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The Impact of Concurrent Gleptoferron and Toltrazuril Injection on Hematological Profile, Health, and Growth in Pre-weaned Piglets

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Abstract. This study sought to assess the effects of simultaneous Gleptoferron and Toltrazuril administration on hematological parameters, health status, and growth performance in pre-weaned piglets. A total of 54 piglets aged 1 to 3 days were randomly allocated into three groups: a control group receiving a placebo injection, a group administered iron (Ferdex), and a group injected with Gleptoferron in conjunction with Toltrazuril (Forceris). Hematological markers, morbidity, mortality, and body weight growth were evaluated during a 20-day duration. The findings indicated that the administration of Gleptoferron and Toltrazuril markedly elevated red blood cell (RBC) counts, hemoglobin concentrations, and hematocrit levels relative to the iron-only group, without an increase in total white blood cell (WBC) counts, suggesting the absence of post-injection infection. Moreover, pigs administered Forceris did not develop hypochromic anemia and had a reduced incidence of diarrhea (6.7%) with no mortality, indicating enhanced gut health and excellent coccidiosis prevention. Significantly, pigs administered Forceris exhibited a weight gain of 9 kg at 20 days of age, in contrast to 7.9 kg in the iron-only cohort. The results indicate that the simultaneous use of Gleptoferron and Toltrazuril provides more advantages in avoiding anemia, improving growth, and fostering better health outcomes in pre-weaned piglets than iron supplementation alone. This efficient and pragmatic approach has significant ramifications for enhancing production in pig breeding operations.

Keywords: Gleptoferron; Toltrazuril; Hematological profile; Pre-weaning piglets; Growth performance

I. INTRODUCTION

Breeding pig farming is a farming enterprise that generates piglets. The success of this company is mostly dependent on the number and quality of piglets produced until the weaning stage. A sow of standard quality produces more piglets, which increases the success of the company. Many reproductive pig farm entrepreneurs see more than 10 children born per sow; yet, after the weaning phase, only 5-6 survive with a body weight below the standard, less than 5 kg per head. Because the break-even point ratio agreed upon by reproductive pig entrepreneurs in 2022 is that the piglets that can be weaned are at least 7 with standard conditions, this situation is quite harmful to the reproductive pig farming company. Given the high production expenses of raising pigs, particularly the 2022 feed price.

Iron deficiency, essential for hemoglobin (Hb) production, is a significant factor contributing to reduced piglet survival due to its potential to cause anemia. Hemoglobin levels will decrease to 70 g/L if piglets do not get iron supplementation until they reach 6 days of age (Wei and Kwan, 2021). Reports suggest that more than 30% of individuals born have had sub-anemia characterized by low iron levels; some research estimates an incidence as high as 40%. Iron is necessary for development at a rate of 7-16 mg per day. This need is not fulfilled by the colostrum and milk produced by the sow.

In the absence of external iron intake, hemoglobin levels will decline when the piglets reach six days of age. Particularly around the age of 1-2 days, oral iron consumption is inefficient due to inflammation of the small intestine caused by E. coli, Clostridium perfringens infection, or coccidia species, which impedes iron

absorption from milk or colostrum. Coccidia sp infection is frequently overlooked by reproductive pig breeders, with approximately 69% of pre-weaning piglets affected (Niestrath et al. 2002; Lysanne et al. 2019).

Farmers are more concerned with E. coli infection prevention. Often ignored, coccidia sp infection. Because they consume sporulated oocysts in the surroundings, piglets are usually infected with coccidia in the first week of life. Very resistant in the environment, oocysts will be expressed by piglets within 5-6 days following infection. Only a small number of farmers administer Fe injections and orally treat coccidia with sulfa. Though the latter approach is quite inconvenient since, apart from injecting iron (Fe), it also includes sulfa drug cengkok, this action can be quite taxing for farmers and may cause stress. Though these activities are quite unrealistic, several studies found that administering sulfa and Fe injections at the age of one, two, or three days can help to overcome anemia. That calls for a study on the technique of giving iron and sulfa types that are practical yet safe and effective. Veterinary medicine is evolving quickly; now, a product has been created that injects iron III as Gleptoferron with toltrazuril to prevent anemia and coccidia. There has, however, been no report on whether the use of this medication prevents anemia more than iron injection by itself. That calls for study.

This study aimed to find out how giving piglets aged 1 to 3 days iron III (Gleptoferron) injection together with toltrazuril affected the blood picture (total erythrocytes, hemoglobin levels, PCV values), health, and weight gain of pre-weaning piglets. Naturally, a safe, affordable, and simple dose for farmers to administer would be desirable, with the expectation that reproductive farmers generating post-weaning piglets do not suffer losses.

II. METHODS

Ethical Clearance and Animal Model

The Animal Ethics Committee of the Faculty of Veterinary Medicine has approved all procedures used in this study under certificate Number: B/283/UN14.2.9/PT.01.04/2023. From Mr. Dewa Putra's breeding farm in Susut Village, Susut District, Bangli, the animal model in this study used piglets aged 1-3 days.

Research Procedure

Using a simple randomized complete design with three treatments and six replications, each replication comprising three piglets aged 1–3 days with an average weight of 1.5 kg, experimental research is conducted, yielding a total of 54 piglets. Before being treated, all piglets used as research samples were weighed first. While treatment group 1 piglets received an injection of 100 mg iron (Ferdex) (P1), the control group piglets (P0) were given a placebo aquadest injection. Treatment group 2 piglets received an injection of Gleptoferron + Toltrazuril (Forceris) (P2). All treated piglets had their blood drawn and put in EDTA-containing tubes after 21 days of treatment; they were then sent to the BBVET Denpasar Laboratory for routine hematological examination comprising Total Erythrocytes, hemoglobin (Hb) levels, and Packed Cell Volume (PCV) values.

A total of 54 piglet samples, all from Mr. Dewa Putra's breeding farm in Susut Village, Bangli, ranging in age from 1 to 3 days with an average body weight of 1.5 kg, were used as test animals in the study. The piglet pens are not separate from the sow pens; rather, the piglets join their mothers to create one combined flock. The iron used is Ferdex (PT. Medion Bandung, Indonesia), composed of 100 mg per 1 ml. The Forceris brand (Ceva Animal Health Pty Ltd, Australia) combines Gleptoferron and Toltrazuril with a composition of 355.2 mg of Gleptoferron, 30 mg of Toltrazuril, and 133.4 mg of Iron III in each 1 ml. One point five ml per piglet is the advised dose.

One day before treatment, the piglets' initial weight data were recorded; 35 days later, the weight data following treatment were recorded. Weight gain is the difference between the final weight and the initial weight. Hematology data were gathered following 21 days of treatment injection.

Clinical symptoms like diarrhea provided morbidity data; mortality was noted from the beginning of treatment to the end of the study. Isospora suis data isolation was done by taking rectal swab fecal samples, which were then put in transport media to be transported to the microbiology lab every week following treatment until the end of the study. BPVET Denpasar carried out hematological tests.

Statistical Analysis

The T-Test (Steel and Torre, 1980) was used to examine body weight gain and hematology data, total erythrocytes, Hb levels, and PCV values. Descriptive analysis was done on the Isospora suis parasite presence, mortality, and morbidity.

III. RESULT AND DISCUSSION

Hematological profile

The impact of the combined injection of gleptoferron and toltrazuril on the blood profile and performance of 2-day-old piglets is seen in Tables 1-5. Table 1 indicates that the forceris injection produced a total White Blood Cell (WBC) count that was considerably lower than that of the

pigs administered Rhenovit (P<0.05). This situation suggests that the cohort of pigs administered Rhenovit likely encountered an infection, resulting in an elevation in WBC, albeit remaining within the normal range. White blood cells are inflammatory cells that contribute to combating bacterial infections in the bloodstream. In the cohort of pigs administered Forceris, the total white blood cell count remained unaffected by infection, maybe attributable to the anti-coccidial drug toltrazuril. If this is accurate, it is highly advantageous for the cohort of pigs administered Forceris, since their gastrointestinal system remains free from inflammation, facilitating adequate iron absorption.

Piglets suffering from inflammation caused by illness have significantly compromised iron absorption, potentially resulting in anemia. Anemia will significantly impede the growth of beginning-phase piglets, leading to stunted growth as seen by reduced body weight. Numerous illnesses capable of inducing inflammation in the digestive system of pre-starter phase piglets include E. coli, Clostridium perfringens, and Isospora suis. Isospora suis infection in piglets during the beginning phase is challenging to treat with strong medicines. Consequently, the prevention of Isospora suis is essential for piglets in the beginning phase. Currently, the administration of toltrazuril for the prevention of Isospora suis infection is highly successful.

Table 1 indicates that the administration of Forceris to piglets at 2 days of age yielded total RBC and Hb levels of 7.3 x 10^6/µL and 12.2 grams%, respectively, at 20 days of age, which were markedly superior to those of the piglets treated with Rhinovit, which exhibited total RBC levels of 4.8 x 10^6/µL and Hb levels of 9.2 grams%. This cohort of pigs exhibits a total red blood cell count that is below the usual range. The standard total RBC count for piglets ranges from 5.0 to 8.0 x 10^6/µL, while the hemoglobin level is between 10.0 and 16.0 grams%. The cohort of pigs administered Rhinovit at 2 days of age exhibited anemia. Anemia is characterized by a reduction in total red blood cell (RBC) count, hemoglobin (Hb) levels, and packed cell volume (PCV) values, as indicated by laboratory findings. If any one of the three characteristics of anemia exhibits a decline, it might be classed as anemia. The anemia manifested is hypochromic anemia, resulting from iron deficiency (Table 4). This disease may result from diarrhea in the cohort of pigs administered Rhinovit. The prevalence of diarrhea among pigs administered Rhinovit was 50%, but the prevalence among those administered Forceris was at 6.7% (Table 4). Consequently, it is imperative to provide Rhinovit at 21 days of age. In the cohort of pigs administered Forceris, anemia did not manifest, therefore obviating the necessity for a further dose at 21 days of age.

Iron deficiency (Fe) frequently occurs in piglets, who are born with sub-anemia and diminished Fe reserves. Neonatal iron insufficiency may impede development throughout the weaning period. A contributing cause to low survival rates in piglets is iron (Fe) deficiency, which is essential for hemoglobin (Hb) synthesis and may result in anemia. If piglets do not get iron supplementation until they are 6 days old, their hemoglobin levels will decline to 70 g/L (Wei and Kwan 2021). Reports indicate that over 30% of neonatal pigs exhibit sub-anemia conditions characterized by low iron levels, with further research revealing effects as high as 40%. The iron required to facilitate development is 7 to 16 milligrams per day. This iron is derived from the body's iron stores and its absorption in the small intestine (Derkach, 2021).

This anemia may be attributed to the inferior quality of iron in Rhinovit or the group of piglets suffering from enteritis, leading to less iron absorption. The significantly elevated WBC count indicates a bacterial infection in contrast to the cohort of piglets administered Forceris, which comprises third-generation iron effective in hemoglobin synthesis and Toltrazuril that inhibits the proliferation of Isospora suis and other bacteria, thereby preventing enteritis and optimizing iron absorption.

Enteritis in piglets, caused by bacterial or coccidia infections, is typically shown by diarrhea, which may result in dehydration that varies from mild to severe, contingent upon the diarrhea's intensity. An elevated PCV value in a full blood examination indicates dehydration. The blood test findings indicated that the PCV value in the Forceris 40% group of piglets was substantially lower than that in the Rhinovit group, which was 47.2% (P<0.05). The PCV value of 47.2% in the group of piglets administered Rhinovit approaches the top limit of the normal PCV range, which is 32-50% (Table 1). This condition may be presumed to induce dehydration as a result of diarrhea. Diarrhea manifested in the cohort of pigs administered Rhinovit at a concentration of 50%. Table 5.

Table 2 presents the differential white blood cell profile, detailing the percentages of neutrophils, eosinophils, lymphocytes, monocytes, and basophils. The neutrophil percentage in the Rhinovit-injected piglet group was 50.05%, markedly above the 39.40% seen in the forceris-injected group (P<0.05) (Table 2). The proportion of neutrophils in both cohorts of piglets exceeded the typical neutrophil percentage of 36.6%. Neutrophilia refers to an elevated neutrophil count, indicating a bacterial infection or a state of stress. The infection severity in the group of pigs administered Rhinovit is greater than that in the group injected with Forceris. Leukocytosis, indicative of bacterial infection, is observed in both the Rhinovit and Forceris-injected piglet groups,

albeit to a lesser extent (Table 1). The incidence of diarrhea symptoms in the group of pigs administered Rhenovit was 50%, but in the group receiving Forceris, it was just 5%.

The incidence of diarrhea-related illnesses in the cohort of piglets administered Rhinovit was intensified by coccidia infection, as evidenced by a significantly elevated eosinophil percentage of 6.35% (Table 2), beyond the usual threshold of 1.6%. The presence of eosinophilia accompanied by diarrhea symptoms suggests that the piglets are afflicted with coccidiosis (Winaya et al. 2022). In contrast to the cohort of piglets administered forceris, which had an eosinophil percentage of 1.4%, indicating the absence of coccidia infection.

Table 1
The effect of Forceris injection on the blood profile of 20-day-old piglets

Supplement dosage in feed	Variable			
	WBC ($x10^3$ /µL)	RBC($x10^6$/µL)	HB (g%)	PCV (%)
F (n=20)	13.330^a	$7,3^A$	$12,2^A$	$40,0^A$
Rh (n=20)	$22,185^b$	$4,8^B$	$9,2^B$	$47,2^B$
Normal physiological values of pigs (Jain,1986)	8,7-37,9	5,0-8,0	10,0-16,0	32-50

*Information: F = Forceris, Rh = Renovit.
**Superscript letters that differ in the same column indicate a significant difference (P<0.05).

Table II
The effect of a combination injection of gleptoferron and toltrazuril on the hematological profile of 20-day-old piglets

Parameter	Control	Gleptoferron + Toltrazuril	Normal physiological values of pigs (Jain,1986)
WBC ($x10^3$/µL)	22.18^b	13.33^a	8.7-37.9
RBC ($x10^6$/µL	4.8^a	7.3^b	5.0-8.0
HB (g/dL)	9.2^a	12.2^b	10.0-16.0
PCV (%)	47.2^b	40.1^a	32-50
MCH (Pg)	-	-	-
MCHC (fL)	-	-	-
Neutrophil (%)	50.5	39.4	-
Eosinophil (%)	6.35	1.40	-
Lymphocyte (%)	51.15	43.0	-
Monocyte (%)	13.05	6.80	-

*Information: WBC = white blood cells; RBC = red blood cells; HB = hemoglobin; PCV = packed cell volume.
**Superscript with different letters in the column direction indicates a significant difference.

Table III
Differential white blood cell count

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Neutrophil (%)	Rh	20	50.05	13.717	3.067
	F	20	39.40	13.272	2.968
Eosinophil (%)	Rh	20	6.35	2.681	.599
	F	20	1.40	1.353	.303
Lymphocyte (%)	Rh	20	51.15	14.673	3.281
	F	20	43.00	13.845	3.096
Monocyte (%)	Rh	20	13.05	3.348	.749
	F	20	6.80	4.372	.978

Table IV
Red blood cell index

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
MCH (Pg)	Rh	20	21.150	1.4151	.3164
	F	20	20.750	1.8426	.4120
MCHC (fL)	Rh	20	65.885	5.1849	1.1594
	F	20	68.605	4.8768	1.0905

Tabel V
Average weight gain of 20-day-old piglets after administration of Forceris or Rhenovit

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Initial Weight(Kg)	Forceris	30	1.5950	.42474	.07755
	Rhenovit	30	1.7483	.42598	.07777
Final Weight(Kg)	Forceris	30	10.5970	.57725	.10539
	Rhenovit	30	9.6067	.79695	.14550
Weight gain over 18 days of treatment	Forceris	30	8.9950	.57381	.10476
	Rhenovit	30	7.8617	.65859	.12024

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Red Blood Cell (RBC) Index

The RBC Index reflects the dimensions of red blood cells as shown by the Mean Corpuscular Volume (MCV), which is macrocytic, signifying a deficiency of hematological components such as vitamin B12, folic acid, and vitamin B6 in the presence of anemia. Normocytic anemia indicates hemorrhage; microcytic anemia implies the animal is afflicted by chronic disease or parasitic infection. The Mean Corpuscular Hemoglobin Concentration (MCHC) index assists in determining hemoglobin concentration. Iron (Fe) is theoretically the major component of hemoglobin. Hyperchromic anemia indicates an elevated circulating concentration of iron; normochromic anemia denotes bleeding; hypochromic anemia indicates iron deficiency.

This research exclusively addressed MCHC, as the treatment used iron (Fe). Piglets injected with Rhinovit had a reduced MCHC of 66 fL compared to those injected with Forceris, which had an MCHC of 68.6 fL ($P<0.05$) (Table 3). The MCHC indicates that the piglets administered Rhinovit are experiencing hypochromic anemia; hence, the Rhinovit injection should be reiterated at 21 days of age.

Piglet Health

Morbidity and death, as presented in Table 4, were seen in pigs administered Forceris or Rhinovit injections. The blood profile of weaned piglets is typically reported as follows: total WBC (10.98 x 10^3/mm), total RBC (5.74 x 10^6/mm), hemoglobin concentration (11.44 g/dl), packed cell volume (PCV) (36.16%), platelet count (318 x 10^6/mm), neutrophils (36.6%), eosinophils (1.6%), lymphocytes (65.4%), and monocytes (6.8%) (Jain, 1986; Ardana et al. 2015; Merdana et al. 2018).

Mass of piglets

The impact of Forceris or Rhinovit injection administered at 2 days of age on body weight is seen in Table 5. The table indicates that the piglet group receiving Forceris and Rhenovit had body weights of 1.6 kg and 1.7 kg, respectively, which were not statistically significant ($P>0.05$). Following the administration of Forceris or Rhenovit at 2 days of life, the body weight increment at 20 days of age was 9 kg for Forceris, greatly surpassing the 7.9 kg seen in the Rhenovit group ($P< 0.01$). Table 5 shows that Gleptoferron is a high-quality iron formulation, offering an ideal complement for the growth of neonatal piglets. The consistent supply of 200 mg of iron as gleptoferron is acknowledged as an efficient method to avert iron deficiency anemia in pigs. Sperling, Daniel, et al. (2018). In the absence of anemia, growth will accelerate, leading to increased body weight.

Table 5 indicates that the cohort of pigs administered Forceris exhibited much superior development rates relative to the cohort injected with Rhenovit. This results from the influence of forceris on the digestive system, which can alleviate enteritis induced by bacteria and/or coccidia. Conversely, the cohort of pigs administered Rhenovit exhibited enteritis, marked by a notable diarrhea incidence of 50%. Diarrhea symptoms may result from bacterial and/or coccidia infections, as evidenced by leukocytosis, neutrophilia, and eosinophilia. Enteritis significantly impairs iron absorption, leading to diminished hemoglobin synthesis and subsequent anemia. Low hemoglobin levels or anemia diminish the oxygen

transport capacity for glucose metabolism in the mitochondria, elevate cardiac workload, and eventually impede development.

IV. CONCLUSION

Based on the research results, it can be concluded that the combination injection of Glicoferon and Toltrazuryl in two-day-old piglets significantly increases the number of red blood cells (RBC), hemoglobin levels, and hematocrit values. Additionally, this injection does not cause an increase in the total white blood cell (WBC) count, indicating no post-injection infection. Piglets that received the treatment also did not experience hypochromic anemia, indicating no deficiency in hemoglobin. The recorded morbidity rate of 6.7% without any further mortality cases confirms the safety of using this combination. Overall, the administration of Glicoferon and Toltrazuryl to two-day-old piglets has been proven to increase weight gain up to 9 kg by the age of 20 days, a result that is better compared to piglets that only received iron injections.

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