Evaluation of nutritional quality of a novel pea protein

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ABSTRACT: A rat feeding study was conducted to determine the nutritional quality of a novel pea protein using the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) methodology. 30 weanling male Sprague-Dawley rats were randomly assigned to one of three diets: protein-free feed (Control), high nitrogen casein feed (Casein), or pea protein. True Digestibility of the protein feeds was 98.0±1.2 percent for Casein and 97.3±1.6 percent for pea protein. The estimated PDCAAS of pea protein was 85 percent for children 3 to 10 years and 93 percent for adults. The pea protein evaluated in this study has a protein quality similar to that of casein, eggs, and soy and much higher than that of common vegetable-based protein sources.

INTRODUCTION

Adequate dietary protein intake in the human diet is vital for promotion of optimal health including weight control (1), bone health (2, 3), cardiovascular health (4), glycemic control (5), and muscle maintenance (6-8). Most research on the physiological effects of dietary protein consumption has focused on protein quantity (e.g. total daily protein consumption, protein consumption in relationship to body weight, or proportion of protein intake relative to other macronutrients). However, there is accumulating evidence that protein quality also plays an integral role in influencing factors such as appetite, energy metabolism, and skeletal muscle protein synthesis (9-11). The nutritional quality of a protein is determined by the ability of the protein to meet the nitrogen and amino acid demands of an organism. The most widely accepted method for assessing protein quality is the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) (12). Despite some limitations, the PDCAAS offers a uniform methodology to assess the quality of different proteins. The FAO/WHO report on protein quality evaluation considered this methodology for measurement of digestibility and concluded that values for true faecal digestibility obtained in rat trials corresponded closely with values obtained from human studies (13). A PDCAAS score of 100 percent represents a protein that, after digestion, provides 100 percent or more of the indispensable amino acids required by the organism.
Pea protein is an excellent source of protein with high bioavailability, excellent digestibility, and, unlike animal proteins, offers beneficial long-term effects on human health and global ecology (14, 15). The purpose of this study was to evaluate the nutritional quality of a novel pea protein isolate.

MATERIALS AND METHODS

Animals

This study utilized 30 weanling male Sprague-Dawley rats (age: 21-28 days, weight: 50-70 g) from the same colony. The experiment was conducted in accordance with guidelines for the care and use of laboratory animals at Shanghai Daan Centre for Medical Laboratory (Shanghai, China).

Diets

The rats were randomly assigned to one of three diets: protein-free feed (Control), high nitrogen casein feed (Casein), or NUTRALYS® pea protein soluble grade (Roquette Frères, Lestrem, France). The composition of each feed is described in Table 1. The Casein and pea protein feeds each contained 100 g protein/kg.

Component	*Percentage*		
	Protein-free	*Casein*	*Pea protein*
Corn starch	80.3	69.8	68.8
NUTRALYS® pea protein	—	—	11.5
Casein	—	10.5	—
Corn oil	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0
AIN 76 Mineral mixture	3.5	3.5	3.5
AIN76-VX Vitamin mixture	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
tert-Butylhydroquinone	10^{-3}	10^{-3}	10^{-3}

Table 1. Feed composition.

The protein content of the Casein and pea protein groups were above the 10 percent threshold recommended by the FAO/WHO for digestibility studies. The pea protein concentration was 11.5 percent, which adjusts to 10 percent when accounting for the 86.8 percent protein content. Casein was selected as the control protein in this study since it is widely utilized for digestibility trials due to the slow release of amino acids and excellent nitrogen retention.
The pea protein under investigation is a vegetable protein extracted from yellow pea (*Pisum sativum*). Peas are cleaned and ground in a dry process to produce pea flour. The flour is then hydrated and the pea starch and internal fibre are extracted separately. The protein fraction is then coagulated for further purification and, finally, carefully dried in a multi-stage spray dryer. The resulting highly purified pea protein isolate contains on average 85 percent protein, 7 percent fat, 3 percent carbohydrate, and 5 percent ash on a dry

matter basis. The diet administrated to the rats consisted of 10 percent corn oil, 5 percent cellulose, vitamins, minerals, preservatives and an adjusted percentage of corn starch depending on the protein composition of the diet.

Methods

Animals were kept in a temperature and humidity controlled room with a 12-hour light/dark cycle. Animals were housed in individual stainless steel screen-bottom cages lined with filter paper with removable food cups. Water was provided *ad libitum* throughout the day.

At baseline, the animals were weighed. After an acclimation phase of 2 days in which the rats were fed a commercial laboratory rat chow diet (AIN-76), animals were fed 15 g dry weight of their respective diets for 9 days. Feces from each rat's cage over the final 5 days of feeding were collected, weighed, and frozen until analysis. Uneaten food from each rat's cage over the final 5 days of feeding was collected and weighed to attain an accurate estimate of food consumption.

In order to determine protein quality of each feed using PDCAAS, the True Digestibility (TD) of each feed was first determined, defined as the difference between intake of nitrogen and output of fecal nitrogen. This is expressed as a percentage of nitrogen intake, where fecal nitrogen is corrected for metabolic fecal nitrogen loss as measured using a protein-free diet as shown below:

$$\text{True Digestibility} = \frac{\text{Nitrogen Intake} - (\text{Fecal Nitrogen} - \text{Fecal Metabolic Nitrogen})}{\text{Nitrogen Intake}}$$

In order to calculate nitrogen intake, the dried weight of the uneaten food was subtracted from the initial amount of food given and multiplied by the percent nitrogen of the respective feed. Fecal nitrogen was calculated as the weight and nitrogen content of the feces using the Kjeldahl method.

Amino acid score

Amino acid composition of protein-containing feeds was determined by hydrolyzing the protein into its component amino acids and then separating the amino acids using high performance liquid chromatography (HPLC). First, a sample of feed containing ~10 mg nitrogen was digested in 400 µg 6 M HCl refluxed at 100°C for 24 hours in a sealed nitrogen-purged ampoule.

The digest was collected, passed through a nitrogen-free filter, and diluted to 500 µl with distilled, deionized water. A 5 µl aliquot was then dried under a vacuum and the residue was redissolved into an eluant appropriate for HPLC. Following HPLC, amino acids were derivatized. Methionine and cysteine were then oxidized to methionine sulfone and cysteic acid using performic acid prior to acid hydrolysis.

Amino Acid	*Amino Acid (mg/g crude protein)*			*Amino Acid Score*	
	Pea protein	*3-10 yr**	*Adult**	*3-10 yr*	*Adult*
Histidine	25	16	15	1.56	1.67
Isoleucine	45	31	30	1.45	1.50
Leucine	84	61	59	1.38	1.42
Lysine	72	48	45	1.50	1.60
Methionine+Cysteine	21	24	22	0.88	0.95
Phenylalanine+Tyrosine	91	41	38	2.22	2.39
Threonine	39	25	23	1.56	1.70
Tryptophan	10	7	6	1.43	1.67
Valine	50	40	39	1.25	1.28

Table 2. Amino Acid Score for pea protein.
*FAO/WHO/UNU (2008) Reference Values.

The amino acid score was then calculated from an amino acid profile as follows:

$$\text{Amino Acid Score} = \frac{\text{mg of amino acid in 1 g of pea protein}}{\text{mg of amino acid in 1 g of reference protein}}$$

The denominator is obtained from the FAO/WHO (2008) reference values for pre-school children aged 3 to 10 years and adults, respectively (16).

Protein digestibility-corrected amino acid score

Of the amino acid scores derived for the feed, the amino acid with the lowest amino acid score is the limiting amino acid (Methionine+Cysteine for pea protein) (Table 2).

This limiting amino acid score is then multiplied by the TD, resulting in the PDCAAS score.

$$\text{PDCAAS} = \frac{\text{mg of first limiting amino acid in 1 g study product protein}}{\text{mg of the same amino acid in 1 g reference protein}} \times \text{TD}$$

Data analysis

A minimum of 10 animals per feed group is recommended to conduct a PDCAAS study (17) and this served as the basis for the chosen sample size in this study. The TD values of each feed group are reported as mean ± SD.

RESULTS

The mean daily feed consumption was 11±2g with no significant differences among groups. True Digestibility of the protein feeds was 98.0±1.2 percent for Casein and 97.3±1.6 percent for pea protein. The estimated PDCAAS of pea protein was 85.1 percent for children 3 to 10 years and 92.8 percent for adults. Despite the fact that Methionine+Cysteine is the limiting amino acid in pea protein, the addition of 0.3 percent methionine to pea protein resulted in no appreciable change in PDCAAS (data not shown).

DISCUSSION

Protein Source	PDCAAS (%)
Casein	100
Egg white	100
Soy protein	100
Beef	92
Fruits	76
Vegetable	73
Legumes	70
Cereals	59
Whole wheat	42

Table 3. Protein digestibility-corrected amino acid scores of pea protein in comparison to other food proteins (12).
Pea protein PDCAAS = 93%

The PDCAAS of pea protein is similar to that of casein, egg white, soy protein, and beef and is much higher than non-animal protein sources such as vegetables, legumes, and cereals (Table 3). Furthermore, the digestibility of the pea protein under study is greater than that of other pea protein concentrates (18-20).
Although vegetables are generally low in calories and fat and high in fibre, they are not generally considered rich protein sources. Pea protein isolate, however, allows consumption of a vegetable source that is a rich source of high quality protein. Another distinct advantage of pea protein over other vegetable protein isolates is that it contains no commonly occurring anti-nutritional factors of vegetables based on testing using the AOCS method Ba 12-75 (21), which are known to diminish nutritional potential by interfering with the intake, digestibility, or bioavailability of its nutrient (22, 23).
The results of this animal study are congruent with a study by Mariotti and colleagues who demonstrated that raw purified pea protein had excellent nutritional value in humans (24). A growing body of evidence suggests that increasing protein intake to levels greater than the current RDA of 0.8 g/kg/day is beneficial for active individuals and strength and endurance athletes (range=1.4-2.0 g/kg/d depending on activity and intensity) (25, 26) as well as the elderly (1.0-1.5 g/kg/d) (27, 28). Protein is more satiating than the isocaloric ingestion of carbohydrate or fat (29-31), an effect influenced by peripheral hormone secretion and modulation of neuronal pathways in the brain stem and hypothalamus that regulate satiety (32).
In active individuals and athletes, additional protein consumption allows for faster recovery from exercise, strengthens immune defenses, and maintains body lean mass (26). In the elderly, higher protein consumption improves muscle mass, muscular strength, physical function, immunity, wound healing, blood pressure, and bone strength (27). Incorporation of high quality vegetable-based protein sources has several health and ecological advantages compared to traditional animal-based proteins. Long-term consumption of large amounts of animal-based proteins is associated with a number of health risks largely due to excess fat and cholesterol and lack of fibre. In contrast, vegetable-rich diets are associated with lower body fat percentage, blood pressure, and LDL cholesterol as well as lower cancer rates and greater life expectancy (33). Furthermore, the land requirements for production of meat proteins are 10 times greater than for plant-based proteins. Industrial agriculture produces large amounts of animal manure, which causes pollution of drinking water and vegetables. Finally, animal production requires significant energy amounts and contributes to deforestation, overgrazing, and overfishing (34, 35). This study had several limitations. First, metabolic cages were not utilized in this study, which may have contributed to inaccuracies in fecal nitrogen determination. Second, as the impact of the feeding on body weight gain was not an outcome of interest, we did not measure the body weight evolution in this short term study. Third, the results of this animal study may not necessarily carryover in humans and, therefore, human clinical trials with pea protein are warranted. Finally, the clinical benefits derived from ingestion of a high quality pea protein must be determined. In conclusion, the novel pea protein under investigation has a TD and PDCAAS similar to that of casein, eggs, and soy and much higher than that of non-animal protein sources. The pea protein is a high quality protein that may be incorporated into the diet to aid with increasing dietary protein intake while deriving the health and environmentally friendly benefits of a vegetable-based food ingredient.

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