EFFECT OF PROBIOTICS ADDED GOAT AND COW MILK YOGURT CONSUMPTION ON IMMUNOGLOBULIN A (IgA) INDUCTION IN HEALTHY ADOLESCENTS

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ABSTRACT: This study investigated whether daily ingestion of probiotics (*Bifidobacterium bifidum* and *Lactobacillus acidophilus)* added mixture goat and cow milk yogurt over 8 weeks would enhance innate immune function in healthy adolescents. The parameter of the innate immunity assessed was immunoglobulin A (IgA). The trial was performed in 20 healthy adolescents (10 males and 10 females) with 20 controls. IgA was measured at baseline, during and after treatment at -1, 0, 3, 5, 8, +1 and +2 weeks. A statistically significant increase in the IgA level ($p<0.05$) occurred over 8 week treatment. Since the final stage (2 weeks after stop supplement) the subjects' immune responses (IgA levels) returned to baseline levels that were not significantly different to the pre-trial values. In conclusion, the supplementation with probiotics formula has the potential to improve innate immune function in healthy adolescents.

Keywords: probiotics, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, immunoglobulin A (IgA), yogurt

INTRODUCTION: Probiotics are nutritional supplements containing potentially beneficial bacteria usually found in the gastrointestinal tract and are currently used to produce beneficial health effects in variety of conditions and diseases in people throughout the world. Probiotics such as lactobacilli have little or no pathogenicity, and may exist as part of the normal indigenous enteric flora[1]. One of the beneficial effects of probiotics is to prevent infectious diseases. Probiotics also enhance humoral immune responses by increasing Immunoglobulin A (IgA) producing cells and stimulating antibody responses to some specific antigens[2]. Specific conditions for which probiotics are used include prevention and treatment of antibiotic-associated diarrhea and other diarrheal illnesses, genitourinary tract infection prophylaxis, increasing immune function, decreasing cholesterol and lipid level and various immune disorders including atopic dermatitis and food allergy. *Bifidobacterium bifidum* and *Lactobacillus acidophilus* strains were reported to stimulate IgA product *in vitro*, implying the possibility that stimulation of IgA production was elicited by resident bacteria[3]. Immunomodulation by probiotics depends on the contact of these microorganisms or their components with the lymphoid tissue. It has been shown that *Bifidobacterium bifidum* and *Lactobacillus acidophilus* stimulate the systemic immune response (macrophage function and number of immunoglobulin secreting cells) as well as the local immune response (IgA secretion into the intestine)[4]. IgA plays a role in local immunity and has a significant function in creating a barrier against infections by pathogenic bacteria or virus. IgA is also actively produced in the intestine, and contributes to the elimination of infectious pathogens from the gastrointestinal tract. Some of probiotics strains have recently been shown to stimulate IgA production. Therefore, ingestion of probiotics may stimulate active IgA production, thereby reducing infections. Oral administration is the normal route by which probiotics are ingested by consumer[4]. In this study, we performed a feeding trial of probiotics added mixtures goat and cow milk yogurt formula in healthy adolescents to elucidate the influence of the formula on the production of IgA level.

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MATERIALS AND METHODS:

Yogurt preparation

Homogenized and pasteurized mixtures goat and cow milk (1:2) was heated to 45ºC and skimmed milk powder was added with high-speed stirring, to make 180g/l total solids in yogurt. Heating was continued to 80-85ºC, and the 1% yogurt starter culture (*Streptococus thermophillus* and *Lactobacillus bulgaricus*) was added. When the fermentation process reached a pH of 4.5, 2% encapsulated probiotics cultures (*B. bifidum* and *L. acidophilus)* were added. The yogurt mix was distributed in 500 ml plastic cups. Incubation was carried out at 42-43ºC until a pH of 4.5 was reached which time the yogurt was then cooled down in an ice water-bath and stored at 4ºC. Drying the yogurt using freeze dryer, frozen at -18 ºC for 24h and dried out at 25 ºC for 72 h.[6)]

Subject and trial design

Twenty healthy adolescent volunteers (10 males, 10 females) aged between 18-25 years were enrolled in an eight-week dietary supplementation trial. Subjects were drawn initially in response to an advertisement for volunteers. Suitable candidates were then selected and followed by informed consent and consultation with a doctor. Inclusion criteria included general good health and mobility, and agreement to conform with the trial guide-lines or provide notification of non-compliance. Exclusion criteria included any recent history of acute or chronic debilitating illness, any recorded of milk-product intolerance, and disagreement to avoid potentially conflicting nutritional or vitamin supplements during the nine week duration of the trial.

The subjects were made aware that some of the milks which they would consume might contain health-benefiting micro organisms, but in all other aspects were blinded with respect to trial design.

The subjects were received packs of probiotics added mixtures goat and cow milk yogurt powder. which were reformed by adding 100 ml. fresh water when intake every day in the morning for 8 weeks. They were instructed to avoid the consumption of other dairy products during the period of the study.

Blood samples were obtained by venipuncture from the subjects at seven time points throughout the trial. Blood drawn for measurement of serum IgA levels were performed at weeks -1, 0, 3, 5, 8,+1 and +2. Weeks -1 (7 days before day 0) and 0 (day 0) served as baseline collections performed before ingestion of probiotics supplements, week +1 (7 days after stop of intake of food sample) and week +2 (14 days after stop of intake of food sample). Probiotics ingestion began after specimen collection on day 0.

Volunteers were interviewed at each sub-sequent visit about consistency of nutritional habits, exercise routine, stress levels, and ingestion of immune supplements or fermented foods. Volunteers were also asked to report any changes in general health during these visits.

The control group (20 healthy adolescent volunteers) was received 100 ml. of plain mixtures goat and cow milk yogurt as placebo for the same period. They were instructed to avoid the consumption of other fermented dairy products during the period of the study. This research has been approved by the Mae Fah Luang University Research Ethics committee on Human Research, base on The Declaration of Helsinki.

IgA analysis

The total serum IgA level was determined by sandwich-type enzyme-linked immunosorbent assay (ELISA), in which anti–human IgA antibody was coated on an ELISA plate and detected with peroxidase-labled anti-human component of IgA[7)].

Statistical methods

Data from the three repeated experiments were analyzed by a SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). If the significant differences between means were found, the mean comparison with Duncan's multiple range test would be applied. The predetermined acceptable level of probability was 5% ($p<0.05$) for all the comparison.

RESULTS AND DISCUSSION:

Subjects demographic data

The data in Table 1 showed that the subjects ranged from 18 – 22 years old, including 10 men and 10 women (non-pregnant and non-lactating). All subjects considered in good general health,

reported no recent history of using immune modulating supplements and ingesting fermented food, and had no history of difficulty donating blood. All were willing to maintain their current pattern of diet, exercise and fermented food avoidance for the remainder of the experiment.

During study only 1 subject reported diarrhea and over gas in the gastrointestinal tract and 1 reported vomiting to occur once in a while, first day of the study. Thirty nine subject reported never (did not) this situation occurred during the intervention periods. No subject withdrew during the study.

The change of IgA level during probiotics-added mixtures goat and cow milk yogurt consumption

The intake of probiotics-added mixtures goat and cow milk yogurt significantly stimulated the production of serum IgA in healthy adolescents. The variation was significant over the 8 week ingestion ($p< 0.05$). IgA number did not vary significantly in the control group during the same period of the study.

The levels of total serum IgA were shown in Table 2. At the time before intake of probiotics-added mixtures goat and cow milk yogurt sample, serum IgA were 275 ± 2.4 and 274 ± 2.0 mg/dl in male and female in treatment groups, respectively. These levels were not different with the control group before the study. During intake, the levels of serum IgA in the treatment group significantly increased, with the highest levels occurring at 312 ± 4.3 and 309 ± 1.7 mg/dl on week 8, in male and female respectively. This IgA level was about 1.13 fold higher than the initial value. After stop of probiotics-added mixtures goat and cow milk yogurt intake on week +2 (14 days after stop intake of samples), the levels of IgA decreased and were close to the initial level. The levels of IgA in male and female were not significantly different in both treatment and control group.

Similar results have been observed by Yoichi *et al.*[8], that was performed to elucidate the influence of a probiotics formula on intestinal microflora and local immunity in healthy children. They reported that a follow up formula containing viable bifidobacteria was given to seven healthy Japanese children for 21 days. During intake of the formula, the administered strain was detected in feces from five subjects (71%) and fecal bifidobacteria slightly increased[8]. The levels of total IgA and anti-poliovirus IgA during intake of the formula were significantly higher than those before intake. The increase in local IgA levels resulting from the ingestion of probiotics formula may contribute to enhance of the mucosal resistance against gastrointestinal infection. Since secretary IgA plays a central role in local immunity and has a significant function creating a barrier against pathogenic bacteria or viruses, such increase may contribute to reinforce the mucosal resistance to infections[9].

Yasui *et al.*[10] indicated that bifidobacteria were stimulated IgA production *in vitro*, implying the possibility that the stimulation of IgA production was elicited by resident bifidobacteria. The ingestion of the probiotics formula containing some strains of bifidobacteria such as *B. lactis* Bb12 and colonization by the strain could trigger IgA production by the host[10].

The result of Zanini and colleagues[11] indicated that the complex yogurt, containing *Lactobacillus casei* and other strains of lactobacilli and bifidobacteria, and the simple yogurt, containing only *L. casei*, were administered daily to 77 healthy volunteers (41 adults and 36 children) for a period of 4 weeks. Microbiological and molecular analyses of faecal samples indicated that the complex product induced a larger increase in the lactic acid bacteria number, compared with the simple product, without significant alterations of the autochthonous species composition. Of the probiotics strains, *L. casei* showed the best survival rate in faeces. The influences of both products on

Table 1 Subjects demographic data

Demographic data	Treatment group	Control group
Total subjects	20	20
Average age (y)	18 ± 0.8	18 ± 0.7
Place of residence		
University dormitory	12	10
Private dormitory	6	8
Private Home	2	2

Table 2 Serum IgA levels of subjects before, during and after consumption probiotics added goat and cow milk yogurt (mg/dl)

Periods of blood drawn**	Treatment group		Control group	
	Male	Female	Male	Female
-1	275 ± 2.4^{aA}	274 ± 2.0^{aA}	275 ± 2.5^{aA}	275 ± 3.8^{aA}
0	276 ± 2.7^{aA}	274 ± 1.4^{aA}	274 ± 2.1^{aA}	275 ± 1.1^{aA}
3	286 ± 3.2^{aB}	287 ± 4.5^{aB}	276 ± 1.9^{bA}	276 ± 1.6^{bA}
5	297 ± 1.3^{aC}	295 ± 1.2^{aC}	276 ± 2.3^{bA}	275 ± 2.4^{bA}
8	312 ± 4.3^{aD}	309 ± 1.7^{aD}	276 ± 2.1^{bA}	275 ± 1.3^{bA}
+1	302 ± 3.6^{aE}	298 ± 2.3^{aE}	275 ± 3.0^{bA}	274 ± 4.1^{bA}
+2	278 ± 1.8^{aAF}	280 ± 3.4^{aAF}	276 ± 3.9^{aA}	276 ± 1.7^{aA}

*Means in the same row with different small letter superscripts for the different between treatment are significantly different ($p< 0.05$); means in the same column for the different periods with different capital letter superscripts are significantly different ($p< 0.05$). Data are expressed as mean ± SD.

** Weeks -1 (7 days before day 0) and 0 (day0) served as baseline collections performed before ingestion of probiotics supplements, 3, 5, 8 served as 1, 2, 3 weeks after day 0, week +1 (7 days after stop of intake of food sample) and week +2 (14 days after stop of intake of food sample). Probiotics ingestion began after specimen collection on day 0.

the immune system were similar, with increase of Natural Killer cell activity and proliferative response to *Candida albicans* in adults and secretory-IgA in children saliva, indicating that immunomodulating properties were probably supported by common traits in the two products. These results suggest that the probiotics products may trigger the immune response. The action on the immune system was strictly correlated to the presence of the probiotics bacteria in the intestine. The use of probiotics treatment was investigated to achieve higher immune production in healthy adolescence. This suggests that ingestion of the formula containing probiotics stimulated the production of IgA in the gastro-intestinal tract of adolescence and IgA is to eliminate invading pathogens from the gastro-intestinal tract[11].

The mechanisms that lactic acid bacteria use to affect the immune system and produce immunostimulative effects are unknown. Probably, lactic acid bacteria (LAB) alone or their products are absorbed by membranous epithelial (M) cells and transported to deeper lying lymphatic follicles where they are checked by immunocompetent cells. Eventually, LAB and their products are transported for immune analysis to systemic lymphatic tissues-mesenteric lymph nodes or the spleen. LAB were found in Peyer's patches after 6-12 hours and in mesenteric lymph nodes 48 hours after ingestion[12]. The interaction of probiotics with the immune cells associated with the intestinal tissue was studies by Perdigon *et al.*[13]. They observed that this interaction was different for each bacteria strain. Some bacteria antigens were only associated with immune cells in Peyer's patches of small intestine, whereas others interacted with cells of lamina propria of the small intestine and large intestine[13]. Differently mechanisms could influence the composition of the probotics that colonise the digestive tract. The two important are: antagonism among bacteria and local immunity. Disturbances in the ecological balance in the gut lead to the growth of harmful bacteria and to their possible translocation to internal organ, which induces disease[12].

CONCLUSION: One of the beneficial effects of probiotics is to prevent infectious disease. The ingestion of the yogurt containing probiotics stimulated the production of IgA in the gastro-intestinal tract of adolescents and IgA is to eliminate invading pathogens from the gastro-intestinal tract. The levels of serum IgA increased significantly during intake of the probiotics

formula. The balancing action of probiotics upon the intestinal microorganisms involves an augmentation in bacterial components, especially lactobacilli and bifidobacteria that may be beneficial to the host. Orally administered probiotics may pass through the gastrointestinal tract to reach the local lymphatic organ, in the gut. Subsequently, translocation of probiotics can lead to the activation of the local immune system in the gut, which results, in turn, in mucosal antibody production, especially of IgA[14].

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