UNIVERSITI PUTRA MALAYSIA

COMPARISON BETWEEN INJECTABLE TOLTRAZURIL-GLEPTOFERRON AND ORAL TOLTRAZURIL WITH INJECTABLE IRON DEXTRAN IN THE PREVENTION OF SWINE NEONATAL COCCIDIOSIS AND IRON DEFICIENCY ANAEMIA

KOH HAO JIE

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Comparison between Injectable Toltrazuril-Gleptoferron and Oral Toltrazuril with Injectable Iron Dextran in the Prevention of Swine Neonatal Coccidiosis and Iron Deficiency Anaemia” by Koh Hao Jie and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

This thesis is especially dedicated to:

My loving parents

Koh Say Leong and Lee Kwee Lian

My supportive supervisors

Dr. Michelle Fong Wai Cheng

Assoc. Prof. Dr. Ooi Peck Toung

My final year projects teammates

Huam Ze Shiun

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LIST OF ABBREVIATIONS

ADG	Average daily gain
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
F	Faecel score
g/dL	grams per deciliter
Hb	Haemglobin level
IDA	Iron deficiency anaemia
ITG	Injectable toltrazuril-gleptoferron
mg	Milligrams
OPG	Oocyst count per gram
OTI	Oral toltrazuril with injectable iron dextran

ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 -Projek.Tahun Akhir

PERBANDINGAN ANTARA TOLTRAZURIL-GLEPTOFERRON SUNTIKAN DAN TOLTRAZURIL ORAL DENGAN DEXTRAN BESI SUNTIKAN UNTUK MENCEGAH KOKSIDIOSIS BABI NEONATAL DAN ANEMIA AKIBAT KEKURANGAN ZAT BESI

Oleh

Koh Hao Jie

2022

Penyelia: Dr. Michelle Fong Wai Cheng

Penyelia bersama: Prof. Madya Dr. Ooi Peck Toung

Koksidiosis neonatal babi adalah penyakit endemik dan di mana-mana yang disebabkan oleh Cystoisospora suis. Anemia kekurangan zat besi (IDA) berlaku pada anak babi, kerana ia dilahirkan dengan simpanan zat besi yang terhad dalam badan. Kedua-dua keadaan menyebabkan pertumbuhan terbantut, yang membawa kepada kerugian ekonomi yang ketara kepada petani. Kajian ini bertujuan untuk membandingkan kesan antara suntikan toltrazuril-gleptoferron (ITG) dan toltrazuril oral dengan suntikan besi dextran (OTI) untuk pencegahan coccidiosis neonatal babi dan

IDA. Anak babi daripada 15 ekor dalam kumpulan OTI menerima dos oral toltrazuril (20 mg/kg) dan satu suntikan intramuskular besi (200 mg/khinzir) 24 jam selepas bersalin, manakala anak babi daripada 15 ekor dalam kumpulan ITG menerima suntikan intramuskular ITG (45 mg toltrazuril + 200 mg gleptoferron) 24 jam selepas bersalin. 3 anak babi daripada setiap anak ayam (jumlah 90 anak babi, 45 untuk setiap kumpulan) telah dipilih secara rawak untuk pensampelan. Kiraan ookista (OPG), peratusan cirit-birit, skor najis, tahap hemoglobin darah, dan parameter prestasi diperhatikan selama 26 hari dan dianalisis menggunakan Ujian T-Pelajar Bebas dan Ujian Mann-Whitney U. Kumpulan ITG menunjukkan trend kiraan ookista yang lebih rendah dan peratusan cirit-birit, pemarkahan najis yang lebih baik tanpa skor F4, tiada anak babi anemia semasa penyapihan dan parameter prestasi yang lebih baik. Walau bagaimanapun, tidak terdapat perbezaan yang signifikan ($p > 0.05$) antara kedua-dua kumpulan. Gabungan toltrazuril dan gleptoferron suntikan tunggal menyediakan terapi metafilatik yang berkesan sebagai alternatif untuk mencegah koksidiosis neonatal babi dan anemia kekurangan zat besi dalam anak babi neonatal, dengan faedah pengurangan intensiti buruh dan campur tangan pengendalian tekanan.

Kata Kunci: *Koksidiosis, Cystoisospora suis, Anemia Kekurangan Zat Besi, Toltrazuril, Iron dextran, Gleptoferron*

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999- Final Year Project.

COMPARISON BETWEEN INJECTABLE TOLTRAZURIL-GLEPTOFERRON AND ORAL TOLTRAZURIL WITH INJECTABLE IRON DEXTRAN IN THE PREVENTION OFSWINE NEONATAL COCCIDIOSIS AND IRON DEFICIENCY ANAEMIA

by

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2022

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Swine neonatal coccidiosis is an endemic and ubiquitous disease which caused by *Cystoisospora suis.* Iron deficiency anaemia (IDA) occurs in piglets because they are born with limited iron storage in the body. Both conditions cause retarded growth, which leads to significant economic losses to the farmers. This study aims to compare the effects between injectable toltrazuril-gleptoferron (ITG) and oral toltrazuril with injectable iron dextran (OTI) for the prevention of swine neonatal coccidiosis and IDA. Piglets from 15 sows in OTI group received an oral dose of toltrazuril (20 mg/kg) and a single intramuscular injection of iron (200 mg/piglet) 24 hours after parturition, while piglets from 15 sows in ITG group received intramuscular injection of ITG (45 mg

toltrazuril + 200 mg gleptoferron) 24 hours after parturition. 3 piglets from each sow (total 90 piglets, 45 for each group) were randomly selected for sampling. Oocyst count (OPG), diarrhoea percentage, faecal score, blood haemoglobin level, and performance parameters were observed for 26 days and analyzed using Independent Student T-Test and Mann-Whitney U Test. The ITG group showed a trend of lower oocyst count and diarrhea percentage, better fecal scoring with no F4 score, no anemic piglets at weaning and better performance parameters. However, there are no significant differences ($p > 0.05$) between both groups. The single injectable toltrazuril and gleptoferron combination provide effective metaphylactic therapy as an alternative to prevent swine neonatal coccidiosis and iron deficiency anaemia in neonatal piglets, with the benefits of reduced labour intensiveness and stressful handling interventions.

Keywords: Coccidiosis, *Cystoisospora suis,* Iron Deficiency Anaemia (IDA), Toltrazuril, Iron dextran, Gleptoferron.

1.0 INTRODUCTION

1.1 Iron deficiency anaemia

Neonatal iron deficiency anaemia (IDA) is a condition associated with reduced red blood cells in the blood due to iron deficiency. It is a very common deficiency disorder during the early post-natal period in fast-growing piglets reared under fully intensive systems as modern farming systems prevent contact to the soil which are the main iron sources in the wild (Egeli & Framstad, 1999). Iron deficiency anaemia causes economical losses in modern farms, as affected piglets may have reduced growth rate and are more susceptible to infectious diseases. The causes of IDA include low body iron reserves at birth due to increased litter sizes, insufficient supplementation of iron, low iron content in sow's milk, high intensity of piglet growth and blood loss at farrowing (Svoboda *et al*., 2005). The piglets will develop iron deficiency (Hb 90-110 g/L) or iron deficiency anaemia (IDA) (Hb < 90 g/L) within 14 days if iron supplements are not given to them (Stojanac *et al*., 2016; Sperling *et al*., 2018). Pigs with anaemia will have lower feed intake (Knight and Dilger, 2018), lower body weight (Loh *et al*., 2001), and higher post-weaning death rates (Stojanac *et al*., 2016). Moreover, Daykin *et al*. (1982), found that piglets with IDA are more prone to diarrhoea caused by *E.coli* infections due to their impaired immunity. Anemic pigs show signs of paleness, lethargy, weakness and retarded growth rate (Taylor, 1989). For decades, iron dextran supplementation has been given parenterally via intramuscular injection at the neck region more preferably. However, large amounts of iron dextran may cause iron toxicity by inducing hepcidin expression, thus decreasing bioavailability of supplemental iron (Szudzik *et al*., 2018). Another comparable alternative iron supplementation to prevent IDA in piglets is gleptoferron. Gleptoferron is a complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid in an aqueous colloidal solution. It has been shown to

reduce anaemic status, increase plasma iron and haematological performances when compared to iron dextran in piglets (Sperling *et al.,* 2018).

1.2 Swine neonatal coccidiosis

Cystoisospora suis, formerly known as *isospora suis* (Barta *et al*., 2005) is a serious and important parasite infection, causing porcine neonatal coccidiosis that affects intensive pig agriculture all over the world (Mundt *et al.,* 2005). *C.suis* mostly affects suckling piglets age from 5-10 days and has characteristics of high morbidity and low mortality but mortality increases with the presence of secondary bacterial infection (Stuart and Lindsay, 1986). Infected suckling piglets show clinical signs in the second and third week of life (Lindsay *et al*., 1992). Infected piglets show clinical signs such as yellow to gray, frothy to pasty rancid-smelling diarrhoea, perineum stained with feces, reduction in weight and depressed (Stuart and Lindsay, 1986), while infected older pigs are usually asymptomatic and act as carriers (Shrestha *et al*., 2015) Economic losses in pig farms are mainly due to mortality, impaired performance, retarded growth, and treatment cost (Stuart *et al*.,1980). Additionally, it is believed that cystoisosporiasis makes piglets more vulnerable to secondary bacterial and viral infections, which raise morbidity, mortality, and administrative costs (Chae *et al*., 1998). Toltrazuril is currently the only effective drug in controlling oocyst excretion and diarrhoea of infected suckling piglets (Mundt *et al*., 2003). Toltrazuril (Pigicox 5%®) is usually given once to piglets by the oral route with dose of 20 mg/kg during the third to fifth day of life. Recently, an alternative injectable toltrazuril and gleptoferron (Forceris®) given once intramuscularly at the neck region of piglets during the first to third day of life at fixed dose of 1.5 ml/piglet corresponding to 45 mg of toltrazuril and

200 mg of iron is widely used. According toJoachim *et al*. (2018), this injectable combination product has also been proven to be more effective in preventing coccidiosis and IDA when compared to oral toltrazuril suspension (Pigicox 5%®), as the injectable toltrazuril has shown to completely suppress oocyst excretion when compared to oral toltrazuril, where low levels of oocyst excretion can be still detected.

1.3 Objective and Justification

The current farm practice is giving injectable iron dextran to piglets during the first to third day of life (Sperling *et al*. 2018) and separate dose of oral toltrazuril during the third to fifth day of life which is quite time consuming, laborious, and incur higher managerial costs (Jaochim *et al*., 2018). Hence, the single injection of the toltrazuril and gleptoferron combination (Forceris®) may be preferable for intensive pig farming. Forceris® (30 mg toltrazuril/ml; 133.4 mg iron/ml as gleptoferron - CEVA) is the first combination product in injectable form that aims to control porcine neonatal coccidiosis and prevent IDA.

The present study is conducted to evaluate and compare the efficacy of injectable toltrazuril-gleptoferron (ITG) and the current farm practice of oral toltrazuril + injectable iron dextran (OTI) in preventing coccidiosis and iron deficiency anaemia in Malaysian pig farm.

The study was conducted with the following objectives:

1. To compare the coccidia oocyst excretion (OPG) between piglets given injectable toltrazuril-gleptoferron (ITG) and oral toltrazuril + injectable iron dextran (OTI).
2. To compare the diarrhoea percentage and faecal score between piglets in the ITG and OTI group.
3. To compare the blood haemoglobin levels between piglets in the ITG and OTI group.
4. To compare the performance parameters, such as average body weight, average daily gain and preweaning mortality percentage between piglets in the ITG and OTI group.

1.4 Hypothesis

Null hypothesis 1: Piglets treated with ITG have similar oocyst per gram (OPG) count when compared to piglets treated with OTI.

Alternate hypothesis 1: Piglets treated with ITG have lower / no OPG count when compared to piglets treated with OTI.

Null hypothesis 2: Piglets treated with ITG have similar diarrhoea percentage and faecal score when compared to piglets treated with OTI.

Alternate hypothesis 2: Piglets treated with ITG have lesser diarrhoea percentage and better faecal score when compared to piglets treated with OTI.

Null hypothesis 3: Piglets treated with ITG have similar blood haemoglobin levels when compared to piglets treated with OTI.

Alternate hypothesis 3: Piglets treated with ITG have higher blood haemoglobin levels when compared to piglets treated with OTI.

Null hypothesis 4: Piglets treated with ITG have similar performance parameters when compared to piglets treated with OTI.

Alternate hypothesis 4: Piglets treated with ITG have better performance parameters when compared to piglets treated with OTI.

2.0 LITERATURE REVIEW

2.1 Etiology of iron deficiency

2.1.1 Low iron storage in new-born piglet

Selection breeding of sow in modern pig husbandry for high prolific reproduction traits exceeds the ability of sows to provide adequate amounts of iron (Fe) to new-born piglets (Szudzik *et al*., 2018). Venn *et al*. (1947) found that a new-born piglet is born with limited iron stores in the body (50 mg Fe), only 5mg of iron are available for synthesis of new haemoglobin. This low iron reserves in the body can only sustain for the first 3-4days (Szudzik *et al*., 2018). Sucking piglets are at risk of developing anaemia 10-14 days after birth if they do not receive any iron supplementation (Csapó, 1995).

Another theory for the explanation by Szudzik *et al*. (2018) is the low efficiency of iron transfer across the placenta from sow to piglets. This is supported by Douglas (1972) which stated the limitation of capacity of direct transferring of iron from blood through epitheliochorial placenta, with only less than 2 percent of iron injected into sows are transferred to piglets during pregnancy. According to Svoboda and colleagues (2005), different methods of supplementation to sows at different pregnancy stages has no significant impact on the improvement of iron storage in piglets. This strongly suggests there is a deficiency in the molecular machinery responsible for transferring iron from sows to piglets, which contributes to one of the factors causing IDA in piglets. However, relevant information and data on this issue is limited and requires further study and investigation.

2.1.2 High growth intensity

The most important factor causing IDA in piglets is the growth intensity in the early postnatal life. Jain (1986) stated that piglets double their body weight from 1.5kg to 3kg and the plasma volume expands by 30% during the first week of life. Normal growth rates for piglets are four to five times their birth weight after three weeks and eight times their birth weight after eight weeks (Radostis *et al*.,1994). Iron requirements are increased because more blood is needed to support the increased amount of tissue being created during high growth intensity as more haemoglobin and iron are needed because of the greater blood volume (Peters and Mahan, 2008). This was confirmed by Kubik *et al*. (2015), who found that the haemoglobin level of largest piglets at weaning is lower when compared to small piglets. Jolliff and Mahan (2011) have similar findings as their research results showed the values of haemoglobin level and packed cell volume (PCV) is lower in heavier piglets during weaning.

2.1.3 Inadequate iron level in sow's milk

According to Brady *et al*. (1978), the iron level of sow's milk ranges from 1.4 to 2.6 to 0.2-4 mg/L and the piglets' absorption of milk iron is 60-90%. New-born piglets require 7-10mg of iron per day during the first 3 weeks of life to perform normal activities and continue to develop without becoming anaemic. Similar results were obtained by Braude and colleagues (1962), in which they stated that 7-16mg of iron per day is required to maintain sufficient concentration of haemoglobin (Hb) and storage iron. However, Venn *et al*. (1947) stated a healthy normal sow's milk is able to provide approximately 1 mg of iron per day for the piglet. This amount is far below their daily requirement, assuming 7 mg of iron is needed per day. Dietary iron addition to sow

feed can only slightly improve the Fe level in milk even with the use of iron from amino acid chelates, (Wei *et al*., 2005).

2.2 Supplement of iron

Piglets have a high risk of getting IDA 10-14 days after birth, and therefore must receive exogenous iron supplementation to prevent IDA. Iron can be given either perorally or parenterally (Sperling *et al*., 2018). The most common practice is giving 200mg of iron-dextran through intramuscular injection at the neck on the third day of life and has been proven effective in prevention of IDA (Morales *et al., 2018*; Sperling *et al*., 2018; Yu *et al*., 2002; Zimmerman *et al*., 1959). Another comparable alternative iron supplementation to prevent IDA in piglets is gleptoferron. Gleptoferron is a complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid in an aqueous colloidal solution. It has been shown to reduce anaemic status, increase plasma iron and haematological performances when compared to iron dextran in piglets (Sperling *et al*., 2018). A new combination injectable product (Forceris®) that contain gleptoferron and toltrazuril reported to be effective in preventing neonatal coccidiosis and IDA simultaneously (Sperling *et al*., 2018; Joachim *et al*., 2018). According to Radostits *et al*. (1994), when a single dose of 200 mg Fe is administered to piglets that weaned at week 4, the iron concentration in the creep feed needs to be at least 240 mg/kg in order to prevent subclinical anaemia. If we use 10mg iron per day as a daily requirement, a dose of 200mg of iron-dextran is able to provide for 20 days until piglets weaned at week 3 (Svoboda *et al*.,2017). Although starter diets were highly enriched with iron, Kubik *et al*. (2015) found that piglets remained anaemic or iron deficient throughout the first three weeks after weaning. This is confirmed by Jolliff and Mahan (2011) as lowest

haemoglobin concentrations are recorded 14-21 days after weaning (31-38 days age). Hence, they believe a second iron injection before weaning may help to maintain post-weaning iron reserves. A second injection at two to three weeks of age may not be cost-effective, according to Radostis *et al*. (1994), who also found that several injections enhance haemoglobin levels without increasing weight gain. This is supported by Hill *et al*. (1999) as one single dose of 200mg iron is able to maintain average daily gain during sucking period but the second dose of 200mg iron does not increase weight gain.

Oral iron can be given as paste, microemulsion, liquid iron in drinking water, oral iron supplied on the floor, or as a dietary supplement (Maes *et al*., 2011). The effectiveness of these techniques is debatable and numerous research discovered a diminished effectiveness in comparison to injections (Stojanac *et al*.,2016). The downside of administering iron to neonatal piglets through their drinking water is that water intake is typically quite low since colostrum and milk consumption make up nearly all of the feed and water intake of new-born piglets (Marchant-Forde *et al*.,2009). Lipinski *et al*. (2011) stated gastrointestinal side effects such nausea, vomiting, abdominal discomfort, and diarrhoea may impair the effectiveness of oral iron therapy. In addition, oral iron administration is labour intensive and time-consuming which level of labour is equal or greater than parenteral administration (Jakobsen *et al*., 2021). According to Jakobsen *et al*. (2021), a new non-invasive method of oral iron administration by adding iron to sow milk replacer and provided ad libitum in a milk cup system has haemoglobin levels similar to parenteral iron supplementation.

2.3 Swine neonatal coccidiosis

2.3.1 Aetiology

Coccidiosis in pigs is normally caused by *Eimeria* species and *Isopora suis (Cystoisospora suis)*. 13 named species of *Eimeria* and 3 species of *Isopora* are known to affect pigs (Levine & Ivens, 1986). The *Eimeria* species affects adult pigs, while *Cystoisospora suis* affects sucking piglets and causing neonatal coccidiosis. *C. suis* is more prevalent in piglets raised in a fully intensive system with confinement, where the warmth and humidity in the farrowing crate enhance the sporulation of *C. suis* oocyst, the *Eimeria* species is frequently detected in pigs raised outdoors or at low temperatures (Henry Too, 2019).

2.3.2 Lifecycle

Every *Cystoisospora* species has a unique host, including C. suis, which develops entirely in pigs. (Worliczek *et al*., 2013 & Lindsay *et al*., 1997). Infection occurred when pigs ingested with feed or water infested with sporulated oocysts. Once in the intestine, the sporulated oocysts undergo excystation to release sporozoites. These sporozoites invade intestinal epithelium, multiply and develop to become merozoites (Harleman & Meyer ,1983). Peak asexual reproduction (merogony) occurs between days 4 and 5 following infection to produce more merozoites (Worliczek *et al*.,2013). In contrast to *Eimeria*, merogonic stages are not categorised by generations but rather by types that are determined by the quantity of nuclei, shape, size, and time of emergence (Lindsay *et al*., 1997). Merogony is followed by gamogony, where macrogametes and microgametes fuse to produce zygotes, which then develop into oocysts. These oocysts released into the intestinal tract are excreted into the

environment in faeces and undergo sporulation in the environment. A sporulated oocyst contained two sporocyst, each sporocyst contained four sporozoites (Harleman & Meyer,1984). Sporulation occurs in 4 days, and it requires suitable temperature (20-37°C), oxygen and humidity (80-85%) (Stuart & Lindsay, 1986). According to Lindsay *et al*. (1992), temperature of heat supply (32-35°C) for the new-born piglets is within the range of temperature (20-37°C) that favours the rapid sporulation (within 12 hours) of oocyst in the farrowing crate. It takes roughly 5-7 days (prepatent period) from the ingestion of an oocyst to its development and release into the intestinal tract (Worliczek *et al*.,2013; Rypula *et al*.,2012). Clinical signs can be seen as early as 3 days after infection with oocysts shedding starting on the fifth day post infection (Mundt *et al*.,2003; Harlemen & Meyer, 1983; Worliczek *et al*., 2007), lasting roughly 5-16 days (Stuart & Lindsay, 1986; Rypula *et al*.,2012). Number of oocyst secretion, severity of diarrhoea varies among individuals, due to factors such as age of infection, individuals health conditions, number of oocyst during infection and duration of exposure (Stuart *et al*.,1982; Muart *et al*., 2006). Oocyst secretion and coccidiosis symptoms peak at 5th-9th and 11th-14th dpi (Harleman & Meyer ,1983; Shrestha *et al*., 2015) because of desynchronization of merozoite development in which some merozoite develop first while some enter development lag phase and develop into microgametes and macrogametes after a few days later (Shrestha *et al*., 2015). Another possible explanation is because of extraintestinal stage re-entering into the intestine from mesenteric lymph nodes, liver and spleen by either active migration or phagocyte (Harleman & Meyer,1984). However, data and research on this extraintestinal stages are still limited, and requires further studies.

2.3.3 Clinical sign

Coccidiosis occurs as early as the fifth day after birth and mainly occurs in neonatal piglets between 7-14 days of age. Predominant clinical signs include fluid or pasty diarrhoea, ranging from yellow to grey rancid-smelling diarrhoea. Most of the time piglets in the infected farrowing unit are damp and covered with liquid faeces, giving the appearance of dirty piglets. As the infected piglets continue to nurse, they become dehydrated, emaciated, and stunted growth (Lindsay *et al*., 1985). Coccidiosis has characteristics of high morbidity and low mortality. Mortality rate tends to vary based on the number of sporulated oocysts ingested, differences in the environment and the presence of other concurrent diseases. Mortality increases with secondary infection by other enteropathogens such as enteropathogenic strains of *Escherichia coli* (E. coli), coronavirus, rotavirus, transmissible gastroenteritis (TGE) virus and *Clostridium perfringens* type C (Stuart & Lindsay., 1986).

2.3.4 Pathology

Gross lesions are only found in the ileum and jejunum. Necropsy examination of severely infected piglets has the characteristic of presence of yellow fibrinonecrotic pseudomembrane loosely adhered to a hyperaemic mucosa. Villous atrophy, villous fusion, crypt hyperplasia, and necrotic enteritis are among the microscopic lesions. The denuded lamina propria of the intestinal villi is covered by metaplastic enterocytes, causing the superficial columnar epithelium to change from cuboidal to flat. Loss of villi and changes in functional absorption ability resulting in diarrhoea and loss of fluid (Stuart *et al*., 1980; Harleman & Meyer, 1982). The degree of severity of coccidiosis is dependent on the number of sporulated oocyst, with 200,000 or more sporulated oocyst

causing severe disease with moderate to extreme mortality (Lindsay *et al*., 1985); while fewer oocyst causes diarrhoea with few or no mortality (Stuart *et al*.,1980).

2.3.5 Diagnosis

Swine neonatal coccidiosis is suspected in sucking piglets between 7-14 days with clinical signs of diarrhoea that are not responding to the antibiotic treatment. The best, quickest and cost-efficient diagnosis method is detecting the presence of oocyst in faeces using faecal floatation and determining the number of oocyst using modified McMaster technique (Joachim *et al*., 2018). The oocysts of *C. suis* have a 'hazy body', a unique characteristic structure between the oocyst walls and the sporont that help to differentiate between oocyst of *C. suis* and *Eimeria* species. Moreover, some oocyst may be seen undergoing a two-celled sporoblast stage which is also a unique characteristic of *C. suis* (Lindsay *et al*.,1982). Mucosal smear of intestine smear for the demonstration of developmental stage. Presence of paired type 1 merozoites is diagnostic. Detection of binucleated type 1 meronts and multinucleated Type 2 meronts in asexual stage, biflagellate microgametes and uninucleate macrogametes in sexual stage are useful to differentiate between *C. suis* and *Eimeria* species but the identification is difficult. Histologic diagnosis of intestinal tissue from jejunum and ileum show blunting and atrophy intestinal villi with presence of type 1 merozoites are diagnostic (Lindsay *et al*., 1983).

2.3.6 Treatment and control

According to Stuart & Lindsay (1986), usage of anticoccidials on sows have little effect in preventing and controlling porcine neonatal coccidiosis as sows are not the major source of coccidiosis for neonatal piglets. Toltrazuril is widely used to treat nursing piglet cystoisosporiasis as a metaphylactic control, it is the only licenced and efficient treatment option for *Cystoisospora suis* infections in suckling piglets (Mundt *et al*., 2003). According to Skampardonis *et al*. (2010), a single oral dose toltrazuril (Baycox 5%®) with dose of 20 mg/kg administered to piglets at third day after birth can reduce clinical symptoms, while also being cost effective (Scala *et al*. 2009). (Forceris®) given once intramuscularly at the neck region of piglets during the first to third day of life at fixed dose of 1.5 ml/piglet corresponding to 45 mg of toltrazuril and 200 mg of iron is more effective compared to oral toltrazuril (Baycox 5%®) suspension (Joachim *et al*., 2018). Toltrazuril is absorbed and metabolized to toltrazuril sulfoxide and toltrazuril sulfone by liver microsomal enzymes. Toltrazuril sulfone has the anticoccidial effect (Lim *et al*., 2010) which reduce the excretion of oocyst by suppressing the development of oocysts by killing asexual and sexual stage of coccidia (Bach *et al*., 2003). Toltrazuril also reduce the risk of diarrhoea (Mundt *et al*., 2003; Joachim & Mundt, 2011). Numerous studies have reported that toltrazuril treatment improves post weaning feed conversion ratios (McOrist *et al*.,2010) and increases weight gain by improving gut health (Scala *et al*., 2009; Maes *et al*., 2007). Moreover, toltrazuril treatment reduces dependence on antibacterial treatment for secondary bacterial infection caused by coccidiosis (Driesen *et al*., 1995). Other treatment using diclazuril and sulphadimidine have been conducted in controlled study, however the results of treatment with diclazuril and sulphadimidine produced no significant

differences as piglets showed similar results with controlled group in term of occurrence of diarrhoea, oocyst excretion and weight gain. (Mundt *et al*., 2003). There is no vaccine currently available for swine neonatal coccidiosis (Mundt *et al*., 2003; Joachim & Mundt ,2011).

According to Stuart & Lindsay (1986), coccidiosis is best controlled through proper sanitation. They recommended the following sanitation procedures: Farrowing crates should be steam cleaned, then disinfected with an ammonia- or phenol-containing solution and let them stand overnight; steam clean the next day. This method is proven useful by Ernst *et al*. (1985) as the incidence of I. suis coccidiosis in litter dropped from 100% to 20%. Farmers should practice good hygiene management by frequently cleaning pens to reduce the number of oocysts in the environment (Harleman & Meyer ,1983).

3.0 MATERIALS AND METHODS

3.1 Farm details

Farm A is located at Tanjung Sepat, Sepang with a population of 1200 standing sow population. It is a farm with an open house system with fully intensive management. Farrowing crates are made with concrete and partial slatted flooring **(APPENDIX I)**. Every unit consists of a manual feeder and a nipple drinker. Sows are fed manually by workers 3 times a day. Nipple drinkers are supplied with treated tap water. Pens are washed with water by workers 2 times a day.

3.2 Assigning ITG and OTI groups

Thirty healthy sows were selected randomly from a farm with a good recording system. Pregnant sows (Large white + Landrace) were moved to the farrowing unit two weeks before farrowing to acclimatise to the housing conditions. Animals were fed with conventional feed and water was provided ad libitum. These sows were divided into Group A (OTI) and Group B (ITG). Each group consists of 15 sows, with similar parity distribution. Only healthy piglets with normal behaviour (active, suckling milk) from each sow were recruited for this study. All piglets from Group A sows were treated with 1.0ml oral toltrazuril and 1.1ml iron dextran complex intramuscular injection at the neck. Farm A was using Pigicox 5% and Ferro 2000 20%. All piglets from Group B sows were given a single intramuscular injection of 1.5mL Forceris® per piglet at the neck **(APPENDIX II)**. Both group piglets are given respective injections on day 1. The piglets were monitored for any post injection reactions (inflammation, swelling, fever, hypersensitivity and bruise) at 1st and 24th hour post-injection. Any local reaction or adverse events will be recorded and followed up. All routine farm piglet management

protocol (castration, tooth-clipping, tail docking, ear-notching etc.) was carried out as usual. Any treatment given to the piglets or litter will be recorded.

3.3 Blood haemoglobin level

3 random piglets per litter were selected for blood haemoglobin measurement. These 3 piglets were chosen based on the principle of following: 3 unhealthy and small piglets were not chosen, 3 largest and dominant piglets were not chosen, choose randomly from the remaining piglets. 45 piglets were selected from each group with a total of 90 piglets were selected for blood haemoglobin measurement. Blood sample was collected from the auricular vein using microcuvette and read with HemoCue HB201+ machine on day 1 and day 26. Results from the HemoCue machine were recorded. **(APPENDIX III)**

3.4 Diarrhoea percentage

Diarrhoea percentages were recorded at 2 time points which were on day 10 or 11 and day 16 or 17. Each individual piglet in each pen was observed for incidences of diarrhoea by looking at diarrhoea staining at the perineal region **(APPENDIX IV)**. The number of piglets having diarrhoea will be divided by the total number of piglets in the pen, and converted to the percentage of diarrhoea for that pen.

$$\text{Diarrhoea percentage} = \frac{Piglets\ with\ diarrhea\ staining}{Total\ piglets/pen} \times 100\%$$

3.5 Faecal scoring

Faecal scores were recorded at 2 time points which were on day 10 or 11 and day 16 or 17. Faecal score for each pen was determined by observing faecal consistency in each pen at 2 time points: day 10 or 11 and day 16 or 17. Faecal samples were collected manually from the piglets by stimulating defecation **(APPENDIX V)**. These fresh faecal samples were scored based on a faecal scoring system with Faecal score (F) 1 represents firm and well-formed faeces, F2 pasty faeces, F3 semi pasty to watery consistency and F4 watery faeces. F1 and F2 were grouped into category 1 which is normal while F3 and F 4 were grouped into category 2 which will be considered as diarrhoea in this study **(APPENDIX VI)**

3.6 Coccidia oocyst excretion (OPG)

Fresh faecal samples that were collected previously for the purpose of faecal scoring at 2 time points: day 11 or 12 and day 16 or 17 were used to determine the number of oocyst excretion in faeces. Three different pieces of faeces will be collected from each farrowing pen and pooled into one sample. A total of 15 samples were collected and kept in the chiller before sending to Parasitology Laboratory of Faculty of Veterinary Medicine for faecal oocyst counts per gram (OPG) using simple floatation and modified McMaster technique.

3.7 Performance parameters

Performance parameters, such as birth weight is recorded at day of life (dol) 1, weaning weight, average daily gain (ADG) and preweaning mortality percentage were

collected at dol 26 and analysed. Sow parity and number of litters were recorded and analysed as well.

3.7.1 Average body weight

All piglets of ITG and OTI groups were weighed at the same time the products were administered on day 1, The same group of piglets in both groups were followed until 26 days, and their body weights were measured again. The piglets in the same crate were put into one plastic basket and weighted using a portable digital weighing scale **(APPENDIX VII)**. Average body weight was then calculated based on the formula below:

$$\text{Average body weight} = \frac{Average\ Weight\ on\ day\ 26 - Initial\ Weight\ on\ day\ 1}{Number\ of\ days\ to\ final\ weight\ (26\ days)}$$

3.7.2 Average daily gain (ADG)

Average body weight taken on day 1 and day 26 are used to determine the ADG based on the formula below:

$$\text{ADG (gram)} = \frac{Average\ weight\ on\ day\ 26 - Average\ weight\ on\ day\ 1}{Numbers\ of\ days\ to\ final\ weight\ (26\ days)}$$

3.7.3 Preweaning mortality percentage

The number of piglets in a pen in both groups were recorded as they were weighed on the same day. The number of piglets in a particular group were then

recorded again when they reached 26-days-old during the measurement of their final weights. The preweaning mortality percentage was then calculated based on the formula below:

Preweaning mortality (%) = $\frac{No.of\ piglets\ (day\ 1) - No\ of\ piglets\ (day\ 26)}{No\ of\ piglets\ (day\ 1)}\ x\ 100\%$

3.8 Statistical analysis

The IBM® SPSS Statistics 28 statistical software was used to analyse the data obtained. Data was subjected to a test of normality first before testing with Independent Student's T-Test and Mann-Whitney U Test. Statistical significance was recorded at $p < 0.05$ and 95% confidence interval.

4.0 RESULTS

4.1 Sow parity

The comparison of the sows parity between the OTI and ITG groups are shown **(Fig. 4.1).** The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

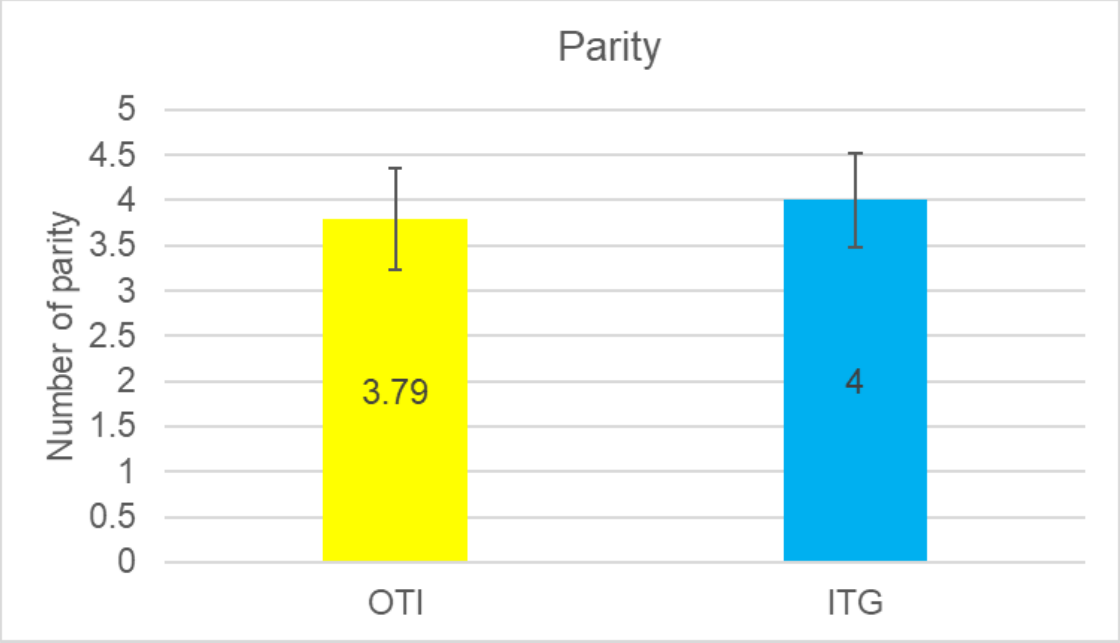

Figure 4.1: Comparison between sow parity of OTI and ITG groups

4.2 Litter size

The comparison of the litter size between the OTI and ITG groups are shown **(Fig. 4.2)**. The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

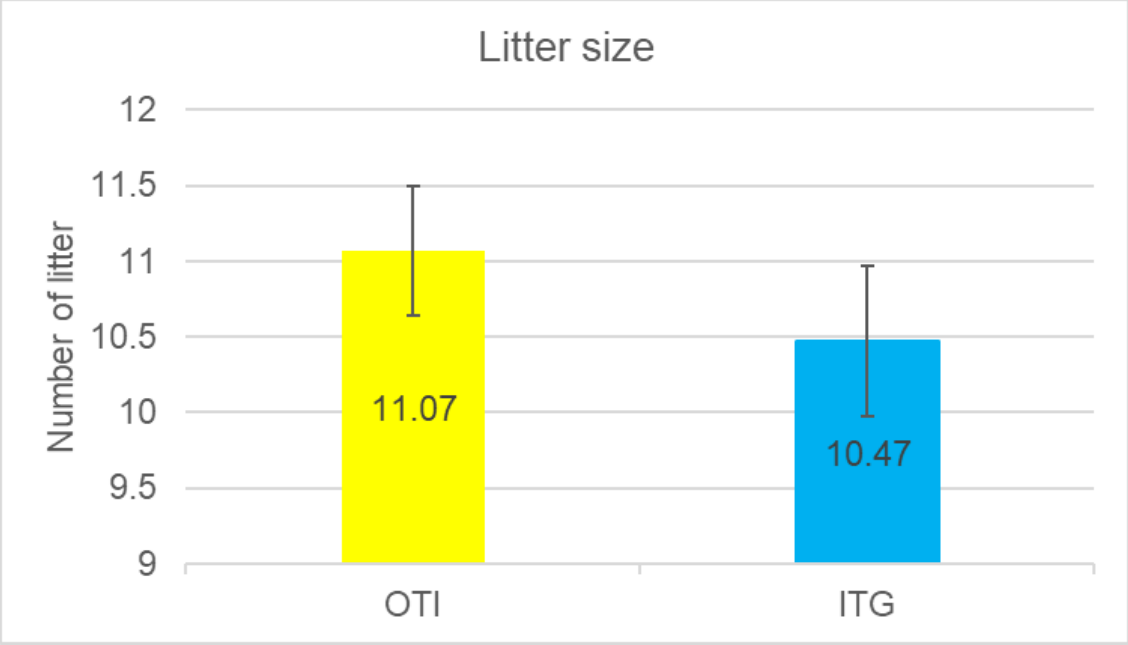

Figure 4.2: Comparison between litter size of OTI and ITG groups

4.3 Injection reaction (Swelling) post 24 hours

Only two injection reactions were observed post 24 hours, which were swelling and bruising **(APPENDIX VIII)**. The comparison of the percentage of swelling between the OTI and ITG groups are shown **(Fig. 4.3)**. Both groups of piglets have a similar percentage of swelling, but the ITG group has a slightly lower percentage of swelling (1.47%). The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

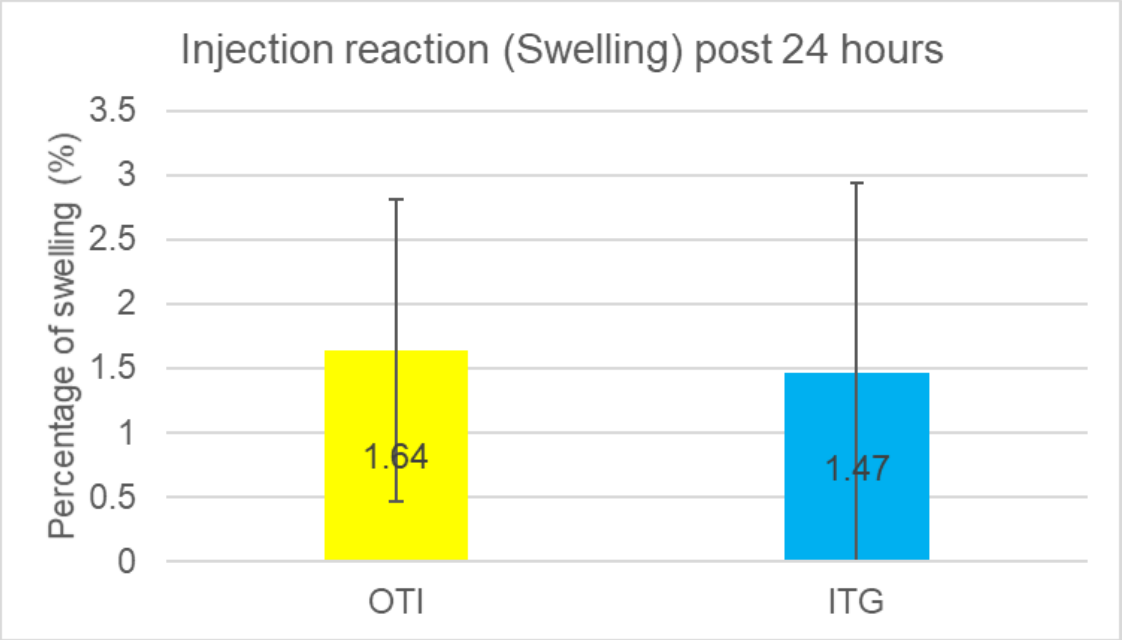

Figure 4.3: Comparison between injection reaction (swelling) post 24 hours of OTI and ITG groups

4.4 Injection reaction (Bruising) post 24 hours

The comparison of the percentage of bruising between the OTI and ITG groups are shown **(Fig. 4.4)**. Both groups of piglets have low incidence of bruising, but the ITG group has a lower percentage of bruising (1.13%) compared to the OTI group (4.56%), with a decrease of 3.43 percent.The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

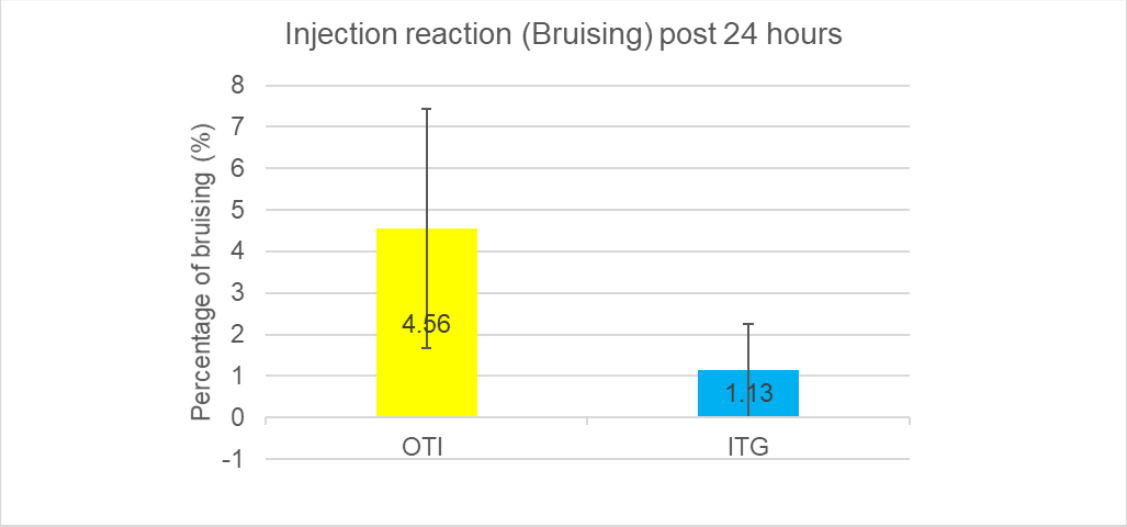

Figure 4.4: Comparison between injection reaction (bruising) post 24 hours of OTI and ITG groups

4.5 Average oocyst count (OPG)

The comparison of the average oocyst count (OPG) between the OTI and ITG groups are shown **(Fig. 4.5).** Data for coccidia oocyst excretion was analyzed using descriptive analysis. Average oocyst count for the OTI group was increased from 2390 to 7825 while the ITG group decreased from 5368.75 to 525.

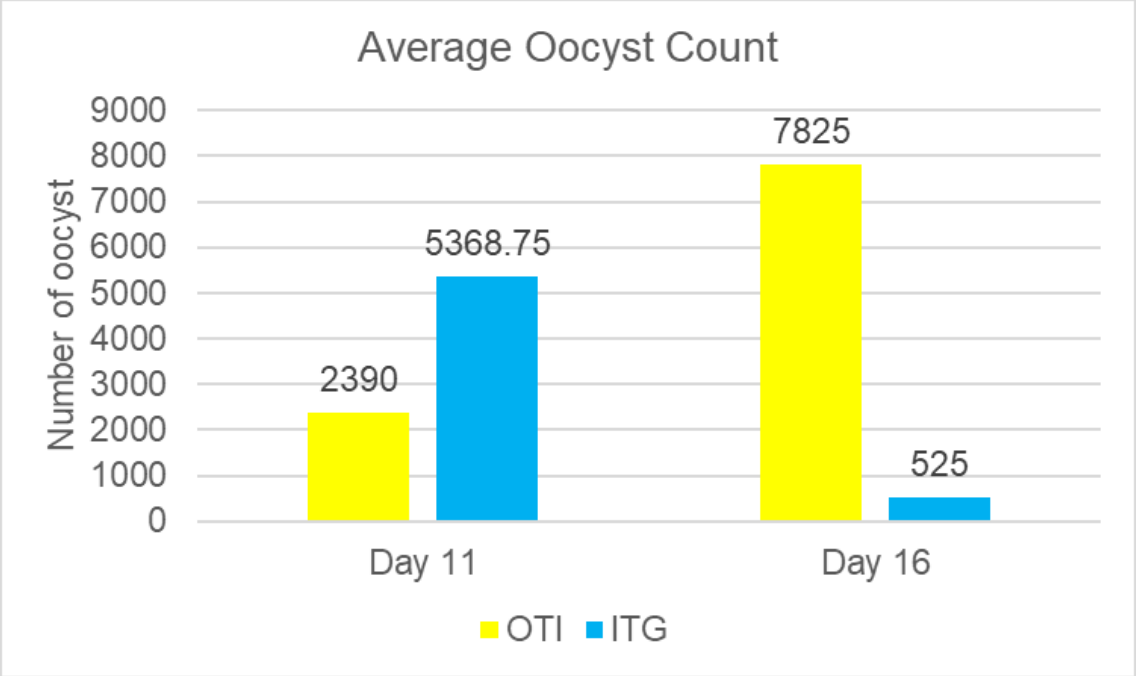

Figure 4.5: Comparison between average oocyst count (OPG) of OTI and ITG groups on day 11 and day 16

4.6 Diarrhoea percentage on day 11

The comparison of the diarrhoea percentage between the OTI and ITG groups on day 11 are shown **(Fig. 4.6).** Piglets in the ITG group recorded lower diarrhoea percentage (12.84%) compared to the OTI group (18.54%) on day 11, with a decrease of 5.7 percent.

The data was not normally distributed and was analyzed using the Mann-Whitney U Test. There were no significant differences between groups ($p>0.05$).

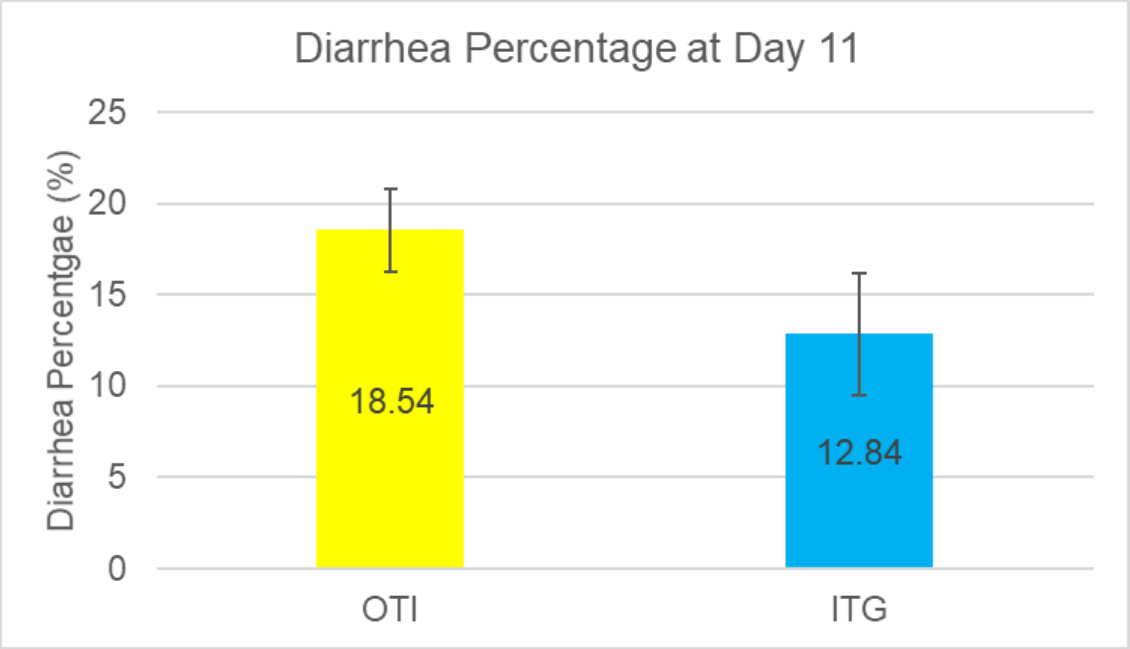

Figure 4.6: Comparison between diarrhoea percentage of OTI and ITG groups on day 11

4.7 Diarrhoea percentage on day 16

The comparison of the diarrhoea percentage between the OTI and ITG groups on day 16 are shown **(Fig. 4.7).** Piglets in the ITG group recorded lower diarrhoea percentage (7.94%) compared to the OTI group (18.51%) on day 16, with a decrease of 10.57 percent.

The data was not normally distributed and was analyzed using the Mann-Whitney U Test. There were no significant differences between groups ($p>0.05$).

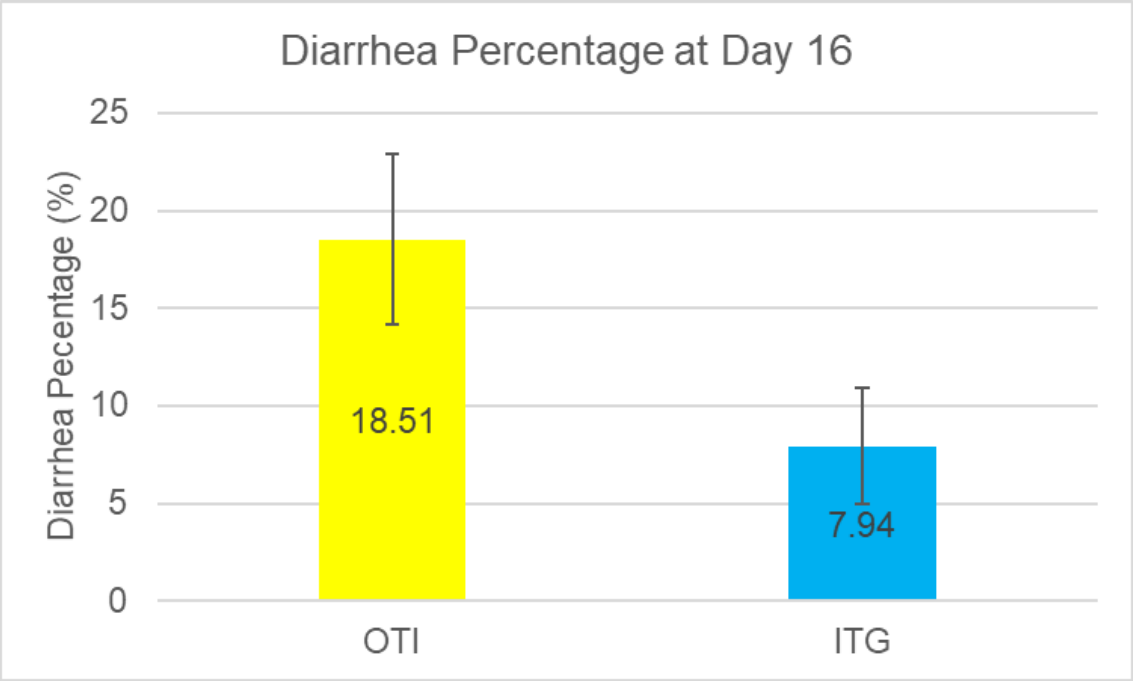

Figure 4.7: Comparison between diarrhoea percentage of OTI and ITG groups on day 16

4.8 Faecal score percentage on day 11

The data for the faecal score percentage on day 11 between the OTI and ITG groups are shown **(Fig. 4.8.1, 4.8.2 and Table 4.8.1).** Data for faecal score percentage was analyzed using descriptive analysis. Piglets from the ITG group had better faecal scores when compared to the OTI group, with the majority of piglets (86.67%) in the normal category and lesser piglets (13.33%) in the diarrhoea category when compared to the OTI group with lesser piglets (61.29%) in the normal category and more piglets (38.71%) in the diarrhoea category. In the ITG group, 56.67% and 30% of the piglets had F1 and F2, respectively, while the piglets in the OTI group had 41.94% and 19.35% of F1 and F2, respectively. In the diarrhoea category, only 13.33% of the piglets from ITG groups had F3, and none of the piglets (0%) had F4 which was the watery diarrhoea, while the piglets in the OTI groups had 12.90% and 25.81% of F3 and F4 respectively. The most obvious comparison between the groups was the percentage of F4. The ITG group had 0% of F4 while the OTI group had 25.81% of F4.

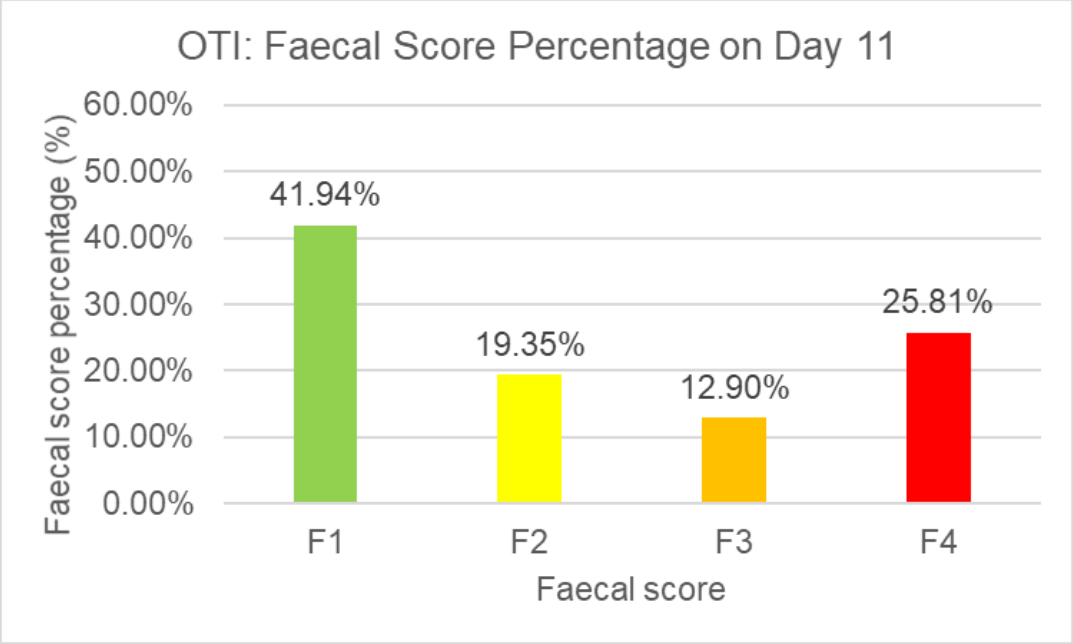

Figure 4.8.1: Faecal score percentage of the OTI group on day 11

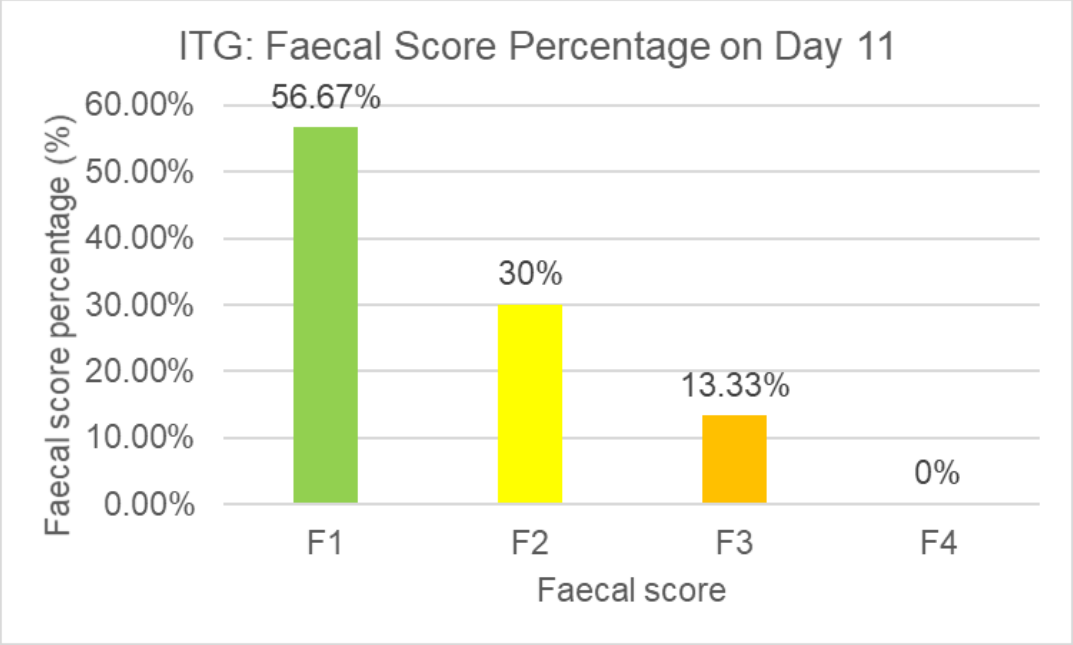

Figure 4.8.2: Faecal score percentage of the ITG group on day 11

Table 4.8.1: Faecal score percentage and total percentage of the OTI and ITG groups

Faecal score	Category	OTI		ITG	
		Faecal score percentage (%)	Total percentage (%)	Faecal score percentage (%)	Total percentage (%)
F1	Normal	41.94	61.29	56.67	86.67
F2		19.35		30	
F3	Diarrhoea	12.90	38.71	13.33	13.33
F4		25.81		0	

4.9 Faecal score percentage on day 16

The data for the faecal score percentage on day 16 between the OTI and ITG groups are shown **(Fig. 4.9.1, 4.9.2 and Table 4.9.1).** Data for faecal score percentage was analyzed using descriptive analysis. Piglets from the ITG group had better faecal scores when compared to the OTI group, with the majority of piglets (96.67%) in the normal category and lesser piglets (3.33%) in the diarrhoea category when compared to the OTI group with lesser piglets (83.33%) in the normal category and more piglets (16.66%) in the diarrhoea category. In the ITG group, 43.44% and 53.33% of the piglets had F1 and F2, respectively, while the piglets in the OTI group had 63.33% and 20% of F1 and F2, respectively. In the diarrhoea category, none of the piglets (0%) from ITG groups had F3, and only 3.33% of the piglets had F4 which was the watery diarrhoea, while the piglets in the OTI groups had 3.33% and 13.33% of F3 and F4 respectively. The most obvious comparison between the groups was almost all piglets (96.77) having normal categories and only 3.33% of piglets had diarrhoea issues in the ITG group.

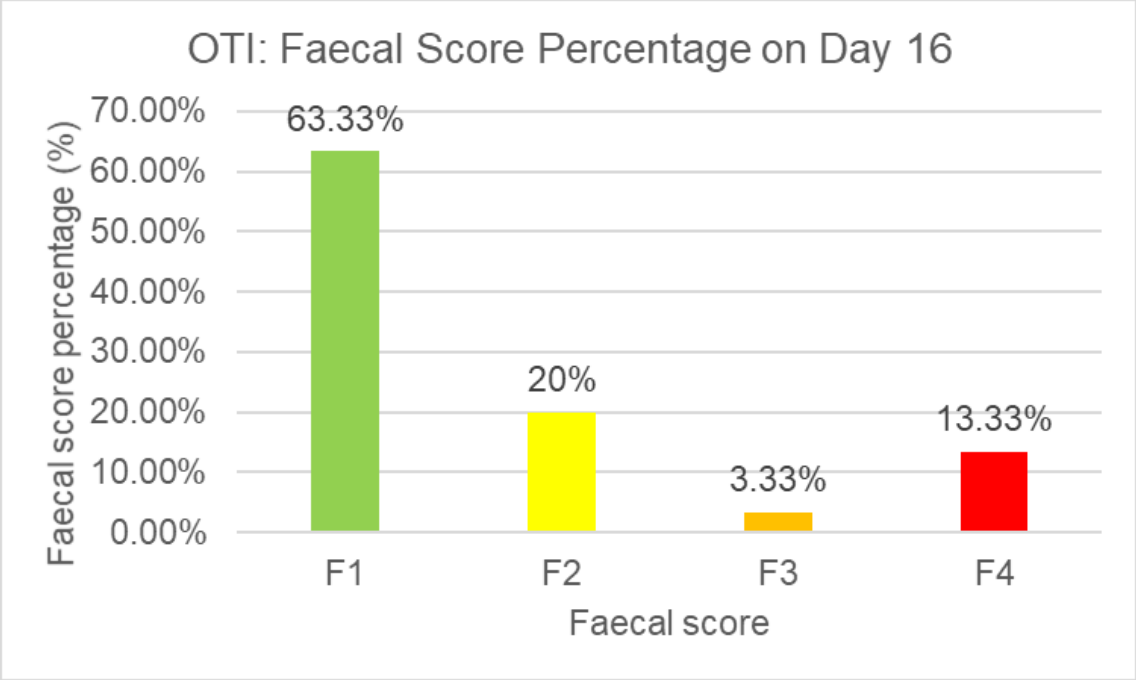

Figure 4.9.1: Faecal score percentage of the OTI group on day 16

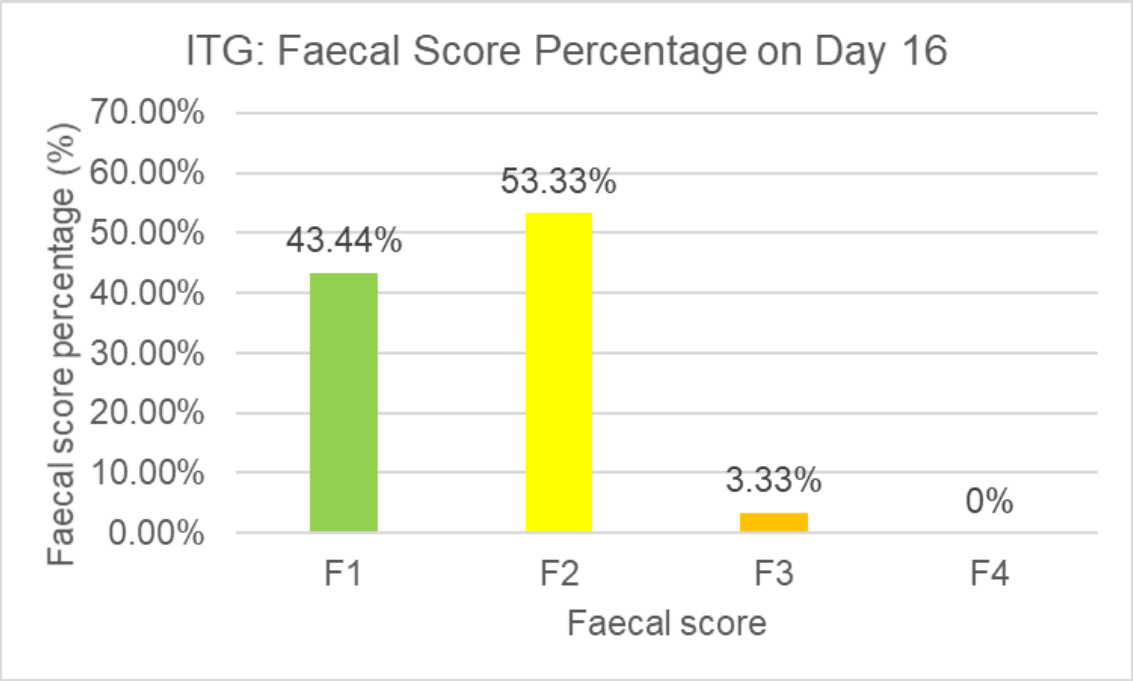

Figure 4.9.2: Faecal score percentage of the ITG group on day 16

Table 4.9.1: Faecal score percentage and total percentage of the OTI and ITG groups

Faecal score	Category	OTI		ITG	
		Faecal score percentage (%)	Total percentage (%)	Faecal score percentage (%)	Total percentage (%)
F1	Normal	63.33	83.33	43.44	96.77
F2		20		53.33	
F3	Diarrhoea	3.33	16.66	3.33	3.33
F4		13.33		0	

4.10 Total mean of haemoglobin level

The comparison of total mean of haemoglobin level between the OTI and ITG groups on day 1 and day 16 are shown **(Fig. 4.10).** The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p > 0.05$).

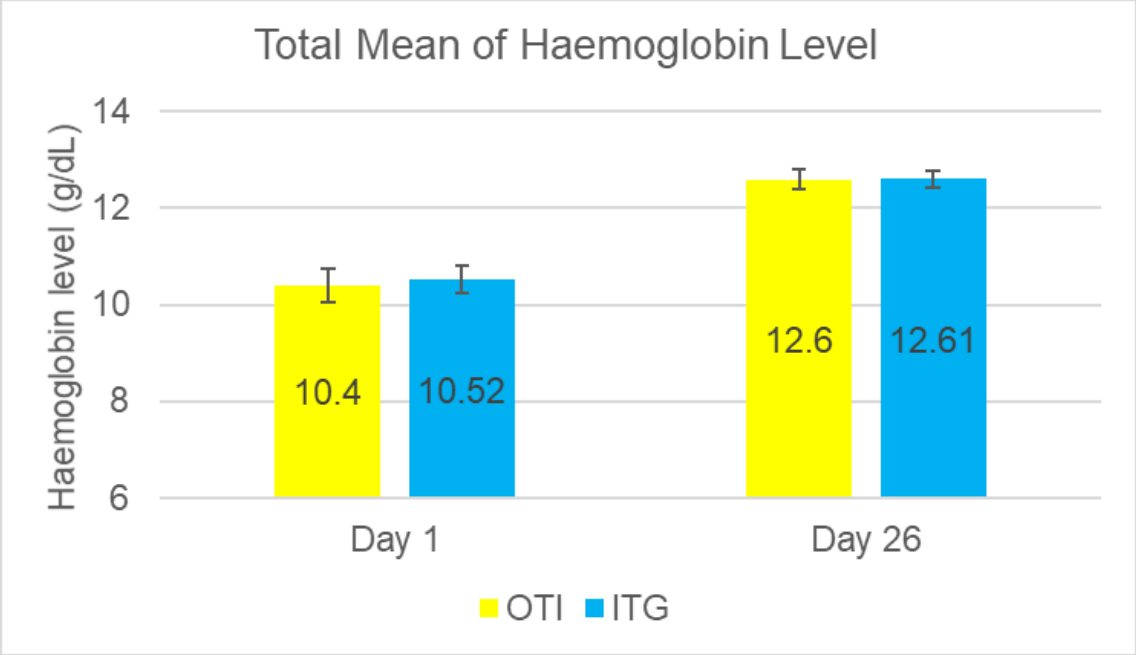

Figure 4.10 Comparison between total mean of haemoglobin level on day 1 and day 26 of the OTI and ITG groups

4.11 Individual and percentage of blood haemoglobin level

The comparison of the individual and percentage of blood haemoglobin level on day 1 and day 26 between the OTI and ITG groups are shown **(Fig. 4.11.1, Fig. 4.11.2 and Table 4.11).** Data of blood haemoglobin level on day for both groups were served as a baseline for comparison on day 26.

Both groups were effective in preventing IDA as both ITG and OTI had more than 90 percent of piglets having normal blood haemoglobin level. However, the ITG group gave better result with 95.56% of piglets having normal haemoglobin level, 4.44% having iron deficiency and 0% of piglets having anaemic status compared to the OTI group with 91.11% of piglets having normal haemoglobin level, 8.89% having iron deficiency and 2.22% of piglets having anaemic status.

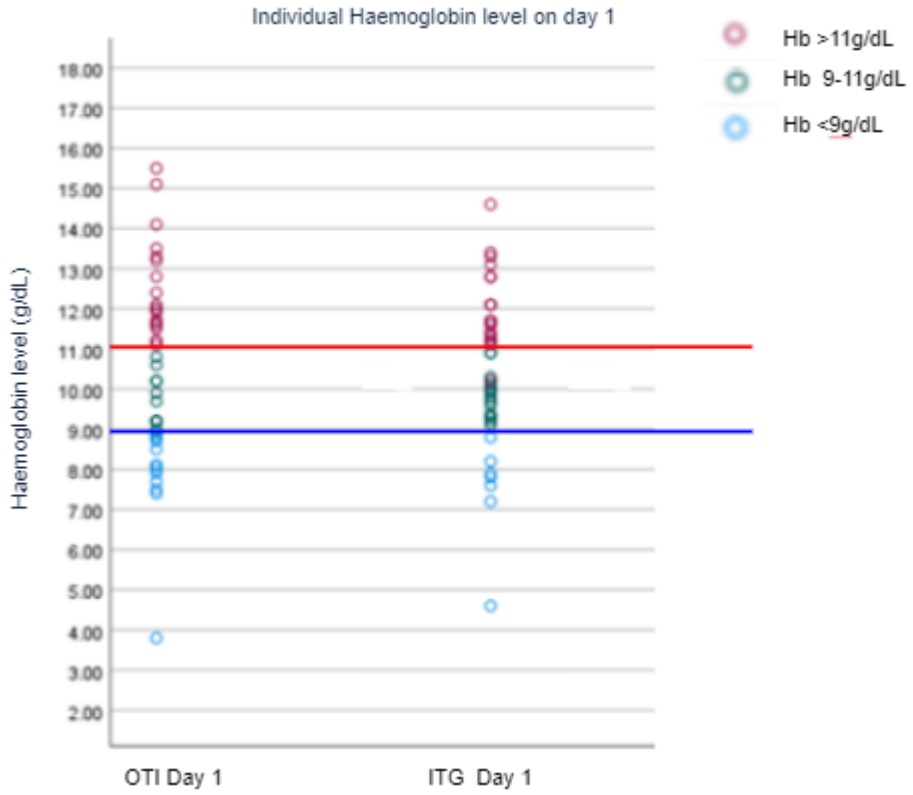

Figure 4.11.1: Comparison between individual blood haemoglobin level on day 1 of the OTI and ITG groups. Categories of red: normal level (> 11 g/dL), green: iron deficiency (9-11 g/dL), blue: anaemia (Hb < 9 g/dL).

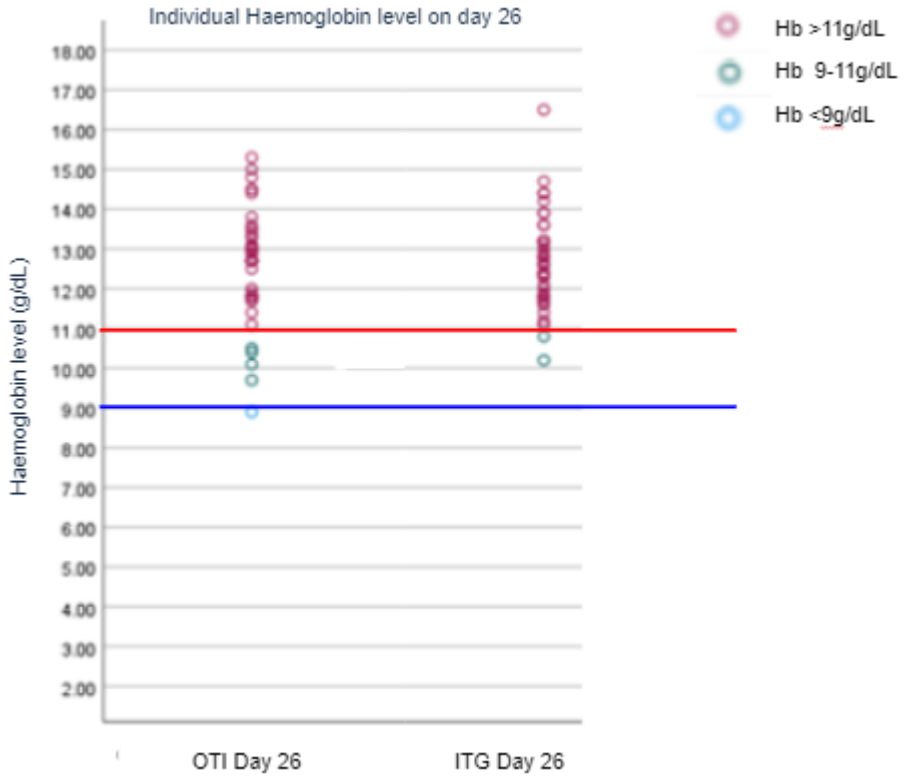

Figure 4.11.2: Comparison between individual blood haemoglobin level on day 26 of the OTI and ITG groups. Categories of red: normal level (> 11 g/dL), green: iron deficiency (9-11 g/dL), blue: anaemia (Hb < 9 g/dL).

Table 4.11: Individual blood haemoglobin level and the respective percentages in 3 different categories for ITG and OTI groups on day 1 and day 26

	Number of piglets and the respective percentage (%)			
Group / Category	OTI Day 1	OTI Day 26	ITG Day 1	ITG Day 26
Hb < 9 g/dL	14 (31.12)	1 (2.22)	7 (15.56)	0 (0)
Hb 9-11 g/dL	11 (24.44)	4 (8.89)	16 (35.56)	2 (4.44)
Hb >11g/dL	20 (44.44)	40 (91.11)	22 (48.89)	43 (95.56)

4.12 Average body weight on day 1

The comparison of the average body weight on day 1 between the OTI and ITG groups are shown **(Fig. 4.12)**. Both groups of sows have similar average body weight on day 11.

The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

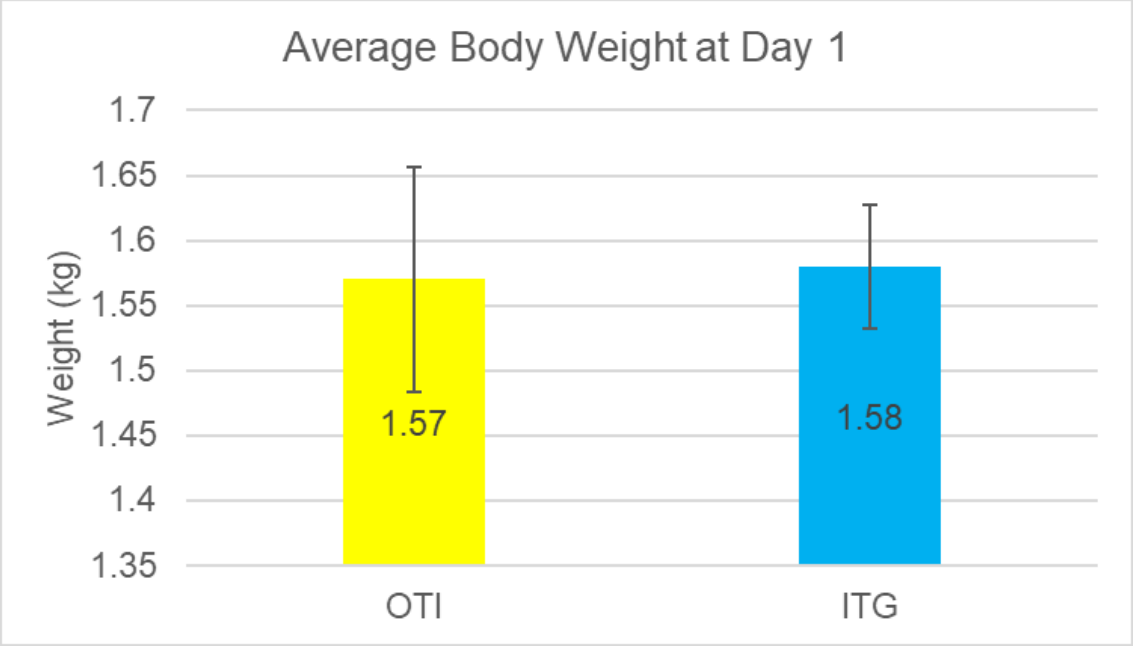

Figure 4.12: Comparison between average body weight on day 1 of the OTI and ITG groups

4.13 Average body weight on day 26

The comparison of the average body weight on day 26 between the OTI and ITG groups are shown **(Fig. 4.13)**. Both groups of sows have similar average body weight on day 26 but piglets from the ITG were slightly heavier (5.79 kg).

The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

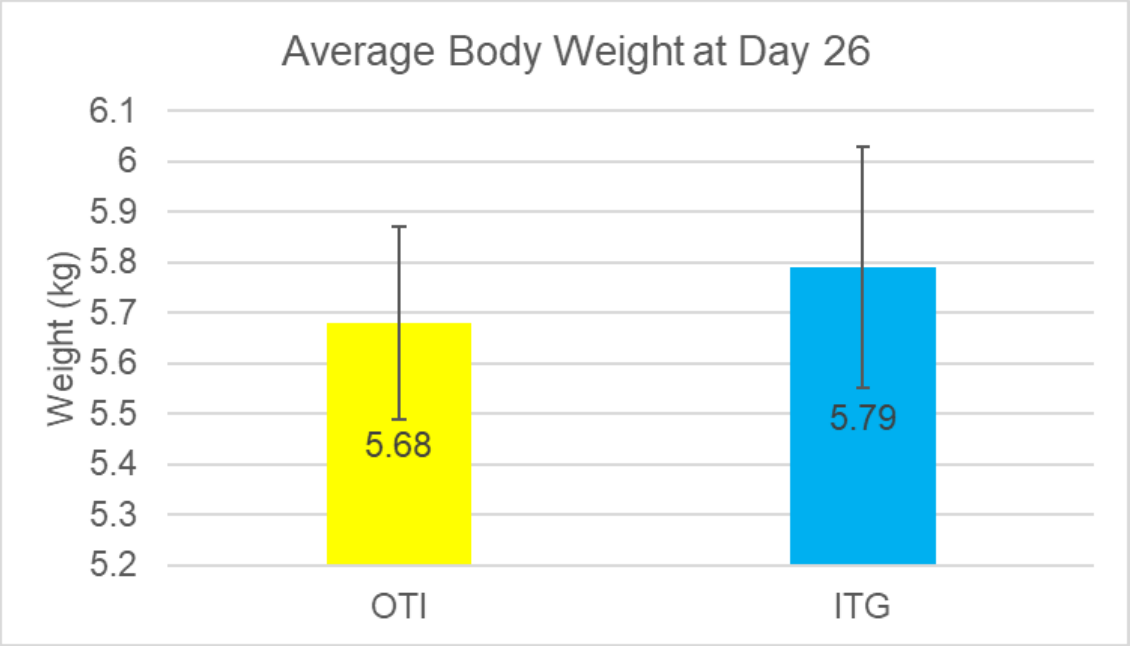

Figure 4.13: Comparison between average body weight on day 26 of the OTI and ITG groups

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4.14 Preweaning mortality percentage

The comparison of the mean preweaning mortality percentage between the OTI and ITG groups are shown **(Fig. 4.14).** Piglets in the ITG group recorded lower preweaning mortality percentage (8.09 %) compared to the OTI group (12.59 %), with a decrease of 4.50 percent.

The data was not normally distributed and was analyzed using the Mann-Whitney U Test. There were no significant differences between groups ($p>0.05$).

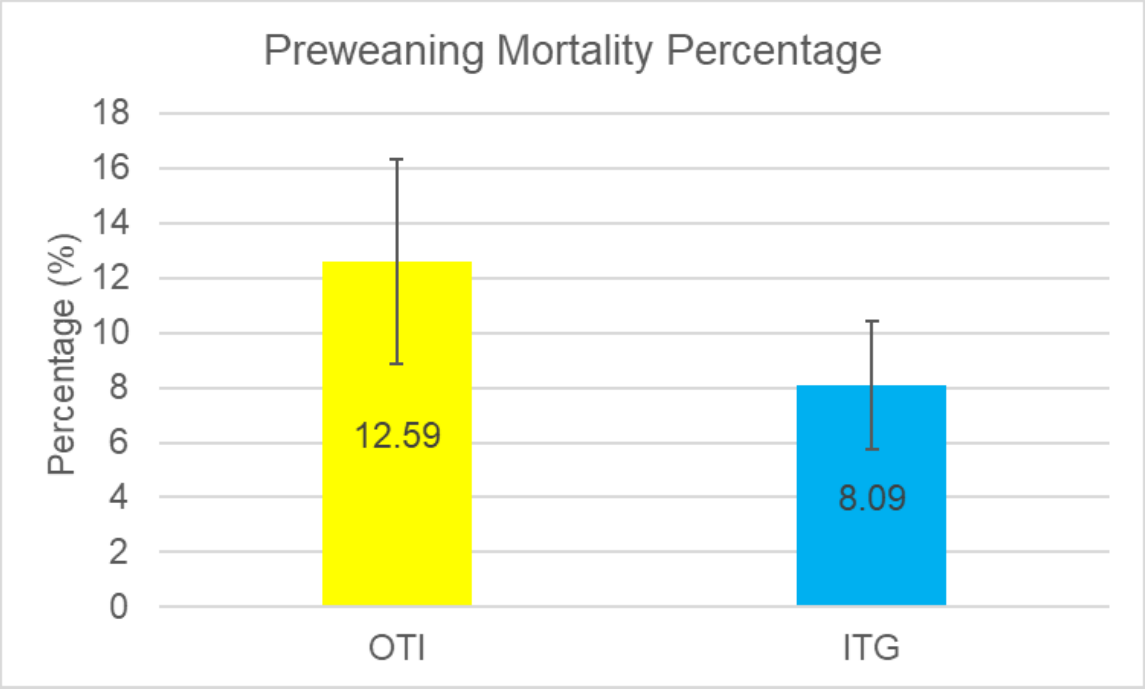

Figure 4.14: Comparison between the mean preweaning mortality percentage of the OTI and ITG group

4.15 Average daily gain (ADG)

The comparison of the average daily gains between the OTI and ITG groups are shown **(Fig. 4.15).** Piglets in the ITG group recorded slightly better average daily gain (162.77 g) compared to the OTI group (152.74 g), with an increase of 10.03 grams.

The data was normally distributed and was analyzed using the Independent Student's T-Test. There were no significant differences between groups ($p>0.05$).

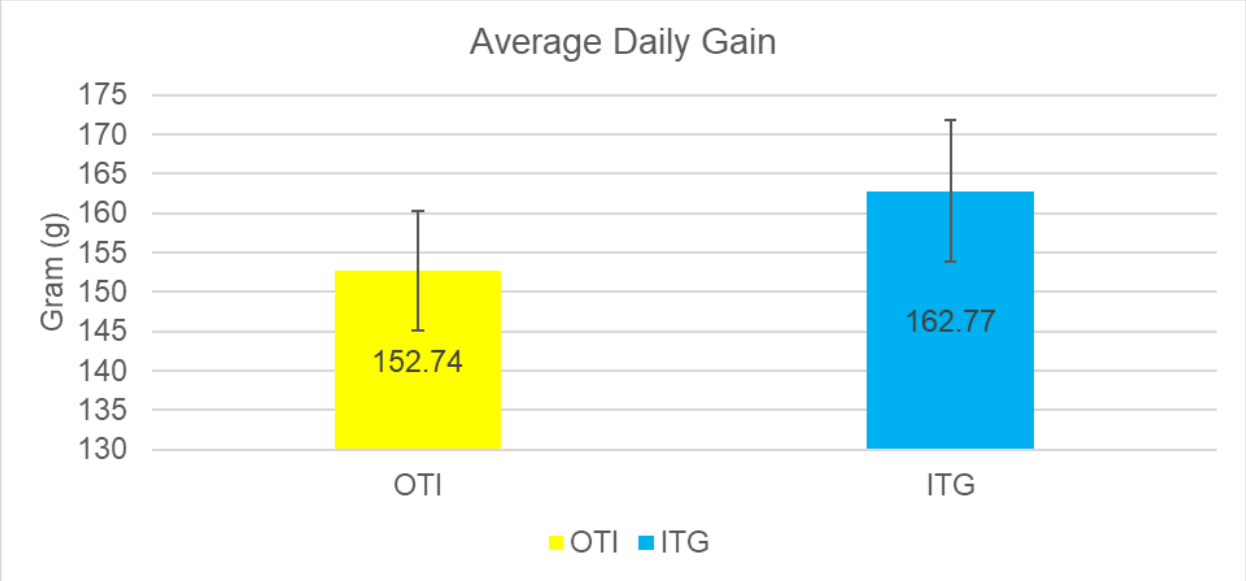

Figure 4.15: Comparison between the average daily gain of the OTI and ITG group

5.0 DISCUSSION

5.1 Blood haemoglobin level

Piglets raised in a fully intensive system lack the access to the soil, which serve as a natural resource of soil for the piglets (Egeli & Framstad, 1999). Piglets will have high risk of getting iron deficiency or IDA 10-14 days after birth, and therefore must require exogenous iron supplementation as metaphylactic therapy to prevent IDA (Sperling *et al*, 2018). For decades, injection of 200mg iron dextran intramuscularly within the first 3 days has been performed on a routine basis (Morales *et al., 2018*; Sperling *et al*., 2018; Yu *et al*., 2002; Zimmerman *et al*., 1959). However, selecting breeding in current swine production for characteristics of higher prolificacy and higher growth performance may suggest that the actual iron requirement might be higher than the current iron requirement we practice. Therefore, modern pigs probably need a higher dose of iron or an exogenous supply that has a higher bioavailability and absorption rate to prevent IDA effectively (Morales *et al.,* 2018).

A few haemoglobin concentration ranges have been used as references from a few literature reviews. Piglets have normal iron status when the Hb concentration level is more than 11 g/dL, iron deficiency when Hb concentration level between more than 9 g/dL but less than 11 g/dL and anaemia when Hb concentration fall below 9 g/dL (Bhattarai & Nielsen, 2015; Morales *et al*., 2018; Perri *et al*., 2017).

In this present study on day 26, the majority of piglets in both the OTI and ITG group had normal iron status (>11 g/dL) indicating that both OTI and ITG were effective in preventing IDA. The ITG group had better haemoglobin level with 95.56 % of piglets having normal haemoglobin level, 4.44% of piglets having iron deficiency and 0% of piglets having anaemia. According to Sperling *et al*. (2018), gleptoferron had been

shown to reduce anaemic status, increase plasma iron and haematological performances when compared to iron dextran in piglets. This was because the gleptoferron in the ITG provides better absorption and bioavailability of iron in the circulation. According to Morales *et al*. (2018), gleptoferron has 4.6 times higher iron absorption in the circulating system, and therefore provides more iron for sustainable supply in the body. Similar data were also recorded by Pollman *et al*. (1983), in which piglets receiving gleptoferron had higher levels of haemoglobin at weaning which indicated gleptoferron had better bioavailability of iron in circulation.

5.2 Average coccidia oocyst excretion (OPG)

In this present study, average coccidia oocyst excretion (OPG) and percentage of diarrhoea of piglets in ITG group was lower than OTI group on day 11 and day 16. Faecal scoring of piglets in the ITG group was generally better than the OTI group.

Injectable toltrazuril in the ITG provides higher and more sustained concentration in plasma and intestinal tissue by 33% (EMA *et al*., 2019), therefore providing a longer time frame of protection to the piglets (Karembe *et al*., 2021). Oral toltrazuril results in faster absorption and metabolization of toltrazuril to toltrazuril sulfoxide and toltrazuril sulfone and excreted in faeces (Lim *et al*., 2010). Oral toltrazuril provides a shorter time frame of protection, toltrazuril sulfone may decline to ineffective levels before infection takes place. Additionally, oral toltrazuril may indirectly promote antiparasitic resistance, possibly as a result of underdosing due to the risk of piglets regurgitating oral toltrazuril (Karembe *et al*., 2021). Since *C. suis* is endemic in farms, we cannot predict the actual time of infection, therefore using product such as Forceris® with prolonged effective drug concentration as well as early administration at

day 1 after birth greatly increase the efficacy of anticoccidial drug (Joachim *et al*., 2018).

Average coccidia oocyst excretion (OPG) in ITG was decreasing from 5369 in day 11 to 525 in day 16 while increasing in the OTI groups from 2390 in day 11 to 7825 Significant reduction of average coccidia oocyst excretion in ITG was similar to finding from Joachim *et al*. (2018) and Kreiner *et al*. (2011). As both ITG and OTI were using toltrazuril which proved to be an effective anticoccidial, the explanation for the increase of OPG in the OTI group was due to sampling error. Sampling error occurred during faecal collection as one fresh faeces sample was collected after the piglet defecated on the floors after manual stimulation. This particular sample had 73200 OPG count. Although the upper part of faeces was only collected and leaving the lower part behind, there was still a possibility the lower part of bottom faeces that was contaminated with sporulated oocyst was collected along with the upper part.

5.2 Diarrhoea percentage

Percentage of diarrhoea for the ITG groups were lower than the OTI groups on day 11 and 16, reflecting the previously reported findings from Joachim *et al*. (2018). In the present study, 12.84% and 7.94% of piglets still showed diarrhoea at day 11 and day 16 respectively. Diarrhoea is the clinical sign of neonatal coccidiosis. However, diarrhoea is not only caused by *C. suis,* it can also be caused by other enteropathogens such as rotavirus, *Cl perfringens* (Vítovec *et al*., 1991), *E. coli* and other enteropathogens (Ruiz *et al*., 2016). Unfortunately, no additional diagnosis methods were performed to determine the exact cause of diarrhoea due to budget

restraining. Therefore, the sole cause of diarrhoea was assumed to be caused by *C. suis* due to the presence of oocyst detected from modified McMaster technique.

5.3 Faecal score

The ITG group had better faecal scoring percentage on both day 11 and day 16 with lesser percentage of piglets having faecal score F3 and F4 which were the diarrhoea category. On both days, piglets in ITG had 0 % of piglets having faecal score F4, which was watery diarrhoea compared to the OTI group having 26.81% and 13.33% on day 11 and day 16 respectively. 0 % of watery diarrhoea in the ITG group indicated injectable toltrazuril was better than oral toltrazuril in terms of improving faecal scoring. Lower incidence of watery diarrhoea reduces the intervention of using antimicrobial treatment and therefore reduces the managerial cost of the farmer (Driesen *et al*., 1995).

5.4 Average daily gain and preweaning mortality percentage

Although there were no significant differences ($p > 0.05$) for average daily gain and preweaning mortality percentage of OTI and ITG groups, the ITG group had slightly better average daily gain, with daily gain of 162.77 gram per day and lower preweaning mortality percentage, 8.09%. Similar data were recorded by Pollman *et al*. (1983), who observed piglets treated with gleptoferron were heavier and with 2.6% lower mortality rate. This might be because of the effect of gleptoferron which provided better absorption and bioavailability of iron in the circulation as iron plays important roles in transportation and storage of oxygen and parts of components for many enzymes. Iron assists transformation of ADP to ATP by activating succinate dehydrogenase in Krebs

cycle. Iron plays a vital role for the immune system as Iron-containing catalase and peroxidases eliminate potentially harmful metabolic by products. Iron also plays a role in cellular development and proliferation by synthesis of DNA (Svoboda *et al*., 2005). Stuart *et al*. (1980) demonstrated the severity of coccidiosis is dependent on the number of infected sporulated oocysts. As injectable toltrazuril provides higher and more sustained concentration in plasma and interstitial tissue, it helped to reduce the number of coccidia in both sexual and asexual stage more effectively. Hence, reducing the chances of severe villous atrophy that later lead to emaciation and mortality.

5.5 Post injection reactions after 24 hours

Post injection reactions are important to determine the safeness of the products. Only two reactions were observed for post 24 hours injection reactions, which were swelling and bruising. There were no significant differences between the two groups, with both ITG and OTI groups having similar swelling percentages, 1.64% and 1.47% respectively. The ITG group had a lower bruising percentage which was 1.13% compared to OTI groups 4.56%. Since there were no other severe adverse reactions, the ITG group was safe to use. Joachim and his colleagues (2018) stated the ITG group was safe to use as no animal from the ITG group showed adverse reactions that required veterinary interventions.

6.0 CONCLUSION

The results of comparison between ITG and OTI groups demonstrated ITG (Forceris) at a fixed dose of 1.5ml/piglets (45 mg toltrazuril plus 200 mg gleptoferron) when administered at once at either day 1,2 and 3 days old was effective in preventing swine neonatal coccidiosis and IDA. Although there were no significant differences between the ITG and OTI groups in term of diarrhoea percentage, blood haemoglobin level and performance parameters, the ITG group using Forceris® with combination of injectable toltrazuril and gleptoferron had lower average oocyst excretion, diarrhoea percentage and preweaning mortality, with better blood haemoglobin level, faecal scoring and average daily gain. Additionally, usage of Forceris was concluded to be safe and convenient in preventing both coccidiosis and IDA at the same time. It is presumed to reduce labour force, labour cost, time and stress exposed to the piglets.

7.0 RECOMMENDATION

The total sample size of both ITG and OTI groups is best to have more than 384 piglets to improve the accuracy of the study in order to generate solid results. Secondly, this farm trial study has to be carried out in more farms as different farms have different oocyst burdens in the environment and also practice different husbandry management. Thirdly, routine washing floor must be done one hour prior to the faecal collection in order to reduce the possibility of sampling error. Workers are advice to practice good biosecurity by not stepping into the most contaminated farrowing crate and moving from that farrowing crate to nearby farrowing crate.

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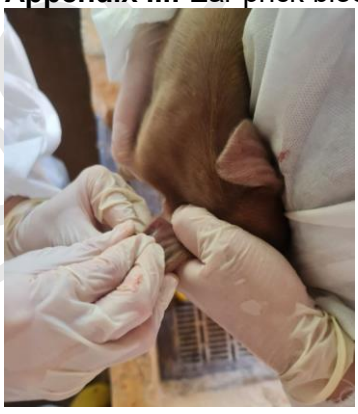
APPENDICES

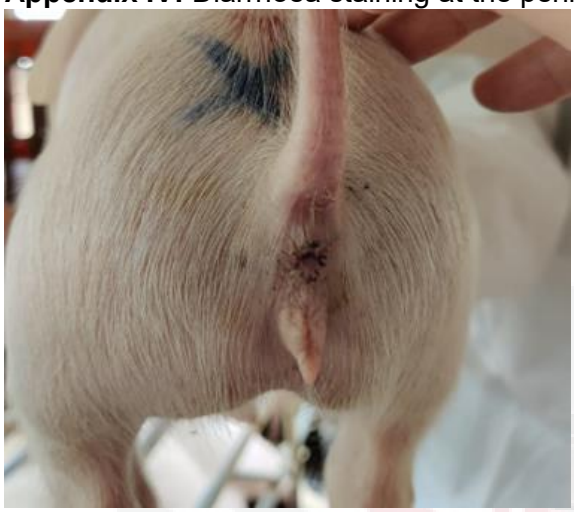
Appendix 1: Farrowing crates made with concrete and partial slatted flooring

Appendix II: Intramuscular injection of Forceris® at neck

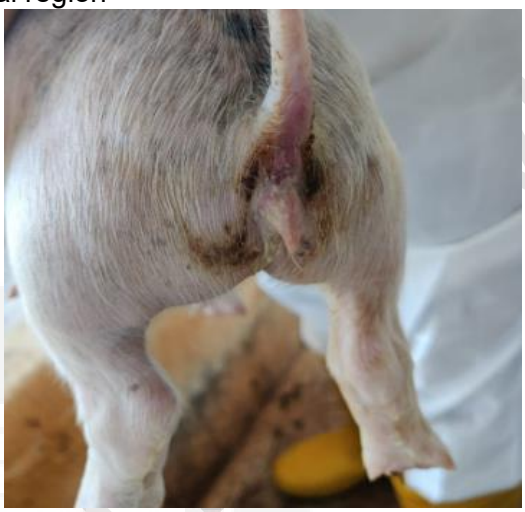
Appendix III: Ear prick blood sampling using 25G needle



Appendix IV: Diarrhoea staining at the perineal region

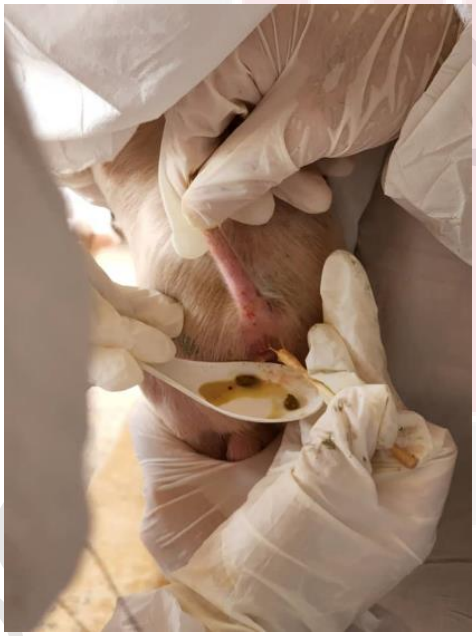


Normal



Diarrhoea

Appendix V: Manuel faecal collection by stimulating defecation using cotton bud



Appendix VI: Summary of faecal scoring (Pérez-Calvo *et al*.,2019)

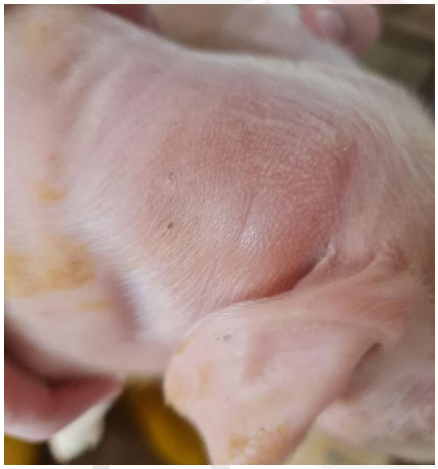
Normal		Diarrhoea	
Category 1		Category 2	
Faecal Score 1 Firm and well formed	Faecal score 2 Pasty	Faecal score 3 Semi pasty to water consistency	Faecal score 4 Watery diarrhoea
			
Texture: -Firm -Varies in hardness Shape: -Sausage	Texture: -Varies in softness -Like toothpaste Shape: -Sausage shape to small puke	Texture: -Mush Shape: -Tends to level with surface -Does not flow through or flows slowly through slatted floors	Texture: -Varies from gruel to water Shape: -Levels with surface -Flows through slatted floor

Appendix VI showed the faecal scoring system. From left to right are F1, F2, F3 and F4.

Source: Pérez-Calvo *et al*., (2019). The measurement of volatile organic compounds in faeces of piglets as a tool to assess gastrointestinal functionality. *biosystems engineering*, *184*, 122-129.

Appendix VII: Body weight measurement using portable digital weighing scale

Appendix VIII: Post 24 hours injection reactions



Swelling reaction post 24 hours injection

Bruising reaction post 24 hours injection