

Soil worms (*Lumbricus rubellus*) as feed additives for piglets' growth, blood profile and immunomodulators



Anak Agung Gde Oka Dharmayudha^{1*}, Ida Bagus Komang Ardana¹, Ketut Budiasa²,
I Made Merdana², I Wayan Nico Fajar Gunawan¹

ABSTRACT

Background: Before the weaning phase, piglets often experience diarrhea and anemia caused by infection with germs such that after weaning, their growth rate reduces, they become pale and also prone to other illnesses which may lead to death. This study aims to determine the effect of earthworm flour (TCT) on growth, morbidity, mortality, blood profile, and antibody titer as an immunomodulator.

Method: Ninety piglets were used before weaning which was divided into four groups, each consisting of 18 members, namely the group that was not given TCT as a control (P0), the one that was given TCT from the age of 20 to 50 (for 30 days total) days with a concentration of 1% in the feed (P1), the concentration of 1.5% (P2), the concentration of 2% (3) and concentration of 2.5% (4). At 14 days old, Hog Cholera vaccination was administered by intramuscular injection, and it was repeated at the age of 28 days. Bodyweight was weighed at the beginning of the study, which was at 21 days old, then repeated at 28, 35, 42, 49 and 60 days after treatment to determine performance. Morbidity and mortality were observed from 21 to 60 days old. At 50 days, blood was collected and stored in a vacuum tube containing an anticoagulant (EDTA) for profile examination. The Hog Cholera antibody titer assessment was performed using the ELISA method.

Result: The TCT concentration of 1.5% in piglet feed significantly increased body weight, decreased morbidity, prevented morbidity, elevated WBC, RBC, Hb, PCV, and neutrophils, reduced eosinophils and lymphocytes but still within a normal range. Therefore, it is judged that the preparation of 1.5% is effective and safe, and the % CP value of hog cholera is positive with a rate of 72.9%, which is significantly higher than the control (66.64%).

Conclusion: Earthworm flour with a concentration of 1.5% in feed is harmless, potent in increasing body weight and is indicated as an immunomodulator.

Keywords: piglets, earthworm flour, performance, hematological profile, immunomodulator.

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¹Laboratory of Veterinary Clinical Diagnostic, Clinical Pathology and Radiology, Faculty of Veterinary Medicine, Universitas Udayana, Bali, Indonesia;

²Laboratory of Veterinary Pharmacology, Faculty of Veterinary Medicine, Universitas Udayana, Bali, Indonesia;

*Corresponding author:
Anak Agung Gde Oka Dharmayudha;
Laboratory of Veterinary Clinical Diagnostic, Clinical Pathology and Radiology, Faculty of Veterinary Medicine Udayana University, Bali, Indonesia;
oka.dharma@unud.ac.id

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INTRODUCTION

The Indonesian government has banned antibiotics as feed additives (AGP; Antibiotic Growth Promoter) with the issuance of Act Number 18 of 2009 in conjunction with 41 of 2014. Prohibition of the use of AGP is regulated in *Permentan* Number 14/2017 regarding the classification of medicine for animals. However, this stimulant has selective toxicity, and its minimal concentration can inhibit growth or kill other microorganisms. However, it is not toxic to host cells. Theoretically, AGP works by maintaining nutrients from microbial destruction, increasing nutrient absorption by creating a barrier in the

intestine, reducing the production of toxins from gut bacteria, and decreasing the incidence of subclinical infections in the gastrointestinal tract.¹

With the prohibition of antibiotics as AGP, surveys from several broiler and pig farms in Bali discovered that there had been very large losses. Broiler farms experienced significantly reduced growth, increased FCR, high morbidity and mortality. The complaints were almost the same from the piggeries, where piglets experienced diarrhea and became pale before and after weaning, which inhibited growth and even resulted in death. To overcome these problems, universities such as Udayana and the animal medicine industry began to study natural ingredients that have

similar efficacy to substitute antibiotics. One of them is Earthworm Flour (*tepung cacing tanah*; TCT) which has been proven to have Lumbricin 1 as an anti-bacterial factor. One type of earthworm containing anti-bacterial factors is the *Lumbricus rubellus*.²

Research showed that the optimum inhibitory growth of *S. pullorum* was 75%.³ Previous study also reported that TCT of *L. rubellus* at 75% can inhibit *S. pullorum* optimally and *Escherichia coli* at 50%.^{4,5} This effect is mediated by the bioactive component 'lumbricin' (0.1 g/g earthworm flour), a peptide compound with complete amino acid constituent, especially proline.² Furthermore, it inhibits the growth of *Escherichia coli*, *Salmonella enteritidis*,

Staphylococcus and *Streptococcus aureus* in vivo.^{4,5} It is reported that *Lumbricus rubellus* has two anti-bacterial factors named Lumbricin 1 and 2. Both function like lysozyme with hemolytic activity and pattern recognition of protein called celom cytolytic factor (CCF) identified in the earthworm, *Eisenia phoetide*.⁶ Chitosan is added as its presence optimizes the bioactive ability found in earthworm flour to increase the anti-bacterial activity of TCT. The bioactivity of 'lumbricin' in TCT is enhanced by adding 0.5% chitosan to inhibit *E. coli*.^{2,4}

Protein in earthworms has different antimicrobial mechanisms than antibiotics that kill microorganisms without damaging body tissues in two ways. These are first by hindering the metabolic pathways that can produce essential nutrients needed by microorganisms or second by inhibiting specific enzymes required for cell wall formation. Meanwhile, the antimicrobial mechanism of earthworm proteins involves creating pores in cell walls of bacteria. That causes its cytoplasm to become exposed to the external environment, disrupting the activity in the organism and leading to death. This mechanism will be more difficult for the bacteria to resist since damage targets the cell structure.⁶

Earthworm flour also contains 63.06% protein; 18.5% fat; and 12.59% carbohydrates.⁷ *Lumbricus rubellus* also contains nine essential and eight non-essential amino acids. The essential ones are arginine, histidine, leucine, lysine, isoleucine, valine, methionine, phenylalanine, and threonine. At the same time, the non-essentials contained in the soil are cysteine, glycine, serine, tyrosine, alanine, proline, aspartic and glutamate acid.⁸

The TCT is usable as the primary source of protein for animal feed and serves as a substitute for fish flour, which has become more difficult to find. Earthworm flour is superior to the fish type as the protein content is 72% which is far higher than the one contained in the counterpart by 22.65%.⁹ In addition, the TCT is not fatty but easily digestible and contains a couple more amino acids compared to the fish product. It acts as a growth promoter that maximizes nutrients' absorption in the

digestive tract, leading to a growth spurt and streamlined feed consumption. Worm flour significantly influences the total number of hemocytes and phagocytic activity.¹⁰

The use of TCT as a food additive to the level of 1.5% in broiler feed improves the performance of the chicken and the animal health in terms of blood profile and protein digestibility.¹ TCT is also used as a growth promoter to 1.5% of the total ratio since it contains Lumbricin as an anti-bacterial factor. However, the use of TCT as growth promoters in piglets has not been reported. Therefore, this study investigates the effect of earthworm flour as growth promoter to improve performance and health as observed from the hematological profile. Also, examine the function of an immunomodulator and reducing morbidity and mortality rate for piglets after weaning.

Based on the background, the objective of this study is to determine the effect of earthworm flour in feed to improve animal performance and health as observed from the blood profile, the Hog antibody titers post-Cholera vaccination, and morbidity and mortality rates of piglets after weaning.

MATERIAL AND METHODS

Piglet as Model Animal

This study used 90 male Landrace piglets age 20 days, weighing 1.6-2.1 kg, healthy and weaned. All the animals belonged to Ms Ni Nyoman Sudiartini located in Banjar Dalem, Petang Village, Petang District, Badung Regency, Bali.

Treatment of Model Animal

A total of 90 piglets that were still with their mothers were randomly divided into five groups, each consisting of 18 piglets. The control group (P0) was given starch as a placebo. The treatment group consisted of four groups given starter feed mixed with different earthworm flour concentrations from 14 days before to 14 days after weaning, at ages 20 to 50. Treatment group 1 (P1) was given 1% earthworm flour; treatment group 2 (P2) was given 1.5% earthworm flour; treatment group 3 (P3) was given 2% earthworm flour; treatment group 4 (P4) was given 2.5% earthworm flour starting. Feed was given according to the need based on age, but drinking water

was administered *ad libitum*. Vaccination was given at 14 days and repeated at 28 days to prevent Hog Cholera infection. The piglets were weighed at 21 days and then repeated at 28, 35, 42, 49 and 60 days. Weight gain was measured by reducing the final body weight with the initial.

The morbidity and mortality rate of the animals was calculated from daily observations, from the age of 21 to 60 days. The blood of the 50-day-old piglets was blood drawn and then collected in vacuum tubes that contained anticoagulants (EDTA) to examine blood profiles using Manual ABC VET Automated Blood Counter, Made in Germany at the Veterinary Clinical Pathology Laboratory, Udayana University. Blood was also collected in a tube without EDTA to evaluate antibody titer of Hog Cholera through the ELISA method in Denpasar Veterinary Center. The data obtained were subjected to Variance Analysis, and when real effects were observed, the process continued with Duncan's Multiple Range Test.

RESULT AND DISCUSSION

Pig Performance

The addition of earthworm flour in feed given to piglets from the age of 21 to 50 days showed that not all doses significantly increased piglet weight gain. The use of TCT as an additive to 1.5% in broiler feed improves the performance (Table 1).

Duncan's test results showed that the average weight gain of 60-day-old piglets that were given extra earthworm flour additives at a dose of 1% in feed was not significantly different from controls ($P > 0.05$). However, when the dose was increased to 1.5%, the bodyweight also augmented significantly compared to controls ($P < 0.05$). Furthermore, when the dose was increased to 2% or 2.5%, the weight gain is significantly lower than control ($P < 0.05$) (Figure 1). Figure 1 indicates that the different dose of earthworm additive in feed and the various weight gain. This means that the flour has a similar effect to low-dose antibiotics as AGP.

When attributing TCT dosage level to piglets' health condition, it was discovered that the addition of this flour at 2% and 2.5% caused quite a high frequency of

Table 1. Pig Weight and Day-60 weight gain.

Treatment	Pig Weight Week (days)						Body Weight Gain at 60 th Days
	III (21)	IV (28)	V (35)	VI (42)	VII (49)	VIII (60)	
P0	4.6 ^a	6,8 ^b	8.6 ^b	10.5 ^b	13.7 ^c	16.1 ^c	11.5 ^c
P1	4.8 ^a	7.5 ^c	8.5 ^c	11.1 ^c	14.1 ^d	16.5 ^d	11.7 ^c
P2	4.8 ^a	8.3 ^d	9.4 ^d	12.9 ^d	15.8 ^e	18.4 ^e	13.6 ^d
P3	4.8 ^a	6.9 ^b	8.5 ^b	11.2 ^c	12.7 ^b	15.7 ^b	10.9 ^b
P4	5.4 ^b	6.0 ^a	7.0 ^a	8.7 ^a	10.5 ^a	12,0 ^a	6.6 ^a

Note: The same letter in the same column states that it is not significantly different (P> 0.05)

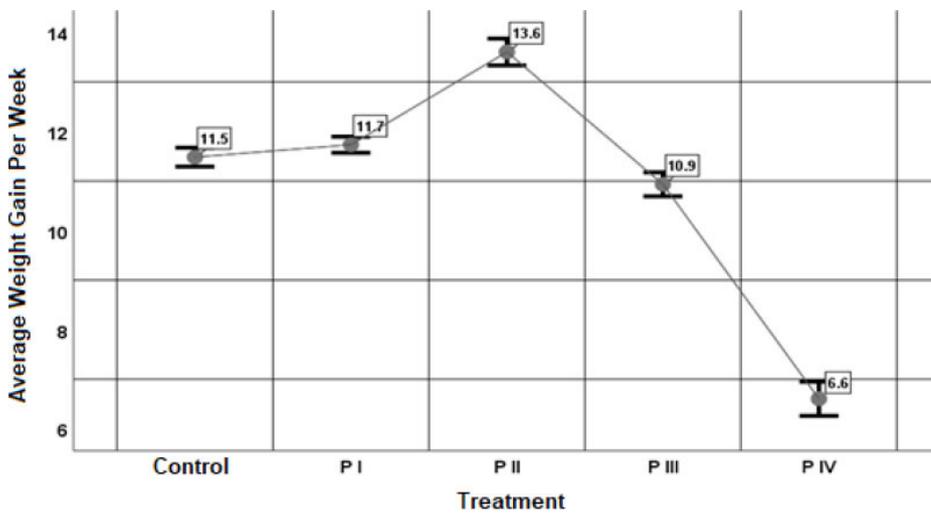


Figure 1. Shows the Effect of adding Earthworm Flour (TCT) in feed for 30 days on Weight Gain at Day-60. Control (P0), 1% TCT in feed (P1), while 1.5% is P2, 2% P3 and 2.5% is P4.

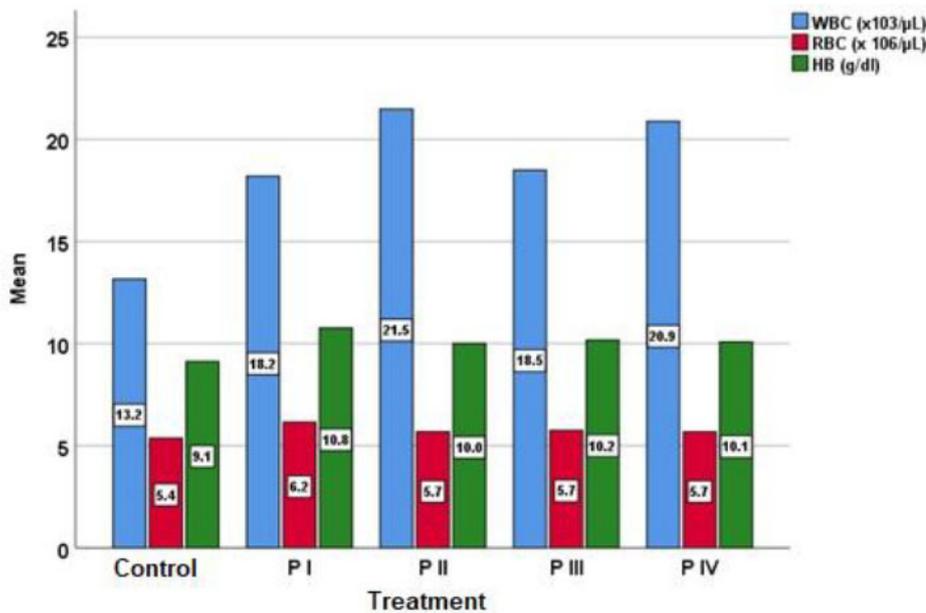


Figure 2. The Graph Shows the Influence of Earthworm Flour (TCT) in feed for 30 days on blood profile at day-60. Control (P0), 1% TCT in feed (P1), 1.5% (P2), 2% (P3) and 2.5% (P4).

diarrhea as presented in Table 2. The morbidity of pigs that received 2% TCT was 50% and at the level of 2.5% the incident increased to 100%. It is not yet understood why an additional dose of the TCT resulted in diarrhea. This condition caused poor piglets' performance as indicated by lower body weight than in the control.

Blood Profile

Table 3 shows that earthworm flour mixed with feed significantly increased total white blood cells (WBC), hemoglobin (Hb), and Packed Cell Volume (PCV) compared to controls. The flour contributed to the increase in Hb, white and red blood cells, as seen in Figure 2.

Conversely, the effect of TCT administration on leukocyte differential count showed variable results (Table 4). The results of leukocytes differential count showed that giving TCT as feed additive increased neutrophils but significantly reduced lymphocytes compared to controls (P <0.05), however, the change is still within the normal range. Meanwhile, the addition of TCT in feed does not affect the percentage of eosinophil and monocyte cells, as shown in Figure 3.

Hog Cholera Antibody Titer

The results of the effect of giving earthworm flour to Hog Cholera antibody titers in piglets are presented in Table 5.

Table 5 shows that the addition of earthworm flour significantly affected the increase in Hog Cholera antibody titers compared to the controls (P <0.05). This was indicated by the positive percentage value of the control (% PC) in the very high treatment, which ranges from 70.75-72.9%, but only the medium was not significantly different (P> 0.05).

Furthermore, this percentage was only 66.64% in the controls, however, the treatment and control group both showed the same seropositive value. According to the Instruction Manual VDPProR CSFV AB C-ELISA_480T, when % PC value \geq 40%, it is seropositive hence, piglets are protected against Hog cholera virus. But

when % PC value $<$ 40%, it is seronegative or the antibody is not protective against Hog cholera virus, therefore, the piglets are infected.

DISCUSSION

Male pigs have a more significant increase in body length than females and are also

longer than them in size. This is caused by androgen hormone in male pigs that stimulates bone growth. In addition, male animals often consume more food than females.¹¹ The growth patterns of piglets correspond with human infants, which are logistic.¹² This may be because, from the time of birth, a piglet gets enough colostrum from its mother. It has been discovered that colostrum accelerates the growth of intestinal mucous villi, and increases the size and weight of the intestine. This happens to elevate nutrient absorption up to 50% on the first day of birth and then increases to 100% when given up to 10 days after birth, while at this time, piglets grow faster naturally. Vitamins and hormones contained in colostrum play a role in stimulating the natural growth of animals. The rate at which landrace pigs grow is also influenced by the intensive handling of feeding and disease.¹³ Sex has a significant effect on body length but none on the body size of the pigs. There was a significant interaction ($P < 0.01$) between sex and age on their length and body circumference, which have a logistical growth pattern in both sexes. The body length of the males has a higher growth rate than in the females, while the increase in the circumference of the male landrace pigs is lower than in the females.¹²

Success parameters for raising a piglet can be measured from growth, including weight gain, feed consumed, and efficient use. One external factor for achieving great performance is by providing high-quality feed.¹³ Besides containing protein, carbohydrates, fats, vitamins and minerals, this type of feed is also equipped with additives such as a growth booster. One of the plant-enhancing additives is antibiotics, often called AGP.¹⁴ However,

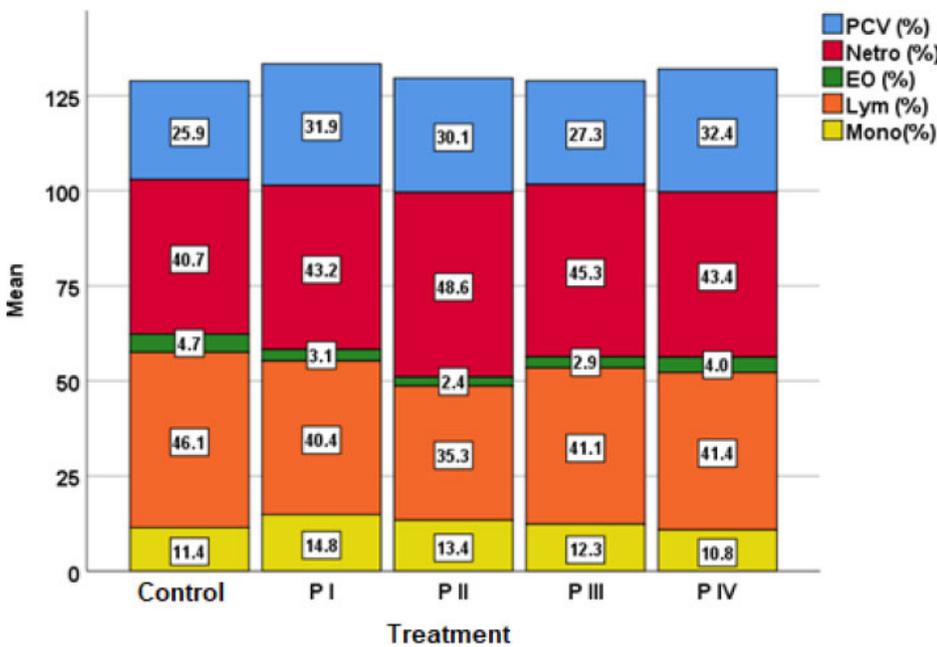


Figure 3. The Graph Shows Effect of Earthworm Flour (TCT) in feed given for 30 days on blood profile at day-60. Control (P0), 1% TCT in feed (P1), 1.5% (P2), 2% (P3) and 2.5% (P4).

Table 2. Morbidity and mortality rate of piglets during treatments of TCT given at different dosage levels.

Treatment	N	Morbidity (%)	Mortality (%)	Note.
0% TCT /Feed	N=18	5 Piglets (27.8 %)	0	Diarrhea
1% TCT/Feed	N = 18	3 Piglets (16.6%)	0	Diarrhea
1.5% TCT/Feed	N = 18	1 Piglet (5.6%)	0	Mild Diarrhea
2 % TCT Feed	N = 18	9 Piglets (50%)	0	Diarrhea
2.5 % TCT /Feed	N =18	18 Piglets (100%)	0	Diarrhea

Table 3. Effects of Earthworm Flour as Feed Additive on Blood Profile in Piglets.

TCT Dose in feed	Variable			
	WBC($\times 10^3$ /mm)	RBC($\times 10^6$ /mm)	HB (gr %)	PCV (%)
0 % TCT in feed (n=18)	13.16 ^a	5.36 ^a	9.14 ^a	26 ^a
1% TCT in feed (n=18)	18.2 ^b	5.8 ^b	10.8 ^c	32.1 ^b
1,5 % TCT in feed (n=18)	21.5 ^d	5.7 ^b	9.9 ^b	30 ^b
2 % TCT in feed (n=18)	18.5 ^{bc}	5.8 ^b	10.2 ^b	25.7 ^a
2,5% TCT in feed (n=18)	20.5 ^{cd}	5.7 ^b	10.1 ^b	32.3 ^b
Normal pig blood profile (Jain,1986)	8.7-37.9	5.0-8,0	10.0-16.0	32-50

Note: the same letter in the same column shows no significant difference ($P > 0.05$)

Table 4. The Effect of Earthworm Flour as Feed Additive on Leukocyte Differential Count in Piglets.

TCT dose in feed	Variable			
	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
0 % TCT in feed (n=18)	40.6 ^a	4.7 ^c	46.2 ^c	11.4 ^a
1% TCT in feed (n=18)	42.4 ^a	3,1 ^{ab}	40.4 ^b	16.1 ^b
1,5 % TCT in feed (n=18)	48.7 ^b	2,9 ^a	35.4 ^a	13.5 ^{ab}
2 % TCT in feed (n=18)	45.5 ^{ab}	2,8 ^{ab}	41.2 ^b	12.3 ^{ab}
2,5% TCT in feed (n=18)	43.4 ^{ab}	4 ^{bc}	41.3 ^b	10.3 ^a
Normal range	16.6-73.1	0.0-11.1	12.5-70.1	0.0-17.0

Note: the same letter in the same column shows no significant difference (P> 0.05)

Table 5. ELISA Test Results of Hog Cholera antibody for 60 days old piglets that have been given Earthworm flour in feed for 20 days of age.

TCT dose in feed	Variable
	Hog Cholera Antibody (% PC Value)
0 % TCT in feed (n=18)	66.64 ^a
1% TCT in feed (n=18)	70.75 ^b
1,5 % TCT in feed (n=18)	72.9 ^b
2 % TCT in feed (n=18)	68.35 ^a
2,5% TCT in feed (n=18)	72.37 ^b

Note: the same letter in the same column shows no significant difference (P> 0.05)

their use has been banned because it was allegedly thought to cause resistance in the human body for those consuming animal meat. As an alternative, natural materials are used for the same purpose. Earthworm flour contains the active ingredient Lubricin 1, which supposedly has antimicrobial properties.² The use of TCT as additives has been shown to affect broiler performance positively.¹

The blood profile of pre-weaned ones was reported as total WBC ($10.98 \times 10^3/\text{mm}$), total RBC ($5.74 \times 10^6/\text{mm}$), hemoglobin concentration (11.44 g/dl), Packed Cell Volume (PCV) value (36.16 %), platelet levels ($318 \times 10^6/\text{mm}$), neutrophils (36.6%), eosinophils (1.6%), lymphocytes (65.4%), and monocytes (6.8%).¹⁵ The significant increase value in WBC, Hb, and PCV possibly occurred because elevated protein intake in each treatment feed after adding TCT increased protein digestibility, causing the elevation in the blood profile. The use of earthworm flour as an additive to 1.5% in broiler feed improved livestock health as seen from the blood profile and protein digestibility.¹ Protein plays a role in the formation of WBC and RBC.¹⁶ The complete nutrient content of earthworm flour caused the increase detected. TCT contains energy, protein, vitamins A, E, B1, B6, B12, and C, folic acid as well

as trace minerals including iodine and selenium, iron, calcium, magnesium, chromium, potassium, sodium, sulfur, zinc, and phosphate.¹⁷ Vitamin B12 and folic acid are anti-anemia, they both aid in preventing anemia which also help to improve the immune system and increase red blood cells. Lactotransferrin plays an important role as a transporter and bacteriostatic factor, while protein enzymes such as lysozyme regulate glycoprotein metabolism. Additional nutrients contained in TCT are hormones, enzymes, amino acids and others that are very useful for the formation of blood cells in piglets. Albumin hydrolysate, casein and protease-peptone fraction provide essential amino acids for protein synthesis. Nutrient composition of earthworms includes 60-72% crude protein, 7-10% fat, 8- 10% ash, and 900-1400 calories g energy.¹⁸

The resistance of immune substances increased the white blood cells in the body with some active ingredients of earthworm flour. The number of normal WBC cells in pig blood ranged from 8.7 to $37.9 \times 10^3/\text{mm}$ (Jain, 1986). However, there was no indication of bacterial infection even though an increase in the number of WBC was observed after the addition of TCT to the feed but it was in the normal range. The

normal values of neutrophil is 16.6-73.1%, eosinophils 0.0-11.1%, lymphocytes 12.5-70.1%, and monocytes 0.0-17.0. The TCT addition did not affect the eosinophils and monocyte values.¹⁹

The formation of antibodies is dependent on the number of blood lymphocytes which perform a particular role in their production.¹⁹ In treatment groups given TCT, the percentage of lymphocytes was significantly lower than in the controls. This means that the earthworm flour was not able to stimulate an increase of the percentage and then the opposite occurred, but, it is still in the normal range of lymphocytes. Therefore, it does not interfere with the formation of antibodies. The increase in % PC Value that occurred in piglets which received TCT as feed additive may be through another unknown mechanism. A research performed by Liu et al. (2004) reported that the *Lumbricus rubellus* was able to stimulate immunity. Earthworm flour contains 1.24% arginine, which functions to increase the activity of thymus gland, hence elevating the number of T lymphocyte, and destroying the causes of infection.⁷

The results of this study showed that giving TCT increased the antibody titer of Hog Cholera significantly (P < 0.05). It could be considered as an immunomodulator according to the mechanism of action. There are three processes involved, which in turn result in normal antibodies. They include immunorestitution which restores the immune system, but immunostimulation is the one that stimulates the system and immunosuppression reduces the immune response.¹⁹ The mechanism of action of TCT as an immunomodulator is still unknown, therefore, there is need for further research.

CONCLUSION

The use of earthworm flour as a feed additive to the level of 1.5% in piglets increased weight gain, improved the health of the piglets as seen from the blood profile, and has the potential to be an immunomodulator.

DISCLOSURE

Ethics Statement

This experiment was performed according to the guidelines of the Animal Ethics Committees (AEC), Faculty of Veterinary Medicine, Universitas Udayana Approval Protocol No. 2976A/UN14.2.9/PD/2019

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors Contribution

IBKA designed the study, analyzed and drafted the manuscript. KB and AAGOD collected the data. IMM and IWNFG interpreted the data. All authors took part in preparing and checking of final manuscript.

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