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Preparation and Evaluation of Weanling Food Consisting of Sprouted Wheat and Stillage Sludge Protein Isolate

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Abstract

Availability of animal protein due to low production and high cost is limited. Therefore, vegetable protein being cheeper source, may be used in the preparation of weaning food for infants. The present study was carried out to prepare a weaning food consisting of sprouted wheat and protein isolate from an unconventional source, like stillage sludge. The chemical evaluation of weaning food was conducted. Amino acid profile of the protein isolate showed the chemical score was 36.42. However thyronine was the limiting amino acid. Results of biological trial conducted on weanling Albino rats showed the digestibility, 96.40, 97.83. Net protein utilization (NPU) 76.34, 78.8, biological value 79.20, 80.54 and protein efficiency ratio 4.0, 4.6 of skim milk diet respectively.

Introduction

The importance of protein for normal growth and development, and for maintaining good health has been sufficiently emphasized. Unfortunately it is lacking in human diet through out the world especially in developing countries like Pakistan. According to a survey conducted in Punjab, the consumption of protein from animal origin by human being was 16.3 gm against the minimum recommended requirement of 24 gm/capita/day (Anonymous, 1987). Scientists of new area are trying their best to solve this problem through non conventional food sources like stillage sludge which is a waste product of sugar industry. It is available in tune of 16000 tons/Annum in the country. Use of this waste product would not only reduce the pollutant, but also serve as a source of energy for the production of low cost high quality microbial biomass protein which can be fed to human beings. But this protein source is limited in lysine which can be improved by culturing *Brevi bacterium flavum* on it (Yawar, 1998).

Commercial weaning foods which are available in the local market are costly. It is imperative to prepare low cost high quality weaning food by supplementing the protein isolate with sprouted wheat.

A study has therefore been planned to enrich the stillage sludge with lysine through fermentation first, then prepare its protein isolate and consequently be used in the preparation of weaning food, with following objective:

- To prepare lysine enriched biomass from stillage sludge
- To isolate the protein from the stillage sludge biomass
- Biological evaluation of weaning food consisting of protein isolate and sprouted wheat

Materials and Methods

Stillage sludge was procured from distillery of Shakarganj Sugar Mills Ltd., Jhang. It is produced in the fermentation tank during the continuous process of ethanol production. Collected material was kept in the refrigerator. Proximate analysis of stillage sludge was carried out using AOAC (1990) methods.

Biomass production: Lysine enriched biomass was produced by fermenting with Brevi bacterium flavum at pH 7 and 35°C temperature following the method described by Yawar (1998). The biomass was dried in an air oven at 100°C for one hour and then at 60°C to constant weight. The dried biomass was ground and subjected to chemical analysis following AOAC (1990) methods. The lysine content of sludge and biomass were determined by Chaves *et al*. (1988). RNA content was also determined by method of Munro and Fleck (1966).

Protein Isolation: The biomass thus produced was subjected to protein isolation by modifying the method of Basit (1996). The isolated protein dried at 60°C under vacuum to constant weight. The protein isolate thus obtained was ground and subjected to proximate analysis following AOAC methods.

The protein isolate was analyse for its amino acid profile (Moore and Stein, 1954). RNA contents was estimated by following the method Munro and Fleck (1966). Nitrogen (%) was estimated by Micro kjeldahl method (Hiller *et al*., 1948). The nitrogen (%) was multiplied with 6.25 to obtain crude protein percentage. The estimation of true protein was carried out by Munro and Fleck (1966).

Preparation of weaning food: Three diets A, B and C were prepared the composition of the diets are shown in Table 1. The diet A was served as control diet which contained skimmed milk. The diet B was served as experimental diet which contained protein isolate and sprouted wheat. The diet A and B were isonitrogenous and isocloric. The diet C was a protein free diet.

Biological Evaluation: The protein isolate was supplemented with sprouted wheat. Its quality was

Table 1: Composition of experimental diet

Ingredients	Diets		
	A	B	C
Biomass protein isolate	-	11.11	-
Skimmed milk	20.00	-	-
Sprouted wheat	-	35.5	-
Corn Starch	55.1	28.4	75.0
Soybean oil	5.0	5.0	5.0
Glucose	10.0	10.0	10.0
Potato starch	5.0	5.0	5.0
Vitamin & Minerals mix	5.0	5.0	5.0
	100.0	100.0	100.0

A = Control; B = Biomass protein isolate with sprouted wheat; C = Non protein

evaluated on weanling Albino rats following method described by Pellet and Young (1980).

Feeding Trial: Twenty four weanling albino rats were selected at 21 days of age. The rats were fed on stock diet for 7 days before the start of experiment and then divided into 6 groups, 4 rats each, with a weight difference of ±5g. Three diets were prepared as shown in Table 1. The two groups of rats were put on each diet. The diets were fed for ten days.

The split feed and faecal were dried and analyzed for nitrogen determination. At the end of experiment, the rats were killed. Each group of carcass was weighed after oven drying at 105°C to a constant weight. The dried carcass was analyzed through Kjeldahl's method (Hiller *et al*., 1948). The quality of protein of the diets were expressed in terms of digestibility of percentage, net protein utilization (NPU), biological value (BV) and protein efficiency ratio (PER) following the method of Pellet and Young (1980).

Protein efficiency ratio (PER)

True Digestibility (TD):

where

$$PER = \frac{\text{Gain in body weight}}{\text{Protein intake of test diet}}$$

$$TD\ \% = \frac{\text{I-(Fn Fk)}}{\text{I}} \times 100$$

I = Intake of nitrogen by rats fed on test diet
Fn = Faecal nitrogen of rats fed on test diet
Fk = Faecal nitrogen of rats fed on nitrogen free diet

Net protein utilization (NPU)

$$NPU = \frac{\text{B-(Bk-Ik)}}{\text{I}} \times 100$$

where
B = Total body nitrogen of rats fed on test diet
Bk = Total body nitrogen of rats fed on nitrogen free diet
Ik = Nitrogen intake of rats fed on nitrogen free diet
I = Nitrogen intake by rats fed on test diet

Biological Value (BV)

$$B.V = \frac{\text{Net protein utilization}}{\text{True digestibility}} \times 100$$

Results and Discussion

The study was under taken to prepare a weaning food consisting a protein isolate from fermented stillage sludge and sprouted wheat.

Chemical Composition of Stillage sludge: The chemical analysis of the sludge is given in Table 2, which reveals that it contain 20.78 percent crude protein, high concentration of ash 28 percent, and minor percentage of crude fat 0.45 percent. However quite considerable level of nitrogen free extract 50.42 percent. Percentage of crude fibre is zero. RNA content was 1.88 percent.

Chemical Composition of Biomass: The chemical composition of biomass is given in Table 2, which shows that it contain 29 percent crude protein which increase from 20.78 to 29 percent after fermentation. It contains high concentration of ash 30 percent. It has 0.01 percent of crude fat and crude fibre 0.5 percent level of nitrogen free extract decreased RNA while RNA contents increase from 1.8 to 1.9522.

Lysine estimation: Lysine contents of the biomass and stillage sludge are shown in Table 2.

The inherent lysine concentration of stillage sludge was estimated to be 300 mg/mL. When it was fermented with *B. flavum*. Its lysine content was increased from 150 mg/100 ml to 700 mg/100 mL.

Protein Isolation: The protein isolate was prepared by isoelectric point by adjusting the pH. The percent protein isolated was then determined using Kjeldahl method. Based on that observation and inferences the procedure for protein precipitation was developed by solubilizing protein in a solution and then adjusting its pH to isoelectric pH (with 1 N H_2SO_4. By this method, the protein isolate obtained

containing 40 percent protein. Results of the present study are in line with the work of Hashmi (1987) who prepared protein isolate from biomass of rice polishing. He obtained protein isolate at isoelectric point technique and its contained 37.6 percent protein.

Table 2: Chemical analysis of stillage sludge, biomass and protein isolate on dry weight basis

Components	Percentage sludge	% age biomass	% protein isolate
Crude Protein	20.78	29.00	40.00
Ether extract	0.40	0.50	0.45
Crude fibre	0.00	0.01	0.00
Ash	28.00	30.10	29.00
Nitrogen free extract	50.42	40.50	30.60
RNA Contents	1.88	1.95	2.03
True protein	-	25.60	32.80
Lysine	150.0 mgs	300.00 mgs	700.0 mgs

Table 3: Average digestibility, net protein utilization, biological values and protein efficiency ratio of diets with skim milk and with sprouted wheat based protein isolate

	Diet	
	Skim Milk (A)	Sprouted wheat based protein Isolate (B)
Digestibility %	96.40	97.83
Net protein utilization	76.34	78.80
Biological value	79.20	80.54
Protein efficiency ratio	4.00	4.60

Amino acid analysis: Amino acid profile of protein shows that almost all amino acids were present in protein isolate. The chemical score of protein isolate was 36.42. However threonine was the limiting amino acid.

Biological Evaluation

Feeding trial: The quantity of protein isolate obtained form the fermented sludge mixed with sprouted wheat and evaluated by feeding to weanling albino rats, at 10 percent protein level along with standard skim milked milk diet and protein free diet.

Weight Gain: The average weight gained per group of rats fed on skim milk and sprouted wheat based protein isolate lets were 28 g and 36g respectively after ten days of leeding trial. Where, as protein free diet group lost 41 g ring the trial.

Feed Consumption: Rats fed on skim milk diet consumed feed (329.5 g) and rats fed on sprouted wheat with protein isolate consumed maximum (332.0 g) while the least feed was consumed by rats fed on protein free diet (269.8 g).

Protein Efficiency Ratio (PER): The average protein efficiency ratio for diet A and B were 4.0 and 4.6 respectively. The results of present study are quite better than Surfraz (1990) who enriched the wet feed by fermenting it with *Arachniotus* sp. and fed the biomass to broiler chicks replacing 50 percent of vegetable protein of basal chick diet. He obtained PER value 1.82 of ration containing biomass protein.

Digestibility: The data on average digestibility of protein of standard and experimental are shown in Table 3. Average protein digestibility of skim milk and protein isolate diets were 96.40 and 97.83 respectively. The present findings are still better than Cardoso and Nicoli (1981) who reported that mycelia' protein from Phanerochaete chrysosporium grown on vinass contained about 23 percent protein rich lysine, when tested on weanling male rats gave digestibility 64.28 percent.

Net protein utilization: The data on net protein utilization for controlled and experimental diets are shown in Table 3. The average NPU value for diets A and B were 76.34 percent and 78.80 percent respectively. The result of the present study are quite higher than Hashmi (1987) who evaluated protein quality of fungal protein biomass obtained from rice polishing fermented with Trichoderma harzianum. The NPU value of biomass protein, protein isolate, residue supplemented with skim milk were 37.5, 37.0, 45.0 and 67.5 percent respectively.

Biological value: The data obtained on biological value for controlled and experimental diets shown in Table 3. The average biological value for diet A and B were 79.20 and 80.54 percent respectively. The results were supported by Roshkova (1979) who tested the protein quality of biomass and tested the protein quality of biomass produced with candida strains 2672 M and 2673 M on acetate and methanol respectively using weanling rats. The biological values of the products were found to be 68.4 and 69.12 percent respectively.

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