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RESEARCH ARTICLE

The Role of Cowmilk's Colostrum as the Source of Passive Immunity and Growth Factor for Improving the Haematological Status and Titre of Antibody against Hog Cholera in Pre-Weaning Piglets

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Abstract

Background: pre-weaning piglets often suffer from anemia, and infected with the bacteria so as growth slows even death. Aim: The purpose of this study was to determine the effect of cow colostrum administration on hematology profile and anti-body titers as an immunomodulatory effect to prevent germ infection. Methods: Seventy two piglets were divided in 4 groups in this research. Group who were not given bovine colostrum as a control, group who were given cow colostrum dose of 3 ml per animal, groups of piglet given bovine colostrum dose of 6 ml per animal, and a group of bovine colostrum piglet given a dose of 9 ml per animal. At the age of 7 days vaccinated against Hog Cholera and repeated at 14 days. Besides, at the period of 20 days all the piglets to have blood drawn and then accommodated in a vacuum tube which already contains anticoagulant (EDTA) for examination hematological profile is checked with Automated Manual VET ABC Blood Counter, made in Germany. Finally at the age of 30 days all piglet blood drawn and then accommodated in the tube for examination Hog Cholera antibody titer by ELISA. The data were analyzed with statistical test. Result: The results showed bovine colostrum supplementation at a dose of 1-5 days of age piglet 3-9 ml/piglet can raise the profile hematology and Hog cholera antibody titer. Conclusion: Addition of cowmilk's colostrum in pre-weaning for the first five days of life could improve both the hematological profile and immunomodulatory set.

Keywords: *Piglet, Bovine colostrum, Hematology.*

Introduction

In piglets under 21 days old, the immune system has not fully developed and thus making this critical period extremely vulnerable to death [1]. This is still considered the most common and the most vital etiology above any other causes and is hard to deal with which makes breeders often complain of having their piglets died. It has been reported that the only way to prevent infection in newborn livestock with the immature immune system is by increasing specific immunoglobulin (immune compounds that are naturally inherited from its mother) concentration either through transplacental transmission of immunoglobulin during pregnancy or via colostrum given during the first three days after delivery. Unfortunately, piglet mothers could not transfer their immunoglobulin to their fetus via placenta since the placenta is too thick to penetrate. Therefore, it is understandable that piglets were born without having any inherited protection towards various kinds of infectious agents in the enclosure environment. The only source of immunoglobulin for these newborn animals is the maternal colostrum. Maternal antibodies or immunoglobulins are naturally inherited from the mother through

placenta or colostrum; it can also be given in an artificial form. Explicitly, in piglets, the maternal antibodies could never pass through the placental barrier, hence the only route of transfer of the antibodies is via colostrum. Colostrum is a thick fluid discharged by maternal mammary glands during the first 2-3 days of life. Colostrum possesses extremely important compounds that play significant roles in preserving health and stimulating the growth of the piglets. Colostrum is one of the methods of transfer of maternal antibodies that enhance piglet's immunity and protect it from environmental pathogens.

In addition, colostrum also provides sufficient amount of nutrients, including protein, carbohydrate, fat, vitamins, and minerals [2], that are required for normal growth and morphological development of the piglets [3].Rich and complete nutrient contents help to induce optimal growth and development of all body systems, including normal hematological parameters.

Normal hematological parameters of pre-weaning piglets are as follow: total leukocytes (10.98 x 103 /mm), total erythrocytes (5.74 x 106/mm), hemoglobin level (11.44 g/dl), hematocrit (36.16%), platelet count (318 x 106/mm), percent neutrophil (36.6%), percent eosinophil (1.6%), percent lymphocyte (65.4%), and percent monocyte (6.8%) [4]. In the first 24 hours of life, piglets should at least be given a total of 200 grams of colostrum to suppress the risk of pre-weaning mortality; however, this amount is often unreached.

It has been reported that newborn piglets usually receive less than 100 grams colostrum per day and therefore predisposes to the occurrence of diarrhea and growth delay. Newborn piglets must be given their maternal colostrum in order to prevent perinatal mortality. Accordingly, artificial colostrum administration provides an alternative source to meet the requirement and one of which is synthesized from cowmilk's colostrum.

Cow milk's colostrum contains a high amount of modulating protein and other peptide substances capable of preventing inflammation [5].Experts have disclosed that immune factors contained within the colostrum include IgA, IgM, IgG, and IgE that can protect piglets from bacterial, viral, fungal, parasitic invasion.

The previous study had reported specific biological activities of cowmilk's colostrum, both growth stimulation, and immune response. If the piglets do not receive any colostrum, it puts them in a state of immune deficiency that increases the likelihood of infection [6].Previous studies have reported that cowmilk's colostrum carries a significant number of anti-H. Pylori immunoglobulin that works actively against H. Pylori colonization in mice [7].

It has been reported that cowmilk's colostrum can also reduce mortality rates in piglets infected by E. Coli [8].Administration of commercial cowmilk's colostrum (Pigstrum) in piglets aged 2-4 days old for 2 ml/piglet is considered adequate to reduce mortality rates and increase their body weight [9]. Studies have demonstrated that supplementation of 6-9 ml cowmilk's colostrum per piglets for 1-5 days is optimal to induce the development of intestinal villi and escalation in intestine length and weight that further stimulate growth and reduce morbidity and mortality in pre-weaning piglets [10].

This is owing to growth factor contents that consist of the following types of growth factor: Insulin-like growth factor responsible for energy production and storage in the form of meat or muscles so that the piglets can achieve rapid growth and development [11].Epithelial growth factor plays essential roles in controlling gastric acid secretion and triggers the growth of epithelial cells and fibroblasts in tissues that is also another aspect of optimal growth in piglets [12].

Reports have demonstrated that colostrum is able to escalate the growth of intestinal villi, increase intestine size and weight, and increase intestinal absorption of nutrients from the ingested food by 50% of the regular absorption rate in the first day of life and is doubled if colostrum is consistently given for consecutive 10 days after delivery that facilitate a more rapid increase in intestine weight that is 90% faster than the average growth [13]. Vitamins and hormones in the colostrum take part in inducing piglets' growth naturally.

Therefore, the piglets can typically experience rapid growth.

It has also been reported that maternal antibodies obtained from colostrum are the passive immunity that protects piglets from infection; however, the protection is temporary. Maternal antibodies enhance newborn piglets' immunity by interfering with the growth of pathogenic organisms or facilitating pathogens eradication through opsonization. On the contrary, maternal antibodies decrease the efficacy of vaccines, particularly active vaccines, by coating antibody that engulfs the epitope of B cells, subsequently interrupting B cells initiation [14].

The critical disadvantage of this is that highly circulating maternal antibodies cause neutralizing reaction with the vaccine antigens administered resulting in reduced effectiveness of the vaccine to stimulate an active production of antibodies [15]. Thereof, piglets with considerable antibody levels will show lower antibody titer following vaccination.

If the piglets receive artificial colostrum in the purpose of growth stimulation, it is not clear whether it leads to decreased post-vaccination antibody titer; hence, studies have to be undertaken to elucidate this issue. Ardana et al [4].Reported that a 30-day-old piglet that had been vaccinated at 7 days post-partum and received a booster at 14 days postnatal had low mean anti-Hog Cholera antibody titer of 41.5% PC value. This titer of antibody possesses significant protective effects.

Methods

Piglets as Animal Models

This study involved piglets that were given commercial cowmilk's colostrum supplementation as the animal subjects. This study was performed at a pig farm owned by Mr Yance located in Tuka Village, Saling, North Kuta, Badung, Bali. Routine blood examination was performed at Veterinary Clinical Pathology Lab of Faculty of Veterinary, Udayana University, while the measurement of anti-Hog Cholera antibody titer was conducted at Veterinary Center Denpasar. This study used a total of 72 healthy piglets aged one-day-old weighing about 1.6-2.1 kg that was breastfed.

Experimentation

A total of 72 piglets, along with their mothers were randomized into four different groups. Each group consisted of 18 piglets used for the following experiments: group one only received aquadest/placebo as the control group; group two received 3 ml cowmilk's colostrum for the first five days of life; group 3 received 6 ml cowmilk's colostrum for the first five days of life; group 4 received 9 ml cowmilk's colostrum for the first five days of life. At seven days post-partum, all the piglets received Hog Cholera vaccine administered intramuscularly, and at 14 days they received the second dose of Hog Cholera vaccine.

By the age of 20 days old, blood samples were collected in a vacuum tube containing anticoagulant (EDTA) for subsequent assessment of haematological profile using Manual Auto Hematology Analyzer. Finally, at 30 days post-partum, blood samples were collected once again for measurement of anti-Hog Cholera antibody titer using ELISA. The data were analyzed using variance and should an apparent influence exist. The analysis was continued with Duncan's multiple range tests.

Results and Discussion

Hematological Profile

The results regarding the analyses of the effects of cowmilk's colostrum on piglets' haematological profile are presented in Table 1. The impacts of cowmilk's colostrum supplementation on the differential count of the white blood cells showed in Table 2.

Table 1: Effects of colostrum supplementation on piglets' hematological profile

Treatment (colostrum supplementation)	Variables			
	WBC/(x10^3/mm)	RBC(x10^6/mm)	HB (gr%)	PCV (%)
0 ml/piglet aged 1-5 days (n=18)	7.06^a	5.29^a	14.78^a	37.1^a
3 ml/ piglet aged 1-5 days (n=18)	8.68^{ab}	6.28^{bc}	15.10^a	38,1^{bc}
6 ml/ piglet aged 1-5 days (n=18)	9.59^{ab}	6.0^b	16.40^a	37,1^a
9 ml/ piglet aged 1-5 days (n=18)	11.20^b	6.65^c	17.82^a	37.57^c

Annotation: the same letter written in the same column indicates no significant difference (p>0.05)

Table 1 demonstrates that supplementation of cowmilk's colostrum in newborn piglets significantly increases the WBC and RBC populations as well as PCV concentration (P <0.05). The effects of administration of 9 ml cowmilk's colostrum showed an increase in total WBC (11.20 x 10^3/mm) and total RBC (6.65x10^6/mm) that were significantly higher than control: 7.06 x 10^3/mm and 5.29 x 10^6 /mm for total WBC and RBC of the control group, respectively (P >0.05); however, the results were not so different with those piglets receiving 3 and 6 ml cowmilk's colostrum per day (P >0.05).

As for the PVC, the 9 ml group did not show any significant difference with the 3 ml group, but the difference was statistically significant when being compared with the control group. The present study shows improvement of the hematological profile of the piglets receiving cowmilk's colostrum but still within the normal limits. The followings are the normal hematological parameters of pre-weaning piglets: total leukocytes (10.98 x 103 /mm), total erythrocytes (5.74 x 106/mm), hemoglobin level (11.44 g/dl), hematocrit (36.16%), platelet count (318 x 106/mm), percent neutrophil (36.6%), percent eosinophil (1.6%), percent lymphocyte (65.4%), and percent monocyte (6.8%). This increase is caused by the excessive amount of nutrient contents within the colostrums [4].Colostrum contains maternal antibodies, energy, protein, vitamins (vitamin A, E, B1, B6, B12, and C), folic acid, as well as trace minerals (iodine and selenium),[2] iron, calcium, magnesium, chromium, potassium, sodium, sulphur, zinc, and phosphate. Vitamin B12 and folic acid are the anti-anemic compounds. Folic acid and vitamin B12 serve to prevent anemia that further improve the immune system and increase the number of red blood cells.

The lactotransferrin content of the colostrum plays important roles as a transporter and bacteriostatic factor. Protein enzymes, such as lysozyme, regulate glycoprotein metabolism. Chemical studies of the cowmilk's colostrum show high dry compound (30-40% higher than milk), especially higher protein level (twice more than milk). The other substances contained in the colostrum, include hormones, enzymes, amino acids, and other nutrients that also contribute to the synthesis of blood cells. Albumin hydrolyzate, casein, and protease-peptone fraction provide essential amino acids for protein synthesis.

Table 2: Effects of colostrum supplementation on the differential count of leukocyte in piglets

Treatment (colostrum supplementation)	Variable			
	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
0 ml/piglet aged 1-5 days (n=18)	41.4[a]	3.1[c]	47.2[a]	6.4[a]
3 ml/ piglet aged 1-5 days (n=18)	42.1[a]	0.0[a]	57.5[b]	8.3[a]
6 ml/ piglet aged 1-5 days (n=18)	37.9[a]	1.3[b]	58.3[b]	7.4[a]
9 ml/ piglet aged 1-5 days (n=18)	48.3[b]	1.2[b]	48.6[a]	8.7[a]

Annotation: the same letter written in the same column indicates no significant difference (p>0.05)

Table 2 presents the results indicating that cowmilk's colostrum supplementation at a dose of 9 ml resulted in an increase in neutrophil count compared with the 3 and 6 ml groups (P >0.05). In contrast, this supplementation resulted in lower eosinophil count compared to the control group (p >0.05). On the other hand, it resulted in higher lymphocyte count in piglets receiving 3 and 6 ml colostrum compared to the control group and the 9 ml group (P >0.05). Lymphocyte concentration can be enhanced by increasing zinc, vitamin C, vitamin E, and selenium intake. Colostrum also contains the aforementioned minerals [2].

The study results did not show any effects of colostrum on monocyte.

Immunomodulator

The results on the effects of cowmilk's colostrum on anti-Hog Cholera antibody among piglets are presented in Table 3. Table 3 shows that colostrum supplementation possesses a significant effect on the elevation of an-Hog Cholera antibody titer in piglets compared with controls (P <0.05). This is indicated by the high positive control value (%PC value) in the experiment groups that ranged between 64.461% - 69.331%; no significant difference was found between the three experiment groups (P >0.05).

Whereas, the %PC value in the control group was only 53.578 %.

Table 3: ELIZA analysis of the anti-Hog Cholera antibody in 30-day-old piglets who received colostrum supplementation

Treatment (Colostrum supplementation)	Variable
	Anti-Hog Cholera Antibody (%PCvalue)
0 ml/piglet aged 1-5 days (n=18)	53.578[a]
3 ml/ piglet aged 1-5 days (n=18)	64.461[ab]
6 ml/ piglet aged 1-5 days (n=18)	69.331[b]
9 ml/ piglet aged 1-5 days (n=18)	67.840[b]

Annotation: the same letter written in the same column indicates no significant difference (p>0.05), %PC <40% = seronegative, %PC>40% = seropositive

Although all the groups showed seropositive values, according to the Instruction Manual VDPro^{R} CSFV AB C-ELISA_ 480T, the %PC value of ≥ 40% is considered protective for piglets against Hog Cholera virus, while %PC value of <40% is considered seronegative and does not possess adequate protection against Hog Cholera virus that puts the piglets in a significant risk of Hog Cholera infection.

The high %PC value in piglets receiving cowmilk's colostrum is perhaps because naturally, colostrum contains substances that can be used for constructing immunity and it is also rich in immunomodulatory components owing to the bioactive contents, including antibodies against various gastrointestinal and respiratory pathogens, as well as growth factors, vitamins, cytokines, and proteolytic factors, lipid, and other glucolytic substances [16].

The bioactive compounds can induce the release of interleukin-12 in macrophages that subsequently stimulates T helper cells proliferation « cell-mediated immune response [17].These compounds also increase the synthesis of interferon that facilitates phagocytosis of environmental pathogens by macrophages (Luis Ocampo and Ivan Sanchez, 2012). Cowmilk's colostrum also induces the release of IL-12 by CD14+ monocytes and unsubstantially induces the production of IFN-gamma. IL-12, IFN-gamma, and other cytokines involved in the polarization of T-helper 1 (Th1) cells are required for optimal immune response against intracellular pathogens, including bacteria and virus [16].Cowmilk's colostrum also affects the stimulus-induced production of IFN-gamma: increases the production of IFN-gamma as a response to weak antigenic stimulation and inhibits IFN-gamma production as a response to intense antigenic stimulation. Immunomodulatory effects of cowmilk's colostrum partially result from colostrinin and proline-rich polypeptides that act on cytokines production to mediate the Th1 response required for immune response [4].

Conclusion

Addition of cowmilk's colostrum in pre-weaning piglets at a dose of 6-9 ml daily for the first five days of life leads to improvements in haematological profile and also possesses immunomodulatory properties.

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