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The effect of dietary sericin on rats

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ABSTRACT: Dietary proteins have been reported to be beneficial for lipid metabolism, tissue growth, development, and repair, as well as immune response. The interactions of dietary proteins and their digested products may regulate the physiology and metabolism of the gastrointestinal tract. However, proteins are largely digested before reaching the large intestine. The present study investigated the use of the non-dietary protein, silk sericin, in rats. Rats were fed either with casein or sericin proteins. Their body weight, food consumption, and complete blood count were measured. The results showed that during the experimental periods, rats fed with casein and sericin diets had no significant changes on body weight, food consumption, or complete blood count. Sericin-fed rats had a significantly decreased CD8a and CD80 positive cells when compared with standard casein protein. In conclusion, dietary proteins may have a differential impact on the leukocyte profile. The mechanisms underlying these changes are not clear but they might be due to the different amino acid compositions of the proteins studied.

KEYWORDS: silk sericin, dietary proteins, systemic immunity

INTRODUCTION

Sericin is a natural macromolecular protein synthesized in the middle part of silk gland of silkworm, *Bombyx mori*. This protein consists of a group of polypeptides with molecular mass ranging from 20–400 kDa. Pharmacological activities of sericin includes anti-oxidant [1], anti-coagulant [2], anti-cancer activities [3–5], tyrosinase inhibition [6], liver and gastric protection [7,8], as well as cholesterol lowering [9,10]. Sericin promotes wound healing process without causing inflammation [11] and is widely used in tissue engineering [12,13]. In cell culture, sericin stimulates mitosis [14], promotes cell proliferation [15], protects against cell death [16], and activates immune system [6].

Evidence suggests that dietary proteins are necessary for regulation of immune system [17]. A deficiency of dietary protein or amino acids can cause impaired immune function and increase the susceptibility to infectious disease [18]. Thus there is growing interest in the role of amino acids in the immune function [19]. In particular, dietary whey proteins have the immunomodulatory effects in mice [20]. Like whey proteins, soy and casein enhance the immune system function [21,22]. Soy protein contains some phytochemicals, such as isoflavones, which influence the signal transduction process of macrophages and cytotoxic T lymphocytes, thus influencing both non-specific and specific immune response [23]. Apart from dietary whey proteins, soy proteins and casein proteins can enhance immune response. Interestingly, sericin which is a non-dietary protein has been reported to activate immune system [6]. The immune system function can be divided into innate and adaptive immunity. The innate immune system acts as a first line of defence by preventing the entry of infectious agents or by eliminating invading pathogens [24]. Protection against infectious agents can be achieved by a combination of innate and adaptive immunity. Due to the complexity of the immune system, the effects of dietary proteins on the immune response is difficult to assess. To evaluate the possibility of using sericin as a dietary protein, the present research investigated the effects of dietary sericin in rats.

Table 1 Amino acid composition of casein and sericin.

Amino acid	Assay (g/100 g)	
	Casein[a]	Sericin[b]
Ser	5.3	33.4
Asp	6.4	16.7
Glu	19.7	4.4
Gly	3.3	13.5
Thr	4.9	9.7
Lys	6.4	3.3
Tyr	4.1	2.6
Arg	2.6	3.1
Ala	4.6	6.0
Val	7.1	2.8
His	2.6	1.3
Leu	8.7	1.1
Iso	–	0.7
Phe	4.2	0.5
Trp	–	0.2
Pro	11.6	0.7
Cys	0.2	0.2
Met	2.6	0.04

[a] Based on Okazaki et al[25].
[b] Based on Nantong Dongchang Chemical Industrial.

Table 2 Experimental diets.

Ingredients	Experimental diets (%)	
	Casein	Sericin
Casein	4.0	–
Sericin	–	4.0
Fat	2.0	2.0
Fibre	2.0	2.0
Crude protein	12.0	12.0
Basal diet mixture[a]	80.0	80.0

[a] Based on standard rat diet CP082 (Perfect companion group co. Ltd, Bangkok).

MATERIALS AND METHODS

Dietary proteins

Casein and sericin diets were produced by the Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. Silk sericin was extracted with deionized water from raw silk yarns of silkworm *Bombyx mori* under high pressure and high temperature condition. The specific extraction condition is under a pending Thai patent (application number 080 595). The protein extract was dried at 130 °C, grounded and sieved through 0.75 mm screen. Table 1 shows the amino acid compositions of casein and sericin determined by an amino acid analyser. Compositions of diet formulations are shown in Table 2. The experimental diets were prepared by mixing standard rat diet with casein or sericin at the level of 4% w/w. All other chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals and experimental design

Male Spargue-Dawley rats in the weight range of 120–160 g were obtained from National Laboratory Animal Centre, Mahidol University, Nakhon Pathom, Thailand. These rats were housed in stainless steel cages under hygienic conditions in the departmental animal house at room temperature of 24 ± 2 °C and a humidity of $50 \pm 10\%$ with a 12 h light-dark cycle. The experimental protocol was approved by the ethical committee for animal care of Naresuan University (protocol number 51 040 022, approved on 14 October 2008). Before initiation of the experiments, rats were adapted to the laboratory condition for one week. After one week of acclimatization, the rats were randomized into two groups. Each group contained 6 rats. Briefly, one rat was taken from the cage and assigned to Group I, then another rat was taken and assigned to Group II, and continued alternating until each group had 6 rats. Rats in Group I were fed with casein diets and Group II were fed with sericin diets. The diet and water were provided ad libitum. Duration of the experiment was 20 weeks. Food consumption and body weight were recorded weekly throughout the experimental period. At the end of experiment, the rats were fasted overnight, anaesthetized with pentobarbitone sodium (40 mg/kg body weight) and then sacrificed. Blood samples were immediately collected with the use of heparin as anti-coagulant for immediate analysis of leukocyte subpopulations.

Collection of blood and detection of surface markers

Blood samples were obtained from these rats by cardiac puncture and were collected into two microcentrifuge tubes containing heparin sodium. Whole blood samples were stained with antibodies specific for CD2, CD3, CD4, CD8a, CD11a, CD25, CD45, CD54, CD80, and CD86 (all from Becton Dickinson, USA). After incubation with the antibodies, the blood samples were lysed with 2 ml of $1 \times$ RBC Lysis buffer (BioLegend) and the cells were added with 200 µl of FACS buffer. All samples were analysed by FACS Calibur using CellQuestPro software (Becton Dickinson, USA).

Statistical analysis

The percentages of each cell type were obtained and the comparison between two groups was made using Student's t-test. The p values of < 0.05 were considered statistically significant.

Table 3 Body weight and food consumption. Values are mean ± SD.

Treatment	n	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Food consumption (g/day)[a]
Group I Casein	6	237 ± 7	503 ± 28	265 ± 29	18.2 ± 0.9
Group II Sericin	6	235 ± 28	484 ± 41	254 ± 18	19.4 ± 2.7

[a] Values from the last month of the experiment.

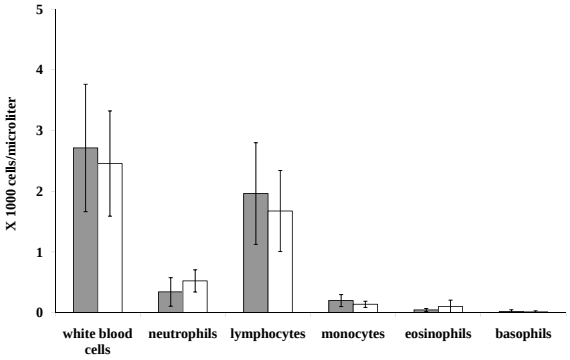

Fig. 1 Complete blood count of rats fed with casein (grey histogram) and sericin (white histogram). Bars are mean ± SD.

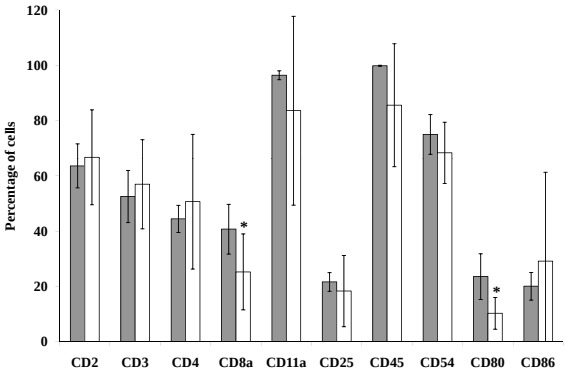

Fig. 2 Leukocyte subpopulations. Percentages of leukocyte subpopulations of rats fed with casein (grey histogram) and sericin (white histogram). $*p < 0.05$ as compared with casein diet group. Bars are mean ± SD.

RESULTS

Effect of sericin on body weight and food consumption

Table 3 presents the initial, final body weights, body weight gain and food consumption of casein-fed rats (Group I) and sericin-fed rats (Group II). The body weights and body weight gain of both casein and sericin diet were similar. No statistically significant difference was observed in food consumption between both groups of rats.

Effect of sericin on complete blood count

The average values of complete blood count of rats fed with casein and sericin diet are shown in Fig. 1. There was no change in complete blood count of the two groups of rats. These results also suggest that the type of protein (casein or sericin) has no effect on the pattern of these cells. The leukocyte profile of the rats was analysed based on cell surface markers on the peripheral blood leukocytes. Fig. 2 shows leukocyte subpopulations. There were similar percentages of the majority of leukocyte subpopulations in rats fed with casein and sericin diets. However, a significant reduction was found in CD8a positive cells and CD80 positive cells in the rats fed with sericin diet (Group II) as compared with casein diet group (Group I).

DISCUSSION

In this present study, rats fed with sericin diet had similar body weight and, by visual observation, the animals appeared to be in good health during the entire experimental period. Sericin has previously been shown to have anti-tumour effect, particularly in colon cancers[3–5]. Sericin may enhance systemic immunity implicated in elimination of tumour cells. In the present study, however, the number of CD8a positive cells and CD80 positive cells decreased. CD8a is a surface marker of cytotoxic T cells and NK cells, which are responsible for immune response in elimination of tumour cells as well as viral infection[24]. Although there was a reduction in the percentages of CD8a positive cells, no significant alteration in CD4 positive cells was found. This suggests that sericin might have an effect on the function of cytotoxic T cells but it may not affect helper T cells. Despite the essential role of CD8 cell in anti-viral immunity, CD4 T cells can also play important roles for control of viral infection as well as cancer[26,27]. There was a reduction of CD80 positive cells without changes in CD86 positive cells. The co-stimulatory molecules such as B7-1 (CD80) and B7-2 (CD86) play an important role in the induction of T cell-mediated anti-

tumour immunity[28]. It should be noted that rats in group I and II are normal rats, therefore it is difficult to draw a conclusion regarding the changes in these surface markers. However, the mechanism underlying the reduced CD8a positive and CD80 positive cells in the present study is unknown. It is possible that different amino acid compositions in these diets may have an impact on these alterations.

Casein protein and its fractions may enhance or suppress innate and adaptive immunity, depending on the purity of protein[29]. Purity of proteins has been considered to be involved in separation techniques. Although casein and sericin have mostly the same pattern of amino acids, sericin might have different immunomodulatory properties than that of casein. In addition, sericin has been shown to lower the level of inflammation in tissue engineering, making them a promising candidate in future biomedical applications[6]. Interestingly, sericin protein contains more serine, aspartic acid, and glycine than casein protein, amino acids reported to affect immune function[25]. Evidence suggest that glycine is essential for the proliferation and anti-oxidative defence of leucocytes and plays a role in regulating cytokine production and immune function[30]. In addition, aspartic acid is essential for the recycling of the citrulline produced by iNOS into arginine in activated macrophages. Thus aspartic acid contributes to the modulation of immune function[31,32]. The role of dietary proteins on immune modulation, such as cytokine production, is complex and requires further investigations.

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