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Protein Quality Analysis of Baby Porridge, Soybean Protein Concentrate, Rebon and Casein Against Weight Gain in Experimental Rats

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ABSTRACT

Protein quality is assessed based on the speed of growth that occurs after consuming and digesting something protein and the amount of nitrogen absorbed or used by the body. Complete protein can ensure good body growth and can maintain or replace damaged body tissues while incomplete protein although it cannot guarantee growth but is able to maintain body tissues. The purpose of this study to determine high-quality protein food sources can be seen from the value of protein quality indicators, including Protein Efficiency Ratio (PER), Biological Value (BV), Net Protein Utilization (NPU), Net Protein Ratio (NPR), True Digestibility (Dt) and Apparent Digestibility (Da). The research design developed in this study is an experiment using experimental mice with indicators of weight gain and protein quality in some foods. The results showed that the average weight gain (8.5 gr) of the largest rats was rats given baby porridge in their diet, as for weight loss (-2.4 gr) of rats given soy protein concentrate (KPK) in their diet. Furthermore, from the assessment of protein quality, it is known that the highest PER value is baby porridge (10.03), the highest BV value is soy protein concentrate (KPK: 0.99), the highest NPU value is soy protein concentrate (KPK: 1.28) and casein (1.23), the highest NPR value is baby porridge (0.13) while the Dt value (protein / casein 1.36) and Da for all types of protein tested are almost the same.

INTRODUCTION

Protein quality indicates the ability of protein to meet the nutritional needs of animals and humans for non-essential nitrogen and essential amino acids. The difference in protein quality can be obtained by knowing the chemical score, protein efficiency ratio, biological value and net protein utilization. The methodology for determining protein quality is based on studying nitrogen balance or mouse growth. By the late 1960s the relationship between energy intake and protein had been tested and human protein requirements had been assessed. Determination of protein quality is a subject considered assuming that protein deficiency is widespread, and an interesting consideration in the treatment of malnutrition with protein-rich diets. During this period many researchers oriented towards discovering new protein sources and developing techniques for evaluating protein quality.

Protein Energy Deficiency (KEP) is a state of malnutrition caused by low consumption of energy and protein in daily food or caused by certain disease disorders, so that it does not meet the nutritional adequacy rate (Ministry of Health RI, 1999). KEP itself is more often found in preschool children (Sukirman, 1974 in Sutanto, 1994). Children with early malnutrition have a higher chance of long-term physical growth retardation, suboptimal mental development, and premature death than normal children. Malnutrition can also result in physical growth retardation which in turn is associated with a high risk of death (Karyadi, 1971). The relationship between KEP and infectious diseases can be explained through the body's defense mechanism, namely in toddlers who have KEP there is a lack of energy and protein input into the body so that the body's

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ability to form new proteins is reduced. This then causes the formation of cellular immunity to be disrupted, so that the body suffers from infection prone attacks (Jeliffe, 1989).

Along with the development of nutritional science, especially agricultural nutrition, agricultural nutritionists are often faced with determining the quality of protein new mixed foods or new varieties of ingredients. The high protein content of a food ingredient does not guarantee high quality either. Therefore, it is necessary to see the quality through experiments on animals (Sibarani, 1986). Some researchers at this time consider that the quality of protein is very important in matters of nutrition for humans. As it is known that protein is very important for the human body, especially for growth and maintenance. Protein deficiency can cause disruption of growth and even cause very dangerous diseases, especially if accompanied by energy deficiency.

Given the importance of protein for the human body, it is necessary to pay attention to protein intake into the body so that in this case the food consumed should be high quality protein, namely protein that can supply amino acids needed by the body. To find out high-quality protein food sources can be seen from the value of protein quality indicators including Protein Efficiency Ratio (PER), Biological Value (BV), Net Protein Utilization (NPU), Net Protein Ratio (NPR), True Digestibility (Dt) and Apparent Digestibility (Da) and others. Based on the consideration of the large role of protein for humans and the importance of knowing high-quality protein food sources, it is necessary to introduce protein quality determination for nutritionists. Therefore, experiments were carried out as an effort to realize this, which was carried out using experimental animals, namely mice. Using experimental rats, Protein Efficiency Ratio (PER), Biological Value (BV), Net Protein Utilization (NPU), Net Protein Ratio (NPR), True Digestibility (Dt) and Apparent Digestibility (Da) and others can be calculated.

METHODS

Experiment Design

The experimental design used was a Group Randomized Design (RAK) with a mouse experimental unit that was treated as follows:

Po = Standard Ration of Casein
P1 = Non protein Ration
P2 = KPK ration
P3 = Rebon Ration
P4 = Baby porridge ration

Each treatment consisted of 3 mice. The division of rats into treatments was carried out randomly Each group was given rations according to predetermined treatment. Trial rationing is carried out for 10 days.

Materials and Tools

The materials used in this experiment were:

1. For the treatment of experimental animals:
   a) Rations are given *ad libitum* with the composition of:

Table 3. Recommended ration composition for determination of PER (AOAC, 1984)

Mixed ingredients	Amount (%)
Sample (Protein Source)*	X = (1.6 x 100)/ % N sample
Corn Oil	8 - [(X x % fat content) / 100]
Mineral mixtures	5 - [(X x % ash content) / 100]
Vitamin mixtures	1 %
Cellulose	1% - [(X x % crude fiber content) / 100]
Water	5 - [(X x % moisture content) / 100]
Corn starch	To make 100%

- This ration contains 10% protein

Table 4. The composition of the ration of rats for each treatment

Material (gr)	Treatment				
	Baby Porridge	Casein	KPK	Rebon	Non Protein
Casein	-	4,34	-	-	-
KPK flour	-	-	9,48	-	-
Rebon flour	-	-	-	9,48	-
Baby porridge	24,56	-	-	-	-
Mazola	1,65	2,99	1,75	2,79	3,03
Mineral mix	1,06	1,86	1,80	-1,53	1,89
Cellulose	-0,28	0,38	-0,34	0,38	0,38

Vitamin	0,38	0,38	0,38	0,38	0,38
Cornstarch	8,63	26,05	22,93	24,49	30,32
Water	1,89	1,89	1,89	1,89	1,89
Additional water	22,11	22,11	22,11	22,11	22,11
The amount of wet ration	**60,0**	**60,0**	**60,0**	**60,0**	**60,0**

b) Drinking water *ad libitum*

c) Wistar type male rats after weaning age (three weeks old), with a total of 15 heads (3 heads / treatment).

2. Materials for fecal and urine protein analysis: $CuSO_4$ and $KmnO_4$ (1:9), concentrated H_2SO_4, Selenium mix, NaOH 40%, standard HCl, H_3BO_3 3%, methyl red indicator
3. The equipment used in the experiment is:
   a. For animal treatment: health and safety cages, food/ration holders, beverage holders, faecal containers, urine/urine holders, analytical scales, ovens, cups, dough com, spoons/stirrers.
   b. For fecal and urine protein analysis: Kjeldahl flask, Distillation flask, erlenmeyer 100 ml, burette, magnetic stirer, measuring flask 100 ml.

Data type

The data obtained were data on rat weight, protein consumption, nitrogen levels from ration injections, nitrogen levels from feces and nitrogen levels from urine. Observations of rat body weight were carried out every two days (I, II, III and IV). Protein consumption is obtained from observing the rations eaten every day multiplied by the percent protein content in each ration. The intic nitrogen content is obtained from the amount of protein consumed for 10 days divided by the nitrogen protein conversion factor. The nitrogen content of feces and urine for 10 days was analyzed using the Kjeldahl semi-micro method.

Principle of Experiment

The experiment was carried out using the *bioassay* method. With physical preparation such as preparing rats, food preparation such as rations and drinks both standard and control, conducting experiments and calculations such as body weight, remaining rations, water content, feces, urine then determined the values of PER, BV, NPU, NPR, Dt and Da according to the formula.

Data calculation and analysis

Protein and Nitrogen consumption calculation

1. Calculate the weight of the initial and remaining rations in each ration treatment
2. Calculate the moisture content of the initial ration and the remaining ration the next day using the usual oven method (direct heating)
3. The initial Net weight is obtained from the weight of the initial ration times the weight of the initial ration moisture content while the residual Net weight is obtained from the weight of the residual ration times the weight of the residual moisture content
4. The ration eaten by the rats represents the difference in the weight of the initial net ration minus the weight of the residual net ration.
5. The amount of mouse protein inject is obtained from the weight of the ration inject times the protein content of the ration
6. The number of mouse nitrogen injections is obtained from the number of mouse protein injections divided by the protein nitrogen conversion factor

Protein quality analysis used is Protein Efficiency Ratio, Biological Value, Net Protein *Utilization, Net Protein Ratio, Digestibiity* and *True Digestibiity*.

RESULTS

Changes in Mouse Weight

After conducting experiments with different food treatments in experimental rats for 10 days, changes in rat body weight were obtained as listed in Table 5.

Table 5. Changes in rat weight with each treatment

Day Weighing	Treatment				
	Non protein	Baby porridge	Protein/casein	KPK	Rebon
I	63.77	56.37	39.9	49.6	53.57
II	59.7	62.3	42.97	45.17	59.9
III	58.4	71.07	46.23	43.8	62.17
IV	56.57	81.53	50.63	42.73	64.57

Average Weight Gain	-2.40	8.39	3.58	-2.29	3.67

From Table 5, it can be seen that the body weight of rats given rations without protein (non-protein) and KPK decreased by 2.40 g and 2.29 gr respectively, while the body weight of rats with rations of baby porridge, casein, and rebon increased by 8.39 gr, 3.58 gr, and 3.67 gr, respectively. The highest weight gain occurred in rats treated with baby porridge, which was 8.39 grams. In the treatment of baby porridge, the baby porridge used is a mixture of three flavors, namely vegetables, chicken team and vegetable fish soup. So that the nutritional content is sufficient and more diverse. This is in accordance with Roger's (1979) statement that another criterion for the growth and development of rats is the adequacy of nutrients in the ration so that it has a positive effect on rat weight gain. In addition, another influencing factor in weight gain is the digestibility of food. An increase in the amount of ration eaten by rats leads to an increase in the speed of digestion flow rate (*ingesta*). *The ingesta* will react with digestive enzymes in a relatively shorter time, resulting in a decrease in digestibility (Mc Donald, 1973). This is what likely caused the weight loss of mice given KPK rations. The increase in rat weight in each treatment can be seen in the rat weight gain chart below (Figure 2).

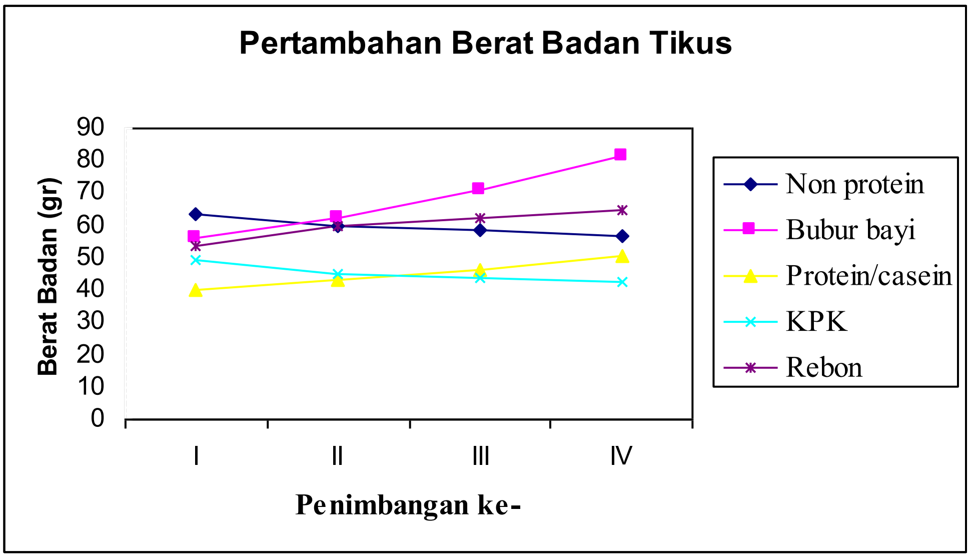

Figure 2. Rats weight gain chart

According to Smith and Mangkoewidjojo (1988) the growth speed of rats is 5 g / day. Thus, from the results of the experiment, only the treatment of baby porridge meets these standards. The difference in the increase in body weight of mice may be influenced by preferences for the rations given and the appetite of mice. With less ration consumption, it will result in energy reserves and the formation of mouse body cells tend to be less so that the rat's weight gain becomes lower. This was clearly seen in rats fed KPK rations. Soybean Protein Concentrate (KPK) is the result of soybean extraction which in the manufacturing process uses fat solvents which are chemicals. The chemicals commonly used in this extraction process are hexane solvents (ethanol). This solvent leaves a very pungent odor that can reduce the appetite of mice. Therefore, this low appetite will reduce the consumption of experimental rats so that there is no significant weight change and even tends to decrease.

Protein Quality Assessment Analysis Results

***Protein Efficiency Ratio* (PER)**

Assessment of protein quality from weight gain to the amount of nitrogen injection through *Protein Efficiency Ratio*. The calculation of the increase in body weight of mice during a particular treatment and compared with the amount of protein consumed from the ransu illustrates the size of the PER or protein *efficiency ratio*. Based on the experimental results, the highest PER value was found in baby porridge, which was 10.03; protein/casein 7.04; and rebon 6.37. While the PER value in KPK is not too much different from the PER value in non-protein. The PER value in KPK tends to be low because KPK is obtained through the extraction of soybean flour, where most of the protein is insoluble under these conditions, because the use of acidic solutions at isoelectric pH can reduce *unfolding*, aggregation, and loss of functional properties.

Table 6. Value PER Each Treatment

Treatment	PER value
Non Protein	-7.37
Baby porridge	10.03

Protein/Casein	7.04
KPK	-3.64
Rebon	6.37

Based on theory, casein is the most important milk protein because it has a PER value of 2.5, so the results of casein experiments are very different. Similarly, rebon protein has a PER value that is very different from the animal protein theory which has a high PER value (Muchtadi, 1989). However, based on the experimental results, the highest PER value was obtained from the treatment of baby porridge. The high value of PER is due to its composition containing sufficient and diverse nutrients, one of which is casein protein. This may occur due to miscalculations, inaccurate measurements or disturbances in the metabolism of mice so that the amount of protein consumed is used more for maintenance function than for growth function.

***Nett Protein Ratio* (NPR)**

The NPR calculation is the same as the PER, but the NPR calculation includes measurements of the weight loss of mice fed a non-protein diet. Based on the experiments conducted, NPR results were obtained as written in Table 7.

Table 7. NPR Value of Each Treatment

Treatment	NPR value
Non Protein	-
Baby porridge	0.13
Protein/Casein	0.04
KPK	-0.14
Rebon	0.04

The highest NPR value is baby porridge which is 0.43 while the KPK has a negative NPR value of -0.14. Therefore, the results of this experiment are in accordance with the statement of Wolzak *et al* (1981) in Hudson (1983) that there is a high positive correlation between PER and NPR values for the same food group. This is indicated by the high PER and NPR values of baby porridge. The presence of a negative NPR value indicates that rats that consume KPK experience weight loss.

Biological Value* and *Apparent Digestibility

The calculation of BV values was determined by study of nitrogen balance using mice. The results of *the calculation of Biological Value* in this experiment are presented in Table 8 below:

Table 8. BV Value of Each Treatment

Treatment	BV value
Non Protein	-
Baby porridge	0.89
Protein/Casein	0.90
KPK	0.99
Rebon	0.58

Based on Table 8, it can be seen that the highest *Biological Value* is found in the KPK ration and the lowest in the rebon ration. KPK with the highest BV value indicates that KPK contains amino acids that are more easily absorbed by the intestine well compared to amino acids found in other ration proteins.

Net Protein Utilization

After processing of data on N consumption and fecal N and N urine from both non-protein diet rat groups and certain protein diets, the NPU value of each protein diet was obtained as shown in Table 9.

Table 9. NPU Value of Each Treatment

Treatment	NPU value
Non Protein	-
Baby porridge	1.08
Protein/Casein	1.23
KPK	1.28
Rebon	0.50

Net Protein Utilization is an indicator in determining protein quality by comparing absorbed nitrogen with injectable nitrogen. Based on the results of experiments on rats, the highest NPU was in the KPK of 1.28. It can also be seen that rebon has a lower NPU value compared to other rations even though according to the theory that the quality of animal protein is higher than the quality of vegetable protein.

Based on the results of data analysis of the amount of feces and urine of rats in each ration treatment, it was seen that urine in rats with rebon consumption had a high proportion compared to rats treated with baby porridge, KPK and kesein, so this made the BV and NPU values in rebon low. Although based on the theory, animal protein has a higher BV and NPU value compared to vegetable protein.

***True Digestibility (* Dt) and *Apparent Digestibility* (Da)**

Like the amino acid composition of proteins, the *digestibility* of proteins is also an important factor in determining the quality of the protein. From the experiments carried out, true digestibility (Dt) and *apparent digestibility* (Da) were produced as listed in Table 10.

From Table 10 it is known that the Dt of all proteins has no significant difference as well as the value of Da, except in the rebon treatment. Based on the theory of animal protein has a higher BV and NPU value, so it can be said that rebon contains much higher protein than protein in other treatments. This is because rebon is one of the fishery products that contains high protein with amino acids that are easily digested. The value of Da is lower than Dt, this is due to the correction of nitrogen loss in mice given non-protein treatment in the Dt calculation while in the calculation of Da this correction was not carried out.

As it is known that measuring protein quality is very difficult to do precisely. Many factors affect the quality of protein, including amino acid composition and digestion of the protein. In addition, protein quality is also influenced by the composition and adequacy of the diet as a whole and on the physiological character, nutritional status, health status of individuals who consume protein (Hudson, 1983). Likewise, the determination of protein quality in experimental animals, the factors mentioned above may also play an important role in the results of the experiment.

Table 10. Dt Value of Each Treatment

Treatment	Dt value	Value Da
Non Protein	-	-
Baby Porridge	1.21	0.98
Protein/Casein	1.36	0.98
KPK	1.29	0.98
Rebon	0.86	0.52

Based on the calculation of protein quality from experiments on rats that have been carried out, it can be seen that the values of protein quality indicators (PER, BV, NPU, NPR) are very much different from the values obtained from various literature.

Hudson (1983) mentions various factors that can affect protein utilization and can affect protein quality in general in humans which can also be interpreted in mouse experiments as shown in Table 11.

Table 11. Factors Affecting Protein Utilization and Protein Quality

Diet	Total protein, total food energy, amino acid composition (both deficiencies and excesses), digestibility, fiber and other food constituents.
Subject	Age, sex, physiological status, (growth, pregnancy, breastfeeding), activity, infections, injuries and emotions.
External	Feeding frequency, social, economic, hygiene, and sanitation.

In experiments on mice conducted, efforts have been made to determine protein quality based on standards set by AOAC (1975) with the hope that the value of various protein quality indicators can be obtained optimally by minimizing various things that may be *confounding factors*. In fact, the experiment that has been carried out this time has not given the expected results. In general, the values of protein quality indicators measured are far different from the results that have been published in various literature, especially on PER, BV, NPU, NPR values. The factor that greatly determines the occurrence of differences in results is suspected is the weakness of the subjects who conducted the experiment, among others, in terms of:

- Improper weighing of rats
- Determination and calculation of the consumed ration
- In collecting urine, there may be wasted due to improper position of the holding bottle
- In the collection of feces, there may be wasted feces so that the weight becomes reduced or the possibility of mixing with the fallen ration so that the weight increases
- Less careful in analyzing urine and fecal protein levels so that the results are not right

In addition, influencing factors are the presence of processes that are difficult to overcome such as the presence of lipid components and the possibility of nitrogen gas being produced in the body from the activity of intestinal microflora in substantes such as nitrites which ultimately count as nitrogen from proteins and affect the results and interpretation.

In fact, experiments on mice as an *in vivo* measurement can cause *an underestimate* in determining protein quality for humans. In general, humans require less protein for growth and more for maintenance than mice. In addition, rats are exposed to amino acids containing sulfur and lysine higher than humans. The difference in needs between rats and humans in such a way can lead to rapid growth rates in mice (Hudson, 1983). Thus, the data obtained from the measurement method on mice actually cannot be used as an accurate prediction of protein quality for humans.

CONCLUSION

Based on the results of the analysis, it was concluded that the greatest increase in rat weight was rats given chicken porridge in their diet, as for the weight loss of rats given soy protein concentrate (KPK) in their diet. Protein quality assessment is known that the highest PER value is baby porridge, the highest BV value is soy protein concentrate (KPK), the highest NPU value is soy protein concentrate (KPK) and protein / casein, the highest NPR value is baby porridge, the highest Dt value in protein / casein and Da for all types of protein tested is almost the same.

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