In vivo (rat assay) assessment of nutritional improvement of peas (*Pisum sativum* L.)

S.A. Nagra[1] and N. Bhatty[2]

تقييم التحسُّن في القيمة التغذوية للبازلاء في الأحياء (بمقايستها في الجرذان)

سعيد أحمد ناغرة، نكهت بتّي

الخلاصة: استهدفت هذه الدراسة معرفة القيمة التغذوية للبازلاء في شكلها النيِّئ والمطهي، وعند إضافة لحم الدواجن أو الضأن أو البقر إليها. وبيَّنت الدراسة أن البازلاء الغضَّة تحتوي على نسبة 3% من الليزين، وأن هذه النسبة انخفضت إلى 0.6% بعد الطهي. ولوحظ تحسُّن مهم إحصائياً في نسبة الكفاءة البروتينيّة PER في وجبة البازلاء بعد الطهي (P<0.05). كما لوحظ تحسُّن يُعْتَدُّ به إحصائياً في القابلية للهضم، وفي صافي الاستفادة من البروتين NPU (P<0.05). وقد أدَّت إضافة لحم الدجاج أو الضأن أو البقر إلى البازلاء المطهية إلى تحسُّن يُعْتَدُّ به إحصائياً في نسبة الكفاءة البروتينيّة (P<0.05). وبيَّنت الدراسة ارتفاع قيمة كلٍّ من نسبة الكفاءة البروتينيّة، والقابلية للهضم، وصافي الاستفادة من البروتين، في الوجبات المضاف إليها لحم الضأن أو البقر بنسبة 15 – 20%.

ABSTRACT This study was conducted to determine the nutritional value of peas (*Pisum sativum* L.) in raw and cooked form and when supplemented with chicken, mutton or beef. Peas had 3.0% lysine, which decreased to 0.6% on cooking. Protein efficiency ratio (PER) of the raw pea diet improved significantly on cooking ($P < 0.05$). True digestibility (TD) and net protein utilization (NPU) also showed significant improvement ($P < 0.05$). Supplementation of cooked peas with 15% poultry meat, mutton or beef improved PER significantly ($P < 0.05$). Higher PER, TD and NPU values were observed in diets supplemented with 15%–20% mutton or beef.

Évaluation *in vivo* (dosage chez le rat) de l'amélioration nutritionnelle due au petit pois (*Pisum sativum L.*)

RÉSUMÉ Cette étude a été menée afin de déterminer la valeur nutritionnelle du petit pois (*Pisum sativum L.*) cru et cuit et complémenté par de la viande de volaille, de mouton ou de bœuf. Le petit pois contient à l'origine 3,0 % de lysine, teneur qui tombe à 0,6 % à la cuisson. Le coefficient d'efficacité protéique (CEP) du régime alimentaire basé sur la consommation du petit pois cru a montré une amélioration significative après cuisson ($p < 0,05$). La digestibilité vraie (DV) et l'utilisation protéique nette (UPN) ont également mis en évidence une amélioration significative ($p < 0,05$). Une supplémentation en petits pois cuits avec 15 % de viande de volaille, de mouton ou de bœuf a entraîné une amélioration significative du CEP ($p < 0,05$). Des valeurs supérieures de CEP, de DV et d'UPN se sont avérées associées à des régimes alimentaires supplémentés de viande de mouton ou de bœuf à hauteur de 15 à 20 %.

[1]*Biochemistry Section, Institute of Chemistry, University of the Punjab, Lahore, Pakistan (Correspondence to S.A. Nagra: sanagra@gmail.com).*
[2]*Department of Rural Home Economics, University of Agriculture, Faisalabad, Pakistan.*
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Introduction

Peas have been a staple diet of man and livestock since prehistoric times. In certain regions, people have always relied on peas and other pulses to provide protein to complement the cereals in their diet [*1*]. They are now grown in temperate regions all over the world, including higher elevations of the tropics. Cultivation is favoured in dry areas where the weather is cool and moisture is abundant during early growth, but where rainfall is minimal during the later stages of development. Peas are an important part of the crop rotation in many countries of Central America and Europe as well as India, Mayanmar and Pakistan. Green peas have become an important green vegetable in many developed countries. They offer a bulk source of seed protein for man and animals from a relatively short growing season compared with other legumes [*2*]. Pakistan produces about 16 000 tonnes of peas annually and the area under cultivation is about 3.2 thousand hectares [*3*].

This study is a continuation of earlier investigations through which we established the effect of cooking and supplementation with different kinds of meat on the nutritional improvement of mash (*Vigna mung*), mung (*Vigna radiate*), masoor (*Lens esculenta*), lobia (*Phaseolus vulgaris*) and gram (*Cicer arietinum*) [*4–8*]. To assess the extent of improvement in the nutritional quality of peas (*Pisum sativum* L.) by cooking and supplementation with different kinds of meats, biological trials were conducted on albino rats.

Methods

Formulation of diets

Peas (*Pisum sativum* L.) were procured from the local market and dried in a hot air oven at 105 °C for around 4 hours. Flour was obtained by grinding and sieving through a 20 mm mesh sieve. The flour was stored in airtight jars at room temperature until use. Similarly, flour was obtained and stored after cooking the peas by a conventional method as described by Bhatty et al. [*4*]. Briefly, the peas were put in a pot, covered with fresh water to 2.5 cm above the surface and boiled (100 °C) on a natural gas cooker for 40 minutes at high heat, then simmered for 30 minutes. At this stage peas became tender.

Maize starch, corn oil and casein (Merck DGaA, Darmstadt) used for the preparation of the standard diet (protein content 84%) were also purchased from the market.

The experimental diets were prepared using raw and cooked peas. Diets were also prepared by replacing 10%, 15% and 20% of the protein of cooked peas with the same amount of protein derived from lean meat: poultry (chicken), mutton or beef. The composition of the diets is shown in Table 1. The casein diet served as a standard and a nitrogen-free diet was used to determine the endogenous nitrogen. The mineral mixture used in the preparation of the experimental diets was prepared according to the formula of Oser [*9*] and the vitamin mixture according to Miller and Bender [*10*].

Biological assay

Biological evaluation was done by measuring the protein quality of diets containing peas in raw and cooked form with and without supplementation with meat.

Albino rats of the *Sprague–Dawley* strain were used. During gestation and nursing, the mothers were fed a balanced stock diet. Litters born to different mothers within 24 hours were taken to be of same age. Weaning was done at 21 days of age. The rats were then put on stock diet for 7 days prior to the experiment. They were arbitrarily divided into experimental units

Table 1 **Composition of experimental diets**

Diet	Ingredients (%)[a]						
	Maize starch	Casein	Raw peas	Cooked peas	Poultry meat	Mutton	Beef
Standard	56.1	12.5	–	–	–	–	–
Nitrogen free	68.6	–	–	–	–	–	–
Raw peas	22.8	–	45.9	–	–	–	–
Cooked peas	25.1	–	–	43.5	–	–	–
Cooked peas + 10% poultry meat	27.7	–	–	39.1	1.8	–	–
Cooked peas + 15% poultry meat	29.0	–	–	37.0	2.7	–	–
Cooked peas + 20% poultry meat	30.4	–	–	34.7	3.6	–	–
Cooked peas + 10% mutton	27.5	–	–	39.1	–	2.0	–
Cooked peas + 15% mutton	28.7	–	–	37.0	–	2.9	–
Cooked peas + 20% mutton	29.9	–	–	34.8	–	3.9	–
Cooked peas + 10% beef	27.7	–	–	39.3	–	–	1.8
Cooked peas + 15% beef	28.9	–	–	37.0	–	–	2.7
Cooked peas + 20% beef	30.3	–	–	34.8	–	–	3.6

[a]*In addition, each diet contained glucose 15.0%, corn oil 5.0%, vitamin mixture 3.5%, mineral mixture 3.1%, dicalcium phosphate 2.5%, calcium carbonate 2.0%, choline chloride 0.15% and inositol 0.1%.*

of 2 rats each in such a way that the initial weight of the rats in each cage was 90 g; 3 experimental units were randomly allotted to each diet. The rats were fed the allotted diet *ad libitum* for a period of 10 days. During this period fresh, clean water was made available at all times and room temperature was maintained at 24–27 °C. The weight of each replicate was recorded daily. The faecal matter from each cage was collected daily, dried to a constant weight and stored in glass bottles for nitrogen determination. At the end of 10 days trial, all the rats were killed with an overdose of chloroform and their cranial and abdominal cavities were opened. The carcasses of each group, inclusive of intestinal contents, were weighed before and after drying at 105 °C to constant weight. The dried carcasses were run twice through a domestic mincer and stored in airtight bottles for estimation of body nitrogen. The nitrogen content of the diet, faeces and carcasses of each group was determined by Khjeldahl's method [*11*]. Data obtained was used to determine the protein efficiency ratio (PER), true digestibility (TD) and net protein utilization (NPU) [*10*].

Table 2 **Proximate composition of peas**

Proximate principals	Raw peas %	Cooked peas %
Moisture	8.0	8.0
Crude protein	23.7	23.2
Ether extract	2.3	2.5
Crude fibre	6.8	6.5
Nitrogen-free extract	62.4	64.6
Ash	4.8	3.2

Samples of peas were analysed for proximate composition (moisture, crude protein, ether extract, crude fibre, total ash and nitrogen free extract, according to standard methods [*11*]. Samples of the

3 kinds of meat were analysed for protein content only.

Amino acid analysis

The amino acid analysis of peas was carried out by the method of Spackman, Steir and Morre using a Beckman Model 120C amino acid analyser (Beckman, Fullerton, California) [*12*].

Statistical analysis

The data obtained for PER, TD and NPU were used for analysis of variance using a completely randomized design. The analysis was computed using *SPSS-400*. Multiple comparisons of means were made using Fisher protected least significant difference (PLSD) test [*13*].

Results

The change in the proximate principals of raw and cooked peas is given in Table 2. Cooking resulted in a slight reduction in crude protein, crude fibre and ash. The lysine content of raw peas was 3.0%, which decreased to 0.6% after cooking.

Comparison of experimental diets containing peas only with standard casein diet (Table 3) indicated that the PER of cooked peas was very close to that of the standard casein diet whereas the diet containing raw peas had a PER value almost half of the cooked pea diet. Other biological parameters, TD and NPU, also showed improvement when peas were cooked.

Biological evaluation of experimental diets is given in Table 4. On average, inclusion of 15% protein from poultry meat yielded comparatively better results in terms of PER, TD and NPU as it is cheaper than the other 2 meats.

Discussion

Chemical composition

Proximate composition of raw peas in our study was 8.0% moisture and 23.7% protein. Augustin and Klein [*14*] reported similar amounts of moisture and protein in raw peas. Ali-Khan and Youngs [*15*] showed the protein content to be 22%–23% in field peas. The variation in crude protein content is a reflection of varietal differences and may be attributed to genetic and environmental factors.

In this study, ether extract was 2.3% in raw peas. Augustin and Klein reported a lower value [*14*] and other reports of fat content range from 1.0% to 3.1% [*2*]. These variations could be due to variety differences. Raw peas had 6.8% crude fibre. Augustin and Klein [*14*] reported a much higher fibre content.

Table 3 **Comparison of experimental diets containing peas only and standard casein diet**

Parameter	Diet					
	Standard		Raw peas		Cooked peas	
	Mean	SD	Mean	SD	Mean	SD
Protein efficiency ratio	2.1[a]	0.05	01.4[b]	0.08	2.0[a]	0.04
True digestibility %	89.5[a]	0.4	74.7[b]	0.6	79.8[c]	0.7
Net protein utilization %	55.8[a]	0.4	41.6[b]	0.3	46.3[c]	0.7

[a,b,c]Means with different superscripts in a row are significantly different at P < *0.05. SD = standard deviation.*

Table 4 **Biological evaluation of experimental diets containing cooked peas and different supplementary levels of poultry meat, mutton and beef**

Parameters	Level of supplementation (%)					
	10		15		20	
	Mean	SD	Mean	SD	Mean	SD
Protein efficiency ratio						
Poultry meat	2.1[a]	0.02	2.2[a]	0.05	2.2[a]	0.03
Mutton	2.2[a]	0.02	2.2[a]	0.03	2.2[a]	0.03
Beef	2.1[a]	0.04	2.2[a]	0.02	2.2[a]	0.03
True digestibility %						
Poultry meat	81.7[a]	0.4	84.1[b]	0.6	85.8[b]	0.59
Mutton	81.8[a]	0.4	86.5[b]	0.7	87.6[b]	0.3
Beef	77.9[a]	0.4	81.4[b]	0.6	84.4c	0.8
Net protein utilization %						
Poultry meat	46.4[a]	0.7	53.2[b]	0.7	54.2[b]	0.8
Mutton	43.6	0.3	49.7[b]	0.6	52.1[b]	0.6
Beef	45.9[a]	0.4	48.5[b]	0.6	50.5[b]	0.4

[a,b,c]*Means with different superscripts in a row are significantly different at* P < *0.05*
SD = standard deviation.

Nitrogen free extract in raw peas was 62.4% [*14*]. Savage and Deo reported nitrogen free extract in the range 60.0%–71.7% [*2*]. Pea seeds had 4.8% ash; other researchers have reported ash contents of 2.4%–4.1% [*16*] and 1.0%–3.4% [*2*].

Raw peas contained 3.0% lysine, providing well above the recommended requirement (12 mg/kg body weight per day) [*17*], making peas an ideal supplement to a cereal based diet. Legumes are considered a good source of lysine and as such provide this essential amino acid to enhance the nutritive value of the protein in mixed diets [*18*]. Savage and Deo reported lysine content at 6.22%–12.3% in peas [*2*]. El-Refai, Gouda and Ammar showed that in general the amino acid content changed only slightly during storage except for small decreases in lysine, cystine, methionine and tryptophan [*19*]. Sarwar, Sosulski and Bell concluded that field peas were superior to soybean when blended with wheat flour or supplemented with additional amino acids [*20*].

A slight lowering was observed in proximate crude protein content of peas after cooking. Other studies have found similar changes [*19,21*].

James and Hove reported that improvement in nutritive value on cooking was a result of the destruction of anti-nutritive factors [*22*]. Manan et al. observed that cooking peas resulted in considerable reduction in the phytic acid content of Pakistani varieties, without any loss of total phosphorus [*23*]. The nutritive value of peas considerably improved on cooking, suggesting that other water soluble and or heat labile anti-nutritive factors might be more important than phytic acid in affecting the overall nutritive quality of seeds. It was observed that cooking affected the amino acid profile. All amino acids showed losses during cooking of peas.

Biological evaluation

Protein efficiency ratio (PER)

Raw peas had PER 1.4, which increased significantly on cooking to 2.0. James and Hove showed a similar increase, 1.87 to 2.21 [*22*]. The improvement in nutritive value on cooking could be due to destruction of anti-nutritive factors. Shah also reported a significant increase in the body weight gain of rats due to cooking of the whole seed [*24*]. Supplementation of a diet based on cooked peas with different types of meat also showed significant improvement over a diet containing raw peas, irrespective of the kind of meat. However, supplementation with different types of meat did not improve the PER significantly over that of the diet containing only cooked peas.

True digestibility (TD)

The TD of protein of peas increased significantly on cooking from 74.7% to 79.8%. It has been reported that protein TD of autoclaved peas increased from 85% to 88% [*20*]. Goodlad and Mather, however, claimed that there were only minor effects of cooking on the digestibility of non-starch polysaccharides and their constituent sugars [*25*]. Fleming and Vose showed that the *in vivo* digestibility of raw and cooked starch from peas was high in rat experiments [*26*]. The increase in digestibility on cooking may be due to the elimination of trypsin and chymotrypsin inhibitors.

In our study, TD also increased significantly when the pea-based diet was supplemented with meat, and increased with increasing level of supplementation. The TD of the diet containing peas supplemented with 20% mutton was significantly higher than the digestibility of other diets.

Net protein utilization (NPU)

The NPU of the diet containing raw peas was 41.6% and on cooking it significantly increased to 46.3%. Shah showed NPU values of 42.4%–46.8% in raw peas and 49.0%–52.0% in cooked peas [*24*]. The NPU values were significantly higher when were supplemented with 20% poultry meat. The NPU also increased with the increase in the levels of supplemental mutton and beef beyond the 10% level. Bell and Youngs reported that biological value of pea protein concentrate alone was low but was considerably improved by the addition of methionine [*27*]. Shah reported a non-significant increase in biological value on cooking; reduction in biological value was suggested as being due to the destruction or leaching of essential amino acids during the cooking process [*24*].

Conclusion

Overall, supplementation of peas with 15% poultry meat optimally enhances the protein quality. Cooking alone can also be used for the improvement of protein quality of peas.

Although this study was conducted on laboratory rats, it provides a rationale for the supplementation of peas with small quantities of poultry meat for the nutritional rehabilitation of poorly-fed communities.

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Technical consultation to review the regional food-based dietary guidelines

The World Health Organization Regional Office for the Eastern Mediterranean organized the above-mentioned technical consultation in Cairo, Egypt, from 2 to 4 April 2007.

The objectives of the consultation were:

- to review the draft regional food-based dietary guidelines under preparation;
- to incorporate additional relevant food and dietary information from Member States; and to
- finalize the content and format of the regional food-based dietary guidelines.

Experts from Egypt, Islamic Republic of Iran, Lebanon, Pakistan, Qatar, Saudi Arabia, Syrian Arab Republic, Tunisia, United Arab Emirates, United Kingdom, as well as WHO concerned staff, participated in this consultation.