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Protein Quality of Anchovy, Mackerel and Canned Sardine Samples

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ABSTRACT

The protein nutritive value of anchovy, mackerel and canned sardine samples together with casein as a reference formulation were evaluated. Proximate composition, protein quality and protein digestibility were determined. Procedures for evaluation included Protein Efficiency Ratio (PER) using the rat bioassay and *in vivo* Apparent Digestibility (AD). Rats fed with canned sardine diet had the highest mean body weight (154.8±12.28g) while rats fed with anchovy diet had the lowest mean body weight (145.27±15.89g) with significant differences between all the groups. Mean body weight of rats fed with selected fish diet was higher compared to rats fed with casein diet. For PER value, canned sardine has the highest value (2.48), followed by anchovy (2.46) and mackerel (2.34). PER value for all selected fish is lower than that for casein (3.14). Mackerel had the highest value of *in vivo* AD (96.99%), followed by casein (96.96%), canned sardine (96.88%) and anchovy (91.29%). In conclusion, among the types of fish compared, sardine had the highest protein quality while mackerel showed the highest digestibility.

INTRODUCTION

Formulated diet plays an important role as the 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 as high and low quality protein. 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 are needed for the functioning of human body systems. Plant proteins are often considered to be of lower quality than animal proteins because they have a lower content of certain essential amino acids. Nevertheless, protein from either source provides amino acids to humans as important materials for protein synthesis and as a source of energy. Generally, protein from animal foods (e.g., dairy products, eggs, meats, fish and poultry) are of higher quality than protein from plant foods (e.g., pasta, rice, fruits and vegetables). Protein quality of fish meal varies widely and its nutrient composition depends on many factors (Davis *et al.*, 2004).

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During the past 50 years, growing concerns about food quality have led scientists to look for methods of measuring and defining the quality of proteins, and the officially approved assay for protein nutritional quality is the rat-based Protein Efficiency Ratio (PER) bioassay (AOAC, 1980). The rat PER assay is easy to conduct and has been used extensively. The PER is the standard used by the U.S. food industry to evaluate the quality of protein in foods and also used to calculate the U.S. Recommended Daily Allowance (USRDA) for protein shown on food tables in the United States (Endres, 2001).

The Protein Efficiency Ratio (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 an attempt to compensate for the difference in food intake, they calculated PER formulation based on gain in weight per gram of protein eaten.

The US Food and Drug Administration has suggested that the use of PER with casein as a reference model for labeling protein foods (Henley, 1992). However the use of PER to estimate human protein requirements has been criticised by some authors (Young & Pellett, 1991). The present study was designed to compare the PER values and *in vivo* digestibility of three types of locally available fish using formulated casein as the reference.

METHODS AND MATERIAL

Proximate Analyses

Proximate analyses of samples were determined according to standard methods described by the Association of Official Analytical Chemists (AOAC, 2000). Nitrogen was determined using the micro-Kjeldahl procedure. The protein content was calculated by the following formula:

$$\%\ \text{protein} = \frac{\text{mL (titrate)} \times 0.1 \times 1.6 \times 6.25}{\text{Weight of sample}}$$

The oven method was used to determine moisture and ash content. The moisture content was calculated using the formula:

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

For determination of fat content, the Soxhlet method was used. It was calculated as follows:

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

Rat Diet Preparation

Each sample was ground using a meat grinder with 8mm grinder plate. Diet formulation was done using the procedures for PER as outlined in AOAC 1984 with casein from Animal Nutrition Research Council (ANRC) as reference protein. Other components included in the diet were vitamin mix AOAC (CA 40055), corn starch, cellulose, sucrose, mineral mix (USP XVII) and cooking palm oil (VESAWIT). Calculation of percentage of ingredient in diet formulation was based on the proximate analyses of the test protein. The total rat diet prepared for each protein source for PER assay was as follows:

Diet requirement
= χ g/day x number of days x number of rats per treatment
= 16 g/day x 28 days x 8 rats
= 3584 g

Table 1. Rat diet composition with 15% test protein (g/kg feed)

Diet Composition	*Casein*	*Anchovy*	*Mackerel*	*Canned Sardine*
Test Protein	488.22	491.04	448.98	675.11
VESAWIT palm oil	319.07	306.15	285.16	156.49
Ash Mixture (AOAC)	193.18	176.68	185.27	150.58
Moisture	155.13	183.01	181.05	160.91
Vitamin mixture (AOAC)	40.00	40.00	40.00	40.00
Cellulose	40.00	40.00	40.00	40.00
Sucrose	1402.20	1469.90	1049.77	1388.46
Corn Starch	1402.20	1469.90	1049.77	1388.46

Data are presented in the mean values (n=2)

Table 2. Proximate analysis of formulated rat diets

Analyses	*Casein*	*Anchovy*	*Mackerel*	*Canned Sardine*
Crude Protein (%)	15.60±0.28	15.76±0.67	15.24±0.42	15.83±3.26
Moisture Content (%)	6.60±0.14	6.25±0.07	5.80±0.71	5.20±0.28
Ash (%)	5.35±0.25	5.35±0.48	5.60±0.29	5.33±0.29
Crude fat (%)	7.52±0.49	8.31±0.12	7.81±0.01	7.44±0.28
Nitrogen (%)	2.50±0.05	2.27±0.01	2.40±0.50	2.71±0.28

Data are presented in the mean values (n=2)

After diet preparation for each type of fish and reference protein (casein), the proximate analyses were repeated to ensure the diet formulation was done correctly (Table 1). Each type of diet formulation (anchovy, canned sardine, mackerel and casein) were fed to 8 male rats (Sprague-Dawley Strain) obtained from the animal laboratory at Universiti Kebangsaan Malaysia Bangi, Selangor.

The composition of the rat diet, each with 15% of the test protein is given in Table 1 and the content of the main nutrients of the formulated diets is given in Table 2.

Rat Bioassay

Protein quality study of each samples were conducted for four weeks to determine protein efficiency ratio (PER) and *in vivo* apparent protein digestibility. The 28 days old rats were placed in individual cages and distributed into four treatment groups. The weight for rats used was between 39 – 85.5 gram with a mean of 69±11.42 g. Prior to feeding the experimental diets, the rats were placed on an adaptation diet for 3 days.

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, faeces and the spilled food were collected daily, dried in oven (100°C) for an hour, then analyzed for moisture content before weighing. The PER is calculated using the formula:

$$PER = \frac{\text{Increase in Body Weight (g)}}{\text{Weight of Protein Consumed (g)}}$$

In Vivo Apparent Protein Digestibility (AD)

Food consumption and fecal output data were recorded daily for eight day (day 10 -18) of the 28 day to determine the *in vivo* protein AD. It was calculated as follow:

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

Statistical analyses

All statistical computations were performed with ANOVA procedure followed by Duncan of Statistical Packaged for Social Sciences (SPSS) version 11.0.

RESULT AND DISCUSSION

Proximate analyses

Data on proximate and chemical analyses (calculated on dry matter basis) of the three fishes are shown in Table 3. The data indicated that the crude protein of mackerel (89.09%) was the highest followed by anchovy (81.46%) and canned sardine (59.25%).

Rat bioassay

In rat bioassay, all rats survived until the end of the observation study and gained positive body weight (Figure 1). From the results, the rats fed a diet of canned sardine fish had the highest mean body weight (154.8±12.28g) compared to other treatments. The lowest mean body weight (145.27±15.89g) was recorded in the rat fed a diet of anchovy. However, the mean body weights for all the three type of fish diets showed no significant difference (p>0.05) for mean body weight.

PER values for rats fed diets of reference casein, anchovy, mackerel and canned sardine are shown in Table 4. The results of PER values obtained are 2.48, 2.46, 2.34 and 2.31 for canned sardine, anchovy, mackerel and casein respectively. Canned sardine has the highest PER value followed by anchovy and mackerel. Casein diet has the lowest PER value compared to the fish diets.

The three selected fish diets showed higher protein efficiency ratio compared to casein (2.31) which is normally around 2.5 (Chapman & Mitchell, 1959). According to Pallert & Young (1980) casein which is not enriched with sulfur-containing amino acid especially metionine has a lower PER value. This study followed the AOAC procedure (2000) which does not mention the enrichment of casein with methionine. This may explain the low PER value of casein in this study (Babji & Selvakumari, 1989).

Dellinger *et al*. (1996) stated that the PER value of mackerel mixed species is 3.55 and the PER value of sardine is 3.55. The results obtained in this study for canned sardine and mackerel are compar-

Table 3. Chemical composition of anchovy, mackerel and canned sardine (dry matter basis)

Chemical composition (%)	*Anchovy*	*Mackerel*	*Canned Sardine*
Crude protein	81.46±0.98^{b}	89.09±1.4^{a}	59.25±1.27^{c}
Ash	4.75±2.25^{b}	3.28±0.37^{b}	7.32±0.40^{a}
Crude fat	2.82±0.25^{c}	7.76±0.28^{b}	24.22±1.51^{a}
Moisture content	3.46±0.30^{b}	4.22±0.13^{b}	5.79±0.37^{a}

[1]Data are presented in the mean values (n=2)
Values in different letters with in same row are significantly different (p<0.05)

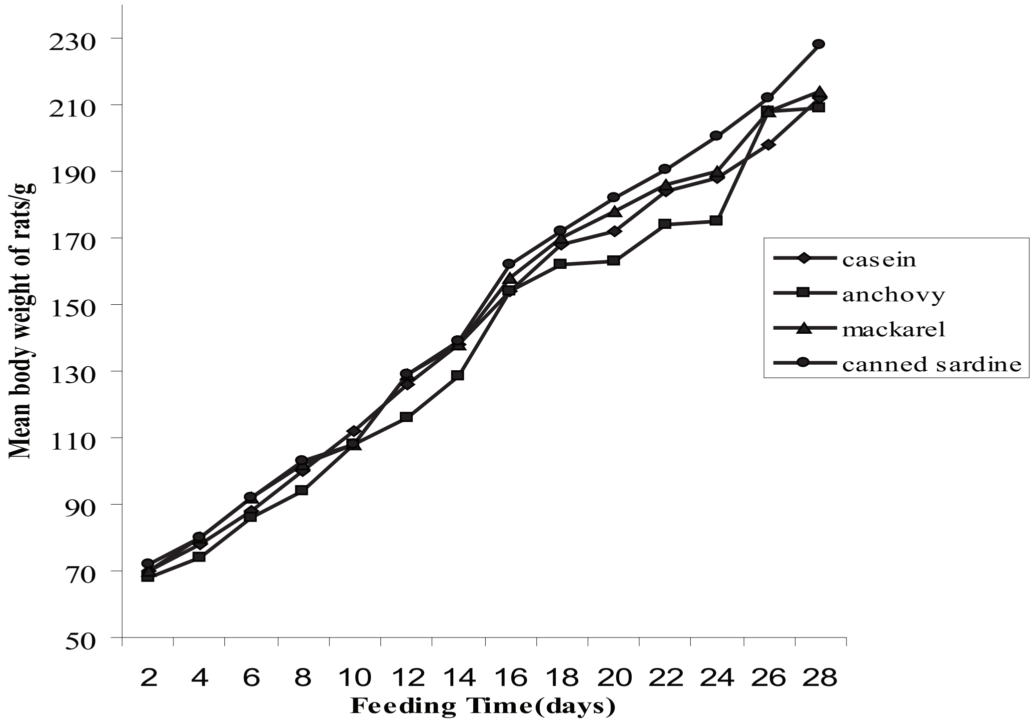

Figure 1. Mean body weight of rats fed with formulated diet of casein, anchovy, mackerel and canned sardine

Table 4. PER values of casein, anchovy, mackerel and canned sardine[1]

Protein source	*Increase in weight (g) (mean±sd)*	*Total feed intake (g/rat/28 days (g)*	*% protein in feed (N x 6.25)*	*Protein consumed (g/rat/28 days) (g)*	*PER*
Casein	145.99±20.22	404.50	15.60	63.12	2.31
Anchovy	145.20±15.89	373.80	15.76	58.91	2.46
Mackerel	144.81±18.81	405.26	15.24	61.76	2.34
Canned Sardine	154.80±12.28	393.94	15.83	62.36	2.48

[1]Mean and standard deviation from 8 rats

atively lower than the values reported by Dellinger *et al.* (1996). This may be due to the practices of variable methods or usage of different fish species. The diet preparation also plays an important role in determining the PER value. The sardine used in this study had undergone an ultra heat temperature for sterilization purpose, whereas the mackerel was boiled at 100°C prior diet preparation. Thus, the protein quality could have been denatured by exposure to the high heating temperature, thus affecting protein quality.

The U.S. Environmental Protection Agency & Toxicology Surveillance for Risk Assessment (EPA, 1999) has also stated

Table 5. Percent i*n vivo* AD of anchovy, mackerel, canned sardine and casein[1]

Protein source	*Weight of feed consumed g/rat/8 days (mean±sd)*	*% nitrogen in feed*	*Total nitrogen consumed g/rat/8 days (mean±sd)*	*Feces dry weight g/rat/8 days (mean±sd)*	*Nitrogen in dried feces g/rat/8 days (mean±sd)*	*Total nitrogen in dried feces g/rat/8 days (mean±sd)*	*% apparent digestibility (mean±sd)*
Anchovy	113.75 ± 1.70	2.52 ± 0.27	2.87 ± 0.23	8.20 ± 0.84	3.03 ± 0.12	0.25	91.29
Mackerel	118.8 ± 4.07	2.44 ± 0.01	2.88 ± 0.08	8.98 ± 1.27	3.01± 1.27	0.27	96.99
Canned sardine	115.97 ± 2.07	2.53 ± 5.66	2.93 ± 0.71	9.28 ± 0.99	3.11 ± 0.99	0.29	96.88
Casein	118.95 ± 0.07	2.50 ± 0.10	2.97 ± 0.11	8.54 ± 0.85	3.01 ± 0.06	0.26	96.96

[1]Mean and standard deviation from 8 rats

that the PER value of fish is 3.55 which is higher than beef (2.30) and milk proteins (casein = 2.50) and close to that of egg (3.92).

Mazid *et al*. (1997) reported that protein quality affects PER values. They also found that the dietary protein content was similar among diets, but different PER values suggest differences in dietary protein quality. Nevertheless, PER results must be interpreted carefully because this parameter assumes that all protein is used for growth (Tacon, 1989).

Table 5 shows the *in vivo* AD of anchovy, mackerel, canned sardine and casein. Results shows that mackerel has the highest percentage of digestibility (96.99%) followed by casein (96.96%), canned sardine (96.88%) and anchovy (91.29%).

Digestibility of protein is related to the quality of the protein in the feed. This is supported by the study conducted by Albrektsen *et al.* (2006) which showed that a fish meal with a high quality protein produces higher AD value compared to a vegetable meal which has low quality protein.

Carolina *et al*. (2005) has also shown that feeding of flour-based diets mixed with mechanically deboned chicken and fresh chicken breast meat gives a high PER, thus resulting in high true digestibility value.

CONCLUSION

Among the samples of selected fish studied, it was observed that canned sardine had the highest value of PER value, reflecting higher protein quality than the casein reference protein. The mackerel-based diet had the highest percentage of *in vivo* digestibility while the anchovy had the lowest percentage. In rat bioassay, all rats gained positive body weight. The rats fed diets of canned sardine fish had the highest mean body weight compared to the other diets.

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