Effect of defatted wheat germ supplemented cookies on the protein quality parameters of rats.

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Abstract: The biological assay of DFWG supplemented cookies was carried out to assess the changes in physical parameters like feed intake and body weight along with protein quality parameters i.e. true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER) and biological value (BV) in the rats. The effect of diets was observed to be significant on feed intake and body weight of rats. The diets containing DFWG supplemented cookies along with casein were consumed higher than that comprised of 0% DFWG. The highest feed intake (31.43 g/day) was found in the rat group fed on casein diet followed by the rat group fed on diet prepared from supplementation of 15% DFWG (31.17 g/day). Similarly, the rats group fed which were fed on both of these above said diets showed relatively higher overall body weights at the end of study period. The body weight of rats increased progressively with the increase in the duration of experiment, while the maximum increase was observed during first 6 days. The rat group fed with protein free diet showed decline in the overall body weight rather than increase throughout the experimental period. The true digestibility of the diets was found to be the highest in the rat group fed on diets containing casein (93.45%) and cookies containing 15% DFWG flour (92.61%). Moreover, non-significant differences existed between both of these diets for true digestibility (TD). The lowest true digestibility was recorded in the rats group fed on cookies of control treatment. Similar trend was observed among different diet groups for net protein utilization (NPU), biological value (BV) and protein efficiency ratio (PER) of rats. The rats fed on casein and 15% DFWG supplemented cookies possessed significantly higher values for NPU, PER and BV while the diet containing 0% DFWG showed the lowest values for these nutritional parameters. The protein quality parameters improved by increasing the level of DFWG supplementation in the cookies.
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Introduction

The wheat grain consists of three distinct parts, the bran, the germ and the endosperm. The distribution of these parts in wheat kernel is as: 82 to 83% endosperm, 12 to 14% bran and 2 to 4% germ (Posner, 2000). The wheat germ is the embryo of wheat, which has reproductive function and separated during milling from endosperm because it adversely influences the keeping quality as well as the processing quality of the wheat flour. In the recent years, with the development of new technologies, the most commercial milling of wheat into flour aims at the maximum extraction of the endosperm without minimum germ contamination. Resultantly, there is a substantial deposit of the wheat germ in the world. Keeping in view the total wheat production of Pakistan, 0.5 million tones wheat germ production in Pakistan was estimated during the year 2007-08 (GOP, 2008).

The wheat germ presently is mainly used in animal feed formulations. So, the wheat germ, a rich source of nutrients is not amply, rationally, and efficiently being utilized (Ge *et al*. 1999). Most of the nutrients in the wheat grain with the exception of starch are concentrated in the germ. It is an excellent source of vitamins, minerals, dietary fiber, calories, proteins, and some functional micronutrients (Shurpalekar and Rao, 1977). The wheat germ not only possesses proteins of high biological value but also its oil is of desirable fatty acid profile and richest natural source of tocopherols (Barns and Tayler, 2006). Due to its composition, the wheat germ is praised as "the natural nutrient treasure-house and life source of mankind" because of its high nutritive value and palatability. The wheat germ oil comprises of high content of polyunsaturated fatty acids among which about 80%, mostly linoleic (18:2) and linolenic (18:3) fatty acids (Wang and Johnson, 2001). These fatty acids are essential and also are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes (Coultate, 1995).

On the other hand, after extraction of oil from wheat germ, the defatted wheat germ is also highly nutritive value protein material, which contains about 30% protein (Ge *et al.* 2000). The cereals possess some essential amino acids in relatively low concentrations. The first limiting amino acid in wheat is lysine, which demands that wheat should be supplemented with high lysine-rich proteins to provide a balanced diet (Pichardo *et al.,* 2003). The endosperm contains higher gluten content which is low in lysine however; albumins and globulins are rich in lysine and are located mainly in the bran and the germ parts of wheat kernel (Lookhart and Bean, 2000).

The amino acid content of defatted wheat germ protein is higher than the FAO/WHO pattern for essential amino acids (Semenyuk and Dyakonova, 1986). The lysine content of defatted wheat germ has been 5.3 to 6.3% and it also possesses higher levels of minerals (Matz, 1991). The improvement in functional properties like foaming, emulsifying, water absorption and oil absorption capacities; texture, nutritional value and flavor of processed foods have also been attributed to the defatted wheat germ. Therefore, defatted wheat germ is a potential nutritious food supplement; in particular, a source of natural high-quality protein, which makes it a good enrichment component in many foods. Thus, the incorporation of defatted wheat germ protein in bakery products can provide the required essential amino acids to cope this deficiency.

In Pakistan, little or no efforts have been made to eliminate or reduce the risk of diseases through diet therapy. There is a need to introduce such raw materials rich in nutrients in daily diet chart which can reduce the threat of protein deficiency, cancer and cardiovascular diseases. The wheat germ utilization in food products has not yet been explored. Keeping in view the nutritional importance and availability of wheat germ, the research study has been undertaken to achieve the following objectives:

- To analyze the effect of defatted wheat germ supplemented cookies on the protein quality parameters of rats.

Materials and methods

The research work was conducted to characterize the wheat germ and assess its suitability for the preparation of cookies. The defatted wheat germ (DFWG) after extraction of wheat germ oil (WGO) was characterized for physico-chemical and functional properties and its suitability in wheat flour at different proportions to make the cookies. The biological assay of DFWG supplemented cookies was carried out to assess the changes in physical parameters like feed intake and body weight along with protein quality parameters i.e. true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER) and biological value (BV) in the rats.

Procurement of raw materials

The wheat germ was the courtesy of Sunny Flour Mills, Lahore and straight grade flour was purchased from local market. The wheat germ and flour samples were packed in polypropylene bags and stored at room temperature for further analysis.

Extraction of WGO

The oil was extracted from wheat germ through solvent extraction technique by using n-hexane. The extracted oil was heated at 40°C to remove the last traces of solvent. The crude oil recovered in this way was kept in desiccator over anhydrous calcium chloride for twenty-four hours, so that the traces of moisture (if present) could be removed. The percentage recovery of oil was calculated by the formula:

$$\text{Wheat germ oil (\%)} = \frac{\text{Wt.of oil (g)}}{\text{Wt.of wheat germ sample (g)}} \times 100$$

3.4.9.2. Housing of rats

Table 1. Composition of diets (DFWG supplemented cookies)

Diets	Ingredients								
	Cookies (g)	Corn starch (g)	Corn oil (g)	Glucose (g)	Min. mix (g)	Vit. mix (g)	Casein (g)	Total (g)	Protein (%)
A	84.67	0.33	-	5.0	5.0	5.0	-	100	10
B	78.74	6.26	-	5.0	5.0	5.0	-	100	10
C	73.80	11.2	-	5.0	5.0	5.0	-	100	10
D	69.11	15.89	-	5.0	5.0	5.0	-	100	10
E	-	67.13	5.0	5.0	5.0	5.0	12.87	100	10
F	-	80.00	5.0	5.0	5.0	5.0	-	100	0.04

A = Cookies supplemented with 0% DFWG
B = Cookies supplemented with 5% DFWG
C = Cookies supplemented with 10% DFWG
D = Cookies supplemented with 15% DFWG
E = Casein
F = Protein free diet

Nearly 60 young male Sprague Dawley rats weighing 60 to 80g were housed in six individual stainless-steel screen cages and allowed free access to food and water. The cages were maintained in a room where temperature of 25°C and 50% relative humidity were maintained. The rats were fed on basal diet for a period of one week and then divided randomly into six groups having ten rats in each. The cookies prepared from selected samples were mixed with all other ingredients of the diet in such a way that 10% of the protein was available from the final diets (Table 1). The three best treatments of cookies along with control, casein diet and protein free diet were fed to 6 groups of rats for 10 days. The rats were fed on the freshly prepared diets and watered individually in separate cages on daily basis up to 10 days. At the end of the experimental period, animals were fasted overnight, anesthetized with diethyl ether and sacrificed.

Feed intake

Net feed intake per group of rats was measured on daily basis by excluding leftover and collected spilled diet during the entire period to observe the impact of individual experimental diet.

Body weight

The gain in body weight of individual rats in each group was recorded on daily basis throughout the experimental period to find out the effect of individual diet on body weight at the end of study.

True digestibility (TD)

The faeces were collected after two days feeding trials from each cage then dry weight and nitrogen content of faeces were determined. The nitrogen intake was calculated from the feed consumption and then TD was calculated by the formula of Miller and Bender (1955).

$$\text{True digestibility (TD)} = \frac{I - (F - FK)}{I} \times 100$$

Where:

I = Nitrogen intake by rats on test diets

F = Faecal nitrogen of rats on test diets

FK = Faecal nitrogen of rats on protein free diet

Net protein utilization (NPU)

The nitrogen content of diets, faeces and carcass of each group including protein free group were determined by micro Kjeldahl's method. NPU of diets were then determined according to the method of Miller and Bender (1955) as given below:

Where:

$$\text{Net protein utilization (NPU)} = \frac{B - (BK - IK)}{I} \times 100$$

B = Total body nitrogen of rats on test diets

BK = Total body nitrogen of rats on protein free diet

I = Nitrogen intake by rats on test diets

IK = Nitrogen intake by rats on protein free diets

Protein efficiency ratio (PER)

The PER is defined as weight gain of rats per gram of crude protein fed and gives an indication of the efficiency of protein utilization. The PER of diets was calculated as under:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total weight gain}}{\text{Total protein intake}} \times 100$$

Biological value (BV)

The biological value (BV) of diets was calculated by using the following formula:

$$\text{Biological value (BV)} = \frac{NPU}{TD} \times 100$$

Statistical analysis

The data were interpreted by analysis of variance (ANOVA) using M-Stat C software package as described by Steel *et al.* (1997). ANOVA analysis tested the significance of the differences between samples at 5% level of significance. The Duncan's Multiple Range (LSD) test was used to determine the level of significance that existed between the mean values.

Results and discussion

Biological study of WGO based cookies

The cookies containing 5%, 10% and 15% DFWG along with control (0% DFWG) found to be the best, based on the scores of physical and sensory evaluations were subjected to protein quality assay through a rat bioassay. The reference diet casein (E) along with the diet containing cookies prepared from 15% DFWG supplementation excelled in body weight, feed intake, true digestibility, net protein utilization, protein efficiency ratio and biological value as compared to all other experimental diets. Protein free diet (F) was also provided to the rats to check the effect of this diet on declining of rat growth. The biological assay was carried out on Sprague dawley rats to study the following biological parameters which are discussed below.

Feed intake

It is obvious from the statistical results given in Table 2 that variation in diets showed a significant effect on feed intake of rats. The daily feed intake values of different rat groups are presented in Table 3. The results showed that the reference casein diet (E) and the diet which was prepared from the cookies containing 15% DFWG (D) were more preferred by the rats. Whereas, the rat group fed on diet A (0% DFWG) consumed 24.65 g diet/day, which was significantly lower than the groups of rats fed on cookies prepared from incorporation of DFWG flour. It is evident from the Table 3 that non-significant differences existed among the feed intake pattern of rat groups fed on diets E and D. The diet prepared from the cookies having no protein (F) was least preferred (16.55 g/day) by the rats. The results also indicated that the feed intake of rats increased as the amount of DFWG increased in the diets. The present findings which showed higher consumption of DFWG supplemented cookie diets are in agreement with the previous results of Arshad *et al.* (2007) who reported that the cookies prepared by incorporation of defatted wheat germ were excessively consumed by the rats compared to the control cookies. Similarly, Attila *et al.* (2001) fed the rats with the diets having wheat germ and stated that rat groups showed preferring attitude towards diet supplemented with wheat germ compared to control diet.

Body weight

The analysis of variance for body weight of rats indicated that experimental diets exerted significant effect on the body weight of rats (Table 2). The body weights of rats fed on diets based on DFWG supplemented cookies at the expiry of the experiment has been given in Table 4.33 and illustrated in Figure 1. The data manifested significantly higher body weight of the rat groups fed on casein (E) diet and diet D (15% DFWG). The rat group fed on casein diet (E) attained 78.41g average weight while the rats provided with diet D (15% DFWG) possessed 77.17g at the end of 10 days, but these two groups showed non-significant difference with each other. It is evident from Figure 4.5 that gain in body weight of rats fed on different diets was consistent. The rats gained the body weight continuously up to 6 days and then beyond this period the increase was recorded minimum up to the expiry of the experiment (10 days). The data interpreted in Figure 4.5 also indicated that the rat group which was fed on protein free diet showed linear decline in the growth and the body weight of the rats in this group reduced significantly with the progress of study period. The results of the present study are in concordance with the reason that body weight of rats increased as the feed intake increased. Similar was the case in present study that the rat groups fed on diets D (15% DFWG) and E (casein) consumed more feed compared to the other groups which resulted in the higher mean body weight for these groups at the end of study. The results demonstrated by Chignola *et al.* (2002) showed that rats fed on wheat germ diet achieved higher body weight compared to the control diet. The researchers related the increase in body weight to the higher levels of protein present in wheat germ relative to the wheat flour and this supports the results found in the present study.

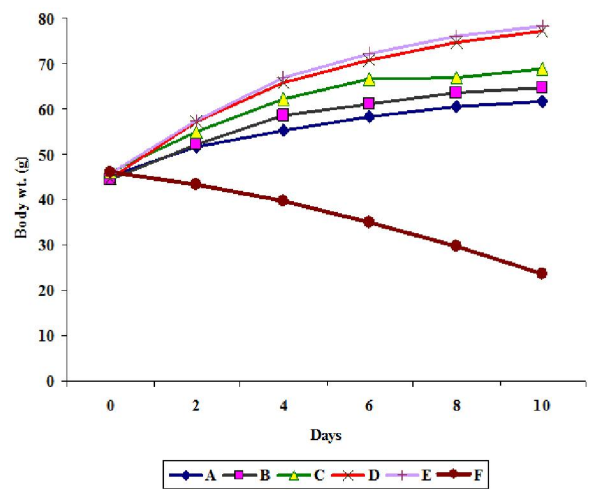

Figure 1. Effect of different diets on the mean body wt. of rats

A = Prepared from cookies supplemented with 0% DFWG

B = Prepared from cookies supplemented with 5% DFWG

C = Prepared from cookies supplemented with 10% DFWG

D = Prepared from cookies supplemented with 15% DFWG

E = Casein diet

F = Protein free diet

Table 2. Mean squares for protein quality parameters of rats fed on DFWG supplemented cookies

SOV	df	Feed intake	Body weight	TD	NPU	PER	BV
Diets	4	2.432**	5.602**	287.170**	166.823**	1.791**	220.732**
Error	10	0.0210	0.0131	1.130	0.751	0.00001	1.474

**Significant ($P < 0.01$)

TD = True digestibility

NPU = Net protein utilization

PER = Protein efficiency ratio

BV = Biological value

Table 3: Protein quality parameters of diets containing cookies supplemented with DFWG fed to rats

Diets	Protein quality parameters*					
	Body weight (g)	Daily Feed Intake (g)	TD (%)	NPU (%)	PER	BV
A	61.75±0.8[d]	24.65±0.5[c]	69.46±2.35[d]	47.36±1.18[d]	1.11±0.06[d]	63.18±2.80[d]
B	64.73±1.0[c]	27.03±0.5[b]	75.80±3.10[c]	50.17±1.64[c]	1.63±0.06[c]	65.80±2.80[c]
C	68.95±1.2[b]	27.80±0.6[b]	88.35±3.25[b]	61.05±2.07[b]	1.72±0.06[b]	78.56±2.82[b]
D	77.17±1.4[a]	31.17±0.7[a]	92.61±3.30[a]	64.28±1.40[a]	2.83±0.04[a]	80.58±3.05[b]
E	78.41±1.3[a]	31.43±0.7[a]	93.45±3.30[a]	65.08±1.45[a]	2.88±0.10[a]	85.36±3.25[a]
F	23.5±0.9[e]	16.55±0.5[d]	-	-	-	-

Means in the same row bearing the same letter are not significantly different ($P < 0.01$)
*Values are averages (10 rats per diet) ± standard error
Body weights are the values of average body weights of one rat per group after 10 days
Daily feed intake values are average daily feed intakes per group of rats during 10 days
PER = Protein efficiency ratio; NPU = Net protein utilization; BV = Biological value; TD = True digestibility
A = Prepared from cookies supplemented with 0% DFWG
B = Prepared from cookies supplemented with 5% DFWG
C = Prepared from cookies supplemented with 10% DFWG
D = Prepared from cookies supplemented with 15% DFWG
E = Casein diet
F = Protein free diet

True digestibility (TD)

The statistical results for true digestibility of different diets presented in Table 2 indicated that true digestibility differed significantly among different experimental diets. The true digestibility presented in Table 3 indicated that the true digestibility of different diets ranged 69.46% to 93.45%. The true digestibility among the cookies enriched with DFWG flour differed significantly and was found higher in the reference diet E (93.45%) followed by diet D (90.61%), while the lowest true digestibility was recorded in experimental diet A (69.46%). The results in Table 3 further substantiated that the experimental diets D (15% DFWG) and E (casein) were statistically at par with respect to true digestibility.

The results further showed that true digestibility of the diet increased with the increase in level of DFWG in cookies. These results provide good information that improvement in the nutritive value of cookies can take place as a result of DFWG addition. The increase in true digestibility with the increase of DFWG flour may be attributed to the valuable amino acid profile of DFWG flour as compared to wheat flour. Lasztity *et al.* (1990) compared the essential amino acid content of white wheat flour, whole wheat flour and wheat germ. They observed that the protein in wheat germ was extra rich in lysine, the amino acid that is deficient in the rest of grain. The increase in true digestibility is also supported by Gupta and Eggum (1998) who reported that the true digestibility of defatted wheat and corn germs is 85% and 76%, respectively, which confirmed the increase in this attribute was higher through DFWG with rat feeding trials in present study.

The present results are in line with the findings of Buraque *et al.* (1979) who found an increase in true digestibility when wheat flours were supplemented with 4% detoxified cottonseed flours for chapattis. The true digestibility of Saudi breads has also been reported maximum of 89.7% in diets containing sorghum protein in the blends (Nanm, 2004).

Net Protein Utilization (NPU)

The statistical results regarding net protein utilization of different diets prepared from cookies supplemented with DFWG flour shown in Table 2 indicated that net protein utilization differed significantly by the differences in experimental diets. The net protein utilization of different experimental diets ranged from 47.36 to 65.08% (Table 3). It is obvious from the results that the reference diet E (Casein) obtained the highest value (65.08%) of NPU followed by experimental diet D (15% DFWG), while lowest NPU value (47.36%) was observed in the diet A (0% DFWG). The statistical results for NPU also depicted that diet D and E yielded statistically at par for this attribute.

The results of the present study demonstrated that increase in DFWG flour in the diets improved net protein utilization. The results further showed that diet containing 15% DFWG was found similar to casein diet with respect to NPU and can be used as replacement of casein. This increase in NPU may be due to the presence of higher amounts of essential amino acids particularly, lysine and threonine which are limiting in the wheat flour. The improvement in net protein utilization with lysine is well documented by Estevez *et al.* (1987), who reported increase in net protein utilization from 52.35% to 67.50% by the incorporation of 15% chickpea flour in the cookie's formulation. These results are further supported by the findings of Anwar (1980) who reported 48.16%, 48% and 43% NPU values for bread. In the present research findings, it has been observed that an increase in net protein utilization occurred as a result of enrichment of wheat flour with different DFWG levels.

Protein efficiency ratio (PER)

The statistical results pertaining to protein efficiency ratio of different diets indicated that protein efficiency ratio was significantly affected due to the differences in experimental diets (Table 2). The protein efficiency ratios of different diets are presented in Table 3. The protein efficiency ratio ranged from 1.11 to 2.88. It is evident from the results that a significant difference with respect to PER was observed among the experimental diets prepared from cookies enriched with different levels of DFWG flour. The protein efficiency ratio was the highest (2.88) in the experimental diet E (casein) followed by the diet D (15% DFWG) but both these diets were statistically at par for PER. The minimum PER value (1.11) was possessed by the rats fed on experimental diet A (0% DFWG flour).

The results revealed that there was a significant increase in protein efficiency ratio in diets when supplemented with DFWG flour at different levels. The protein efficiency ratio of the proteins is dependent on essential amino acids present in the samples as well as the ability of human or animal to digest and utilize these amino acids (Jansen, 1978). The research workers (Phimphilai *et al.,* 2006) have reported that defatted wheat germ addition enhanced the protein efficiency ratio when added at different levels in the wheat flour. The results of the present study are in close agreement with the studies reported by Zhu *et al.* (2006) who described that protein efficiency ratio of defatted wheat germ protein exceeded 2.0, describing a protein of high quality.

Biological value (BV)

The analysis of variance for biological value of different diets enriched with DFWG (Table 2) revealed that biological value varied significantly by the differences in experimental diets. The biological value of different experimental diets ranged from 63.18 to 85.36 (Table 3). The biological value differed significantly in different diets supplemented with DFWG flour. The highest biological value was found in the reference diet E (casein) and the lowest in the diet A (0% DFWG). The difference for BV among experimental diets E (Casein) and the diet D (15% DFWG) was found to be non-significant. The results on biological value showed that there was a significant increase in the biological value of cookies when enriched with DFWG flour whereas it was observed to be the highest when wheat flour was enriched with 15% DFWG.

The improvement in biological value of diets with DFWG flour is well supported by Siddique *et al.* (1996) who observed an improvement in the biological value of chapatti supplemented with chickpea flour. The present results are supported by the findings of Anwar (1980) and Kausar (1976) who reported biological value for wheat flour as 56, 92, 56, 50, 67 and 63 respectively. Thus, the results for biological value of cookies found in the present study are in agreement with the previous findings of the above-mentioned researchers.

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