

## IMMUNOMODULATOR, GROWTH PERFORMANCE AND HEMATOLOGICAL PROFILES OF VIUSID VET SUPPLEMENTATION ON PIGLETS

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**Background:** Immunity system plays important roles in maintaining health status in human as well as in animals. This work aims to study the effect of supplementation of food supplement Viusid vet 90 on growth performance, morbidity and mortality rates, hematological profile, and on antibody titer which may indicate its possible role as an immunomodulator. **Methods:** Sixty (60) piglets were used as the experimental animals divided into 2 groups, namely group without Viusid supplementation as the control group and that with Viusid supplementation starting from 3 days of age until 13 days of age (10 days of treatment). The dose of supplementation in the feed was 5 cc of Viusid given twice a day. At day 7 of age, the piglets were vaccinated intramuscularly with Hog cholera vaccine and it repeated at day 14 of age. The animals were weighed at the beginning of the experiment and again at Day 15 and then at the end the experiment at Day 30. At 20 days of age all animals were bled using vacuum tube containing anticoagulant EDTA. Subsequently the bloods were examined for hematological profiles using Manual ABC VET Automated blood counter. Finally, at 30 days of age they were all bled again for the assessment of their antibody titer against Hog cholera following ELISA method. **Results:** supplementation of Viusid vet 90 to piglets improves performance by reducing morbidity and mortality rates. As hematological profile and differential leucocyte count did not show any abnormality then it may be concluded that supplementation of Viusid is effective and safe for piglets. Moreover, the percentage Plate Count (PC) value for Hog cholera was seropositive (54.69%) and significantly higher than control (41.32%) and this could indicate that Viusid vet 90 act as a strong immunomodulator.

**Keywords:** piglet; Performance; morbidity; mortality; hematological profile; immunomodulator

### INTRODUCTION

It has been known for long time that immunity system plays important roles in maintaining health status in human as well as in animals. Its decreasing function leads to less ability of the body to defend from infiltration of disease agents, particularly in newborn babies. In human babies under 5 years of age, such problems occur due to bad conditions of the body and its environment such as loss of appetite, nutrient imbalance, and so forth. As the results, antibody production do not optimum; the phagocyte and macrophage cells less able to capture and kill the infiltrated disease agents. Moreover, stress due to flue, cold, diarrhea, and

other illness will also reduce the immune system function.<sup>1</sup>

Immunity in the newborn babies is normally delivered from mothers through breast milk. However, there are some food supplies that can stimulate the development of immunity, such as flavonoids, spirulina, zinc, honey, omega-3, probiotic, meniran, calcium, and vitamin C. Therefore, food supplements particularly those with immunomodulator property should be included in the ration of the baby. Djajakusumah (2010) mentioned the role of glycyrrhizic acid as an immunomodulator.<sup>1</sup> Its effect is less powerful than that of cortisone so that it can prevent stress longer by blocking 11- $\beta$ -hydroxysteroid dehydrogenase type 2 enzyme.<sup>2</sup> Glycyrrhizic acid is a triterpene glycoside of roots of licorice plant (*Glycyrrhiza glabra*); it can inhibit the activity of Phospholipase A and cause the release of prostaglandin E2 in tissues that, in turn, increase the movement of leucocyte to inflammation area and stimulate

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phagocytosis. Moreover, this substance can also stop replication of virus as it inhibit the activity of viral phosphorylating P kinase; as the results, virion escapes from the capsid and, therefore, there are no virus penetration to the cells.<sup>2</sup>

Combination of Glizigen-Viusid is effective in curing genital warts disease caused by human papilloma virus.<sup>3</sup> Glizigen contains active substance glycyrrhic acid and act as an antiviral drug that is produced by Catalyst Laboratory, Spain. On the other hand, Viusid is food supplement that can stimulate the immune system, effectively control viral infection, and act as an antiinflammation.<sup>3</sup> Viusid vet also produced by the same producer in Spain and contains various active substances, such as maleic acid, glycyrrhizic acid, amino acid glycine, glucosamine, piridoxin, calcium panthotenate, ascorbic acid, vitamin B12 and folic acid.<sup>4,6</sup>

Various active substances contents in Viusid may help improving the immune system of human babies. The work reported in this article was performed to study the effect of supplementation of Viusid in diet of the piglet animal model on growth performance, morbidity and mortality rates, hematological profiles, as well as its effect as an immunomodulator.

## MATERIALS AND METHODS

This study applied a control group design using piglets as an animal model in assessing the effect of food supplement "Viusid vet 90". A total of 60 piglets at 3 days of age weighing 1.6 – 2.1 kg were randomly assigned into 2 groups; the treatment and control groups. The treatment group orally received Viusid vet 90 at a dose of 5 cc twice a day for 10 days, starting from 3 days of age. On the other hand, the control group is just supplied with aquadet as placebo.

At 7 days of age, they are all vaccinated against Hog cholera and the vaccination was repeated at 14 days of age. Then at 20 days of age, all animals were bled for assessment of hematological profiles using Manual ABC Vet Automated Blood Counter, made in Germany. Finally, at 30 days of age blood samples were again withdrew from all experimental animals for examination of Hog cholera antibody using ELISA. During the course of experiment, the animals were monitored for their morbidity and mortality rates. Body weights were recorded at day 15 and 30 days of age. The data collected were analysed statistically using T test and considered significant at  $p < 0.05$

The present experiment was conducted at a private piggery field owned by Bapak Yance at Tuka village, Dalung, Kuta, Bali, from October 1 – November 1, 2014. Blood examination was performed at the Laboratory of Veterinary Clinic Pathology, Faculty of Veterinary Medicine,

Udayana University whereas data for antibody titer was obtained from "Balai Besar Veteriner", Denpasar.

## RESULTS AND DISCUSSION

### Growth Performance and Health Status of the Piglets

Data supplementation of Viusid vet 90 to piglets starting from 3 days of age till 15 days of age, in regards to their growth rate and morbidity and mortality rates, are presented in Tabel 1

Tabel 1

Growth performance and health status of piglets following supplementation of Viusid vet 90

Parameter	Groups		p*
	Control (n=30)	Treatment (n=30)	
Initial body weight (Kg)	1.76±0.12	1.77±0.12	0.79
Body weight at 15 days of age (Kg)	5.14±0.74	5.6±0.55	0.03
Body weight at weaning (30 days) (Kg)	9.07±1.28	9.78±1.28	0.08
Weight gain (Kg)	7.26±1.28	8.01±1.22	0.06
Morbidity rate	33.33±3.84	6.72±4.30	0.003
Mortality rate	10.00±2.36	0±0.00	0.001

\*significantly difference ( $p < 0.05$ )

### Hematological Profiles

Results of assessment of hematological profiles of piglets following Viusid vet 90 supplementation are presented in Table 2 and 3.

Tabel 2

Hematological profiles of piglets following supplementation of Viusid vet 90

Parameter	Groups		p*
	Control (n=10)	Viusid vet 90 (n=15)	
WBC ( $\times 10^3/\text{mm}$ )	10.98± 2.42	11.03±3.29	0.98
RBC ( $\times 10^6/\text{mm}$ )	5.74±0.65	5.58±0.94	0.66
HGB (g/dl)	11.44±0.54	11.57±1.25	0.83
HCT (%)	36.16±0.40	35.33±5.18	0.73
PLT ( $\times 10^6/\text{mm}$ )	318.00±44.94	351.07±243.43	0.62

\*significant difference at  $p < 0.05$

Tabel 3

Profile of differential white blood cells of piglets following supplementation of Viusid vet 90

Variable	Groups		p*
	Control (n=10)	Viusid vet 90 (n=15)	
Neutrophil (%)	36.60±9.53	33.27±14.84	0.65
Eosinophil (%)	1.60±1.83	1.40±1.77	0.83
Lymphocyte (%)	65.40±6.47	57.60±15.09	0.28
Monocyte (%)	6.80±3.03	7.60±2.67	0.58

\*significant difference at  $p < 0.05$

### Immunomodulator

Result of the current study on the effect of supplementation of Viusid vet 90 to piglets on antibody titer against Hog cholera can be seen in Table 4.

Table 4

Titre antibody against Hog cholera of piglets at 30 days of age after supplementation of Viusid vet 90.

Parameters	Groups		p*
	Control (n=10)	Viusid vet 90 (n=15)	
Antibody against Hog cholera (%PC value)	41.52±4.63	54.69±11.44	0.001

\*significant difference ( $p < 0.01$ ), %PC < 40% = seronegative, % PC > 40% = seropositive.

### DISCUSSIONS

#### Growth Performance and Health Status of the Piglets

Tabel 1 shows that in regard to body weight, supplementation of Viusid vet 90 led to a significant increase ( $p < 0.05$ ) in body weight of the animals at 15 days of age, but weight gain and body weight until weaning did not differ significantly ( $p > 0.05$ ) compared to the control group. It is true that weight gain is quite dependent on feed consumption. Before weaning, piglets mainly live on sow's milk and therefore, no different in body weight was noted between the two groups. But when Viusid supplementation is carried out to grower till finisher, then it may significantly increase body weight. As stated by Fernandez *et al.* (2014), fattening pigs that supplied with Viusid vet powder at 2 gram/kg feed for 70 days has significantly enhanced body weight.<sup>7</sup> Viusid vet, in addition to its glycyrrhizinic acid content, contains amino acid glycine that may stimulate glucagon hormone that functions in glucose metabolism, improve growth rate and immunity system. Its vitamin C content may help in absorption of zinc needed for collagen formation and as an antioxidant. Its other functions are to increased NO bioavailability, improve cardiopulmonary performance, and to get rid of oxidative stress that all of those, in turn, may improve growth rate.<sup>8</sup> Viusid vet formula is specifically designed as booster in improving immune system when given to livestock. Moreover, Viusid vet given to pigs at 1 kg/ton feed or at 1 gram/litre drinking water for 10 days may prevent the occurrence of diseases and stress condition.

The present results (see Table 1) also demonstrated that piglets supplemented with Viusid vet 90 has zero percent (0%) of mortality rate and a significantly lower ( $p < 0.01$ ) morbidity rate compare to control animals. This may indicate that Viusid supplementation may play role in minimizing or even in preventing death of animals.

Other effects that may be noticed during Viusid supplementation include decreases in stress condition, in rehabilitation period, in long term effect of drugs, in the removal of residues in the liver and in the occurrence of hepatitis, and enhancement of immune system. Its glycyrrhizinic acid content may increase concentration of gamma interferone, decrease viral load and inhibit replication of viral DNA and RNA. According to Mustika (2013), supplementation of Viusid vet may decrease ratio H/L in chicken that may be expressed in terms of a decrease in rate of environmental heat stress and metabolic stress in the animals.<sup>9</sup> Similar conclusion was noted in the present study (see Table 3). Moreover, calcium panthotenate in Viusid may be useful for antibody synthesis and thus can reduce infection rate.<sup>10</sup>

#### Hematological Profiles

As shown in Table 2, supplementation of Viusid may maintain normal profiles of red blood cells and white blood cells that can be noticed from the total numbers of WBC, RBC, HGB, HCT, and PLT that did not differ significantly from those of the control group. Moreover, the same conclusion can be made concerning the differential white blood cells; there were no differences in percentage of neutrophil, eosinophil, lymphocyte, and monocyte between the two groups. However, the ratio H/L was lower for the Viusid group compared to the control one (see Table 3). This data showed that Viusid supplementation at least do not have negative effect on hematological profile as it contains vitamin C that may help in absorption of zinc. Moreover, its vitamin B12 content may play role as antianaemia, improve the immune system, increase red blood cells number, and stimulate appetite that all of those, in turn, may improve growth rate and the animal's life.<sup>4,6</sup>

#### Immunomodulator

Table 4 showed that supplementation of Viusid has significantly ( $p < 0.01$ ) increased the antibody titer compared to that of the control group, but both of them indicated the seropositive values. According to Instruction Manual VPro<sup>R</sup> CSFV AB C-ELISA-480T, % PC value  $\geq 40$  % may be categorized as seropositive value means piglets are protective against invasion of Hog cholera virus. On the other hand, % PC value < 40 % indicate seronegative value meaning that piglets are not protective against the same virus.

The high value of %PC of piglets after supplementation of Viusid vet 90 may indicate its effect as an immunomodulator that may be due to its Glycyrrhizinic acid content that play role as a strong antiviral and that may stimulate interleukin-12 production in macrophage.<sup>11</sup> Moreover, it may also increase the production of helper T cells in cell-mediated immune respond and of interferone

that may improve activity of macrophage in killing microorganism.<sup>12</sup> Shamsa *et al.* (2010) stated that glycyrrhizinic acid potentially act as hepatoprotector, immunomodulator and as an antiviral.<sup>10</sup> The effect as an immunomodulator of glycyrrhizinic acid is beneficial to individuals that are unhealthy or under stress condition so that their antibody produced are seropositive. Glycyrrhizinic acid having structure resemble to the structure of steroid may prolong activity of cortisol in the body.<sup>13</sup> Furthermore, its amino acid arginine may function in improving activity of the thymus gland and in increasing the number of lymphocyte T cells and, thus, reducing the occurrence of infection. Finally, the content of piridoxan and calcium panthotenate also play role in enhancing antibody production and in reducing infection

### CONCLUSION

Supplementation of Viusid vet 90 in feed to piglets may reduce morbidity rate and prevent mortality. This product acts as an immunomodulator and may improve growth performance as well as maintain normal hematological profiles of the animals. Therefore, Viusid vet 90 given to piglets is quite beneficial in order to improve growth rate and subsequent production.

### ACKNOWLEDGEMENTS

The authors appreciate Mr. Tani Sunarja, Head of PT. TobbsSales Indonesia for supplying us with Viusid vet 90 that were used in the present study. We also wish to thank Mr. Ketut Panca for allowing us to use various facilities in his piggery during the course of experiment.

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