiotic gern with a the prefer as extract of lac insects, since it grew within their prefer an extract of lac insects, since it grew within the bodies, a special media was prepared. Even with such an additive to the culture media attempts to grow the symbi-

Consulting literature I came across the work of Meyer [2]. He found bactered in the nephreadia of some land molbases. Trying to isolate them he mostly got negative results. He writes (on p. 72) that "it was expected that the smearing of erushed concretions, teeming with bacteria, on suitable medium would produce a confluent growth. Instead however starile plates or a few scattered colonies have been obtained."