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Protein quality of selected edible animal and plant protein sources using rat bio-assay

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Abstract: Protein efficiency ratio (PER) and protein digestibility are important parameters used in protein quality determination. Protein nutritive values of selected protein sources: buffalo meat, casein, soy protein isolate, and tempeh, with sodium caseinate as a reference formulation, were evaluated. Determination of proximate analysis, protein quality and protein digestibility were monitored. Procedures for evaluation of protein quality and digestibility included PER using the rat bioassay and *in vivo* Apparent Protein Digestibility (APD). The rats fed with buffalo meat had the highest mean increase in body weight (102.73g±8.95) while rats fed with tempeh had the lowest mean for increase in body weight (16.34g±9.11). Although the mean for body weight gained showed significant differences between all treatments ($P<0.05$), there was no significant difference ($P>0.05$) found between casein and soy protein isolate for total food intake. For the PER value, buffalo meat had the highest value (2.99), followed by sodium caseinate (2.41), casein (1.93), soy protein isolate (1.52) and tempeh (1.10). The PER value for buffalo meat (2.99) was higher than sodium caseinate (2.41) while the rest of the treatment were comparatively lower than sodium caseinate. For the *in vivo* apparent protein digestibility, tempeh had the highest value (91.41%±3.76), followed by casein (91.34%±3.15), buffalo meat (90.79%±1.44), soy protein isolate (89.52%±2.96) and sodium caseinate (89.47%±2.31).

Keywords: Protein efficiency ratio, apparent protein digestibility, protein quality

Introduction

Formulated diet plays an important role as a source of nutrients, and protein is recognized as one of the most important dietary components (Goytortúa-Bores *et al*., 2006). Protein quality can be classified into two groups: low and high quality proteins. Low quality protein does not contain all essential amino acids required for use in protein synthesis whereas high quality protein contains most of the essential amino acids that is needed for the function of the human body system. Plant proteins are considered to be of lower quality than animal protein because they have lower content of certain essential amino acids. Nevertheless, proteins from either source provide amino acids which are important material for the protein synthesis component of metabolism and as a source of energy. Generally protein from animal foods such as dairy products, eggs, meats, fish and poultry is of higher quality than protein from plant foods such as pasta, rice, fruits and vegetables.

Growing concern about food quality has led scientists to look for methods of measuring and defining the quality of proteins. The rat-based Protein Efficiency Ratio (PER) assay (AOAC 1984) was easy to conduct and had been used extensively. The PER was the standard widely used by the U.S. food industry to evaluate the quality of protein in food and was also used to calculate the U.S Recommended Daily Allowance (USRDA) for protein shown on food tables in the United States (Endres, 2001).

PER is a measure of protein quality which is usually used to calculate protein quality by putting young animals on diets at 10% protein by weight with various test proteins, and monitoring their growth. Osborne et al. (1971) observed that young rats fed with certain proteins gained little weight and ate little protein whereas those which were fed better quality proteins gained more weight and consumed more protein.

In this study, the protein quality of formulated diets were evaluated the using rat bioassay of protein efficiency ratio (rat–PER), as well as *in vivo* digestibility of selected high protein food sources such as buffalo meat, milk casein, soy protein isolate and tempeh, a fermented soy bean product.

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Materials and Methods

Proximate analysis

Protein, fat, moisture and ash were determined using the Association of Official Analytical Chemists (AOAC, 1984). Nitrogen content was determined using the micro-Kjeldahl procedure.

The protein content was calculated by this formula.

$$\% \text{ of Protein} = \frac{(\text{mL of sample} - \text{mL of blank}) \times \text{N of standard acid} \times 0.0140067 \times 100 \times 6.25}{\text{N in diet (g)}}$$

* mL of hydrochloric acid required to titrate sample solution.

The moisture and ash content were determined using the oven method. They were calculated using the formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

$$\text{Ash (\%)} = \frac{\text{Weight of ash with crucible} - \text{Weight of crucible}}{\text{Weight of sample} - \text{Weight of crucible}} \times 100$$

Soxhlet method was used to determine fat content. It was calculated as:

$$\text{Fat (\%)} = \frac{[(\text{Weight of flask + fat}) - (\text{Weight of flask})]}{\text{Weight of sample}} \times 100$$

Rat diet preparation

Formulation of diet was done using the procedures for PER as outlined by AOAC 1984 with sodium caseinate as reference protein. Other components included in the diet were ash mix (USP XVII), vitamin mix AOAC (CA 40055), corn starch, cellulose, sucrose and palm oil. Calculation of ingredient in the diet formulation was based on the proximate analysis of the test protein. After diet preparation for each samples and reference protein (sodium caseinate), the proximate analysis were repeated to ensure the diet formulation was done correctly following the recommendation of AOAC (1984). Each type of diet formulation (tempeh, buffalo meat, sodium caseinate, soy protein isolate, and casein) were fed to 10 rats (Sprague-Dawley Strain) obtained from the animal laboratory at UKM Bangi, Selangor. The total rat diet prepared for each protein source for PER assay was calculated as:

Diet requirement = X g/day × number of days × number of rats per treatment

= 17 g/day × 28 days × 10 rats

= 4670 g

ALACID ™ composing of mineral acid casein and caseinates was obtained from local supplier (PERNIAGAAN USAHA CAHAYA)

Rat bioassay

Approximately 4 four weeks were taken to conduct the protein quality study for each sample to determine PER and *in vivo* apparent protein digestibility. The 28 days old rats were placed in individual cages and randomly assigned by treatment to individual cages. The weight range for the rats used was between 41.4–93.17 g with the mean value of 65.05±2.32 g. Prior to feeding the experimental diets, the rats were placed on an adaptation diet for a period of three days (AOAC, 1984; Babji and Selvakumari, 1989)

PER assay

Food and water were supplied *ad libitum*. Body weight was recorded for 0 day and every two days for 28 days. For determination of feed intake, feces and the spilled feed were collected daily, dried in oven (100ºC) for an hour, and then analyzed for moisture content before weighing (AOAC, 1984). The PER is calculated using the formula:

$$\text{PER} = \frac{\text{Increase in body weight (g)}}{\text{Weight of protein consumed (g)}}$$

Adjusted PER

The PER result for sodium caseinate used as the control was arbitrarily adjusted to a value of 2.5. Other test protein treatments were then similarly adjusted to the standard of 2.5 to permit comparison of PER tests on different types of proteins in this study with those from other studies.

In vivo apparent protein digestibility (APD)

Food consumption and fecal output data were recorded daily for eight days (day 10 – 18) of the 28 days to determine the *in vivo* APD (AOAC 1984). It was calculated as:

$$\textit{In vivo}\ \text{APD (\%)} = \frac{\text{N in diet (g) - N in feces (g)}}{\text{N in diet (g)}} \times 100$$

Statistical analysis

All statistical computation was performed with ANOVA followed by Duncan with Statistical Package for Social Science (SPSS) software version 12.0.

Result and Discussion

Proximate analysis

Data on proximate analysis and chemical analysis were calculated based on dry matter basis as shown in Table 1. The data indicated that the crude protein of soy protein isolate (95.00%) was the highest followed by buffalo meat (88.60%), casein (87.50%) and tempeh (54.68%). The fat content of tempeh (22.41%) was significantly higher ($P< 0.05$) than buffalo meat (11.18%). Casein (11%) had higher moisture content ($P< 0.05$) while there was no significant difference in moisture among other diet composition. Ash content of buffalo meat (4.09%) was significantly higher ($P< 0.05$) than tempeh (1.79%).

Rat bioassay

In the rat bioassay, all rats survived until the end of the observation study and gained positive body weight (Figure 1). As shown in Table 2, the rats fed with the buffalo meat diet had the highest mean body weight (102.73g±8.95) compared to other treatments. The lowest mean body weight (16.34g±9.11) was observed from the group of rats fed with tempeh. The mean in body weight gained for all four types of diets showed significant difference at ($P<0.05$). However, other diets showed no significant difference ($P>0.05$) except for tempeh and buffalo meat for total feed intake (TFI). From the study, it was found that the total consumption by rats for buffalo meat diet was the highest (356.98 g) followed by casein (250.19 g), soy protein isolate (236.29 g) and tempeh (200.37g) and with the reference protein, sodium caseinate of (252.94 g).

Protein efficiency rate (PER)

PER data for rats fed with sodium caseinate as the reference followed by treatment diets of casein, soy protein isolate, tempeh and buffalo meat were shown in Table 2. The results of PER values obtained from the study were 2.99, 2.41, 1.93, 1.52, and 1.10 for buffalo meat, sodium caseinate, casein, soy protein isolate and tempeh respectively. Buffalo Meat had the highest PER value while tempeh displayed the lowest PER value.

The PER value of buffalo meat from this study was 2.99 which was comparable with the rat PER value of beef (3.12), reported by Piva *et al*. (2000). PER value of the reference diet sodium caseinate was 2.41 which is similar to a study conducted by Sindayikengera and Xia (2005). Caseinates provided outstanding nutritional properties, contained all of the essential amino acids but had a protein efficiency ratio of 1.93 in this study. Caseinates generally have a minimum protein content of 90% on a dry solid basis (Hambraeus 1982). Except for buffalo meat, the PER values for the rest of the diets were lower compared to the reference sodium caseinate.

ALACID™ lactic acid casein diet resulted in the PER value of 1.93. This value ~~is~~ was lower when compared to the standard PER value of casein, which was 2.5 (Chapman and Mitchell, 1959). This might be due to the lactic acid casein which had a reduced content of sulfur-containing amino acid, methionine. According to Pallert and Young (1980), casein which was not enriched with sulfur containing amino acid especially methionine had~~s~~ a lower PER value. Therefore, casein enriched with methionine need to be considered in order to obtain a high PER value in an animal study. This is similar to soy protein isolate which Meyer (1967) stated could be measured by the increase in weight. PER value can be improved significantly by the addition of small quantities of methionine.

As for tempeh, the PER value was 1.10. According to Mugula (1991), the protein efficiency ratio of tempeh was 1.61±0.33. This difference in value might be due to practices of variable methods used

Table 1. Proximate analysis of raw materials (dry matter basis)

Diet Composition	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
Casein	87.50 ± 0.06 [c]	1.00 ± 0.01[c]	11.00 ± 0.20[a]	1.80 ± 0.03 [d]
*SPI	95.00 ± 1.71[ab]	0.62 ± 0.25 [c]	0.85 ± 0.08 [b]	3.78 ± 0.02 [c]
Buffalo Meat	88.60 ± 0.27[bc]	11.18± 0.16 [b]	0.61 ± 0.03 [b]	4.09 ± 0.01 [b]
Tempeh	54.68 ± 5.69 [d]	22.41± 0.79 [a]	0.59 ± 0.01 [b]	1.79 ± 0.02 [d]
Sodium Caseinate	95.95 ± 0.49 [a]	0.90 ± 0.69 [c]	0.66 ± 0.21 [b]	4.30 ± 0.04 [a]

Means and standard deviations from 10 rats. Different letter (a-d) in the same column showed significant differences at P <0.05. Data is presented in mean values (n=2).
*SPI = Soy Protein Isolate

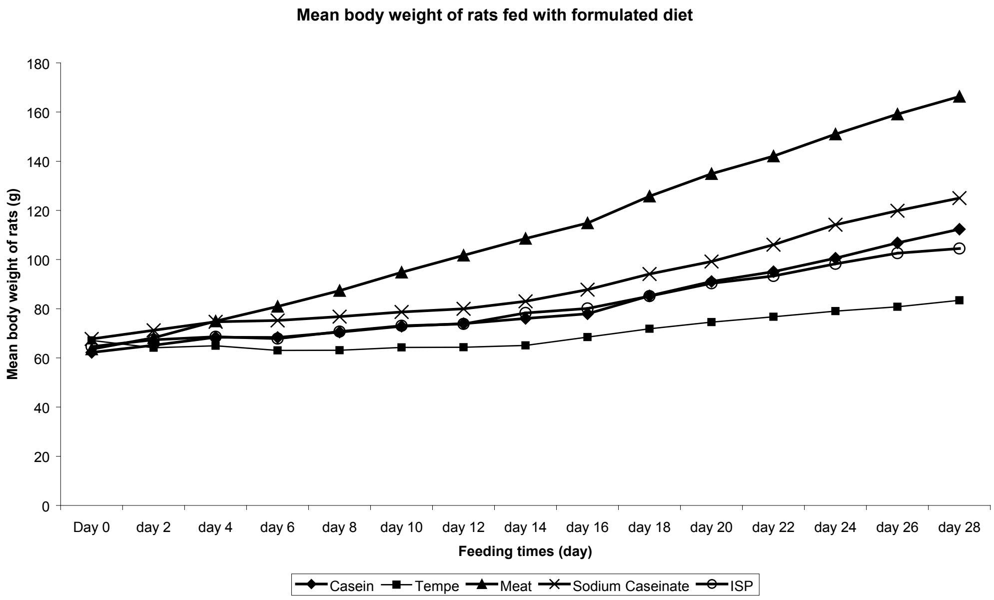

Figure 1. Mean body weight of rats fed with formulated diets of sodium caseinate, casein, soy protein isolate, tempeh and buffalo meat

Table 2. PER values of all diets in the study

Diet	Increased body weight (g±sd)	Total feed intake (g/rat/28days) (g±sd)	% protein in feed (N x 6.25)	Protein consumed (g/rat/28days) (g)	PER	Adjusted PER
buffalo Meat	102.73 ± 8.95[a]	356.98 ± 34.31[a]	9.63	34.38	2.99	3.10
Casein	50.07 ± 23.50[b]	250.19 ± 43.89[b]	10.37	25.94	1.93	2.00
*SPI	34.79 ± 14.94[c]	236.29 ± 26.81[b]	9.71	22.94	1.52	1.58
Tempeh	16.34 ± 9.11[d]	200.37 ± 36.26[c]	7.35	14.72	1.10	1.45
Sodium Caseinate	57.30 ± 14.69[b]	252.94 ± 45.12[b]	9.41	23.80	2.41	2.50

Mean and standard deviation from 10 rats (n=2). Different letters (a-d) in the same column shows significant differences at P <0.05.
* SPI: Soy Protein Isolate

Table 3. Percentage *in vivo* apparent protein digestibility of tempeh, casein, soy protein isolate, buffalo meat and sodium caseinate

Protein sources	Weight of Intake feed (g/rat/28rat) (g±sd)	% N	Total Nitrogen Consumed	Feces Dry Weight (g±sd)	% N	Total in Nitrogen Dried Feces (g/rat/28 day)	% Apparent Protein Digestibility
Tempeh	55.14±12.05	1.40	1.63±0.28	6.16±2.09	2.33	0.14±0.05	91.41 ± 3.76 [a]
Casein	76.60±10.39	1.66	1.27±0.15	5.12±1.33	2.18	0.11±0.03	91.34 ± 3.15 [a]
buffalo Meat	98.66±10.21	1.54	1.52±0.14	7.31±1.53	1.82	0.14±0.03	90.79 ± 1.44 [a]
SPI	59.68±11.37	1.76	1.05±0.20	5.41±0.72	2.10	0.11±0.02	89.52 ± 2.96 [b]
Sodium Caseinate	63.13±12.73	1.51	0.95±0.17	4.80±1.17	2.07	0.10±0.02	89.47 ± 2.31 [ab]

Mean and standard deviation from 10 rats. Different letter (a-b) in the same column shows significant difference at $P < 0.05$.

during diet preparation. Besides that, the PER value also depended on the duration of incubation during tempeh process of fermentation. There was a similar decrease in the rate of growth as the fermentation time increased. Fresh tempe had higher quality of protein when compared with tempeh which were fermented for 72 hours. Since tempeh was made from soy beans, the limiting essential amino acids in soy protein were tryptophan and cysteine (Richard *et al*., 2005).

Apparent protein digestibility (APD)

Table 3 showed the *in vivo* apparent protein digestibility (APD) of casein, soy protein isolate, buffalo meat, tempeh and sodium caseinate reference. Only soy protein isolate showed significant difference ($P>0.05$) when compared to the rest of the diets. Tempeh displayed the highest percentage of digestibility (91.41%), followed by casein (91.34%), buffalo meat (90.79%), soy protein isolate (89.52%) and sodium caseinate (89.47%). Digestibility of protein is related to the quality of protein in the feed. The higher ratio of total essential amino acid to total amino acid contents in samples resulted in rapid growth of rats. The high ratio of essential amino acids to total amino acids in meat products explain their superiority in protein quality when used as new protein sources for rats (Hoffman and Falvo, 2004).

Generally, all plant and animal proteins have approximately the same 20 amino acids. The proportion of the amino acids varies as a characteristic of the protein source. The nutritional quality of any protein relates to its amino acid composition, digestibility, and ability to supply the essential amino acids in the amounts required by the species consuming the protein (Endres, 2001). For plant protein, specifically soy protein is high in protein value, however the protein efficiency ratio is lower compared from animal protein (casein and buffalo meat). The PER for soy protein concentration is 2.1 (Richard, 2005), sodium caseinate is 2.5 (Sindayikengera and Xia 2005), casein is 2.5 (Hambraeus, 1982) and meat is 3.12 (Piva *et al*., 2000).

Conclusion

Among five types of diets, the buffalo meat had the highest value of PER, reflecting higher protein quality than the sodium caseinate reference. Tempeh diet had the highest value for *in vivo* digestibility while the lowest was soy protein isolate. In the rat bioassay, all rats gained positive body weight and rats fed with buffalo meat diet had the highest mean body weight compared to others.

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