

Efficacy of toltrazuril and gleptoferron with dextran on the prevention of coccidiosis and iron deficiency anemia in suckling piglets

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ABSTRACT

The study aimed to evaluate the efficacy of injectable toltrazuril and gleptoferron (combined in one injectable product) in controlling coccidiosis and iron deficiency anemia in piglets. A total of 16 farrowing litters were assigned to 2 treatment groups, 8 litters per group. The piglets in the control group (conventional treatment) were injected with 1 mL of iron dextran (200 mg) and orally administered 1 mL of 5% toltrazuril at 2 days of age (DOA). In the treatment group, the piglets were injected with 1.5 mL of combined product (containing 200 mg of iron and 45 mg of toltrazuril), also at 2 DOA. The mean hemoglobin concentration of 2 DOA piglets in the control group (110.00 ± 17.20 g/L) was similar to that of the treatment group (104.60 ± 15.40 g/L) ($P > 0.05$). However, the mean hemoglobin concentration of the control group (103.30 ± 15.80 g/L) was lower than that of the treatment group (111.60 ± 16.00 g/L) at weaning ($P < 0.05$). Real-time PCR results did not detect oocysts in fecal samples except at 15 DOA. The threshold cycle (Ct) index in the control group at 15 DOA (34.36 - 34.79) was lower than that of the treatment group (35.07 - 36.98). Furthermore, the treatment group had a higher mean body weight at weaning and average daily gain (7.98 kg and 265.20 g/day) than that of the control group (7.40 kg and 225.60 g/day) ($P < 0.001$). In conclusion, combination product provided more benefits for suckling piglets, including controlling coccidiosis and significantly improving weight gain.

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1. Introduction

Toltrazuril is routinely used to control coccidiosis in neonatal piglets on affected farms not only in Vietnam but also worldwide. Its high anticoccidial activity is due to its ability to kill all asexual and sexual stages of coccidia, except the oocyst (Mundt et al., 2003; Joachim & Mundt, 2011). Coccidiosis is a common disease in the swine farming industry globally, caused by a protozoan parasite called *Cystoisospora suis* (formerly *Isospora suis*). This disease is one of the primary causes of diarrhea in suckling piglets, leading to poor productivity and significant economic losses in the pig farming industry (Karembé et al., 2021; Sawhney et al., 2023) and oral formulations have been licensed for many years. Recently, the first parenteral formulation containing TZ in combination with iron (gleptoferron). It is prevalent in all types of farrowing facilities with poor hygiene and management. Toltrazuril is typically administered orally.

Recently, a new injectable combination product (30 mg toltrazuril/mL; 133.4 mg iron/mL as gleptoferron), which can reduce stress and labor costs, has been developed to control coccidiosis and prevent iron deficiency anemia in suckling piglets. A single intramuscular shot (with a recommended dose of 1.5 mL/piglet, equivalent to 45 mg toltrazuril and 200 mg iron) is administered 24 - 96 h after birth (Morales et al., 2018; Hiob et al., 2019; Joachim et al., 2019). Administering toltrazuril by injection ensures accurate and sufficient dosing (avoiding issues where piglets do not swallow or vomit) and combines iron supplementation, reducing stress for suckling piglets and significantly saving labor costs. Although some studies on the pharmacokinetics of combined product

(toltrazuril and gleptoferron) have been conducted by Joachim et al. (2018), experimental data on its efficacy are still limited, particularly in Vietnam. Therefore, the objective of this study was to evaluate the efficacy of combined product in controlling coccidiosis and preventing iron deficiency anemia in suckling piglets during the suckling period.

2. Materials and Methods

2.1. The experimental farm and animals

The experiment was conducted on a farm with a history of coccidiosis, using Pig Improvement Company (PIC) sows crossbred with Duroc boars. Piglets from a total of 16 farrowing litters were randomly selected and divided into two groups, with each group kept separately to prevent the spread of coccidia during the rearing period. Both groups were balanced in terms of sow parity and the average weight of the piglets (Table 1).

The control group: the piglets (2 days old) were injected with 1 mL of iron dextran (200 mg) and orally administered 1 mL of 5% toltrazuril (drop suspension with the pump provided by the supplier).

The treatment group: the piglets (2 days old) were injected with 1.5 mL of the combined product (30 mg toltrazuril/mL; 133.4 mg iron/mL).

Table 1. Body weights of neonatal piglets

Groups	N	Mean body weight ($\pm$ SE)kg	P
Treatment	92	1.54 $\pm$ 0.02	0.565
Control	93	1.52 $\pm$ 0.03	

2.2. Blood sampling và hemoglobin test

A total of 48 piglets (6 piglets - three barrows and three females) in each group were randomly chosen to measure Hb concentration on the farm. Blood was taken from the ear vein of the piglets, two first drops were removed and the test was tested with Hemocue® Hb 201+ system (SE-262 71, Ängelholm, Sweden). Results were displayed in g/L within 15 - 60 sec depending on the hemoglobin concentration. Piglets were classified as follows: Hb levels < 90 g/L were considered to be anemic, Hb levels $\geq$ 90 g/L and $\leq$ 110 g/L were suboptimal and Hb levels > 110 g/L were optimal (Bhattarai & Nielsen, 2015b).

2.3. Fecal sampling and real time PCR

Fecal samples were collected from 5 piglets per litter and then pooled them into one pooled sample (3 - 5 g/sample). Use medical cotton to clean the anus before taking the samples, then use the swab to collect feces inside the rectum with 3 - 5 g/sample. It was put in 15 mL tube and kept cool to deliver to the lab the same day. As 8 litters of each group were examined four times, a total of 32 fecal samples/group were available. The sampling schedule was at 3, 5, 15, and 24 DOA.

DNA extraction

The DNA of *Cystoisospora suis* was extracted from fecal samples following the manufacturer's instruction by using QIAGEN's QIAamp® PowerFecal Pro® DNA Kit.

Real time PCR protocol

The real time PCR reaction mix is based on QIAGEN's QuantiNova Probe RT-PCR kit with the specific oligonucleotides (Samarasinghe et al., 2008). The following oligonucleotides were used: Forward primer: CystoisoITSGF1 (GATCATTCACACGTGGCCCTT);

Reverse primer: CystoisoITSR4 (CCTTTATGCGGCCATTCTGAAGC); and CystoisoITS probe 2 (FAM-GCGTTCGGGGGAAGCTGTG-BHQ1). The total volume was 20 μ L as followed: the reaction mixture consisted of 3.4 μ L distilled water, 1 μ L of each primer (10 μ mol/ μ L), 10 μ L QuantiNova MasterMix (2x), 0.1 μ L ROX, 0.5 μ L probe (10 μ M) and 4 μ L extracted DNA. A thermal cycler was used to perform 95°C for 10 min, 40 cycles (95°C sec, 60°C for 40 sec).

2.4. Produced performance

After farrowing, all neonatal piglets were weighed, those weighing 0.9 kg or more and healthy were selected for raising. At weaning, 6 piglets per litter were randomly weighted at 26 DOA.

2.5. Statistical analysis

Summary statistics were calculated for each variable. This study was performed a two-samples t-Test for body weight and hemoglobin level by using Minitab (17 version).

3. Results and Discussion

3.1. Hemoglobin level

Table 2. Hemoglobin test results

Groups	Age	Optimal		Sub-clinical		Anemia		Total	
		No.	%	No.	%	No.	%	No.	%
Control	2 d old	20	41.67	24	50.00	4	8.33	48	100
	24 d old	15	31.25	24	50.00	9	18.75	48	100
Treatment	2 d old	19	39.58	24	50.00	5	10.42	48	100
	24 d old	29	60.42	15	31.25	4	8.33	48	100

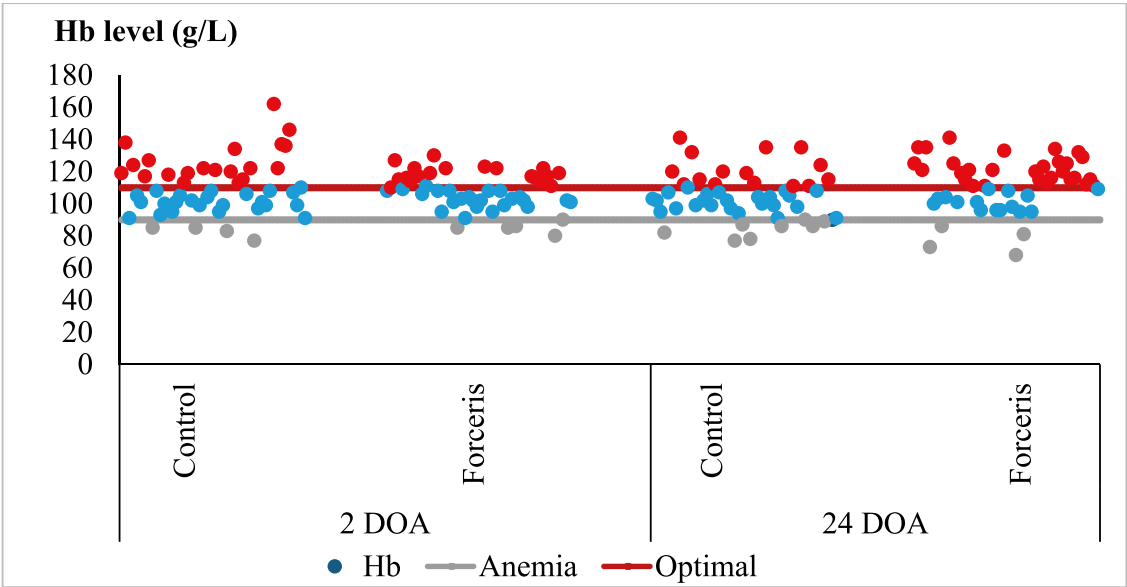


Figure 1. Hemoglobin test results.

Hemoglobin results are shown in Table 2 and Figure 1. The findings indicated that the treatment group showed greater improvement in hemoglobin levels (24 DOA) compared to the control group. At 24 DOA, the hemoglobin (Hb) level of suckling piglets in the treatment group was at optimal levels in 60.42% of cases, compared to 31.25% in the control group. Additionally, the anemia rate in the treatment group (8.33%) was lower than that in the control group (18.75%). Bhattarai & Nielsen (2015a) and Chevalier (2019) confirmed the association

between hematological parameters at weaning and weight gain in piglets. Positive correlations were observed between hemoglobin levels and weight gain, indicating that an increase in hemoglobin corresponds to an improvement in weight gain. The results suggest that combination product performed well in improving Hb levels, consistent with previous reports (Karembe et al., 2021) and oral formulations have been licensed for many years. Recently, the first parenteral formulation containing TZ in combination with iron (gleptoferron).

The study compared the efficacy of gleptoferron and iron dextran in preventing anemia in suckling piglets. Both administered products were safe, but gleptoferron may have greater efficacy in preventing anemia compared to iron dextran. The study highlights the importance of iron supplementation for piglet health and growth. Iron supplementation through parenteral administration is a common practice to prevent iron deficiency anaemia in piglets. Hemoglobin level is the most widely used parameter for detecting anemia and evaluating

the efficacy of iron supplementation. However, Hb values in suckling piglets are more variable, physiological levels and consequently anemia are defined differently. Following the intramuscular administration of 200 mg of iron at 2 DOA, Hb levels in piglets in the treatment group were more optimal, with less anemia observed compared to the control group. It has also been observed that an optimal iron status (Hb > 110 g/L) at weaning can lead to improved growth performance, suggesting a more effective hematinic activity of gleptoferron (Morales et al., 2018; Chevalier, 2019).

3.2. Real time PCR result

Table 3. Real time PCR results

Groups		Results	3 DOA	5 DOA	15 DOA	24 DOA
Control	Positive (%)	Ct = 34.36	-	-	12.50	-
		Ct = 34.79	-	-	12.50	-
	Negative (%)		100	100	75.00	100
Treatment	Positive (%)	Ct = 35.07	-	-	12.50	-
		Ct = 36.98	-	-	12.50	-
	Negative (%)		100	100	75.00	100

Ct: threshold cycle; DOA: days of age.

Diagnosis of coccidiosis is considered a challenging process; it is usually performed by collecting and pooling fecal samples from different piglets of the same litter. The most common diagnostic technique is flotation with oocysts and subsequent counting, often using a McMaster chamber. However, other techniques with higher sensitivity, such as real time PCR, are becoming more popular.

The oocyst detected results in both the treatment and control groups were negative except at 15 DOA. At 15 DOA, the suckling piglets can get a high-risk period for coccidiosis infection with 25% infected proportion (two pooled samples) (Table 3). Interestingly, the

administration of toltrazuril can reduce the infection and harmful effects of coccidia in both experimental piglets. However, the results showed a small difference in the Ct index, with the treatment group having a higher Ct index than the control group (it means that the detection of coccidiosis DNA is lower than the control group) (Table 3). This further confirms that intramuscular administration of toltrazuril using the combined product provides better control of coccidiosis in piglets. This result aligns with the findings of Karembe et al. (2021), which showed a high concentration of toltrazuril in the intestinal tissues of piglets when the combined product was administered at 2 DOA.

Cystoisospora suis is an intracellular parasite that infects intestinal cells, primarily in the jejunum and ileum (Niestrath et al., 2002). The injectable toltrazuril product with an oral toltrazuril product for controlling piglet cystoisosporosis. Both treatments were safe and highly effective in suppressing oocyst excretion, with body weight development significantly improved in the treatment group compared to the control group. In this study, real-time PCR method was used to detect oocyst in fecal samples. The results showed that real time PCR was more sensitivity and specificity to diagnose coccidiosis in swine. The results of this study align with Maes et al. (2007), which reported positive effects of toltrazuril on piglet growth in herds without clinical isosporosis.

3.3. Body weight

Table 4. Body weight at weaning

Groups	N	Mean body weight ($\pm$ SE)kg	P
Treatment	48	7.98 $\pm$ 0.11	< 0.001
Control	48	7.39 $\pm$ 0.10	

The average live weight of neonatal piglets in both groups at the beginning of the experiment was similar (Table 1). At weaning (26 DOA), the mean body weight in the treatment group (7.98 kg) was higher than that of the control group (7.39 kg). The difference in average weaning weight is statistically significant ($P < 0.001$) (Table 4). This result indicates that the combined product can improve weight gain in suckling piglets due to the effectiveness of iron and toltrazuril, as well as the reduction of stress. This leads to healthier and faster weight gain in suckling piglets. This is consistent with the results of Joachim et al. (2018), who compared the effectiveness of the combined product with an oral toltrazuril product. Their study showed that the mean body weight in the treatment group

was higher compared to the control group due to a reduction of coccidia infection and stress. This study also demonstrated that suckling piglets in the treatment group had higher ADG than those in the control group. Growth performance during the suckling period is essential for post-weaning growth, even if the difference is not statistically significant at the start. Improved growth performance may be associated with optimal iron status, enhancing oxygen transport, immune function, vitality, and metabolism (Perri et al., 2016; Chevalier et al., 2021).

4. Conclusions

The optimal Hb levels (60.42%) were higher, and the anemia Hb levels (8.33%) were lower in the treatment group at 24 DOA. The Ct index for coccidia detection using real time PCR was above 35 in the treatment group. Weaning weight gains were significantly higher in the treatment group compared to the control group. The use of a product which combines toltrazuril with gleptoferron, can reduce coccidia infection and increase growth in suckling piglets.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Author contributions

Nguyen Ba Toan and Vo Phong Vu Anh Tuan conceived and designed this study. Nguyen Ba Toan and Huynh Vo An Khang collected data from the swine farm. Nguyen Ba Toan; Vo Phong Vu Anh Tuan and Nguyen Thi Thuong drafted the manuscript. All authors read and approved the final article.

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