

Amino acid methionine, lysine and vitamin choline: A potential feed supplement to reduce mortality, improve immunity, and health of Bali pigs in the growth phase



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ABSTRACT

Introduction: Balinese piglets, separated from the mother and given traditional feeding, can cause prolonged growth. So, it is necessary to provide feed with a complete formula to support the growth process. Previous studies reported that Balinese piglets fed with a mixture of yellow corn, pollard, Aminovite, table salt, and minerals with several formulations and a metabolized energy (ME)/crude protein (CP) ratio 2800 kcal/kg/12% significantly increased body weight. However, his health status has not been reported, both the hematological profile and antibody titer. The purpose of this study was to determine the effect of the amino acid methionine, lysine, and choline (Aminovite) on health, hematological profile, and antibody titer as immunomodulatory effects.

Methods: This study used 72 Balinese piglets after being separated from the mother, divided into four groups of 18 piglets each. The first group (P0) was given basic feed mixtures of corn and pollard as a control. The second group (P1) was fed P0 + 0.5% Aminovite. The third group (P2) was fed P0 + 1.00% Aminovite. The fourth group (P3) was fed P0 + 1.50% Aminovite. Bali piglets are vaccinated with the Hog Cholera vaccine. Furthermore, on the 12th day of the week after treatment (aged 80-90 days), a hematological profile examination will be carried out, and the antibody titer of Hog Cholera will be examined. The data were analyzed using the One Way Anova test and Duncan's Multiple Range Test. The p-value <0.05 is significant.

Result: There was no mortality of piglets in all groups, but the control group had a high morbidity rate (22.2%) than the other groups. Aminovite mixed in the feed significantly increased WBC, total RBC, Hb levels, and PCV values compared to control (p<0.05). Aminovite can also increase neutrophils and monocytes and significantly reduce lymphocytes compared to control (p<0.05).

Conclusion: The addition of Aminovite as a feed supplement in Balinese piglet feed improved the health of Bali piglets seen from the blood profile and increased Antibody titers resulting from Hog Cholera vaccination.

Keywords: piglets, the amino acid methionine, ELISA, Hog Cholera.

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INTRODUCTION

The Balinese pig farming system is still traditional, characterized by feed management, only provided with makeshift feeds and poor maintenance management. Disease prevention measures are also lacking so that the growth of Balinese pigs is prolonged. Previous studies reported that with improved nutrition in feed, the growth of Bali pigs could be increased to 0.35 - 0.5 kg per day in the growth phase. The mixture of corn and pollard added with the amino

acid supplements of methionine, lysine, and choline promotes even better growth. It is just that production costs will increase. The cost of feed is a severe problem in the hog industry, contributing around 65 to 75% of the total production cost.¹

Apart from feeding problems, health problems such as infection in pigs still require attention. Infections that often attack pigs include bacterial, viral, fungal, and parasitic infections such as worms and *Coccidia spp.* Diseases caused by bacteria include *Escherichia coli*, Salmonellosis, Erysipelas, Mycoplasma, and others.

Meanwhile, diseases caused by viruses include Swine Flu, Porcine epidemic diarrhea (PED), Circovirus type 2 (CV type 2), Hog Cholera and others.² Antibiotics can prevent bacterial infections, but there is no cure if there is a viral infection. The only thing that can prevent viral infection is vaccination. The purpose of vaccination is to form immunity or antibodies. The success of vaccination depends on the quality of the vaccine, vaccination program and livestock health.³ Therefore, balanced nutrition is necessary, such as amino acids. According to Sumadi et al., the

addition of Aminovite (methionine, lysine and choline) was 0.5%; 1.0% and 1.5% for 12 weeks resulted in the final body weight being 32.80 kg, respectively; 36.61 kg and 38.31 kg were significantly different from the control pig's body weight, namely 27.69 kg. The higher the Aminovite concentration, the higher the increase in weight of the growing pigs.⁴

However, the addition of amino acids methionine, lysine and vitamin choline has not been reported on the health of pigs seen from the hematological profile and antibody titer to protect new piglets from infection. So, the study aimed to determine the effect of giving the amino acids methionine, lysine and choline in feed on health status seen from the hematological profile, immunomodulatory effects, and to reduce morbidity and mortality of piglets after separation from the mother.

METHODS

Study design and samples

This research uses male Bali piglets after separating from their mother like an animal model. The number of samples used was 72 individuals with a bodyweight range of 11.60 - 14.10 kg. Piglets were obtained from a nursery under Veterinary Wayan Syartama Hadi Nugraha, whose address is Kintamani, Bangli, Bali. The length of the study was two weeks for adjustment of experimental feed and then continued for 12 weeks of research data collection. The pen needed is a fattening pen to raise piglets after weaning, equipped with a place to eat and drink.

Model animal treatment

A total of 72 piglets were randomly divided into four groups. Each group consisted of 18 animals, namely group 1 (P0) as a control given basic feed mixture of corn and pollard and placebo starch; group 2 (P1) was fed with P0 + 0.50% Aminovite treatment; group 3 (P3) was fed with P0 + 1.00% Aminovite treatment; Group 4 (P4) was fed with Aminovite P0 + 1.50 treatment. Aminovite and feed formulations used are those from Sumadi et al. (Table 1). The feed was given according to needs based on age. Meanwhile, drinking water was given by *ad libitum*. In order to prevent the Hog Cholera infection disease, Hog cholera

Table 1. Composition of 1 kg of ingredients and nutrients Aminovite

Materials	Composition		
	Weight (kg)	Nutrient (%)	Nutrient (kg)
Lysine	0,800	79	0,632
Methionine	0,185	99,9	0,183
Choline	0,15	60,5	0,091
Total	1,000	-	-

Table 2. Morbidity and mortality of piglets during the administration of Aminovite supplements in feed

Group; n=18	Morbidity; n (%)	Mortality (%)	Morbidity condition
P0	4 (22,2 %)	0	diarrhea
P1	2 (11,1%)	0	diarrhea
P2	1 (5,6%)	0	cough and dyspnea
P3	1 (5,6%)	0	diarrhea

Table 3. Effect of the addition of the amino acid methionine, lysine, and choline in the feed on the hematologic profile of piglets

Group	Variable			
	WBC ^a (x10 ³ /μL)	RBC ^b (x10 ⁶ /μL)	Hb ^c (gr%)	PCV ^d (%)
P0 (n=18)	16,3	4,8	13,7	26,3
P1 (n=18)	21,4*	5,4*	14,8*	29,1*
P2 (n=18)	17,2	6,5*	14,4	36,9*
P3 (n=18)	16,3	6,6*	15,8*	36,2*
Piglet normal hematologic value ⁵	8,7-37,9	5,0-8,0	10,0-16,0	32-50

*p-value <0.05; ^aWhite blood count; ^bRed blood count; ^cHemoglobin; ^dPacked cell volume

vaccination was administered at 14 days of age and repeated at 35 days of age.

Data collecting and analysis

The data were calculated from daily observations, namely from the start of the study to completion (80-90 days). At the end of the study, the blood was taken and then stored in a vacuum tube that already contained anticoagulants (EDTA) to examine the hematologic profile. The blood samples then were examined with the ABC Manual VET Automated Blood Counter, Made in Germany at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Udayana. Blood is also collected in a tube without EDTA to examine the Hog Cholera antibody titer using the ELISA method at the Denpasar Veterinary Center. The data were analyzed using the One Way Anova test and Duncan's Multiple Range Test. The p-value <0.05 is significant.

RESULTS

Piglet morbidity and mortality

All of the piglets that received Aminovite with concentrations of 0.5%, 1% and 1.5% in primary feed and without additional Aminovite were all sick with varying morbidity (Table 2), with the lowest morbidity in the group given Aminovite 1.5% to 5.6%. The morbidity was relatively high if not given Aminovite (control), namely 22.2% (P0). The symptoms of illness that can be observed are diarrhea, coughing and other respiratory symptoms. Clinical symptoms that appear may be caused by a bacterial, viral or parasitic infection. The mortality in this study was 0% in all experimental groups.

Hematology profile

Aminovite mixed in the feed can increase the total white blood count (WBC), total red blood count (RBC), hemoglobin

Table 4. Effect of the addition of the amino acid methionine, lysine, and choline in the feed on the piglet's leukocyte differential count

Group	Leukocyte			
	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
P0 (n=18)	30.22	3.39	57.78	8.61
P1 (n=18)	37.94*	3.28	48.83*	9.94*
P2 (n=18)	36.56*	2.67	50.50*	10.28*
P3 (n=18)	44.00*	2.67	42.28*	11.00*
Normal value ⁵	16,6-73,1	0,0-11,1	12,5-70,1	0,0-17,0

*p-value <0.05

Table 5. Hog Cholera antibody ELISA test results of 12-week-old piglets that have been given Aminovite in feed

Group	Hog Cholera antibody (%PC value)
P0 (n=18)	46.0
P1 (n=18)	57.2*
P2 (n=18)	73.9*
P3 (n=18)	78.5*

*p-value <0.05

(Hb) levels, and packed cell volume (PCV) values significantly compared to controls ($P < 0.05$) (Table 3). Normally, the blood profile status of piglets was total WBC ($10,98 \times 10^3/\text{mm}$), total RBC ($5.74 \times 10^6/\text{mm}$), hemoglobin level (11.44 g/dl), Packed Cell Volume/PCV value (36.16%), levels of platelets ($318 \times 10^6/\text{mm}$), neutrophils (36.6%), eosinophils (1.6%), lymphocytes (65.4%), and monocytes (6.8%).⁵

The results of the leukocyte differential examination showed that the administration of Aminovite as a supplement in the diet could increase neutrophils and monocytes, significantly reduce lymphocytes, compared to controls ($P < 0.05$) and did not affect eosinophils (Table 4). However, the effect was still in the normal range. Normal values for neutrophils (16.6-73.1%), eosinophils (0.0-11.1%), lymphocytes (12.5-70.1%), and monocytes (0.0-17.0%).⁵

Hog Cholera antibody titer

Aminovite had a significant effect on increasing Hog Cholera antibody titer in Bali piglets compared to controls ($P < 0.05$) (Table 5). This result is indicated by the high percentage of positive control

(%PC value) in the treatment, ranging from 57.2% to 78.5%. The higher the Aminovite dose, the higher the antibody titer. Meanwhile, the percentage value of positive control in the control piglet group was only 46.0%. However, the treatment group and the control group both showed seropositive values. According to the instruction manual VProR CSFVAB C-ELISA_480T, if the %PC value $\geq 40\%$ is a seropositive value or the condition of the piglets is protective against Hog cholera virus attack. If the %PC value $< 40\%$ is a seronegative value or the antibody is not protective against Hog cholera virus attack, piglets are infected with the Hog Cholera virus.

DISCUSSION

Giving Aminovite in the feed does not aim to prevent infection but to increase the body's resistance to maximum growth. The addition of amino acids in the feed can increase the microbial activity of normal flora in the jejunum and cecum, which can improve the intestinal health of pigs. This condition will increase the body's resistance.⁶ So that with a healthy immune system due to the administration

of Aminovite, piglets, even though they are sick, do not cause death.

Routine blood tests are beneficial to determine the health status of livestock and can also help diagnose the cause of disease. Decreased total leukocytes in pigs with symptoms of fever and diarrhea were diagnosed with mild to moderate Hog Cholera disease.⁷ The hematological condition obtained occurred because the intake of amino acids in each treatment feed increased after adding Aminovite and the digestibility increased so that the blood profile would increase. The use of amino acids methionine and lysine in pig feed can improve livestock health seen from the blood profile and increase protein digestibility. Amino acids play a role in forming WBC and RBC.⁸ Pigs, especially those that are growing, really need amino acids, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.⁹ Increasing the amino acid (AA) profile of food by using L-Lys, L-Thr, dl-Met, L-Trp and L-Val helps reduce the CP content of the food, thereby reducing nitrogen excretion while maintaining pig performance. Valine is the fifth limiting AA in a soy-cereal-based diet. The extent to which the CP content in food can be further reduced without compromising performance depends on the subsequent limiting AA requirements. In a soy-cereal-based diet, Ile, His and Leu may become limiting AAs after Val, although information on requirements for these AAs is scarce.¹⁰ With the addition of Aminovite in the feed will support the growth of piglets including the development of their blood profile.

Aminovite in feed on differential cells of blood leukocytes aged pigs. The increase in neutrophils and monocytes is thought to stimulate the formation of inflammatory cells actively. These inflammatory cells are to ward off bacteria that enter the pig's body. Aminovite is very beneficial for pigs that do not receive antibiotic growth promoters (AGP). Eosinophil cells are not affected by Aminovite. That indicates that Aminovite does not cause allergies. Parasitic infections and/or allergies can cause increased eosinophil cells in the blood.⁵

Various pathogens cause production

diseases in pigs, particularly those affecting the digestive and respiratory tracts, resulting in significant economic losses. Other factors such as stress, poor husbandry and nutrition can also contribute to the susceptibility of pigs to disease.¹¹ For this reason, the pig's immune system needs to be improved by vaccination and supported by providing a balanced nutritional intake such as providing vitamins, minerals and amino acids.

The formation of antibodies is influenced by the completeness of essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.⁹ It also depends on the number of lymphocytes in the blood, but the high lymphocyte count does not necessarily mean it will increase antibody titer. Antibody formation is played by lymphocyte cells.⁷ In piglets given additional Aminovite, the lymphocyte percentage was significantly lower than the control. That means that Aminovite cannot stimulate an increase in the percentage of lymphocytes, but the opposite happens, inhibiting the formation of lymphocyte cells. It is just that it is still in the normal range of normal lymphocytes, so it does not interfere with antibody formation. The increase in %PC Value that occurred in Bali piglets that received Aminovite may be through the mechanism of amino acid availability. The availability of methionine and lysine greatly determines the formation of antibodies. The amino acid arginine contained in the feed can increase the thymus gland activity, increase the number of T lymphocytes, and destroy the cause of infection.⁹ The results showed that the administration of Aminovite could significantly increase the Hog Cholera antibody titer ($P < 0.05$) compared to the control.

CONCLUSION

The addition of Aminovite as a feed supplement in Balinese piglet feed improved the health of Bali piglets seen from the hematologic profile and increased antibody titers resulting from Hog Cholera vaccination.

DISCLOSURES

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Conflict of Interest

All authors declared no conflict of interests between authors and any organizations or persons that could influence the objectivity during the study, interpreting the result and writing of the manuscript.

Author Contribution

All authors distributed equally in concepting, designing, supervising the manuscript, conducting the study, analyzing the data, and preparing the manuscript and agreeing to submit this final version to this journal.

Ethical Consideration

The ethic commission has approved this research of the Faculty of Veterinary Medicine, Universitas Udayana.

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