Effect of iron/anticoccidial treatment via injection on growth performance in piglets under the field conditions of a standard commercial Danish pig herd

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Abstract

Control of cystoisosporosis caused by *Cystoisospora suis* in piglets is crucial for decreasing diarrhoea and increasing the zootechnical performance. The aim of this field trial was to compare the effects of toltrazuril (TZL) treatment administered via different routes on the growth performance in suckling piglets. The study was conducted on a commercial Danish pig herd according to a parallel, randomized block design and compared two different treatment protocols: oral administration of TZL and injection of a combination product based on TZL and gleptoferron iron. In total, 763 piglets were included, with 377 piglets treated orally with TZL + iron gleptoferron. Forceris®, a combination product was used for intramuscular (i.m.) administration to 386 piglets. Faecal scoring and parasitological examinations were conducted at 11–14 days of age (DOA) and again at 18–21 DOA. Piglets treated intramuscularly presented a significantly greater average daily weight gain (ADWG), with a difference of 20 g between the groups; the average weight gain was 209 g (196–223 g), whereas the average weight gain in the orally treated animals was 189 g (175–203 g) ($P = 0.035$). Greater oocyst shedding and a frequency of diarrhoea were observed in piglets treated with oral TZL; however, these differences were not significant. This study demonstrated increased treatment efficacy of i.m.-administered TZL for *C. suis* infection, resulting in increased ADWG and is the first reported trial confirming a difference in outcomes versus those of oral TZL treatment under field conditions.

Coccidiosis, cystoisosporosis, injectable toltrazuril, gleptoferron

Cystoisosporosis, a common disease caused by *Cystoisospora suis* that affects pig production not only in Denmark but also worldwide, is most frequently controlled by metaphylactic treatment with toltrazuril (TZL) on positive farms where TZL is registered and available, usually within the piglet's first five days of life. Parasitic infection can lead to diarrhoea, reduced growth, and decreased well-being in piglets (Lindsay et al. 1985; Bach et al. 2003; Mengel et al. 2011), resulting in economic losses for pig producers (Scala et al. 2009; Kreiner et al. 2011; Joachim et al. 2018).

Clinical signs of cystoisosporosis are typically observed in piglets aged 7–21 days (until weaning). Coccidiosis often presents with characteristic yellow-grey diarrhoea, which can become waterier as the disease progresses. Piglets become dehydrated and lethargic and may exhibit reduced growth (Joachim et al. 2019). Morbidity is often high, whereas mortality is moderate (Lindsay et al. 1985).

Faecal examination via the McMaster technique can detect and exactly quantify oocysts in faeces since oocysts have a highly characteristic appearance and can be distinguished from *Eimeria* species (Joachim et al. 2018). The excretion of oocysts is biphasic, usually starting 5–6 days after the infection by parasite. Clinical signs (diarrhoea) can be usually observed shortly before, but also during or after the onset of shedding of oocysts into the

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environment (Joachim et al. 2018). Until recently, cystoisporosis was managed mainly by the oral application of TZL together with improved hygiene in the farrowing house. Recently, new combination products containing TZL and iron for intramuscular (i.m.) application were developed and registered within the European Union (Hiob et al. 2019; EMA 2019).

After the prospect of injection treatment was introduced to Denmark, fast adoption of the treatment strategy was observed among Danish herds (internal data and observation, not published). However, there is a lack of comparative data evaluating the efficacy of different protocols based on robust, controlled field studies under Danish farm conditions.

The background for the present investigation was based on the hypothesis that treatment of cystoisosporosis via different protocols, i.e., intramuscularly with injectable TZL compared to orally administered products would improve efficacy of the control protocol, leading to lower frequency of diarrhoea, oocysts excretion, and consequent better zootechnical performance measured as average daily weight gain (ADWG). Higher and more sustained concentrations of TZL and toltrazuril sulphone have been observed in the plasma and gastrointestinal tract of piglets following i.m. application (Karembe et al. 2021). An improved pharmacokinetic profile may be responsible for the improved efficacy of the product, resulting in increased anticoccidial activity with consequent improvement in the growth potential of piglets before weaning (Karembe et al. 2021; Karembe et al. 2024).

Materials and Methods

Ethics statement

The protocol for the present study was approved by the Danish Medicine Agency (DKMA) case no. 2022031469 on May 2, 2022.

The study was conducted on a Danish swine production farm located on the island of Fyn in Denmark, housing crossbred pigs of Landrace, Yorkshire, and boar Duroc genetics, with a high health status of blue specific pathogen-free from *Mycoplasma hyopneumoniae* + *Actinobacillus pleuropneumoniae* type 12. The facility houses a total of 1,400 sows. *Cystoisospora suis* infection was confirmed before the start of the study via the McMaster method (Joachim et al 2018). Despite the regularly applied control program based on the oral administration of TZL at 3 to 5 days of age (DOA), low to high levels of oocysts were confirmed before start of the study (Table 1).

Table 1. Assessment of coccidia oocyst load from pooled samples collected before the start of the trial.

Age at sampling (DOA)	Level of shedding	OPG
10	Moderate	2,860
	Non-detectable	0
	Low	110
	High	7,810
	Low	110
20	Low	220
	High	20,900
	High	41,580
	Moderate	630
	Moderate	1,730

Oocyst load before the trial (low < 500 OPG/g faeces; moderate 500–5,000 OPG/g faeces; high ≥ 5,000 OPG/g faeces; OPG - oocyst number per gramof faecal sample; DOA - days of age.

Litter equalization was performed, sows were randomized and distributed between the two groups, and all piglets from a particular sow were allocated into one of the two groups. The allocation process was carried out by the site investigator on the farm using the farrowing schedule before they visually inspected the sows. For each sow, a dice was used to choose the colour of the group, and all the parities available were considered for the randomisation based on the randomisation list considering the ascending order of parities. Even numbers on the dice were designated for the blue, oral group, and uneven numbers signified the green, i.m. group.

Within the interval of 24–96 h post farrowing, the piglets were individually weighed via a scale (produced by Bjerringbro Weights Aps, Denmark) with a weight resolution of 10 g. Subsequently, they were earmarked with either blue or green earmark identifiers. Cross-fostering and litter equalization were performed before the initial weighing. The piglets were subsequently allowed to move between sows that had litters with the same earmark colour. Variations in the timing of weighing were solely due to practical considerations for the farm personnel.

The study consisted of two parallel treatment groups. Each piglet received the following treatments: Piglets in the first group (oral group: Cevazuril®, Ceva Sante Animale, France, 424 piglets in 32 litters) received 45 mg of TZL *per os* at 3 to 5 DOA according to standard practices of the farm, and at 1 to 3 DOA they received 200 mg gleptoferron/piglet (Viloferron, Serumwerk Bernburg AG, Germany) i.m.. Piglets in the second group (injectable group: Forceris® Ceva Sante Animale, France, 493 piglets, 37 litters) received 1.5 ml consisting of 45 mg TZL and 200 mg gleptoferron i.m. at 1 to 3 DOA. Piglets were weighed at the end of the trial between 18 and 23 days after the initial weighing as before. The variation in the number of days in trials for individual piglets was caused by the urgent practical need for nursing sows for a large number of newborns. Consequently, the best-looking litters were chosen via visual inspection by the caretaker, who was blinded and did not participate in the administration of the studied substances. This experimental approach was reflected in the statistical evaluation of the results.

Faecal scoring and oocyst counts were performed via quantification of oocysts by the McMaster method (Joachim et al. 2018). Between 11 and 14 DOA and again between 18 and 21 DOA, a total of 10 randomly selected litters from each group were used for faecal sampling and diarrhoea score evaluation. The selected litters from every third pen were included by the investigator which resulted in an even distribution throughout the section. The same 10 pens were scored at two time points at one-week intervals. Faeces were scored on a scale from 1 to 4, with a faecal score (FS) of 1 indicating firm, an FS of 2 indicating pasty, an FS of 3 indicating semiliquid and an FS of 4 indicating liquid faeces, and an FS of 3 or 4 indicating diarrhoea (Joachim et al. 2018). On the same day that faecal scoring was conducted, faecal samples were collected from the same 10 litters, with 5 individual fresh faecal samples of different FS from different locations in the pen collected from the pen floor to obtain pooled samples (Sperling et al. 2022). The faecal scoring was performed on the farm directly on the pen floor by the site investigator.

These samples were kept refrigerated until shipment to the Veterinært Laboratorium, Kjellerup, Denmark, for McMaster analysis. The samples were analysed via the accredited flotation McMaster technique (Joachim et al. 2018).

Inclusion and exclusion criteria

All clinically healthy piglets from sows listed on the farrowing schedule for this week were included in the study. Both male and female piglets were included, with the exclusion of piglets exhibiting obvious malformations or in cases where the personnel responsible for the study on the farm deemed their retention in the study to be in violation of animal welfare standards. One litter in the blue group and three litters in the green group were excluded because the piglets suffered from excessive neonatal diarrhoea in the whole litter at 1–3 DOA. Furthermore, one litter in the green group was weaned to meet the needs of the nursing sows without being weighed and was thereby excluded.

Statistical analysis

The effect of treatment on ADWG was analysed via a linear mixed model in which the effect of litter was included as a random effect and other potential covariates were included as fixed effects. A Type III analysis of variance with Satterthwaite's method was used to test and remove nonsignificant variables, and a *post hoc* analysis of estimated marginal means was applied to compare the treatment groups while adjusting for the effects of other covariates, such as the initial weight of the piglet. The random effect of sow status was compared with the residual variation by calculating the intraclass correlation, i.e., the proportion of the total variation attributed to the effect of sow status. The intraclass correlation is calculated as the random variance at the sow level divided by the total variation (i.e., random effect of sow status + residual variance).

The statistical analysis was carried out in R via the lm4, lmerTest and demeans packages for statistical modelling and subsequent *post hoc* analysis (Bates et al. 2015; Kuznetsova et al. 2017; Core Team 2023).

Results

In total, 377 piglets in the oral group and 386 piglets in the injectable group completed the trial. There appeared to be a slightly greater daily weight gain in the injectable group than in the oral group but also a slightly greater initial weight and slightly greater age at inclusion. The protocol specified that the piglets should remain in the study for 21 days; however, because of practical issues related to the needs of the nursing sows, some piglets were taken out of the study at 18 or 19 days after inclusion. The ADWG varied according to the time of taking out. Piglets that were taken out early had a greater ADWG than those who were in the trial for 21–23 days. Piglets that were taken out early were chosen because of their body size and growth performance. Table 2 shows an overview of the ADWG according to the number of days in the trial.

An overview of the data variables can be found in Table 3. The variables attached to the sows were the number of litters included, the average number of liveborn piglets per litter

Table 2. Average daily weight gain (g) according to the number of days in the trial.

	Day of trial	n	ADWG	SD	Min	25%	50%	75%	Max
Oral TZL	18	24	227.11	56.45	124.44	180.83	241.39	259.31	325.56
	19	27	218.60	54.44	102.63	175.53	223.68	241.32	347.89
	21	310	193.23	63.38	68.10	149.05	188.10	241.43	369.52
	23	16	105.73	40.56	57.83	62.50	114.78	130.11	173.04
Injectable TZL	18	102	253.80	72.97	92.78	206.53	261.94	296.94	452.78
	19	13	183.10	33.27	128.95	159.47	179.47	191.05	261.84
	21	256	192.73	62.10	55.71	148.45	190.24	235.83	342.86
	23	15	100.64	36.59	50.43	62.39	104.35	128.26	160.00

TZL – toltrazuril, n – number of piglets, ADWG – average daily weight gain, SD – standard deviation, Min – minimum, Max – maximum

Table 3. Data overview.

	Oral TZL	Injectable TZL
N. of piglets	377	386
N. of litters	31	34
Average n. of liveborn piglets	17.9	16.5
N. of sows in experiment (# sows in 18/19/21/23 days)	2/2/26/1	9/1/22/2
Average weight at inclusion (g) (SD)	1,791 (387)	1,797 (408)
Average age at inclusion (days)*	2.66	2.75

TZL – toltrazuril, N. – number, SD – standard deviation, * Variable is nested at the sow level.

and the number of sows in the trial for 18, 19, 21 and 23 days, respectively. Furthermore, the variables attached to the piglet, average daily weight gain, average weight at inclusion, average age at inclusion and number of piglets in the trial were 18, 19, 21 and 23 days, respectively.

The initial model of daily gain included investigated groups, age at inclusion, weight at inclusion (initial weight) and days in the trial. Parity was placed as a fixed effect as well as a random effect of the sow. After non-significant effects were removed in the order of least significant variables first via Satterthwaite's method, parity ($P = 0.65$) and age at inclusion (P value = 0.58) were removed. Hence, the final model considered days in the trial as an explanatory variable with a $P < 0.001$ and an initial weight of $P < 0.001$. However, as argued earlier in this section, the decision to wean early was based on the visual inspection of the litters by the caretaker chosen as a consequence of their body size and growth performance. Hence, one might argue that days in the experiment should be disregarded as a variable. When this approach was adopted, the final model included colour and initial weights as well as a random effect of sows. A summary of this model is given in Table 4.

Table 4. The model.

Variable	Estimate	P value
Intercept (g)	18.7 (15.2)	0.220
Injectable TZL (g)	20.0 (9.3)	0.035
Initial weight (g)	0.095 (0.007)	< 0.001
Sow (random effect) (SD)	37.6	
Residual (SD)	52.5	

TZL – toltrazuril, g – gram, SD – standard deviation

Adjusting for differences in initial weight yielded estimated marginal means (95% confidence interval) of 189 g (175–203) for the oral group and 209 g (196–223) for the injectable group. The random effect of sow status accounted for approximately 34% of the total random variation, which is in line with what is usually observed in these types of data.

The average daily weight gain was significantly greater in the green group treated i.m. ($P = 0.035$), with a difference of + 20 g.

Faecal score and oocyst count

The results in Fig. 1 show numerically greater amounts of oocysts per gram of faeces (OPG) in response to oral TZL at both 11–14 DOA and 18–21 DOA (avg. 30,046 OPG and 2,189 OPG, respectively) than in response to i.m. TZL (avg. 7,920 OPG and 1,639 OPG, respectively).

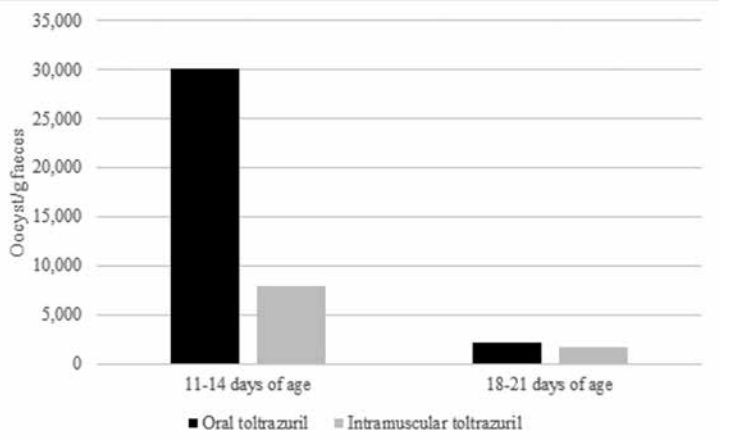

Fig. 1. Average OPG/litter at 11–14 days of age and 18–21 days of age.

OPG - oocysts per gram, g - gram

Similarly, the faecal scores presented in Fig. 2 as the average score per litter (SPL) showed that piglets treated with oral TZL had a firmer consistency of faeces at both 11–14 DOA and 18–21 DOA (average 3.5 SPL and 2.5 SPL, respectively) than piglets treated with i.m. TZL (average 1.9 SPL and 1.7 SPL, respectively).

These differences were only numerical because the sows are the statistical units with only 10 litters per group.

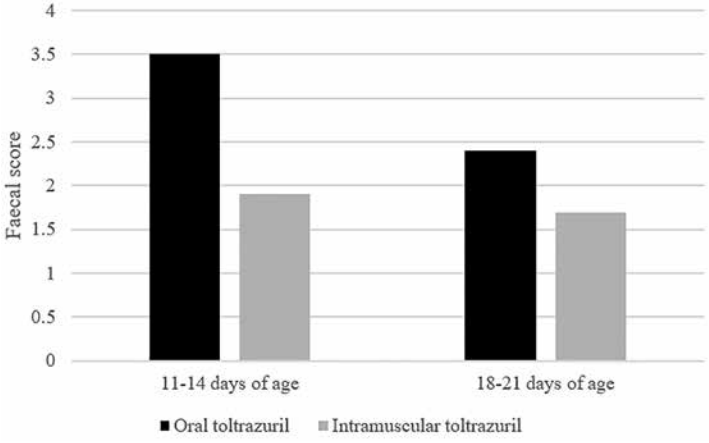

Fig. 2. Average faecal score/litter at 11–14 days of age and 18–21 days of age.

Discussion

Piglets infected by *C. suis* often exhibit reduced body weight, along with different levels of diarrhoea. Previous studies have demonstrated the positive effect of oral TZL treatment on body weight gain in infected piglets (Lindsay et al. 1985; Mundt et al. 2006; Rypula et al. 2012). The present study compared the efficacy of oral administration of TZL with that of i.m. injection of a combined TZL/gleptoferron product.

When the piglets were treated with the i.m. combination, a significant improvement in the ADWG indicator was observed, with a decrease in the number of OPG, especially during the first sampling at the 2nd week of life. Furthermore, a firmer consistency of faeces was observed.

More piglets from the i.m. group reached an optimum weight earlier with their consequent weaning. Since the primary factor for choosing piglets for weaning in connection with the need for nursing sows was the size of their piglets, this finding is consistent with the higher growth rate in the i.m. TZL group. Therefore, a significant portion of the early-weaned piglets belonged to the i.m. TZL group.

The number of days for which each piglet was in the trial was a significant variable, with a $P < 0.001$. However, the decision to wean a litter was based on the size of the piglets, which of course was driven primarily by the daily weight gain. Respecting the farm practice and the field trial set up should be considered as one of the limitations of the study, as optimally data on all piglets should be recorded at the same time.

Furthermore, since piglet growth is expected to increase with age, one could speculate that an even greater difference might have been observed if the piglets had been kept for the entire planned period, resulting in a greater difference in daily growth.

It has previously been shown that TZL administered i.m. as opposed to orally leads to higher relative bioavailability of TZL and the active metabolite toltrazuril sulphone. The i.m. administration was proven to yield relatively higher and long-lasting concentrations of TZL and toltrazuril sulphone in both the plasma and especially in target tissue of the small intestine (Karembe et al. 2021; Karembe et al. 2024), despite the fact that TZL and its active metabolite have no growth promotional effect as was demonstrated previously on coccidia negative farms (Maes et al. 2007). A prolonged effective drug concentration at the target site of infection can be expected to greatly increase the efficacy of anticoccidial drugs, improve the treatment effect and avoid significant affection of intestinal mucosa. These facts may explain less frequent diarrhoea leading to better intestinal mucosa functional status (intestinal permeability, translocation from the gut to the blood, as well as chronic systemic immune activation) (Sperling et al. 2023). The timing of treatment and its effect on early stage of parasite development has been well described. Injectable administration according to SPC may result in greater effect due to control of early infection, especially taking to consideration high parasite pressure confirmed on farm before the start of the trial. In a similar study on Spanish farms, oocysts were detected on every farm and in a considerable number of litters despite the application of TZL, with the estimated overall prevalence (95% confidence interval) of 40 (32–49)%, including 47 (29–65)% in non-medicated litters, 52 (38–67)% in orally medicated litters and 28 (16–40)% in i.m. medicated litters ($P < 0.05$), showing the highest efficacy in litters where piglets were injected (Deak et al. 2024) .

A limitation of our study was the number of litters investigated for the presence of the oocysts, which did not show a significant effect of treatment but only a numerical one. For an evaluation of the parasitological outcome of the treatment protocol, an experimental set up of the trial would be more suitable, following individual piglets and their excretion pattern (Karembe et al. 2021).

Differences in pharmacokinetics between the different treatment protocols may explain the decrease in the number of oocysts, especially at the first sampling point, although owing

to the rather small sample size with the sow as the statistical unit, this difference was not significant. At the same time, the blue group treated orally had at least three times more average OPG per litter, and the faecal samples were observed to be more fluid.

Since the results and effects are likely to vary between herds, an individual assessment of the effect in each herd is advisable. Several factors, such as the initial level of oocysts on the crate floor at farrowing (infection pressure), timing of treatment and consequently, the actual weight of piglets can contribute to the results assuming compliance with the treatment procedure during well-controlled trial conditions. Another aspect which can significantly contribute to control is the biosecurity measures and disinfections effective on oocysts applied on farms (Hinney et al. 2020). It can be assumed that i.m. application offers a more precise and reliable method in field conditions, since piglets may refuse or regurgitate orally administered products, which may lead to underdosing (Hiob et al. 2019).

In conclusion, under the conditions of this field trial, parenteral administration of TZL improved the ADWG (P = 0.035) at weaning in comparison with growth performance of piglets treated by standard protocol based on application of oral TZL.

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