# EFFECT OF PROBIOTIC ADMINISTRATION ON THE LEVELS OF FECAL SECRETORY IMMUNOGLOBULIN A IN PREMATURE INFANTS

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## ABSTRACT

*Secretory immunoglobulin A (sIgA) plays an important role in the defence of the gastrointestinal tract. The level of fecal sIgA is associated with increased neutralization and clearance of viruses. Preterm infant whose delay of intestinal colonization may benefit from strategies to support maturation of humoral immunity and endogenous production of sIgA by early colonization with probiotic. The objective of this study was to evaluate the effect of probiotic on the fecal sIgA level in newborn preterm infants. A randomized double-blind placebo control study of newborn preterm infants (30-36 weeks of gestational age) who were spontaneous delivered and had no asphyxia was conducted in NICU Soetomo Hospital-Surabaya November-December 2006. Both groups were breast fed. Group probiotic was given multistrain probiotic containing $10^7$ cfu of Lactobacillus acidophilus, Bifidobacterium longum, and Streptococcus faecium once daily for 14 days since $2^{nd}$ day of age. The control group was given placebo. Fecal sIgA was determined by an enzyme-linked immunosorbent assay before and after intervention. Subject/s who got Respiratory Distress Syndrome or sepsis during study was dropped out. Statistical analysis used in this study were Chi-square, Paired t - test, Independent sample t - test, Mann-Whitney, Wilcoxon Signed Ranks test, and Multivariate analysis of variance ($\alpha$ = 0.05). Forty seven neonates were enrolled, 7 of them were drop out (2 with RDS, 5 with sepsis). Forty analyzed neonates were divided in probiotic (n=20) and placebo group (n=20). There was no significant difference about basic characteristics between the groups. At first examination, median of fecal sIgA level did not differ significantly between two groups (p=0.512), 0.164 and 0.174 mg/g feces in probiotic and placebo group respectively. There was higher increment of fecal sIgA level in probiotic than placebo group (1.735 vs 1.449 mg/g feces, p=0.003). In conclusion, preterm infants may benefit from probiotic because of the observed clear tendency to increase fecal sIgA secretion.*

***Keywords*****:** *preterm infant, probiotic, fecal sIgA*

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## INTRODUCTION

The incidence of premature babies born in different countries are still high. In Dr Soetomo, got + 18% of all babies born preterm births. (IRD Data Dr Soetomo, 2005) Premature babies have 3-10 times the incidence of infection is higher (Stoll et al, 2004), and mortality due to infection 13% higher than newborns (Gomella, et al, 1999) The process of infection in newborns occurs largely through the mucosa, especially in the gastro intestinal tract, nasopharynx, orofaring, and conjunctiva (Gomella et al., 1999; Bellig et al., 2003; Gottof et al., 2004) Among the various mucosa, gastro intestinal mucosa is the largest (Hogenova et al., in 2004) and is the largest immune organ of the body; but in premature infants mucosa has a resilience that are not perfect. (Mackie et al., 1999; Bourlioux et al., 2003) One of the important components in the intestinal mucosa is resistant microflora, in which colonization of microflora in premature infants is delayed compared to infants. (Gewolb et al., 1999; Millar et al., 2003; Zhang et al., 2005) The delay in the process of colonization of intestinal microflora in premature infants causes less optimal stimulus for activation of intestinal mucosal immune response so that more premature babies are susceptible to infection.

Intestinal mucosal immune responses manifested in the form of humoral as immunoglobulin production, cytokines, and cell phones such as the activation of intraepithelial lymphocytes and lamina propria. (Mayer et al., 2005) From a variety of products gut mucosal immune responses, secretory immunoglobulin A (sIgA) is the main product mucosal immune system, and is the dominant immunoglobulin in mucosal surfaces. sIgA plays a role in gastrointestinal defense mechanisms against dietary and microbial antigens by performing immune exclusion through adhesion obstacles and

potential dangers of antigen invasion into the mucosal tissue and neutralize toxins and microbial pathogens virulent factors. (Mestecky et al., 1999; Woof et al., 2005), so low sIgA levels are a risk factor for infection in the mucosa. (Mayer et al., 2005; Woof et al., 2005) examined levels of intestinal sIgA intestinal fluid is too invasive for a newborn, and studies have shown that sIgA levels in the feces gives a good representation of total sIgA in colon. (Grewal et al., 2000)

The presence of microflora in the intestine is necessary to trigger the development of humoral and cellular immune systems in intestinal mucosal immune system during the neonatal period (Cebra et al., 1999; Stagg et al., 2003) The development of IgA producing plasmablast in intestinal mucosa and production of intestinal sIgA is strongly influenced by the gut microflora. (Cebra et al., 1999) In premature infants, due to the relatively sterile treatment patterns, use of antibiotics, too late to get milk, etc., causing microflora colonization disrupted / delayed. In breastfed babies, the highest levels of sIgA was found in the first days of life, then decreased gradually. (Zierikzee et al., 2006) sIgA concentration was less in infants who are not / too late to get breast milk as often experienced by premature babies, because in addition to containing sIgA, milk contains bifidus factor to trigger the growth of intestinal bifidobacteria. (Mayer et al., 2005)

Based on the above theory, consider a strategy to accelerate the process of colonization of the intestines in premature infants with the administration of probiotic live bacteria. Probiotics are dietary supplements that contain live microorganisms that benefit host health. (Schrezenmeir et al., 2001) After treatment with probiotics, found elevated levels of intestinal sIgA significantly in rats try (Grewal et al., 2000), and children (Fukushima et al., 1998); and increased serum IgA levels in premature infants . (Cukrowska et al., 2002) fecal sIgA research has been conducted on adult patients (Watanabe et al., 2003), and in children (Dion et al., 2004). In newborn infants who do not get breast milk has also proved the existence of a positive influence on fecal sIgA levels after administration of prebiotic and probiotic (Zierikzee et al., 2006), but have never done on premature babies. Therefore in this study, to prove the effect of probiotic in increasing intestinal mucosal immune response in premature infants be examined levels of sIgA from stool specimens as a mirror of the gut mucosal immune responses.

## MATERIALS AND METHODS

This study is a randomized double-blind experimental pre test - post test control group design. Research conducted in the Observation Room Neonatal Intensive Care Unit Emergency Room and Installation of Intermediate Neonates Dr Soetomo, starting from November to December 2007 against premature baby. Airworthiness of ethics has been obtained from the Airworthiness Team Ethics Section of Child Health Dr Soetomo. The inclusion criteria of this study is the baby born in the Delivery Room IRD Dr Soetomo with gestational age 28-36 weeks based on the Ballard Score, born in physiological (vaginal without action), not experiencing asphyxia, not with the oral cavity anatomical abnormality which causes difficulties giving drink, with no major congenital abnormalities, age less than 24 hours, and parents willing to undertake this research by signing a consent form. While exclusive criteria are not breastfed babies, or not to remove feces within 24 hours of the first life. Infants will be excluded from the study if the parent research trip to follow the patient refused to continue the study, patients experiencing sepsis or Respiratory Distress Syndrome (RDS), patients can not be tracked his existence before the research is completed so it can not be observed and completed research, or patient died. Infants who experienced sepsis or RDS remains are followed.

If a parent has signed a letter of consent as research subjects, infants included in groups A or B according to the results of randomization. Baby's general condition is checked, birth weight, and fecal samples were taken out within 24 hours after birth. Intake of fecal done by first installing urine collector for urine does not wet the feces. Feces collected on the plastic, and retrieved using a small plastic spoon immediately after defecation and patients included in the cryotube, then stored in a temperature of-200C in the Laboratory of Microbiology & Virology Faculty of Veterinary Medicine, Airlangga University. Before the stool samples sent to the laboratory, samples were stored temporarily in a refrigerator freezer, and during delivery included in the sample which has been given stereofome cooling to keep the stool is not dry. Samples should be already in the temperature-200C prior to 24 hours after taking stool.

On the second day the baby was given the stocks of packaging sachet of powder that were coded as A or B according to the group, 1 sachet / day for 14 days. Preparations were reconstituted with 5 cc of D5%, given orally using a spoon / speen or per sonde according to the ability of infants to drink. Package code of A or B is given and kept secret by the production department of pharmacy of Dr Soetomo, and recently opened after the study is completed. Recorded history of antenatal care, the presence of maternal infection / febrile before the birth, the premature rupture of membranes, amniotic cloudy / mekoneal. Clinical examination was performed

every day to know the symptoms and signs of infection, weight changes, namely gastro intestinal disorders of gastric retention, vomiting, abdominal distension, and abdominal wall erythema. If found symptoms and signs of infection, complete blood examination, CRP, and blood culture, and the infants excluded from the study but still be observed. On Day 16, repeated stool samples taken in the same way. If the baby has not defecate in the day, making stools done in the next 1-2 days.

After all fecal samples (pre and post) collected from all study subjects, was examined for levels of sIgA by ELISA technique in the Laboratory of Microbiology & Virology, Faculty of Veterinary, Airlangga University.

The statistical test used in this study is the Chi-square test, Wilcoxon Signed Rank Test, Mann-Whitney test, Multivariate analysis of variance, independent t test and Paired t test, with significance level $p < 0.05$. The results then are presented in narrative form, tables and graphs.

## RESULTS

During November - December 2006 found 79 babies born prematurely (15.4%) of 513 infants born in the Delivery Room Dr Soetomo IRD. There were 47 infants who fulfilled the inclusion criteria, 7 of which can not continue the study because two babies had respiratory distress syndrome (one infant from each group), both died, and five babies had severe sepsis (four infants from the placebo group). Forty infants who remained divided in the probiotic and placebo groups according to randomization, each 20 babies. All these infants were examined fecal sIgA on the first day of life. On the second day of treatment given to the provision of a placebo control group and the provision of probiotics in the treatment group, where the contents of both the new regimen known after the study is completed. After 14 days administration of placebo or probiotics, conducted re-examination fecal sIgA levels on days 15-18.

### Sample Characteristics

The basic characteristic of this sample is shown in Table 1, which showed no significant differences in gender, gestational age, Apgar score first and fifth minutes, birth weight, history of premature rupture of membranes, amniotic fluid color, and a history of febrile mothers / intrapartum infection between the two group.

Although the inclusion criteria of gestational age is between 28 to 36 weeks, but in this study no infants with gestational ages based on the Ballard Score less than 30 weeks. If the gestational age disaggregated into 30-33 weeks and 34-36 weeks, also found no significant differences between the two groups ($p = 0.561$).

Table 1. Characteristics of the sample on the basis of the probiotic and placebo groups

| | Probiotic group (n= 20) | Placebo group (n= 20) | *p* |
|---|---|---|---|
| Gender | | | 0.201 |
| Men | 6 | 11 | |
| Women | 14 | 9 | |
| Gestational age (weeks) | 32.6 ± 1.88 | 33.3 ± 1.37 | 0.219 |
| 30-33 weeks | 11 | 10 | 0.561 |
| 34-36 weeks | 9 | 10 | |
| 1 minute Apgar score | 6 (3-8) | 7 (5-8) | 0.327 |
| 5 minute Apgar score | 8 (5-9) | 8 (7-9) | 0.445 |
| Birth weight (g) | 1572.50 (±263.32) | 1742.50 ± 354.39) | 0.093 |
| ≤ 1500 g | 12 | 6 | 0.112 |
| > 1500 g | 8 | 14 | |
| Premature rupture of amnion | 5 | 4 | 1.000 |
| Cloudy/meconeal amnion | 2 | 2 | 1.000 |
| Febrile/infectious mother | 3 | 2 | 1.000 |

* Differences significant at p value <0.05

**Fecal secretory immunoglobulin A levels**

Taking the first stool specimen (before treatment) can not be done simultaneously at the age of 0 days, because the feces that have been discarded before the infant entered the study or the number of stools that are too small. Thirteen samples were taken at the age of one day, six of the probiotic group and seven from the placebo group (p = 0.327).

Stool specimens making replications (after treatment) is conducted varies from age 16 -18 days. If the baby has been discharged from the hospital before the study is completed, preparations for the treatment brought home and asked to remain granted on the baby every day with the monitoring using the phone. Stool specimens were taken by officers to the house of study subjects by following the planned procedure.

SIgA levels of fecal examination performed after all samples collected by using ELISA techniques. Stool sIgA levels are calculated based on the conversion of the Optical Density readings generated from the ELISA reader, and expressed in units of mg / gram of feces. There is a baby who had higher levels of fecal sIgA beginning of a very high compared to the others ie 1, 424 mg / g of feces. Under the normality test, the concentration of fecal sIgA was found abnormal distributed, so statistical analysis for fecal sIgA levels using the Mann-Whitney test and Wilcoxon signed ranks test. Babies who have high levels of fecal sIgA is derived from the placebo group, had a birth weight of 2000 grams, with 36 weeks of gestation, and have been able to drink enough milk from the first day of birth.

Fecal sIgA levels before and after treatment between both groups can be seen in Table 2. It appears that at the initial examination, there were no significant differences in levels of fecal sIgA in both groups (p = 0.512). While on cross examination (after treatment), showed significant differences with fecal sIgA levels between the two groups (p = 0.003), whereby higher levels of the probiotic group 19.7% compared with placebo.

Table 2. Fecal secretory IgA levels before and after treatment between the two groups

| Levels of fecal IgA (mg/g feces) | Probiotic group (n= 20) | Placebo group (n= 20) | *p* |
|---|---|---|---|
| Before treatment | 0.164 (0.018-0.965) | 0.174 (0.018-1.424) | 0.512 |
| After treatment | 1.735 (1.637-3.602) | 1.449 (0.799-2.085) | 0.003* |
| Elevation | 1.704 $\pm$ 0.5492 | 1.336 $\pm$ 0.4543 | 0.027* |

* Differences significant at p value <0.05

Table 3 shows the fecal sIgA levels before treatment than after treatment in each group, where there are significant differences both in the probiotic group (p = 0.0001) or placebo group (p = 0.0001) levels after treatment is higher than before treatment. This increase also can be seen from Table 2, where the average increase in the probiotic group is greater than with placebo (p = 0.027).

Table 3. Fecal secretory IgA levels before and after treatment in each group

| Group | Levesl of fecal IgA (mg/g feses) | | *p* |
|---|---|---|---|
| | Before treatment | After treatment | |
| Probiotic | 0.164 (0.018-0.965) | 1.735 (1.637-3.602) | 0.0001* |
| Placebo | 0.174 (0.018-1.424) | 1.449 (0.799-2.085) | 0.0001* |

* Differences significant at p value <0.05

Figure 1 clarifies the data that increases in levels of fecal sIgA in the probiotic group greater than placebo.

**Confounding factors**

From the 40 infants studied, 28 of them get Ampicilin antibiotic-sulbactam with or without Netylmycin, which all begin on the first day of birth. The number of infants who received antibiotics from each group along with the old gift can be seen in Table 4.

Based on Chi-square test, showed no significant difference in the number of infants who received antibiotics in both groups (p = 1.000). Similarly, duration of antibiotic also did not differ significantly according to independent sample t test (p = .733). All infants in this study to get the milk by giving the start time is different, where the median start time of administration occurred earlier in the placebo group (p = 0.033). However, given the volume of breast milk is still very minimal with average volume began giving 2-5 cc every four hours, which increased gradually in accordance with a baby drinking tolerance. Based Multivariate analysis of variance, either granting or breast feeding antibiotics on study subjects did not affect the difference in the results of fecal sIgA levels between two groups with significance level (p) respectively 0.536 and 0.789.

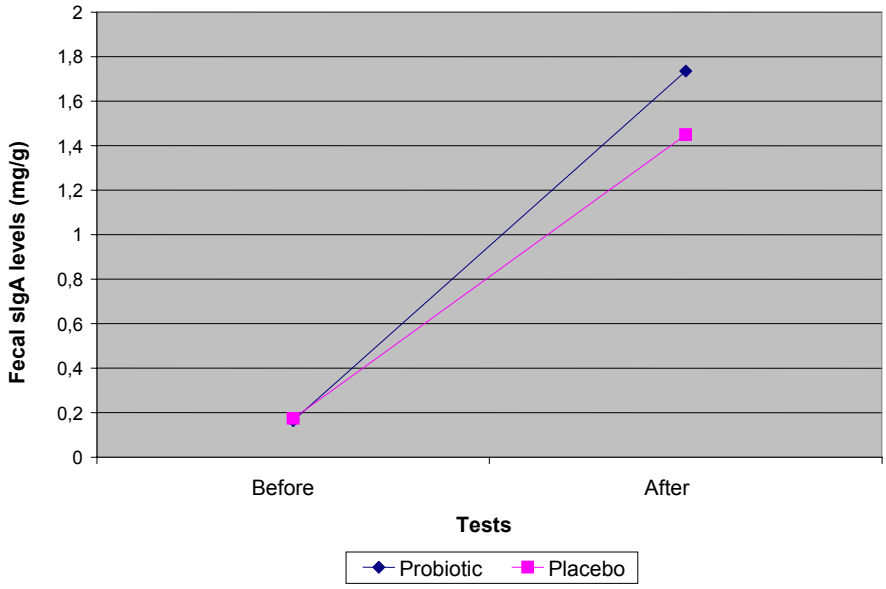


Figure 1. Median fecal sIgA levels before and after the treatment in both groups.

Table 4. Distribution of infants who received both antibiotics and breastfeeded group

| | Probiotic group (n= 20) | Placebo group (n= 20) | *p* |
|---|---|---|---|
| Received antibiotics | 14 | 14 | 1.000 |
| Duration of antibiotics treatment | | | 0.733 |
| 3 hari | 8 | 10 | |
| 7 hari | 6 | 4 | |
| Began receiving breastfeed (days) | 3 (1-4) | 2 (1-5) | 0.033* |

* Differences significant at p value <0.05

**Side effects**

During clinical monitoring study carried out every day to monitor any side effects such as gastro intestinal disorders such as diarrhea, constipation, bloating, gastric fluid retention, vomiting, and symptoms and

signs of sepsis. Complete blood examination, CRP, and blood sugar levels performed on the first day. Asymptomatic hypoglycemia was found in four infants, each group of two babies, but they can be overcome by D10% infusion fluid.

There were four infants with diarrhea (all from the placebo group), but none of which showed growth of bacteria in stool cultures performed. Gastric fluid retention occurs less in the treatment group (5 vs. 9 infants).

Five infants experienced symptoms and signs of sepsis so that the treatment was stopped, and Complete Blood examination, CRP and blood culture. Clinically observed every day until the patient declared cured or dies. One in five infants from the probiotic group (0.05% of the probiotic group) and having symptoms of sepsis from day-7, but the results of blood culture showed no growth of bacteria, including probiotic bacteria. The patient was otherwise improved and discharged from the hospital at the age of 18 days. Four infants of the placebo group (0.2%) experienced sepsis in a row on day-3, 3, 5, and 6. One of four babies died at the age of 10 days, with Enterobacter aeruginosa culture results; three other babies recovered and repatriated at the age of 18.19 and 22 days in good condition, with the results of blood culture of Pseudomonas in one patient.

## DISCUSSION

Characteristics of the subjects in this study did not differ significantly according to gestational age, birth weight, Apgar score first and fifth minutes, infection risk factors such as premature rupture of membranes, amniotic fluid color changes, febrile mothers or intrapartum infection (Table 1), so it can be said that factors could be excluded as the cause of differences in research results. At the age of 0 or 1 day, feces all infants in the two groups was examined sIgA levels. In Table 2 it appears that the median levels of fecal sIgA probiotic group at the first days of life was 0.164 mg / gram of feces, whereas in the placebo group 0.174 mg / gram of feces ($p = 0.512$).

Previous research found that the median age of 0 day fecal sIgA levels in newborns are breastfed 10.918 mg / g feces, whereas in newborns who do not get breast milk, fecal sIgA has not been sought. (Zierikzee et al., 2006) This explains the acquisition of fecal sIgA in infants aged 1-2 days in our study, because all infants in this study breastfed (Mother's Milk), although with a start time and the number of different gift.

In this study there were significant differences regarding the start time of breast feeding, which earlier in the placebo group received breast milk (2 days vs. 3 days, $p = 0.033$). Based on this reality, can be logically accepted that infant fecal sIgA levels in the placebo group at the beginning of the study is higher than the probiotic group although no significant difference, because the baby is getting passively sIgA from human milk. Nevertheless, based on the Multivariate analysis of variance showed that the factors when starting breastfeeding in this study did not affect fecal sIgA levels with $p = .789$. Low levels of fecal sIgA in this study compared to previous studies provide evidence that premature babies have extremely low levels of sIgA in the first days of life.

This supports the theory still weak intestinal mucosal immune response of preterm infants as reflected by the production of sIgA. One factor that may occur in premature infants of a delay in the colonization of the intestine, where normal intestinal colonization is the stimulus for the occurrence of an immune response in the intestinal mucosa. (Cebra, 1999)

Seventy percents of infants in this study have long given antibiotics with different, but found no significant differences regarding duration of and number of infants receiving antibiotics between the two groups ($p = 0.733$ and $p = 1.00$). Homogeneity of the long use of antibiotics may be needed in this study, because antibiotics have a major influence on mucosal immune responses by affecting the existence of the normal intestinal flora, where the old antibiotics can kill the normal intestinal flora and reduce the stimulation of the immune response in the intestinal mucosa. A study showed that giving antibiotics during the first week of reduced number and phenotype of lymphocytes in Peyer's Patches. (Yaguchi Y et al., 2006)

After treatment, there were elevated levels of fecal sIgA in both the probiotic and placebo groups, where the concentration in the probiotic group was higher than placebo. Naturally, intestinal sIgA production will gradually increase with increasing age. (Mayer, 2005) As for other factors that affect the synthesis of sIgA of providing breast milk or formula, the presence of intestinal colonization, the presence of intraluminal infection, and antibiotics. (Woof et al., 2005) In this study, several factors have been controlled by random processes such as breastfeeding and antibiotics. This study uses patented probiotic products multistrain acidophillus consisting of Lactobacillus, Bifidobacterium longum, and Streptococcus fecalis, which are all gram-positive bacteria. This material was chosen as the treatment regimen, because the product contains probiotic bacteria strainnya each of which has been proven in many studies of its superiority as an

immunomodulatory mucosa. (Hoyos AB et al., 1999; Lin HC et al., 2005) With the expected effects of probiotics multistrain imunomodulasi synergy with each other, because specific probiotic strains have effect. But in this study can not be known strains of probiotics are more positive role in increasing the synthesis of immunoglobulin A. Ideally, this study uses several single strains of probiotic strains that can be proved where a particular role, but in Indonesia at the time of the research done, no probiotic products on the market that contain a single strain. Another reason the use of this product in this research because these products have been circulated widely and easily available on the market, well-shaped granules in sachets are easy to be dissolved so that the easy way of administration.

What is the mechanism of probiotics in improving intestinal sIgA levels represented by the examination of feces in this study, it is not done the research. But some suspect that the literature contained in the peptidoglycan cell wall of probiotic bacteria, will serve as antigens in the intestinal lumen, which then stimulates the intestinal mucosal immune response. (Cebra, in 1999) Although this response can be triggered also by the bacterial pathogen, but the benefits derived from antigens of pathogens, probiotics compared very clear, namely that probiotics did not cause infection. Peptidoglycan composing the bacterial cell wall of gram-positive pathogens is acting as-associated molecular patterns (PAMPs) or antigen to be captured specifically by dendritic cells in intestinal mucosa through its surface receptors are pattern recognition receptors (PRR) or Toll-like receptors (TLRs) Toll-like receptors (TLRs), to subsequently be presented on T cells (AJ Stagg, 2003) signals through a different TLR produces different biological responses, and different TLR expression by different subsets of dendritic cells caused DC subset to respond to specific microbial structures. (Stagg AJ et al., 2003; Ezendam J et al., 2005) Of the 11 types of TLR that has been found in humans, TLR 2 and TLR 4 is an act to recognize PAMPs from gram-positive bacteria, including probiotic bacteria in general.

As is known, from a variety of products gut mucosal immune responses, secretory immunoglobulin A (sIgA) is the main product mucosal immune system, and is the dominant immunoglobulin in mucosal surfaces, so it can be said that measuring the levels of sIgA from intestinal fluid or feces can reflect on the competency of the immune response intestinal mucosa. (Mestecky J et al., 1999; Mayer L et al., 2005) This can be described as relatively stable intestinal sIgA in the intestinal lumen. Stability of sIgA This is one of the hallmarks of sIgA has a special structure that is not destroyed by proteolytic enzymes, namely its secretory component (SC). SC is a fragment of the polymeric IgA receptor (pIgR) that play a role in the transport of polymeric Ig (containing chains A) via the epithelial cells of mucosal surfaces that limit, so all have sIgA SC. This component is a protein produced by diglikosilasi and mucosal epithelial cells, stabilizing the structure of polymeric IgA and protects the IgA molecule from proteolytic activity of secretion. (Woof JM et al., 2005) This makes intestinal sIgA was found relatively intact in the feces, so check sIgA in the feces can be used to determine the immune response in the intestinal mucosa. It has been proved by studies in mice try to get that content in feces and intestinal sIgA positive correlation, whereby higher levels of fecal sIgA than sIgA intestinal. (Grewal et al., 2000) More high levels of sIgA in feces is probably due to the accumulation of fecal sIgA sIgA than sIgA are produced along the entire gastrointestinal tract, whereas only a limited measure intestinal sIgA levels in one segment of the gastrointestinal tract.

Based on the fact that the levels of fecal sIgA can reflect intestinal sIgA levels, this study used examination sIgA levels of fecal specimens to identify intestinal mucosal immune response, considering the subjects in this study were preterm infants. to take no action that is too invasive, was examined sIgA from stool specimens as a mirror of the gut mucosal immune responses. This study only found that there are elevated levels of fecal sIgA (which is expected to represent intestinal sIgA) in premature infants born after the administration of probiotic bacteria for two weeks. These results support the idea that the presence of microflora in the intestine is necessary to trigger the development of humoral and cellular immune system in the gut mucosal immune system during the neonatal period (Cebra, 1999; Stagg AJ et al., 2003), so that the colonization of the gut microflora in infants who are not perfect premature need triggered by an early colonization of using probiotics. Similar results obtained in previous research that found increased levels of intestinal sIgA significantly after administration of probiotics in rats try (Grewal et al., 2000), and children (Fukushima et al., 1998).

Security of probiotics in premature infants is still debated. (Millar et al., 2003; Zhang et al., 2005) However, a number of overseas studies using a premature baby as a research subject for the investigation of various things have been done, and none of which found no side effects. (Millar et al., 1993; Kitajima et al., 1997; Uhlemann et al., 1999; Costalos et al., 2003; Lin HC et al., 2005) Similarly, in this study found no side effects such as sepsis feared. This is evident from only the obtainment of a baby in the probiotic group had sepsis from day-7, but from his blood culture showed no bacterial growth. Quite the contrary in the placebo group, there were four infants with sepsis. Two of them suffered severe sepsis baby

started day 3 with a blood culture showed bacteria including Enterobacteriaceae in an infant; two infants experienced severe sepsis day in succession from the 5th and 6th, where the last baby was found on blood culture Pseudomonas. From these data, it was suspected that probiotic bacteria given early after birth has a protective effect against early infection in newborns, which generally occurs in the mucosa (gastrointestinal or respiratory), so that in the probiotic group that experienced no sepsis before day 7; opposite infant sepsis of the placebo group experienced sepsis caused by pathogenic intestinal bacteria in third day.

## CONCLUSIONS

There are elevated levels of secretory immunoglobulin A good stool fed premature infants who were not given probiotics or probiotics, but elevated levels of secretory immunoglobulin A premature infants fed fecal probiotic higher than those not given the probiotic, where probiotics are given for 14 days proved to increase the levels of fecal secretory immunoglobulin A premature infants at 19.7%.

Similar research needs to be done that compare several single strains of probiotic strains of probiotics to determine the most effect on elevated levels of fecal sIgA, as well as examination of probiotic bacteria are fecal culture as well as research that proves more specifically the role of probiotics in enhancing mucosal immune responses, for example by screening plasmablast producing IgA, TLR-2, and TGF-β.

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