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The Effect of Goat Milk Fractions Supplementation on Serum IgE Response and Leukocytes Count in Dinitrochlorobenzene Sensitized Rat

Nurliyani, E. Harmayani, and MHNE. Soesatyo

Abstract**—**In Indonesia, goat milk is often consumed and believed as anti-allergy. The objective of this research was to study the effect of goat milk and their fractions (casein and whey) supplementation on total serum IgE concentrations and leukocytes count in rat sensitized with contact allergen dinitrochlorobenzene (DNCB). Female Wistar rats 6-8 weeks old were divided into four groups: 1) whey, 2) casein, 3) whole milk supplementation and 4) phosphate-buffered saline/PBS (control). The results showed that supplementation of goat milk on rats did not affects on total serum IgE concentrations and number of leukocytes. After sensitized with DNCB, the monocyte percentage in rats was higher ($P<0.01$) than before. In conclusion, goat milk or their fractions supplementation unable to decrease the total serum IgE concentrations and also had no effect on leukocytes count. However, 1% DNCB could increase the number of monocytes, but could not induce the IgE response.

Keywords**—**Dinitrochlorobenzene, Goat Milk Fractions, IgE, Leukocytes.

I. Introduction

IN Indonesia, the dairy goat farm of Ettawah Crossed Bred is increase in recent years along with the vigorous promotion of the health benefits of goat milk. Goat milk is believed to reduce symptoms of allergic disease, which tends to increase in developing countries and a lot of environmental pollution. To avoid environmental conditions which can trigger allergies are very difficult. It is therefore necessary to find alternatives to prevent the occurrence of allergies such as by consuming functional food. Source of functional food can be derived from plants and animals.

Although the chemical composition between goat milk and cow milk are similar, however goat milk products found to be superior in terms of hipoalergenicity, the morphology of fat and fatty acid composition [1]. The content of goat milk fatty acids is very beneficial to health, such as for the treatment of various disorders and diseases of the digestive tract and reduce the incidence of cow milk allergy. The total medium chain triglyceride (MCT), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFAs) in goat milk is higher than cow milk. The content of goat milk MCT (C6 - C14) is 35% and 17% in cow milk. The total of caproic (C6), caprylic (C8) and capric acid (C10) is 15% in goat milk, whereas cow milk is 5% [2]. Capric acid, caprylic and MCT contained in goat milk is beneficial for a variety of clinical disorders including malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, intestinal resection, premature infant feeding, non-thriftiness of children, infant malnutition, epilepsy, cystic fibrosis, coronary by -pass and gallstones. This is caused by metabolic uniqueness that can provide energy directly without deposited in adipose tissue, serum cholesterol lowering activity, inhibition and limiting cholesterol deposition [2]. Goat milk with α s2-casein genotypes causing intestinal and systemic sensitization in guinea pig lower than α s1-casein genotype. Extensively hydrolysed formula based on the Italian dairy goats have been used and recommended as baby food with a CMA[3].

Previous research conducted by [4], suggests that the goat milk powder has a similar effect with cow milk colostrum in reducing the rat epithelial intestinal damage induced by indomethacin. Similarly, growth factor activity of goat milk in cultured cells is higher than cow milk [5]. It is explained that the higher medium chain fatty acids, monounsaturated and polyunsaturated fatty acids in goat milk than cow milk may reduce allergic reactions to cow milk.

Allergic reactions can be classified into four types based on the substance involved in the mechanism and timing of the reaction, namely type I, type II, type III and type IV. Type I allergy (immediate hypersensitivity) is mediated by IgE (reaction occurs 15-30 minutes after exposure to antigen / foreign material). Allergic type II is mediated by IgM or IgG and complement (the reaction to occur a few minutes to several hours). Allergy type III (immune complex hypersensitivity) is mediated by soluble immune complexes and the reaction occurs 3-10 hours of exposure to the antigen. Allergic type IV (cell mediated or delayed type hypersensitivity), reached a peak 48 hours of exposure to the antigen [6]. The skin contact allergies are allergic type IV which can be caused by some low molecular weight chemicals. Types of skin allergens such as dinitrochlorobenzene (DNCB), dinitrofluorobenzene (DNFB) and trinitrochlorobenzene (TNCB) can induce little mononuclear cell infiltration in the larynx or lungs Wistar rats and mice Balb/c. Skin sensitization involves the activation of

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non-specific and specific immunity. Chemical sensitizer acts as a hapten that would be antigenic by conjugation with protein macromolecules in the skin. Sensitization is mediated by the migration of haptenated epidermal Langerhans or dermal dendritic cells from skin to lymph nodes channel [7].

Immune response can be polarized toward the production of T helper 1 (Th1) or T helper 2 (Th2). Allergic contact dermatitis (particularly Th1) is a common allergic disease of the skin, whereas asthma and allergic rhinitis (mainly Th2) most commonly occurs in the respiratory tract. Th1 cells mainly produce IL-2 and IFN-γ is much related to protective immunity and DTH responses, whereas Th2 cells mainly produce IL-4 and IL-5 related to the production of IgE and respiratory allergic [8]. Research conducted by Kuper et al. [9] showed that the contact allergen DNCB did not induce respiratory allergy in Brown Norway rats. Meanwhile, other studies using trimellitic anhydride (TMA), known as respiratory allergens can induce increased serum IgE substantially in mice.

Some previous research, has been studied on the effect of various fibers on serum IgE response in mice [10], while the various doses of *Lactobacillus plantarum* has been investigated their effect on haematologis parameters in rats [11]. The effect of goat milk or their fractions on serum IgE levels and the leukocytes count in DNCB sensitized rat so far not been reported. In addition, there are conflicting results regarding the effect of DNCB induction on serum total IgE. According to Ban and Hettich [8], induction of DNCB may increase total serum IgE in mice, and 1% DNCB as a negative control experiments in mice. Therefore, the purpose of this study intended to study the effect of supplementation of goat milk and their fractions (whey and casein) on serum IgE response and the leukocytes count namely neutrophils, eosinophils, basophils, lymphocytes, monocytes in DNCB sensitized rat. Goat milk or their fractions are expected to be developed as anti-allergic functional food.

II. Materials and Methods

A. Materials

Goat milk of Etawah Crossed Bred from ndonesia as a main material research, microbial rennet (Marzyme, Glengarry Cheesemaking Supply, Canada) for making rennet casein and their byproduct whey, 2.4-Dinitrochlorobenzene (DNCB) as contact allergens (Sigma-Aldrich), acetone and corn oil (4: 1) as the allergen solvent. Chemicals for analysis of serum IgE by using Rat IgE ELISA Kit (Immunology Consulans Laboratory, Inc). Giemsa staining is used for blood sample preparation.

B. Research Design

The study was conducted with female Wistar rat 6-8 weeks old. The animals were acclimated for 5 days before the start of the study. They received standard AIN (the American Institute of Nutrition) -93 diet [12], and drinking water *ad libitum*. The rats were assigned into four groups; each group received the following treatments: whey, casein, whole milk, and PBS (no supplementation) as control. The dose of goat milk supplementation was 0.4 g/day with forced feeding. The rats were sensitized on day 15 with 1% DNCB (150 µl) at the dorsal area of the body, and challenged with 0.5% DNCB (75 µl) [9] at their external ears on the day 24. Blood sampling performed on day 14 (in rat without DNCB) and on day 28 (in rat with DNCB). During the experiment the rats continued to receive the AIN-93 standard feed. All procedures related to animal experiment were conducted following the recommendation of the Ethical Committee of Universitas Gadjah Mada, Indonesia (Ethical Clearence Number: KE/FK/194/EC).

C. Analysis of Total Serum IgE

Analysis of serum IgE in accordance with the instructions on the Rat-IgE ELISA Kit (Consultans Immunology Laboratory, Inc.), as follows: All reagents placed at room temperature, prepared reagents for standard, conjugate and sample dilution, by using buffer 1 part concentrate (5x) and 4 parts aquabidest. Wash solution prepared by diluting 1 part the concentrated wash solution (20x) to 19 parts aquabidest. Further dilution for serum (10x) prepared by pipetting 30 ul serum plus 270 ul of diluent (buffer 1x) and mixed thoroughly. Standard dilution series was made with a variety: 8 ul Rat IgE calibrator was added 817 ul buffer diluent (1x) (the highest standard /S6: concentration of 32 ng / ml). Standard 6 (S6) was transferred to a tube of 300 ml buffer diluent 1x (S5) (concentration of 16 ng / ml), and so on until S1 (1 ng / ml). Standard wells filled with 100 ul standard, and 100 ul buffer diluent 1x filled to the blank well, and then 100 ml of sample pipetted into the sample wells. Plate covered and incubated at room temperature for 60 minutes. Enzyme-antibody conjugate biotin was prepared (for each plate needs 10 ul conjugate plus 990 ul buffer dilution 1x). Plate was washed 4 x by wash solution, and 100 ul conjugate pipetted into the wells, then the plate covered and incubated at room temperature for 60 minutes in a dark room. Plate was opened and washed 4 x, then added 100 ul TMB substrate (teramethyl-benzidine) to all well. Plate were incubated for 10 min in a dark room and added to 100 ul stop solution to all wells, then read on an ELISA plate reader 450 nm.

D. The Number of Leukocytes Counting

Rat blood samples taken by the hematocrit and additional anti-coagulant EDTA (Ethylene diamine tetra acid), and then made preparations of blood smears on object glass. Blood film fixed with absolute methanol, and then staining by 10% Giemsa for 30 minutes. Smear preparations were washed with distilled water and air-dried. Observations and leukocyte counts performed with a microscup magnification of 100x, and the amount of leukocytes calculated as a percentage relative [13].

III. Results

A. Total Serum IgE

The average of total serum IgE in rats fed the goat milk and their fractions before and after treatment with DNCB are shown in Table I.

Supplementation of goat's milk or their fractions in rat had no effect on serum total IgE or the same as control rat. Supplementation of goat's milk or its fractions as much as 0.4 g / head / day did not decrease in serum total IgE.

TABLE I
THE AVERAGE OF SERUM IGE CONCENTRATION IN RAT BEFORE AND AFTER DNCB SENSITIZED (NG/ML)

	Before	After	Average
Supplementation			
Whey	22.918	63.770	43.354
Casein	59.491	66.897	63.194
Whole milk	52.725	52.674	52.699
Control	63.301	69.002	66.412
Average	49.014	63.090	56.202 [ns]

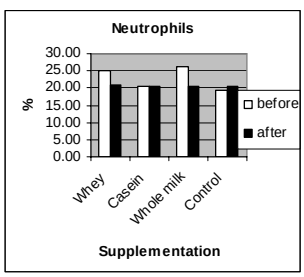
ns : non significant

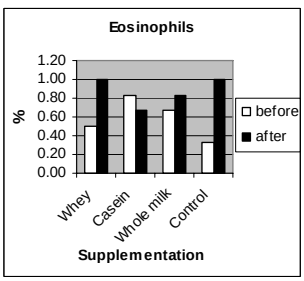
B. Leukocytes Count

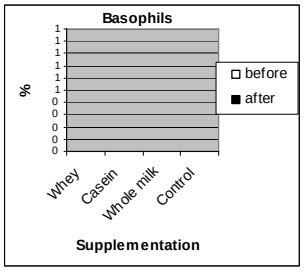
The average of relative percentage of leukocytes that includes neutrophils, eosinophils, basophils, lymphocytes, and monocytes in rat supplemented goat's milk and its fractions, before and after DNCB sensitization can be seen in Figure 1. Goat milk and their fractions supplementation have no effect on the percentage of rat leukocytes. However, DNCB sensitization effect on the number of monocytes, while the number of neutrophils, eosinophils, basophils and lymphocytes are not affected by DNCB treatment. The percentage of monocytes after DNCB sensitized were higher ($P < 0.01$) than before.

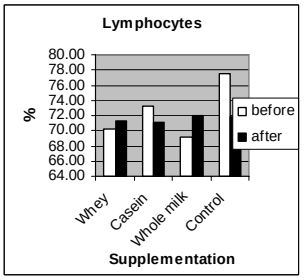
IV. DISCUSSION

As shown in Table I, the treatment of goat's milk whey prior to allergens tend to be the lowest IgE response among the other treatments (casein, whole milk and the controls), although it was not significant. It may be possible in the goat's milk whey contains components as a potential anti-allergic. Because the dose of whey that supplemented is too low lead to the decrease in serum IgE was not significant. According Debbabi et al [14], immunomodulatory properties, among others depending on the dose and the routes of antigen and antigen protein structure, which will affect the local immune response or peripheral. The results of extensive clinical studies in children with CMA in France, found that treatment of goat's milk gives a positive result in 93% of children, so it has been recommended in the nutrition of children due to low allergenicity and digestibility better than cow's milk [2]. Previous research has been done on rats that were given various treatments showed that the pectin fiber provides a lower IgE response than cellulose, so it is possible pectin as an anti-allergic [10].









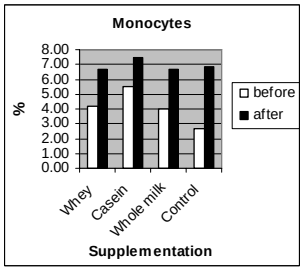


Fig. 1 The average of leukocyte percentage (a) neutrophils, (b) eosinophils, (c) basophils, (d) lymphocytes, and (e) monocytes in rat supplemented with goat milk fractions before and after DNCB sensitized

After DNCB sensitized, apparently serum IgE in rats supplemented goat's milk or its fractions are not significantly increased. These results indicate that 1% DNCB sensitization could not induce IgE response or allergic type I. Contact allergen DNCB is an allergen that can induce allergic responses of type IV or DTH (delayed-type hypersensitivity), and not induce type I allergy is characterized by an increased IgE response. Previous studies using rat supplemented with goat milk whey and DNCB sensitization showed a negative DTH response and also a decline in levels of cytokines IL-4 and IFN-γ [15]. Thus the goat whey whey could reduce inflammatory reactions that commonly occur in allergies. IgE levels depend on several factors, especially the chemical concentration. The fact in previous studies, TMA and DNCB may induce increased IgE, and induction is influenced by the dose given. TMA exposure influence on the development of asthma. Immediate-type hypersensitivity reaction associated with IgE production is distributed systemically and can bind to the receptor surface of mast cells, basophils, macrophages and other cells that present antigen [8]. Results of research conducted by Ban and Hettich [8] showed that the TMA 25% (instead of 1% DNCB) can induce high serum IgE levels, and are indicated by an increase in the number of IgE-producing plasma cells in the lamina propria of the trachea. TMA will lead to a Th2 response, whereas DNCB leads to a Th1 response [8]. These results confirm the opinion Ban and Hettich [8], that 1% DNCB did not induce IgE response.

The higher percentage of monocytes after DNCB sensitized (Figure 1e) is understandable since monocytes would later develop into macrophages in tissue, which acts the body in the first line of defense or as an APC (antigen-presenting cells). Therefore, if there is an antigen / foreign materials including allergen, the number of monocytes will increase. Basophils

were not found in leukocyte count (Figure 1c). These results are in accordance with the opinion of [16], that basophils are detected very low in uninfected rat, which is only 0.06% or 1/1600 of leukocytes), and the highest increase in number after 13 days of initial infection is about 4.5% of total leukocytes (80-fold increase compared to normal rat). Basophils also be increased if there is an allergic type I (IgE-mediated allergy), because metakromatic cell populations identified in several allergic diseases express the high affinity IgE receptor on their surface [17]. Leukocytes are increased if there is infection. Neutrophils would increase if there is an acute infection, whereas lymphocytes would increase if the infection is chronic. Therefore in this study there was no treatment of infection in mice, there was no increase in the percentage of leukocyte components. Previous research conducted by [11] showed *Lactobacillus plantarum* supplementation can increase the percentage of neutrophils and lymphocytes, but not increased basophils and monocytes. Neutrophils are responsible for phagocytosis of microbial pathogens during the first few hours after infecting the tissue.

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