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Probing the Protein Quality of Wheat-Mung Bean Based Weaning Foods Using Rat Bioassay

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Abstract

Protein-energy malnutrition affects children during their vulnerable age of development. Nutritionally balanced weaning foods are the key to attain optimal physical and mental development of the children. In this study, the biological value of proteins in cereal-legume weaning foods was investigated using rat bioassay. The trial was carried out by feeding the respective diets to 5 groups of Sprague Dawley rats (n=6). Feed intake, water intake, body weight was recorded while urinary and faecal outputs were collected and analysed. Weaning food prepared using germinated grains showed the highest values for PER (1.84±0.75), FER (0.04±0.01), NPR (0.95±0.03), RNPR (0.76 ±0.05), PRE (15.2±0.48), AD (97.47±0.12), TD (99.07±0.66), BV (89.67±2.62) and NPU (88.83±2.28) as compared to the other treatments. It was concluded that germination is a promising processing method to enhance the protein quality in weaning foods.

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Introduction

Protein quality refers to the presence of adequate amounts of all the essential amino acids in the proteins of food. According to this, a food that has all the nine essential amino acids in the correct proportion is known as a complete protein. High-quality protein weaning foods are effective for good growth and development (Uauy *et al.,* 2015). Their intake during the early years of childhood is important as it is positively correlated with the weight and height in the later years. Inadequate intake of high-quality proteins can result in protein-energy malnutrition (PEM), which manifests in either marasmus or kwashiorkor, occurring more commonly in children. Malnutrition is a leading cause of child morbidity and mortality. The United Nations Children's Fund states that globally the rate of stunting is 23%, underweight is 16%, and wasting is 7%. Pakistan also has the highest prevalence of all kinds of malnutrition (Di Cesare *et al.,* 2015). According to the latest national survey (Bhutta *et al.,* 2011), 43% of children were stunned, 31.2% were underweight, and 16.8% were wasted. Malnutrition generally occurs during the crucial period of weaning, in which the infant's diet transitions from exclusive breastfeeding to family food (Shah *et al*., 2019).

The effects of malnutrition on infants are so profound and alarming and it can jeopardize the future of infants. Weaning foods should supply a good quantity and quality of protein, as the requirement for protein increases up to three times from the age of 6 months to 2 years (Dewey and Adu-Afarwuah 2008). In developing countries, two-thirds of the population cannot afford animal protein sources, which is why they must rely mainly on cereals and legumes as a source of protein. Tryptophan and lysine are deficient in cereals, while sulphur-containing amino acids are present in good amounts (Ijarotimi and Famurewa, 2006). Contrarily, legumes are a rich source of lysine, but they are deficient in sulphur-containing amino acids (Shah *et al.,* 2017). The combination of cereals and legumes improves the quality of protein in an infant's food. The protein quality is a measure of the efficiency or utilization of proteins by the body. It also depends on the bioavailability of proteins (Jood and Singh, 2001) which may be affected by the presence of antinutrients in cereals and legumes (Khattab and Arntfield, 2009). The bioavailability of the protein depends on the composition and processing methods employed to develop that food. Therefore, in the current study weaning foods prepared using roasting, germination and fermentation were subjected to investigate the protein quality using rat bioassay. Adequate and appropriate nutrition is the key to ensure that children can have a strong immune system as well as good physical and mental development during their early years of life (Asad and Mushtaq, 2012). The aim of the study is to evaluate the quality of protein in the wheat-mung bean based weaning foods developed using three different technologies i.e. roasting, germination and fermentation using rat bioassay.

Materials and methods

Procurement and preparation of the raw materials

Wheat and mung beans were procured from the local market in Lahore. The raw materials were cleaned manually to remove impurities, and then they were packed separately in the zip-locked bags.

The roasting procedure followed was according to the method described by Msheliza *et al.* (2018), the germination procedure followed was according to the method described by Gahlawat and Sehgal (1992) and the fermentation procedure was according to the method described by Adebayo-Oyetoro *et al.* (2012) (Fig. 1).

Treatment plan

T_R = Roasted wheat (70g); roasted mung bean (30g).

T_S = Sprouted wheat (70g); sprouted mung bean (30g).

T_F = Fermented wheat (70g); fermented mung bean (30g).

Biological evaluation

The biological evaluation of cereal-legume based weaning foods was performed by feeding the respective treatments along with the non-protein diet and standard (casein) diet to Sprague Dawley rats. In addition to the treatments, mineral-vitamin mixture, cellulose, sucrose, and corn starch were also combined with the experimental diet (Gahlawat and Sehgal, 1992; Mensa-Wilmot *et al.,* 2001).

Rat bioassay

30 Sprague Dawley rats (males) were housed in the animal house of the Institute of Molecular Biology and Biotechnology, The University of Lahore. The temperature (23±2°C), humidity (55±5%) and 12-hr light-dark cycle were maintained throughout the experimental period.

The rats were given a basal diet for one week and then they were weighed and randomly divided into 5 groups, each group comprising of 6 rats (Onweluzo and Nwabugwu, 2009). The average weight per group was approximately the same (±2g). All the groups were fed the respective diets for 21 days. Feed intake, spilled diet, water intake and body weight were measured daily. The last 7 days were for the collection of faeces and urinary output.

The faeces collected were dried, weighed and powdered for analysis. To avoid losses of ammonia, a few drops of H_2SO_4 were added to the collected urine. The nitrogen of faeces and urinary output was determined by Kjeldahl method (Serrem *et al.,* 2011).

The rules and regulations set by the ethical committee of the University of Lahore were followed while conducting the research and the rights of the research animals were respected.

Protein quality evaluation

The weight gain, spilled diet, faeces, urinary outputs were analysed to work out protein efficiency ratio (PER), the food efficiency ratio (FER), net protein retention (NPR), protein retention efficiency (PRE) apparent digestibility (AD), true digestibility (TD), biological value (BV) and net protein utilization (NPU) as described by Pellet and Young (1980); Ingbian and Adegoke (2007). These parameters were calculated as follows (Gahlawat and Sehgal, 1992):

$$PER = \frac{\text{gain in bodyweight } (g)}{\text{protein intake } (g)}$$

$$FER = \frac{\text{gain in body weight } (g)}{\text{food intake } (g)}$$

$$NPR = \frac{\text{weight gain of test group} + \text{weight loss of protein free group}}{\text{weight of test protein consumed}}$$

$$PRE = NPR \times 16$$

$$AD = \frac{Ni - Nf1}{Ni} \times 100$$

$$TD = \frac{Ni - (Nf1 - Nf2)}{Ni} \times 100$$

$$BV = \frac{Ni - (Nf1 - Nf2) - (NU1 - NU2)}{Ni - (Nf1 - Nf2)} \times 100$$

$$NPU = \frac{BV \times TD}{100}$$

Where; Ni: nitrogen intake of animal fed test diet; NF1: nitrogen excreted in faeces of animals fed test diet; NF2: nitrogen excreted in faeces of animals fed protein-free diet; NU1: nitrogen excreted in the urine of animals fed test diet; NU2: nitrogen excreted in the urine of animals fed protein-free diet.

Data analysis

Collected data was subjected to statistical analysis using SPSS version 25. The results of the study are given as mean values and standard deviations.

Collected data were subjected to one-way analysis of variance (ANOVA) and a P-value of ≤ 0.05 was considered significant. Means were separated using Tukey's HSD test.

Results

Physical parameters of experimental rats

The results for the physical parameters of the experimental rats showed that the type of weaning food and the study duration has significant influence on the water intake, feed intake, gain in body weight among different groups of experimental rats.

Table 1. Effect of treatments on growth study parameters in experimental rats.

Groups	PER	FER	NPR	RNPR	PRE
G1	0.82 ±0.23[d]	0.01 ±0.00[c]	0.58 ±0.02[d]	0.46 ±0.02[d]	9.28 ±0.33[d]
G2	1.84 ±0.75[b]	0.04 ±0.01[b]	0.95 ±0.03[b]	0.76 ±0.05[b]	15.2 ±0.48[b]
G3	0.95 ±0.16[c]	0.03 ±0.00[bc]	0.75 ±0.02[c]	0.60 ±0.03[c]	12 ±0.32[c]
G4	2.35 ±0.25[a]	0.08 ±0.01[a]	1.25 ±1.02[a]	1.00 ±0.00[a]	20 ±0.32[a]

Values given are Means ± SD, and the means with the same letters in a column are not significantly different. PER: Protein efficiency ratio; FER: Food efficiency ratio; NPR: Net protein ratio; RNPR: Relative net protein ratio; PRE: Protein Retention Efficiency; G1: Roasted weaning diet; G2: Germinated weaning diet G3: Fermented weaning diet; G4: Casein diet; G5: Protein-free diet.

Feed intake

Daily feed intake of the rats in the different groups is given in Fig. 2. The demand for the feed increased over time in all the groups. The average daily feed intake started at (12.3±4.5) on the initial day and ended at (57.4±12.5) on the 18[th] day of the study. Rats fed a casein diet showed the highest intake, while the rats fed on a protein-free diet showed significantly low intake. Among the treatment groups, rats fed germinated weaning diet showed the highest consumption, followed by fermented and roasted weaning diets.

Water intake

At the start of the study the water intake was 16ml in Group 1, 19ml in Group 2, 17ml in Group 3, 20ml in Group 4, and 12ml in Group 5 which increased to 20ml, 23ml, 20ml, 24ml, and 15ml respectively, at the termination of the study period (Fig. 3). The highest water intake was noted in rats fed the casein diet, while the lowest was seen in rats fed the protein-free diet. Among the treatment groups, rats fed germinated weaning diet showed the highest intake, followed by fermented and roasted weaning diets. Intake of water increased over time, with the highest consumption in each group towards the end of the study.

Gain in body weight

Fig. 4 illustrates the gain and loss in body weight of the experimental rats. A dramatic increase in the bodyweight of rats fed casein can be seen. The body weight increased from 35g to 39g in Group 1, 47 g in Group 2, 40 g in Group 3, and 61 g in Group 4 at the end of the study period roasted <fermented <germinated <casein). On the other hand, there was a dramatic loss of body weight in the rats that depended on the protein-free diet for survival. Their weight decreased from 35g to 22.67g at the end of the study period.

Growth study parameters of experimental diets

Mean values for the growth study parameters, protein efficiency ratio, feed efficiency ratio, net protein ration, and relative net protein ratio presented significant variations between the treatments and the control (Table 1).

Protein efficiency ratio

In the present study, the casein diet had a PER of 2.35. The PER of the treatments ranged from 0.95 (roasted weaning diet) to 1.84 (germinated weaning diet). The values for another parameter FER, which is the ratio between the gain in body weight and the food intake, ranged from 0.01 (roasted weaning diet) to 0.08 (casein diet). The highest FER in the treatment groups was seen in the germinated weaning diet (0.04).

Net protein ratio

In the present study, NPR showed significant variations between the casein and the treatment groups. Casein's diet showed superiority with an NPR of 1.25. Among the treatment groups, the NPR ranged from 0.58 (roasted weaning diet) to 0.95 (germinated weaning diet). The highest NPR and RNPR recorded was 0.95 and 76% respectively for the germinated weaning diet, which indicated that the germinated weaning diet was more efficient than the other treatments in supporting the growth and maintenance of weanling rats.

Nitrogen balance study parameters of experimental diets

Mean values for the nitrogen balance parameters, apparent digestibility, true digestibility, biological value and net protein utilization presented significant variations between the treatments and the control (Table 2).

Apparent digestibility and true digestibility

The present study shows that the AD (98.91) and TD (99.40) of the casein diet were higher than the treatment diets and thus more digestible, while the roasted weaning diet showed the lowest AD (73.17) and TD (76.87). Among the treatment groups, the geminated weaning diet had the highest AD (97.47) and TD (99.07) and was comparable with the casein diet.

Biological value and net protein utilization

BV of the diets ranged from 71.22% (roasted weaning diet) to 94.68% (Casein diet). The BV of germinated (89.67) and fermented (82.34) weaning diets were better than the roasted weaning diet but lower than the BV of the casein diet. The results show that germinated and fermented weaning diets are capable of supporting growth in infants. NPU ranged from 54.95 (roasted weaning diet) to 94.11 (casein diet). The NPU of the germinated weaning diet (88.83) was significantly higher among the treatment groups followed by the fermented weaning diet (78.41).

Table 2. Effect of treatments on nitrogen balance study parameters in experimental rats.

Groups	AD	TD	BV	NPU
G1	73.17±0.93^{c}	76.87±2.33^{c}	71.22±14.59^{b}	54.95 ±12.89^{c}
G2	97.47±0.12^{a}	99.07±0.66^{a}	89.67±2.62^{ab}	88.83±2.28^{b}
G3	92.97±2.41^{b}	95.16±1.59^{b}	82.34±5.61^{ab}	78.41 ±6.67^{b}
G4	98.91±0.53^{a}	99.40±0.34^{a}	94.68±1.32^{a}	94.11±0.99^{a}

Values given are Means ± SD, and the means with the same letters in a column are not significantly different. AD: Apparent digestibility; TD: True digestibility BV: Biological value NPU: Net protein utilization; G1: Roasted weaning diet; G2: Germinated weaning diet; G3: Fermented weaning diet G4: Casein diet G5: Protein-free diet.

Discussion

After the age of six months, infants require nutritious weaning foods along with breast milk (Dewey and Brown, 2003). However, in most areas of the developing world, the traditional weaning foods lack in protein and other essential nutrients, which puts the infants at risk of malnutrition (Dewey and Brown, 2003; Ijarotimi, 2008). Adequate weaning foods with high protein quality are essential for the body's growth and development. Some factors influence the quality of protein: the balance of amino acids; digestibility of protein; and the infant's need for amino acids.

Feed intake, water intake and body weight gain are all interrelated with each other (Selman *et al.,* 2001). They are usually affected by environmental as well as genetic factors (Smith *et al.,* 2000). In the present study, feed intake increased over time.

In addition to this, the type and quality of the protein in the di*et al*so affects the intake of food (Onofiok and Nnanyelugo, 1998). A high intake of protein containing diets like the germinated weaning diet can be because of its improved palatability and flavour, mainly due to the presence of aromatic amino acids.

Intake of food also depends upon the body's requirements, and the food's potential to meet these requirements (FAO, 2011). The fact that rats fed a protein-free diet consumed the lowest in the present study, shows that the protein-free diet was unable to fulfill the nutritional requirements of the experimental rats.

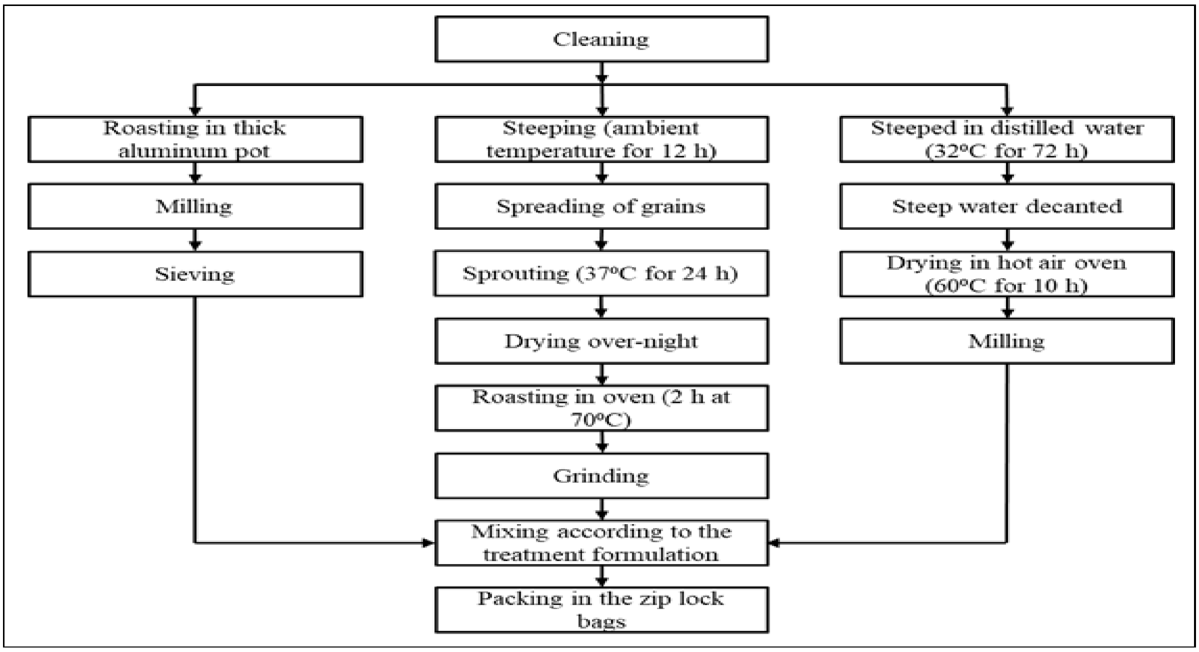

Fig. 1. Flow chart for the production of roasted, germinated and fermented cereal and legume.

This reduced intake of food can cause protein deficiency resulting in protein-energy malnutrition and then death. Studies have reported that the food intake of fortified diets was comparable with unfortified diets but significantly different and higher as compared to the basal diet (Serrem *et al.,* 2011). Observations showed that the feed intake of experimental rats in each group had a positive correlation with bodyweight gain. Oluwole *et al.,* (2018), Gahlawat and Sehgal (1992) and Ingbian and Adegoke (2007),) also reported a similar trend.

Increased water intake seen in rats fed casein and germinated weaning diet is due to a high feed intake. Similar findings were reported by Bachmanov *et al.,* (2002).

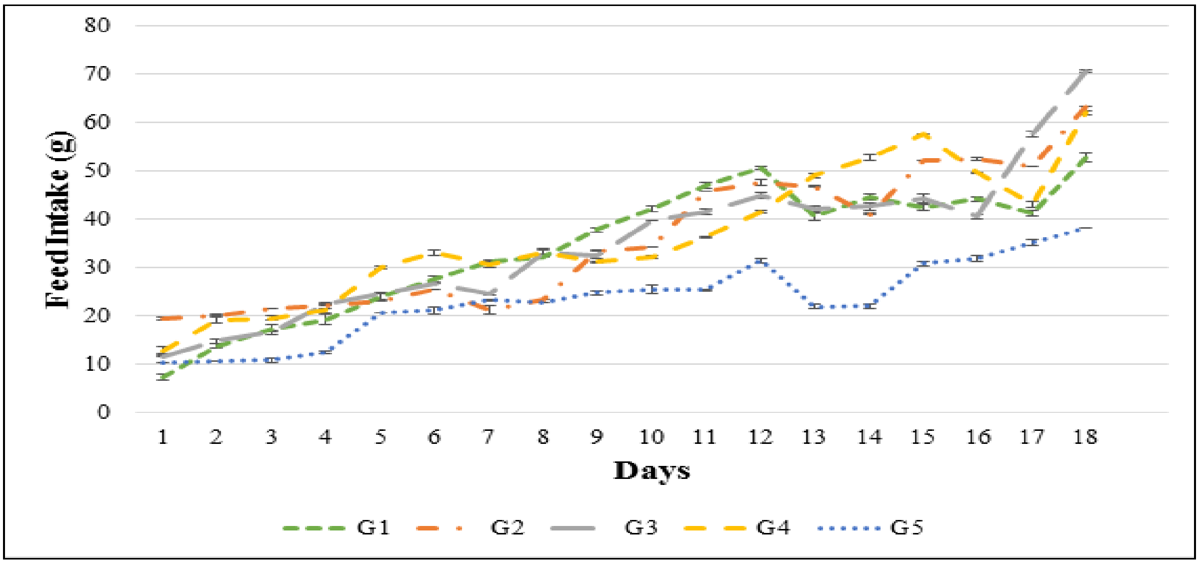

Fig. 2. Feed intake (g) of experimental rats during the trial.

The quality of protein and the amount of feed intake affected the body weight gain. Bodyweight gain in rats fed casein diet was higher as compared to weight gain in rats fed the protein-free diet. Among the treatment groups, rats fed germinated weaning diet showed the highest weight gain followed by fermented and roasted weaning diet. Similar results were reported in previous studies (Gahlawat and Sehgal, 1992; Oluwole *et al.,* 2018). Looking at the effect on body weight over time, this study found the highest gain towards the end of the study, when the feed intake was also the highest. Food and water intake strongly correlate with body weight, and this can be seen in the present study. High body weight gain can also be attributed to a high protein quality of the diet. That is why the rats that depended on the protein-free diet became thinner with time. A decrease in body weight was seen in the rats fed the protein-free diet. This shows that protein is essential for growth and development.

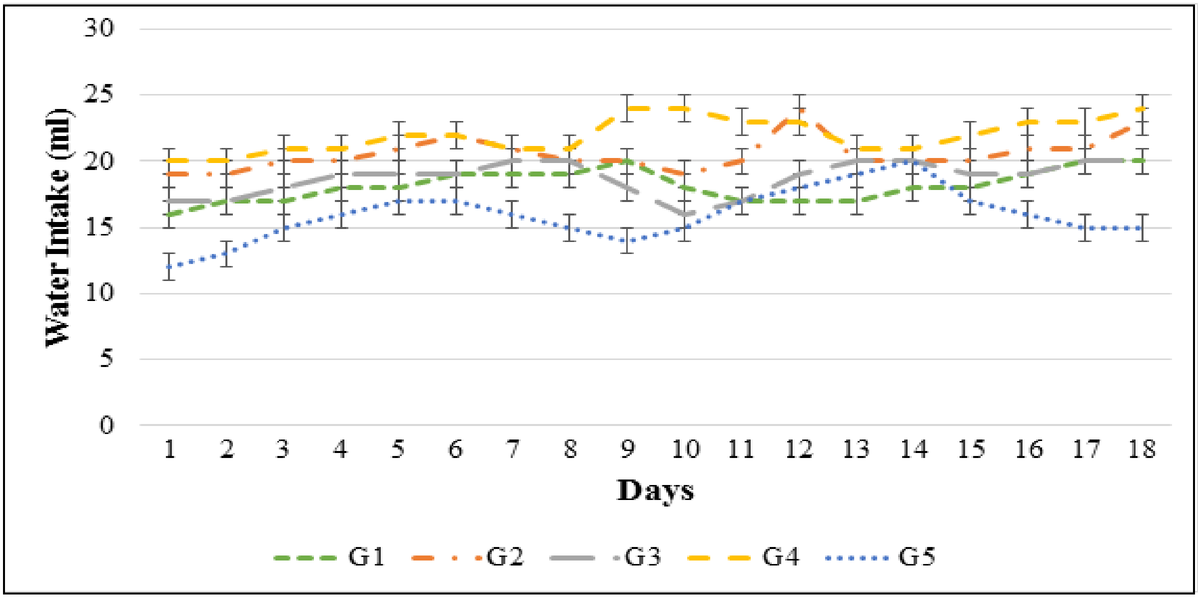

Fig. 3. Water intake (ml) of experimental rats during the trial.

Protein plays an important role in the growth and development of infants. Proteins from the diet are hydrolysed into amino acids and thereafter absorbed in the cells. Bio-efficacy of cereal-legume based weaning foods is important to determine the nutritional quality of the food. That is why the growth-based study is used to assess the quality of protein for human use. PER specifies the relationship between the gain in body weight of the experimental animal and the corresponding consumption of protein. PER is one of the most used methods for evaluating protein quality. A food with a higher PER is considered to have better-quality as compared to the one which has a lower PER. PER is associated with the presence of essential amino acids in the diet as well as the digestibility of these amino acids. Becker (2007), have measured PER in animals by measuring weight gain per unit of protein, for a short period. The PER values for the casein diet reported by other studies are 3, 3.5, and 2.87 (Kalra and Jood, 1998; Gahlawat and Sehgal, 1992; Asemi and Taghizade, 2009). As the results of the present study, Olapade and Aworh (2012), reported a PER of 1.14 to 2.15 in millet and pigeon pea weaning foods, while a PER of 0.08 to 0.32 in extruded weaning foods made from cowpea and fonio. However, the results obtained in the present study were lower as compared to the higher PER reported by Ijarotimi and Keshiro (2012) and Oluwole *et al.,* (2018). While the values for FER, which shows the efficiency of food to support growth, were like the study conducted by Nassar and Sousa (2007). The present study found significant improvements in the PER and FER of the germinated cereal-legume weaning foods among the other treatment groups. The higher PER of the germinated weaning food maybe because of the degradation of carbohydrates and proteins into smaller absorbable forms by the enzymes, which in turn improves and facilitates the digestion and absorption in the experimental rats. It can also be attributed to its improved amino acid profile which contains a good balance of essential amino acids. Moreover, the rats fed germinated weaning diets ate more and gained more bodyweight, which could also be a reason for a higher PER value. The results were consistent with previous findings, which reported significant improvement in the PER of germinated cereals and legumes (Oghbaei and Prakash, 2016). Many studies have shown no significant increase in the PER of fermented foods, neither did any study show a significant decrease. Studies have shown improvement in PER as a result of increased lysine content after the fermentation process. In the present study, the improved PER in fermented weaning diets could be a result of improved lysine content. The lower food intake found in rats fed fermented diet could also be a reason for lower PER value contrary to the rats on the germinated diet. Lower PER values (0.60 to 1.70) have been reported in a previous study, while increased PER values (2.17 to 2.64) were found in extruded weaning foods formulated from green gram, Bengal gram, wheat flour, sesame flour, groundnut, sugar and white milk powder (Fashakin and Ogunsole, 1982). The protein quality of chickpea, corn and cowpea floors was studied after natural fermentation. The researchers found significant improvements in the protein quality of the fermented flours (Hamad and Fields, 1979). In the methods that use heat, a significant reduction in the protein content is observed, as it modifies the structure of globulin and affects various amino acids (Arif *et al.,* 2012). Lower quality and quantity could be the reason for lower PER and FER observed in roasted weaning diet (Nassar and Sousa, 2007; Hayat *et al.,* 2014).

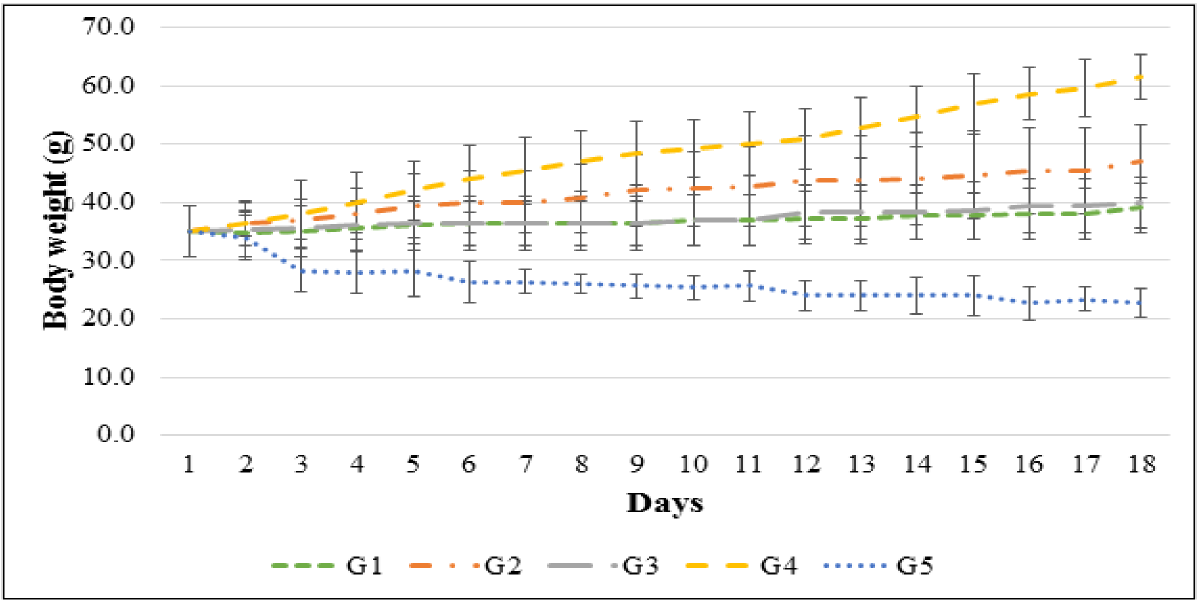

Fig. 4. Body weight (g) of experimental rats during the trial.

NPR shows the change in weight in the experimental animals fed test diet to those fed protein-free diet. As compared to the present study, higher NPR for casein diet were reported in other studies (3.5, 3.65 and 4.3) (Kalra and Jood, 1998; Mensa-Wilmot *et al.,* 2001; Asemi and Taghizade, 2009). The difference in NPR value is associated with the quantity and quality of protein consumed. Higher NPR values were reported in different varieties of soybean, barley and cowpea (Mensa-Wilmot *et al.,* 2001; Jood and Singh, 2001). Higher NPR values seen in germinated and fermented weaning diets as compared to roasted weaning diets may also be due to the enzymatic degradation of proteins into smaller easily absorbable forms. The RNPR values were calculated from the NPR values by correcting casein to 100. In contrast to the PER, NPR and RNPR also take into consideration the maintenance needs of the weanling rats. That is why a germinated weaning diet can be said to be effective and efficient in fulfilling the growth and maintenance requirements of the infants. Methods like AD, TD, NPU and BV use nitrogen retention as the dependent variable in determining the quality of protein. The digestibility of protein is also an important factor in the evaluation of protein quality. The present study results were consistent with the findings of the previous studies (WHO, 1985; Ruel *et al.,* 2003). The average faecal nitrogen output of rats fed casein diet was significantly lower in comparison to the other diets, and this is reflected in the higher result of true digestibility. A higher digestibility suggests higher dietary nitrogen retention in the rats. A study (Kamau *et al.,* 2016) reported AD ranging from 87.82% in sorghum-soy to 97.57% in the maize diet. TD for the casein diet was reported 92% and 99% in other studies (Gahlawat and Sehgal, 1993; Koo and Lasekan, 2007). The main reason behind the difference between the AD and TD of casein and other diets is protein intake and excretion by experimental rats. Improvement in digestibility of protein after germination and fermentation could be because of the partial degradation of complex proteins into simpler absorbable forms. Current study outcomes were consistent with those of previous studies (Kalra and Jood, 1998; Shekib, 1994).

The protein digestibility has also been shown to depend on the limiting and essential amino acids that are found in the protein of the food (Becker, 2007). That is why the evaluation of the biological value is also important. Biological value is a nitrogen balance method that differentiates between ingested protein and absorbed protein. Biological value assesses the proficiency of the protein to support growth and development through nitrogen retention in the body. It calculates the protein absorbed and retained by the body (Francis *et al.,* 2009).

The results of the present study showed that germinated and fermented weaning diets are capable of supporting growth in infants. On the other hand, net protein utilization indicates the protein and biological value of the amino acids bioavailable from food (Becker, 2007). It determines the nitrogen intake that is retained stated as percent nitrogen absorption. The low NPU observed in the roasted weaning diet maybe because of the increased nitrogen excretion and a consequent decrease in nitrogen retention by the body. The higher NPU values suggest that more nitrogen was retained and used by the experimental rats. Onweluzo and Nwabugwu (2009), found superior protein quality due to the germination process. These results suggest germination of cereals and legumes can be an economical and effective method in improving protein digestibility and thus help in developing high protein quality weaning foods.

Conclusion

Protein-energy malnutrition is a complex issue, only a multisectoral approach can be effective in promoting public health. In the present study, the protein quality of formulated cereal-legume weaning foods was assessed. The growth and nitrogen balance study has confirmed that germination of cereal and legume blend provides a nutritionally enhanced product. Germination techniques can be employed to develop acceptable and nutritionally enhanced, high protein quality weaning foods. As germination has shown an improved protein bioavailability and digestibility and thus contributed to the overall protein quality of the food. Moreover, it gives good color and flavor to the food. This is why germinated weaning foods can be used for infant feeding.

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