PS9.04 The different effects of sheep meat and duck meat supplemented diets on rat thyroid hormone and cytokines 30.00

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Abbreviations

Traditional Chinese Medicine (TCM)
sheep meat (SHP)
duck meat (DUK)
control (CON)
interleukin-1β (IL-1β)
interleukin-2 (IL-2)
interleukin-4 (IL-4)
interleukin-6 (IL-6)
interleukin-10 (IL-10)
tumor necrosis factor-α (TNF-α)

Abstract

Background & aims: Sheep meat and duck meat were described as useful agents to cure disease in traditional Chinese medicine for long time, especially because of their different immunoregulation on human body. This study is to explore the mechanisms underlying the different influences of the intake of sheep meat and duck meat diets on serum cytokine using a rat model from the viewpoint of function of amino acid and fatty acid composition of the diets.

Methods: Male rats were randomly assigned to 3 groups, being fed sheep meat supplemented diet, duck meat supplemented diet, or soy bean supplemented diet (CON). After 30 days, to detect the levels interleukin-1β(IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α(TNF-α) with the method of Radioimmunoassay (RIA).

Results: Serum levels of IL-1β, IL-4, IL-10 and TNF-αin sheep meat group were significantly higher than those in duck meat supplemented group ($P<0.05$).

Conclusions: Sheep meat and duck meat diets have different effects on rat serum cytokine, and difference in the amino acid profiles and fatty acid composition of the sheep meat and duck meat maybe explain the phenomenon.

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Short title: Influences of sheep meat and duck meat, Serum cytokine

Key words: Sheep meat; Duck meat; Serum cytokine

I INTRODUCTION

Variation in nutrient status may influence the body's physiological activities, and recent progress in immunology has shown that there are strong interrelations between the endocrine, nervous and immune systems [1, 2]. Control of the phase response is mediated by cytokines. Cytokines are a diverse range of polypeptides and multifunctional molecules that exert numerous important actions to maintain homeostasis via complex interactions between the endocrine, nervous and immune systems [3]. It has been proved that serum levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon (IFN), could be altered by dietary protein, amino acid and fatty acid intake diets [4, 5]. Cytokines and free radicals have the capacity to enhance the production of each other. Interleukin-1and TNF-α stimulate production of oxygen free radicals, hydrogen peroxide and nitric oxide from a range of phagocytic and endothelial cells. Likewise, oxidantscan enhance interleukin-1 and TNF-α production via activation of the nuclear transcription factor NFkB [6]. The over production of cytokines and free radicals can have detrimental effects, e.g., in patients with rheumatoid arthritis and adult respiratory distress syndrome [7].

Sheep meat and duck meat were described as useful agents to cure disease in traditional Chinese medicine for long time [8]. However, the specific physiological changes related to consumption of sheep meat and duck meat have not been well defined and the mechanisms were unexplored. It is very important for nutrition scientists to understand the cause why sheep meat and duck meat can differently influence the body's physiological activities.

The impact of glycine on interleukin production by human monocytes had been investigated, and the result revealed that glycine reduces in a dose-dependent manner the release of proinflammatory TNF-α and IL-1β after endotoxin (LPS) exposure, but accelerates anti-inflammatory IL-10 secretion [9]. In addition to the observed effects of glycine on monocytes, the metabolism of lymphocytes seems to be influenced by the glycine supply. Addition of glycine to lymphocytes-inhibited CD3 stimulated proliferation by about 40% of control by an IL-2-independent mechanism [5]. The function of amino acids must be re-evaluated based on the findings that glycine and glutamine can cause metabolic and even immunological alterations.

There is preliminary evidence that proinflammatory cytokines TNF-♋ and IL-6 may change after an acute fat bolus [10-12]. The fatty acid composition affects established markers of cardiovascular disease and type 2 diabetes mellitus (T2DM) such as dyslipidemia, glucose, and insulin control.

This paper analyzed the amino acid profiles and fatty acid composition of the sheep meat and duck meat supplementation diets and examined the influence of these diets intake on rat serum cytokines in young SD rats. The aim of this study is to explore the mechanisms underlying the different physiological influences of the intake of sheep meat and duck meat on the body using a rat model from the viewpoint of function of dietary amino acid and fatty acid compositions. The hypothesis of this study is that the sheep meat and duck meat supplementation diets with different amino acid profiles and fatty acid composition can affect on the rats' serum cytokines differently.

II MATERIALS AND METHODS

2.1 Animal experiment

Twenty-seven healthy Sprague-Dawley male rats with weights of 160-180g were purchased from Shanghai Slaccas Medical Animal Center (Shanghai, China) and randomly assigned to three groups (n=9, each). All rats were accommodated in an environment controlled room (temperature: 23 ±3℃, humidity: 75 ± 5%, 12 h dark/12 h light cycle) throughout the experiment. The three groups of rats were fed with sheep meat supplemented diet, duck meat supplemented diet and control diet (soy protein supplemented diet), respectively. The ingredients of the three diets were listed in Table 1[13]. The sheep meat was supplied by Caoyuanxingfa Group Ltd. The duck meat was obtained from Tianhui Food Ltd in Shandong Province. All the diets were air dried at 100±5℃ for 72 hours. The amino acid profiles of the diets were analyzed by Hitachi Amino Acid Analyzer 835-50 (Hitachi Co. Japanese), and the result was showed in Table 2. And the fatty acid composition was analyzed and the result showed in Table 3. The rats had free access to tap water and diet. The animal research was undertaken following the guidelines of the Animal Ethics Committee of the Nanjing Agricultural University.

2.2 Body weight, diet consumption

The body weight and food intake of the rats of the CON, SHP and DUK groups were registered on days 1, 10, 20 and 30 of the experiment.

2.3 Serum samples collection

Blood samples were collected on day 30 from the rats by the method of orbital venous plexus penetration. The samples were stored at 4℃ for 1.5 h and then centrifuged at 3000×g for 10 min at 4℃, Then the serum samples were immediately aliquoted and frozen at −70℃.

2.4 Serum cytokine assays

Serum concentrations of IL-1♌, IL-2, IL-4, IL-6, IL-10 and TNF-♋ were measured by radioimmunoassay (RIA) commercial kits from Beijing North Institute of Biological Technology(Beijing, China), coefficients of variance, normal ranges and sensitivity were as follows: IL-1♌: CV intra-assay <12%, CV inter-assay<13.0%, normal range 0.1-8.1 ng/ml, sensitivity 0.1 ng/ml; IL-2: CV intra-assay <7%, CV inter-assay <10%, sensitivity 0.1 ng/ml; IL-4: CV intra-assay <8%, CV inter-assay <15%, sensitivity 0.165 ng/ml; IL-6: CV intra-assay <10%, CV inter-assay<15.0%, normal range 0.1-3.2 ng/ml, sensitivity 0.1 ng/ml; IL-10, CV intra-assay <5%, CV inter-assay <10%, sensitivity 3 ng/ml; TNF-♋💻 CV intra-assay <10%, CV inter-assay<15.0%, normal range 9-590 fmol/ml, sensitivity 6 fmol/ml. All measurements were performed twice and the mean of the two measurements was used for the present analysis.

2.5 Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences version 12.0 for Windows (SPSS Inc.). Statistical comparisons were made using one-way analysis of variance (ANOVA), and data were expressed as means ± standard deviation (SD) at the significance leve of 0.05.

III RESULTS

3.1 Body weight, diet consumption

The initial and final body weights of the three group rats were no significantly different. So did their diet consumption (Table 3).

3.2 Cytokine levels in serum

The total effect of the cytokine composition is often of greater importance than the actions of one or two cytokines alone. As shown in Fig.1, the SHP group had significantly higher serum levels of IL-1♌, IL-4, IL-10, TNF-♋compared with the DUK group (p<0.05), and had significantly higher serum levels of IL-1♌, IL-4 and IL-6 compared with the CON group (p<0.05), especially IL-2, and IL-10, the difference was extremely significant (p<0.01).The levels of IL-2 and IL-10 in the DUK group were higher than the control group (p<0.01 and p<0.05 respectively).

IV DISCUSSION

The present research result indicated that sheep meat and duck meat supplemented diets had no significantly different influence on rat diet consumption and body weight (Table 4), but they had different influence on rat serum levels of cytokines (Figure 1).

Evidence that amino acids can influence immune function is accumulating. A study showed that chicks fed on a methionine-sufficient diet had higher IL-1 activity, growth rate and feed intake compared with chicks fed on a methionine-deficient diet when they received immunogen injections [14]. In rat, dietary methionine deficiency reduced mitogen-induced proliferation of T-cells [15]. Recently, the scientific interest in glycine has gained importance by the finding that an enteral diet supplemented with 5% glycine reduced the mortality of rats challenged with endotoxin (LPS) [16]. In that study, it was shown that the administration of glycine reduced the plasma levels of TNF-♋, indicating that glycine has immunoregulative properties. In present study, the amino acid profiles of the three diets (sheep meat diet, duck meat diet and Control diet) were analyzed and the results were showed in Table 2. It is worth noting that the amino acid profiles of sheep meat diet and duck meat diet were similar except the methionine, proline and glycine. Protein of DUK diet was rich in methionine, its level was 2.38 fold higher than CON diet and 1.21 fold higher than SHP diet. Proline and glycine contents of DUK diet were approximately 10% lesser than SHP diet. In our study, the result showed that rats fed SHP diet had higher levels of IL-1♌, IL-6 and TNF-♋ than the DUK group and the CON group. This indicates that amino acid profiles explained, only in part, different influence of intake sheep meat and duck meat on rat serum cytokines.

The major difference of the dietary lipid in the three diets was showed in Table 3. The saturated fats myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) in SHP diet were higher than in DUK diet. While, monounsaturated fatty acid (MUFA) oleic acid (C18:1) and the polyunsaturated fatty acids (PUFAs) linoleic acid (C18:2) and linolenic acid (C18:3) in SHP diet were lower than in DUK diet. The cytokines IL-6 and TNF-♋ are key mediators of inflammation [17, 18] that are increased in obesity, insulin resistance, and T2DM [17, 19]. Although there is evidence of dietary modulation in response to changes in total fat [20, 21] and fatty acid composition [22] during long-term supplementation, less is known about postprandial response.

So, it is possible that different amino acid and fatty acid composition of sheep meat and duck meat diets during gastrointestinal digestion, have a different impact on rat serum cytokines. Further studies should be undertaken to determine and evaluate the role of amino acid and fatty acid composition of sheep meat and duck meat during digestion, and their modulating mechanisms need to be demonstrated.

V CONCLUSION

The present study has reported the discovery that sheep meat and duck meat diets have different effects on rat serum cytokine, and difference in the amino acid profiles and fatty acid composition of the sheep meat and duck meat maybe explain the phenomenon, and the mechanisms need further researches.

STATEMENT OF AUTHORSHIP

This statement of authorship covers the following article: The different influences of sheep meat and duck meat supplemented diets on rat serum cytokine, written by the following authors:

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest for any of the authors.

ACKNOWLEDGEMENT

Yingjun Zhang, Minyi Han, Weiqing Sun, Haijun Xu and Yongsheng Yang conducted the experiments and wrote the manuscript. All authors (including Ming Huang, Chunbao Li, Guanghong Zhou and Xinglian Xu) contributed in the conception and design of the study, acquisition of data, analysis and interpretation of data. The study was supported by the earmarked fund for modern agro-industry technology research system. The authors thank Wei Fashan, Chang Haijun, Liao

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REFERENCE

1 Gauthier, S. F., Pouliot, Y., & Saint-Sauveur, D. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. International Dairy Journal, 2006;16, 1315-1323.

2 Gill, H. S., Doull, F., Rutherfurd, K. J., & Cross, M. L. Immunoregulatory peptides in bovine milk. The British Journal of Nutrition, 2000;84 (Suppl 1), 111-117.

3 Miyoshi, S., Kaneko, T., Ishikawa, H., Tanaka, H., & Maruyama, S. Production of bioactive peptides from corn endosperm proteins by some proteases. Annals of the New York Academy of Sciences, 1995; 750, 429-431.

4 Bethin K. E., Sherri K. V., & Muglia L. J. Interleukin-6 is an essential, corticotrophin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. The National Academy of Sciences, 2000; 97, 9317-9322.

5 Erich Roth. Immune and cell modulation by amino acid. Clinical Nutrition, 2007, 26, 535-544

6Lamas, S., Michel, T., Brenner, B. M. & Marsden, P. A. (1991) Nitric oxide synthesis in endothelial cells: evidence for a pathway inducible by TNF-a. Am. J. Physiol. 261: C634-C641.

7 Barnes, P. J. (1990) Reactive oxygen species and airway inflammation. Free Radical Biol. & Med. 9: 235-243.

8* Li Shizhen. Bencao Gangmu. Ming Dynasty (1578).

9 Spittler A, Reissner CM, Oehler R, et al. Immunomodulatory effects of glycine on LPS-treated monocytes: reduced TNF-a production and accelerated IL-10 expression. FASEB, 1999; 13:563–71.

10 Nappo F, Esposito K, Cioffi M. Postprandial endothelial activation in healthy subjects and type 2 diabetic patients: role of fat and carbo- hydrate meals. J Am Coll Cardiol 2002;39:1145–50.

11 Lundman P, Boquist S, Samnegard A, Bennermo M, Held C, Ericsson C, et al. A high-fat meal is accompanied by increased plasma interleu- kin-6 concentrations. Nutr Metab Cardiovasc Dis 2006;17:195–202.

12 Blackburn P, Despres J, Lamarche B, Tremblay A, Bergeron J, Lemieux I, Couillard C. Postprandial variations of plasma inflamma- tory markers in abdominally obese men. Obesity 2006;14:1747–54.

13 GUSTAVO BOUNOUS & PATRICIA A. L. KONGSHAVN. Influence of dietary protein type on the immune system of mice. J Nutr 1983; 113:1415-1421

14 Klasing, K. C. & Barnes, D. M. (1988). Decreased amino acid requirements of growing chicks due to immunological stress. Journal of Nutrition 118, 1158-1 164.

15Nauss, K. M., Connor, A. M., Kacancaugh, A. & Newbern, P. N. (1982). Alteration in immune function in rats caused by dietary lipotrope deficiency: effect of age. Journal of Nutrition 112, 2333-2341.

16 Zhong Z, Jones S, Thurman RG. Glycine minimizes reperfusion injury in a low-flow, reflow liver perfusion model in the rat. Am J Physiol 1996;270:G332–8.

17 Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumour necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.

18 Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumour necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111–9.

19 Vidal H. Obesity and inflammation: the adipocytokines. Ann Endo- crinol (Paris) 2003;64:S40−4.

20 Meydani SN, Lichtenstein AH, Cornwall S, Meydani M, Goldin BR, Rasmussen H, et al. Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived n-3 fatty acid enrichment. J Clin Invest 1993;92:105–13.

21 Kelley DS. Modulation of human immune and inflammatory re- sponses by dietary fatty acids. Nutrition 2001;17:669−73.

22 Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 2001;36:1007–24.

*Bencao Gangmu, also known as ***Compendium of Materia Medica***, is a Chinese materia medica work written by Li Shizhen in Ming Dynasty. It is a work epitomizing materia medica (??) in Ming Dynasty. The *Bencao Gangmu* is regarded as the most complete and comprehensive medical book ever written in the history of traditional Chinese medicine. It lists all the plants, **animals**, minerals, and other objects that were believed to have medicinal properties.
(http://en.wikipedia.org/wiki/Ben_Cao_Gangmu)

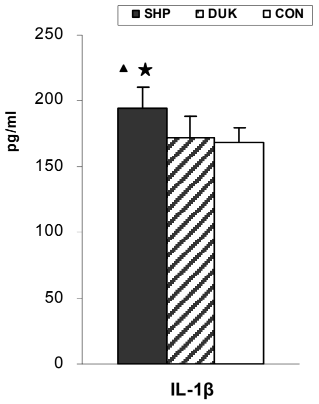

Fig. 1 A

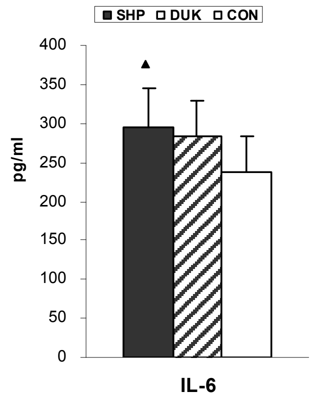

Fig. 1D

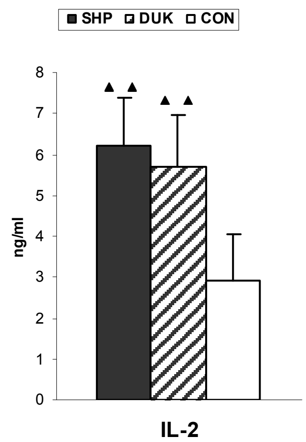

Fig.1 B

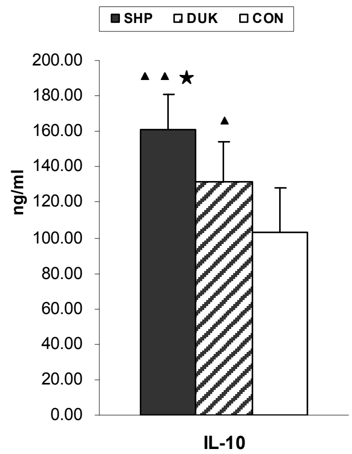

Fig. 1 E

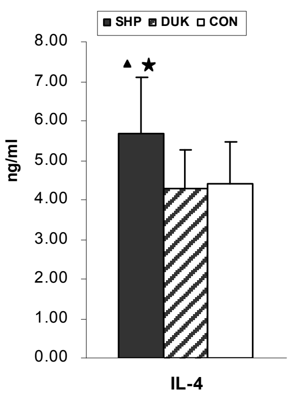

Fig 1 C

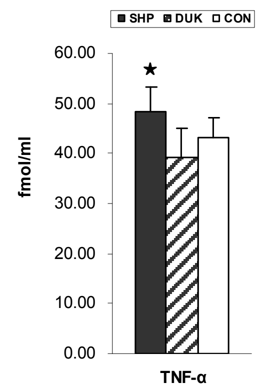

Fig.1F

Figure legends

Fig.1. IL-1β (A), IL-2 (B), IL-4 (C), IL-6 (D), IL-10 (E), TNF-α (F) levels in sera of sheep meat (SHP), duck meat (DUK) supplemented diets and control (CON) groups on 30 days. All values are expressed as mean± SD of 9 rats in each group. ▲ SHP or DUK group compared with control group ($p<0.05$), ▲▲SHP or DUK group compared with control group ($p<0.01$); ★SHP group compared with DUK ($p<0.05$); ★★ SHP group compared with DUK ($p<0.01$).

Table 1 Diet ingredient composition* (%)

Ingredients	Control diet	Sheep meat diet	Duck meat diet
Corn	14.5	25	32
Wheat by-products	14.7	26.7	23.7
Soy bean cake[1]	57.5		
Sheep meat powder[1]		46	
Duck meat powder[1]			42
Soy oil	11		
Vitamin mix	1	1	1
Mineral mix	1	1	1
NaCl	0.3	0.3	0.3
Total	100	100	100
Total protein (100%)	30.4	30.4	30.41
Total Energy(J/Kg)	1.48×10^{7}	1.42×10^{7}	1.51×10^{7}

Note: * The levels of protein of sheep meat powder, duck meat powder and Soy bean cake were 57.86%, 58.13% and 46.8% respectively. The powers contain about 10% water, and the powders of the sheep meat and duck meat were made from fresh sheep meat and duck meat, whose ingredient composition as the following: protein 18.0% and 15.5%; fat: 9% and 7.35%; water: 72% and 76%. The three types of diet had same protein level of 30.4% and approximate total energy. Vitamin mixture provide in milligrams per 100 g diet: ascorbic acid, 31.4; niacin, 5.04; riboflavin, 0.38; thiamin, 0.32; folic acid, 0.063; vitaminB-6,0.25; biotin, 0.032; pantothenic acid, 1.9, choline, 53.2; and per 100g diet: vitamin A,1007 IU; vitamin D, 253 IU; vitamin E, 6.3 IU; vitamin B-12, 1.26 μg ; And phylloquinone, 63μg. The mineral mixture content of ions or cations (expressed in milligrams per 100 g diet) and the actual chemical compounds fed, were : 378 Ca ($CaHPO_4 \cdot 2H_2O$ and $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$); 208 P ($K_2HPO_4 \cdot 2H_2O$) ; 7.7 Fe ($FeSO_4 \cdot 2H_2O$); 44 Mg (MgO); 0.38 Cu ($CuSO4 \cdot 5H_2O$); 2.5 Zn ($ZnSO_4 \cdot 7H_2O$); 0.63 Mn ($MnSO_4$); 840 Cl ($C_5H_{14}ClNO$); 1050 K ($K_2HPO_4 \cdot 2H_2O$); 245 Na (NaI).

Table 2 Amino acid profiles of the diets

	Amino acid	CON diet	SHP diet	DUK diet	SHP/CON	DUK/CON	DUK/SHP
	Essential						
1	Thr	1.19	1.34	1.34	1.13	1.13	1
2	Val	1.31	1.59	1.58	1.21	1.20	0.99
3	Met	0.36	0.7	0.85	1.96	2.38	1.21
4	Ile	1.31	1.33	1.4	1.01	1.07	1.05
5	Leu	2.25	2.54	2.57	1.13	1.14	1.01
6	Phe	1.52	1.31	1.3	0.86	0.85	0.99
7	Lys	1.78	2	1.92	1.12	1.08	0.96
	Non-Essential						
8	Asp	3.63	2.81	2.82	0.77	0.78	1.00
9	Ser	1.50	1.28	1.27	0.86	0.85	0.99
10	Glu	5.87	5.15	5.09	0.88	0.87	0.99
11	Gly	1.24	1.94	1.72	1.56	1.38	0.89
12	Ala	1.26	1.94	1.91	1.54	1.51	0.98
13	Cys	0.47	0.37	0.35	0.80	0.75	0.95
14	Tyr	1.02	0.89	0.9	0.88	0.88	1.01
15	His	0.75	0.78	0.72	1.04	0.96	0.92
16	Arg	2.27	1.9	1.87	0.84	0.82	0.98
17	Pro	1.19	1.38	1.22	1.16	1.02	0.88
	Total	29.21	29.25	28.8	1.00	0.99	0.98

Note: Protein of DUK diet was rich in methionine, its level was 2.38 fold higher than CON diet and 1.21 fold than SHP die. Both SHP diet and DUK diet were rich in threonine, valine, methionine, leucine, glycine, alanine, and levels of them were approximately 10% ~20% higher than that in CON diet, while the levels of phenylalanine, aspartic acid, serine, glutamine, cystine, tyrosine, arginine were over 10% lesser than CON diet.

Table 3 Composition of dietary lipids in the test meals showing the major fatty acid constituents（%）

	CON	SHP	DUK
Saturated Fatty Acid			
C14:0	-	2.3	0.6
C16:0	-	24.6	21.9
C18:0	3.1	16.7	5.2
C19:0	-	0.11	0.8
Unsaturated Fatty Acid			
C18:1	22.35	24.87	47.1
C18:2	54.31	3.1	11.2
C18:3	-	0.6	41.1

Result are expressed as Mean ± SD (n=9). There is no significantly different ($p > 0.05$).

Note: Saturated fatty acid was rich in lipid of SHP diet. Unsaturated fatty acid was rich in lipids of DUK diet and soy oil.

Table 4 Body weight and diet consumption of rats

	CON	SHP	DUK
Initial body weight(g/rat)	170±6.5	173±6.3	179±6.5
Final body weight(g/rat)	380±25.5	382±30.6	368±27.5
Food intake(g/rat per 10 days)	253±22.4	258±23.4	263±24.8

Result are expressed as Mean ± SD (n=9). There is no significantly different ($p > 0.05$).

Poster exhibition parallel session 9: Meat in nutrition

PE9.01 Protein quality of selected protein food using rat bio-assay 1.00

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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 together with sodium caseinate as a reference formulation were evaluated. Determination of proximate analysis, protein quality and protein digestibility were monitored. Procedures for evaluation included protein efficiency ratio (PER) using the rat bioassay and in vivo apparent digestibility. The rats fed with buffalo meat consumed (356.98g±34.31) had the highest mean in increased body weight (102.73g±8.95) while rats fed with tempeh consumed (200.37g±36.26) had the lowest mean in increased body weight (16.34g±9.11). Although the mean in body weight gained showed significant differences between all treatments ($p<0.05$) but for total food intake there was no significant difference found between casein and soy protein isolate. For PER value, meat has 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 meat (2.99) is 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 has the highest value (91.41%±3.76), followed by casein (91.34%±3.15), meat (90.79%±1.44), soy protein isolate (89.52%±2.96) and sodium caseinate (89.47%±2.31).