

Biological Protein Quality of Indigenous and Exotic *Chenopodium Album* (*Bathua*) Cultivars

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Abstract: *Chenopodium album* (*Bathua*) belonging to family Chenopodiaceae is an underutilized crop having high nutritive value especially protein (12-13%) with appreciable amount of lysine which is generally deficient in grains. Beside this it also have medicinal value however the studies on this crop are very limited especially on biological quality so the present study was planned to assess the biological quality of two *Chenopodium album* cultivars i.e. IC 107299 (Ic) and EC 507733 (Ec). The diets were isoproteinous containing 10% protein whereas *C album* cultivars were fed at 10 and 20% levels to 5 groups of male albino Wistar rats (10 each group) for 28 days and biologically evaluated by rat growth and nitrogen balance studies. The results of present investigation revealed that feeding of exotic cultivar resulted in marked increase in food intake, protein intake and body weight gain as compared to indigenous cultivar. The PER of indigenous cultivar fed group was 3.49 and 3.43 whereas exotic cultivar fed group was 3.46 and 3.27 at 10 and 20% level. The nitrogen balance studies also revealed better intake and retention in exotic cultivar fed group. The APD, TPD and biological value are also higher in exotic cultivar fed groups as compared in indigenous cultivar fed group. It was found that although both the diets showed good growth response but still exotic cultivar showed superior growth as compared to indigenous cultivar.

Keywords: *Chenopodium Album*, Biological Quality, True Protein Digestibility, PER, *Bathua*, Pseudo Cereal, Under-Utilized Crop

1. INTRODUCTION

Malnutrition is a major nutrition problem in India and other developing countries which is responsible for about 40-50 per cent of the infant death all over world [Singh et.al 2007]. The major reason for this is increasing gap between food supply and population growth. It is expected that world population reaches 9.3 billion by 2050, so food production increase is essential [Pritchard and Amthor 2005]. In present scenario it is important for diversification towards traditional crops because the current dependence on a few major crops may result in food scarcity. The new crops may provide reasonable productivity and reliability as well as nutritional and medicinal benefits. Cereals provide a good source of dietary fibre and other important compounds with nutritional potentials such as phenolic compounds, antioxidants, minerals and vitamins [Repo et.al. 2007]. Many seeds, which were once used in traditional human and animal diets, have now fallen into disuse due to the

increased availability of commercial food products. However, since some of these seeds are native to and grow well in regions where food shortages and famine are endemic, their agricultural and nutritional potential needs to be re-assessed. From the nutritional point of view it is well known that the bioavailability of animal food protein is significantly superior to vegetable protein sources especially in essential amino-acids [Gamel et.al. 2004]. However some vegetable protein sources such as soyabean, high quality maize, *Chenopodium* seeds and amaranth seeds contain proteins which approach the quality of animal proteins. *Chenopodium* comprises of about 250 species which are herbaceous, suffrutescent and arborescent perennials and belongs to the family Amaranthaceae [Rana et.al.2010]. Several plants of this species are of minor to moderate importance as food crops, both leafy vegetables and pseudo-cereals. These includes Quinoa (*Chenopodium quinoa*), Kaniwa (*C. pallidicaule*), Fat hen (*C album*), good king henry (*C bonus-henricus*) and Epazote (*C ambrosoides*) etc. Like other crops *C album* has attracted renewed interest because of its unique characteristics mainly due to the high nutritional value of protein and its ability to grow under extreme conditions. They are rich source of lysine, natural supplier of essential fatty acid and antioxidants [Wood et.al.1993] and high protein efficiency ratio [Ranhotra et.al.1993] that distinguishes its nutritional quality from that of other cereal grains. Apart from its nutritive value, *C album* also has pharmaceutical properties. Its oil is effective against many intestinal parasites, abdominal pains, eye diseases and is also antihelmintic, laxative and diuretic. The seeds are known to be hypocholesteromic and hypoglycemic. Besides of such important nutritional value of this crop still the studies on this crop especially on indigenous and exotic cultivars are is limited so the present study was planned to evaluate the protein quality of indigenous and exotic *C album* cultivars.

2. MATERIALS AND METHOD

Plant Material

The two cultivars of *C album* indigenous IC 415477 (Ic) and exotic EC 507733 (Ec) were procured from National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Shimla (H.P.) and were reproduced in the farms of KVK Bajaura, Kullu. The seeds were harvested after maturity and then brought to the laboratory of CSKHPKV,

Palampur. The procured seed samples were then cleaned manually to remove adhering dirt, dust and foreign particles. The seeds were grounded in to a fine powder with the help of stainless steel mixer and were stored in airtight plastic containers and stored in a freezer. They were conditioned at room temperature before use.

Preparations of Diets

The diets were isoproteinous containing 10 per cent protein. The indigenous and exotic cultivars of *C album* were incorporated in diets at 10 and 20% level and rest protein content was adjusted at 10% with casein after taking in to account the protein content of Ic and Ec cultivar (13.45 and 12.54% respectively). The composition of diet is given in Table 1. The ingredients were mixed, homogenized and passed through 70 mesh sieves to ensure uniform distribution of vitamin and mineral mixture.

Feeding Experiment

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with standard guidelines and with due approval from the CSKHPKV, Palampur (H.P.) animal ethics committee. Fifty male albino weanling rats weighing 28 ± 5 gm were obtained from the Germ Free Animal House of the College of Veterinary Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar India. The rats were equally divided in to 5 groups with 10 rats in each group and housed in an air-conditioned animal room at a temperature of $22 \pm 2^\circ\text{C}$ with a relative humidity of $50 \pm 5\%$ in individual polypropylene cages. The animal room was on a 12hr daily lighting period cycle. The daily food was restricted at 10g of dry matter whereas normal tap water was provided *ad libitum* and the left meal is weighed next day. Feeding lasted for 40 days however feeding trial for PER lasted for 28 days. Ten days before the expiry of feeding the rats were transferred to metabolic cages and rats were allowed to adapt for 4 days. Feed residues, urine and feces were collected during a 5 day balance period. Urine and feces were collected in 5% sulphuric acid and assayed for nitrogen by the micro Kjeldhal method.

The biological quality of the *C album* was expressed in parameters such as Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), Weight gain, Apparent Protein Digestibility (APD), True Protein Digestibility (TPD), Biological Value (BV), Net Protein Utilization (NPU), Net Protein Ratio (NPR) and Protein Retention Efficiency (PRE) according to the method of Chick *et al.* [8].

Statistical Analysis

The statistical analysis of the data obtained was carried out to establish the difference among various cultivar feeds. One Way Analysis of Variance (ANOVA) were used to evaluate the significant difference of the data at $p \leq 0.05$ using statistical package WINDOWSTAT 8.0 available in the Department of Physical Sciences and Languages, COBS, CSKHPKV, Palampur H.P. The obtained data were interpreted following Sendecor and Cochran [9] and comparisons between groups were made by means of an unpaired student's t-test.

3.RESULTS AND DISCUSSION

Biological evaluation has been recognized as a test criterion for assessing the dietary proteins quality. The data pertaining to the biological quality is given in Table 2.

Feed and Protein consumed

A glance of data in Table 2 shows the feed and protein consumption pattern of rats fed on Diet 1, Ic and Ec cultivar. It is clear from the table that significantly highest feed and protein consumption was there in rats fed at 20% Ec cultivar and lowest in Diet 1 fed group. The maximum feed and protein consumption was in *C album* exotic cultivars fed group which might be due to high acceptability of diets to the rats as compared to casein and indigenous cultivar. Gamel *et al.* [4] also found high consumption of amaranthus (another pseudocereal) when compared to casein fed group thus showing its high acceptance among the animals.

Weight Gain

The weight gain for 10 and 20 percent Ic cultivar fed group was 68.77 and 63.86 g whereas for 10 and 20 percent levels of Ec cultivar fed group was 69.35 and 70.78 g respectively. (Table 2) Weight gain increase in casein fed group this might be due to the reason that better growth is always with good quality protein [10]. The increase in weight when rats fed on two levels of *C album* cultivars might be due to higher feed and protein consumption which might have resulted in the weight gain. However although the feed and protein consumption was higher in two *C album* cultivars but still weight gain was less when compared to Diet 1 fed group which might be due to the presence of anti-nutritional factors which might affect the absorption of many nutrients by binding them whereas Diet 1 contain proportionate amount of all the nutrients which help in supporting growth. White *et al.* [11] while studying the depletion-repletion in rats fed on quinoa found that the weight gain by the rats fed on quinoa was more when compared with casein and milk. The results of present investigation are slightly different from that of White *et al.* which might be due to the difference in varieties which inturn affect the genetic background of the variety and thus influence the nutritional composition. However the results showed that the amount and quality of the food consumed by the experimental animal greatly influenced their growth response.

Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER)

FER represents the weight gain of an animal per weight of feed consumed under certain specific conditions *viz.* a dietary protein level of 10%. This method has proved beneficial in evaluating the quality of feed. The effect of feeding *C album* cultivars on feed efficiency ratio (FER) and protein efficiency ratio (PER) is given in Table 2. Maximum FER and PER was in Diet 1 fed group followed by 10 percent Ic cultivar fed group. The results showed that PER value of *C album* cultivars are closer to that of casein showing good quality proteins in these seeds.

Mahoney *et al.* [12] reported the PER values of 2.1 and 2.7 for whole quinoa flour and cooked quinoa proteins respectively. Whereas, Gross *et al.* [13] reported PER value of 3.0 for quinoa protein. The variation in the data of PER and FER as compared to other workers might be due to difference in quality of protein, fibre content, low diet intake, higher fecal losses and anti-nutrients in diet [14].

Nitrogen Balance

Similarly from data in Table 2 it is evident that the nitrogen intake and excretion was highest in Diet 1 fed group and minimum nitrogen intake, excretion and retention was in 20 percent Ic cultivar fed group. The maximum nitrogen intake, excretion and retention were in Diet 1 fed group this might be due to the reason that these diets are from casein which is a pure synthetic diet containing all essential nutrients. However the incorporation of *C album* in the diets resulted in the decreased nitrogen balance especially in case of indigenous cultivar when compared to control group this might be due to the presence of certain anti-nutrients like tannins, phytates, saponins etc. which affect the utilization of proteins by binding them. Ranhotra *et al.* [7] also reported that the nitrogen balance of casein control group (67.8%) was slightly higher than quinoa fed group (65.0%) giving credence to the present study.

Apparent Protein Digestibility (APD)

Apparent protein digestibility is based on fecal protein losses. Data in Table 2 shows the results on APD of feeding two levels of Ic and Ec cultivar fed groups in comparison with control group. As is clear from Table maximum APD content was present in Diet 1 fed group and minimum in 20 percent *C album* Ic cultivar fed group. The APD was maximum in casein control group and low in other groups which might be due to the reason that grain derived foods are usually less digested than animal derived foods moreover the presence of various anti-nutrients like phytate, tannins etc. in grains, also interfere with the absorption of many nutrients like proteins and thus lowering APD content. Gross *et al.* [13] reported 84% APD for quinoa protein however Ranhotra *et al.* [7] found that the APD of quinoa fed rats was slightly lower than that of casein fed rats. The result are at par with other workers however the slight variation in the results might be due to the varietal differences which lead to genetic variability along with variation in agro-climatic conditions.

Biological Value (BV)

Biological value is the percentage of digestible protein from a test food retained by experimental animal. It is generally used as an index of protein quality and a protein having 70% BV is generally considered capable of supporting growth [15]. As is clear from the Table maximum BV was in Diet 1 i.e. 85.98 percent and minimum in 20 percent Ic cultivar fed group i.e. 72.71 percent. Ruales and Nair [16] reported the BV for raw and washed quinoa as 82.58 and 80.79 per cent. Feeding of *C album* cultivars resulted in marked decrease in BV this might be due to the presence of various anti-nutrients like saponin, tannins, phytate etc. which inhibit the nutrient utilization and thus leading to poor growth in rats.

True Protein Digestibility (TPD)

The maximum TPD was in Diet 1 fed group (96.35 percent) and minimum in 20 percent Ic cultivar fed group i.e. 88.48 per cent. A significant ($p \leq 0.05$) difference was there in TPD when control group was compared with two *C album* cultivars fed groups. The lower TPD of *C album* cultivars fed groups as compared to control group might be due to the presence of various anti-nutrients like tannins, phytates etc. which inhibit absorption and metabolism of different nutrients. According to Onwuka [17] tannins act as an anti-nutrient which provoke an astringent reaction in the mouth and make food unpalatable. They can form complex with the proteins and thus precipitating proteins in the gut thus leading to decrease in the feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility in experimental animals [18].

Net Protein Utilization (NPU)

Significantly higher NPU content was found in Diet 1 fed group and minimum in 20 percent Ic cultivar fed group. The lower NPU in *C album* cultivars fed group in comparison to casein control group might be due to more nitrogen loss through urine and feces. Ruales and Nair [16] reported the NPU content of raw and washed quinoa fed groups as 75.74 and 73.95 percent. The results of present investigation are in accordance to Ruales and Nair however the slight variation might have been due to the varietal differences along with the content of anti-nutrients present which might affect its utilizations.

Net Protein Ratio (NPR)

NPR is the measure of total nitrogen retained in the weanling rat and is a direct measurement of utilization of dietary protein. It is clear from Table 2 that the NPR content ranged between 2.15 to 3.45 with minimum value for group fed on 10 percent Ic cultivar and maximum for Diet 1 fed group. A significant ($p \leq 0.05$) difference was observed when control group was compared with *C album* cultivars fed groups. The NPR of control diets was highest this might be due to the reason that these diets do not contain any plant material therefore no anti-nutritional factors were present in them. Whereas in *C album* cultivars the NPR content was higher in exotic cultivar as compared to indigenous cultivar this might be due to the presence of anti-nutrients that might hinders the protein quality of indigenous cultivar and intum affects the NPR content. Moreover the NPR content is also affected by the level of limiting and essential amino acids present in bound form with other chemical compounds [19].

Protein Retention Efficiency (PRE)

A glance of data in Table 2 shows that maximum PRE was in Diet 1 fed group (55.16%) and minimum in 20 percent *C album* Ic cultivar fed group (34.72%). The lower PRE of indigenous and exotic cultivars fed group in comparison to casein control might be due to the presence of anti-nutrients like phenolic compounds, phytic acid, oxalates etc. According to Gurumoorthi *et al.* [20] the phenolic compounds present in foods decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals. They also lower the activity of digestive enzymes such as α amylase, trypsin, chymotrypsin and lipase and may cause damage to the mucosa of digestive tract.

4. CONCLUSIONS

The protein quality of two *C album* cultivars i.e. indigenous and exotic were biologically evaluated by rat growth and nitrogen balance studies. The results showed that both the *C album* cultivars showed good protein quality however the exotic cultivar showed better growth

response as compared to indigenous cultivar. The various biological parameters showed that BV, APD, TPD, nitrogen balance all showed better response in case of exotic cultivar as compared to indigenous cultivar. So, it can be suggested that *C album* can be used as supplementary food in mixtures to increase the biological value of some other foods.

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Table 1 Composition of Experimental Diets (g/100 g)

Ingredients	Control diet	<i>Chenopodium album</i> diets			
		Ic Cultivar		Ec cultivar	
		10%	20%	10%	20%
Casein	9.81	8.46	7.11	8.56	7.31
Ground nut oil	10.00	9.66	9.38	9.65	9.30
Sucrose	10.00	10.00	10.00	10.00	10.00
Cellulose	5.00	4.77	4.54	4.78	4.56
Mineral mixture*	4.00	4.00	4.00	4.00	4.00
Vitamin mixture*	1.00	1.00	1.00	1.00	1.00
Cholesterol	-	1.00	1.00	1.00	1.00
Choline chloride	0.02	0.02	0.02	0.02	0.02
Starch	60.17	51.09	42.95	50.99	42.81
<i>Chenopodium album</i> cultivars	-	10.00	20.00	10.00	20.00

Note: All diets contained 10% protein including the crude protein from *Chenopodium album* source

All diets contained 10% fat including the crude fat from *Chenopodium album* source

All diets contained 5% fibre including the crude fibre from *Chenopodium album* source

*Based on the National Academy of Science recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Table 2 Effect of feeding Indigenous (Ic) and Exotic (Ec) *Chenopodium album* (*Bathua*) cultivars at two different levels on the biological protein quality

Attribute	Diet 1*	Ic cultivar		Ec cultivar		CD(p≤0.05)
		10%	20%	10%	20%	
Feed consumed (g)	182.03	199.14	187.65	203.51	218.61	23.95
Protein consumed (g)	18.20	19.91	18.76	20.37	21.86	2.39
Weight gain (g)	74.49	68.77	63.86	69.35	70.78	2.87
FER	0.417	0.349	0.343	0.346	0.327	0.040
PER	4.17	3.49	3.43	3.46	3.27	0.40
Nitrogen intake (mg)	672.96	499.20	481.73	536.16	540.36	9.11
Nitrogen excreted (mg)	322.03	194.98	197.03	222.55	222.59	7.54
Nitrogen retained (mg)	350.93	304.22	284.70	313.61	317.77	10.19
APD (%)	84.78	81.55	80.41	82.28	83.72	1.20
BV (%)	85.98	75.62	72.71	78.28	81.22	1.17
TPD (%)	96.35	90.58	88.48	90.27	91.18	1.30
NPU (%)	82.84	68.49	64.35	70.67	74.06	1.66
NPR (%)	3.45	2.15	2.17	2.23	2.51	0.11
PRE (%)	55.16	34.82	34.72	35.78	40.22	1.70

*Diet 1- Control without cholesterol