Investigation of oral rennin (chymosin) supplementation as a farm level protocol to improve the passive transfer of immunity in neonatal piglets
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Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

A total of 20 sows and 20 gilts were used in this trial, while 10 sows and 10 gilts were induced to farrow using injectable prostaglandin F2α. Piglets from both induced and non-induced dams were randomly assigned to one of three treatment groups: no supplementation, oral supplementation with rennet and oral supplementation with saline. Piglets assigned to the rennet and saline treatment groups were administered their assigned treatment via stomach tube, twice in the first 12 hours post farrowing. A blood sample was collected from each piglet 48-72 hours post farrowing.

Oral rennet supplementation of piglets, piglets derived from induced or non-induced dams did not influence serum globulin concentrations in piglets at 48-72 hours, piglet bodyweight (at birth and weaning), growth rate or mortality rate.

Dam parity (sow or gilt) and litter size had a significant correlation with piglet bodyweight at birth and weaning, serum globulin concentrations, growth rate and mortality rate.

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1. Introduction

Pre-weaning mortality is of great concern to the commercial pig industry. Early studies on pre-weaning mortality in Australian piggeries identified pre-weaning piglet mortality rates between 19.7% (Glastonbury 1976) and 11.3% (Spicer, Driesen et al. 1986). These figures have since decreased to 12.5% in 2010 (APL 2012). Studies have shown that there is a negative relationship between: piglet survival rates and litter size; litter size and piglet size at birth; and dam parity and colostrum quality (Glastonbury 1976; Spicer, Driesen et al. 1986; Klobasa, Werhahn et al. 1987; Quiniou, Dagorn et al. 2002; Devillers, Farmer et al. 2007). Despite these associations of pre-weaning mortality in piglets, the absorption of Igs, especially immunoglobulin G (IgG), is important in providing passive protection against disease. Oral supplementation with rennet post-farrowing may promote Ig absorption, and therefore reduce mortality. Rennet is a network of enzymes that occur naturally in the stomach of newborn mammals, the active enzyme being chymosin or rennin, which assists in the release of immunoglobulins (Igs) from the colostrum and milk ingested by causing curd formation. Gamma-glutamyl transferase (GGT) is an enzyme produced by the ductile cells in the mammary gland, thus can be found and measured in the colostrum produced by the dam. This enzyme has previously been used as an indirect measurement of Ig absorption from ingested colostrum (Parish, Tyler et al. 1997; Wilson, Tyler et al. 1999). In a study by Gregory (2003), addition of rennet to colostrum caused 20% fewer calves to have low GGT activity (<500 U/L), and the proportion of calves with low serum GGT activity fell by 60%. Considering the piglet mortality rate in Australian piglet populations, piglets, especially those will low birth body weights, may benefit from oral rennet supplementation immediately following farrowing.

The aim of this study was to investigate the impact of oral rennet supplementation on piglet serum globulin concentrations. Piglets derived from induced farrowing and non-induced farrowing sows and gilts were compared.

2. Methodology

Sow and Gilt Selection

Twenty sows and 20 gilts from an intensive pig herd were sampled. Half of each group (10 sows and 10 gilts) were injected with 1mL of prostaglandin F2α (Lutalyse®, Pfizer, West Ryde, NSW) into the vulval lips. This was performed twice within a 6 hour interval (i.e. first in the morning and then in the afternoon) the day before the estimated due date, which was based upon a mean gestation length of 115 days.

Piglet Treatment Allocation

Either 3 or 6 or 9 piglets from each litter were included in the trial. This number was maximised according to the size of the litter. For example, if there were 5 piglets in the litter 3 piglets were randomly selected and assigned to the trial. The remaining two piglets were excluded from the trial although remained with the litter.

The piglets to be included in the trial within each litter were ranked according to their birth weights. The piglets were weighed within 6 hours of birth. The ranked list of piglets was sequentially divided into groups of 3 piglets starting with the heaviest piglet. The piglets within each group were randomly assigned to one of the three treatment groups.

The three treatments consisted of no oral supplementation, oral 0.9 % saline supplementation and oral rennet supplementation. Piglets assigned to saline supplementation or rennet supplementation groups were fed 4 mL of their

designated oral supplement via a stomach tube, twice in the first 12 hours post parturition. The rennet supplementation consisted of vegetarian rennet (Cheeselinks, Little River, VIC) was diluted 1:100 with saline solution to achieve a final concentration of 2.02 IMCU/mL.

Piglet Blood Sample Collection, Weaning and Serum Testing

A blood sample was collected from each piglet by venepuncture of the anterior vena cava 48-72 hours post parturition. All piglets were weighed at weaning (21 ± SE 0.3 days) and all mortalities from birth to weaning were recorded. All the blood samples were centrifuged and the serum tested using mass spectrometry (Beckman Coulter AU480, Lane Cove, NSW) for globulin level (Globulin level= Total protein- Albumin).

Statistical analysis

Descriptive statistics to determine normal distribution were performed. Mean and 95% Confidence Intervals (CI) were calculated for parity, induction and treatment groups. All factors found to be significant were further tested using a univariate ANOVA, Pearson correlations and Student's T-Test ($P<0.01$) (SPSS 19, IBM, St Leonards, NSW).

3. Outcomes

Piglet serum globulin concentration and treatment group

There were no statistically significant differences in mean serum globulin concentrations in piglets at 48-72 hours post farrowing, between the three treatment groups, when comparisons were made at three levels (Table 1):

(1) treatment (no supplementation, saline supplementation, rennet supplementation).
(2) induction (induced, non-induced).
(3) dam parity (sow, gilt) ($P>0.05$).

Table 1 - Mean ± SE piglet serum globulin concentration (g/L) at 48-72 hours post farrowing for all piglets, piglets derived from sows and gilts, piglets derived from induced and non-induced sows and gilts, and for piglets allocated to the no supplementation (None), rennet supplementation (Rennet) and saline supplementation (Saline) treatment groups (n= number of piglets)

All piglets (n=294) 39.01 ± 0.51											
Sows (n=149) [a] 42.15 ± 0.65						**Gilts (n=145) [a]** 35.78 ± 0.70					
Induced (n=65) [b,c] 40.80 ± 0.94			**Non-induced (n=84) [d,e]** 43.19 ± 0.88			**Induced (n=78) [b,d]** 36.22 ± 0.93			**Non-induced (n=67) [c,e]** 35.27 ± 1.06		
None[ǂ] (n=22)	**Rennet[ǂ] (n=22)**	**Saline[ǂ] (n=21)**	**None[ǂ] (n=27)**	**Rennet[ǂ] (n=28)**	**Saline[ǂ] (n=29)**	**None[ǂ] (n=27)**	**Rennet[ǂ] (n=24)**	**Saline[ǂ] (n=27)**	**None[ǂ] (n=23)**	**Rennet[ǂ] (n=23)**	**Saline[ǂ] (n=21)**
39.55 ± 1.62	41.68 ± 1.64	41.19 ± 1.66	44.56 ± 1.82	42.14 ± 1.67	42.93 ± 1.04	34.52 ± 1.48	38.38 ± 2.09	36.00 ± 1.25	35.00 ± 1.73	33.91 ± 1.98	37.05 ± 1.83

ǂ No treatment groups were statistically significant.
* Same superscript letters denote statistical differences ($P<0.05$).

Piglet birth weight, weaning weight and growth rate

Piglets derived from sows, non-induced sows and gilts and piglets supplemented with saline solution had the highest birth bodyweights for their group, with weights of 1.72 ± SE 0.05 kg, 1.61 ± SE 0.05 kg and 1.64 ± SE 0.06 kg, respectively (Table 2). Piglets derived from sows had statistically different and higher mean birth bodyweight (1.74 ± SE 0.03 kg) compared to piglets derived from gilts (1.46 ± SE 0.02 kg) ($P<0.05$). Piglets from induced sows had a statistically different and higher birth bodyweight (1.82 ± SE 0.04 kg) compared to piglets derived from non-induced sows (1.68 ± SE 0.03 kg) ($P<0.05$), while piglets derived from non-induced gilts had a statistically different and higher mean birth bodyweight (1.49 ± SE 0.03 kg) than piglets derived from induced gilts (1.45 ± SE 0.03 kg) ($P<0.05$) (Table 2). All remaining comparisons were not significant ($P>0.05$).

Table 2 - Mean ± SE piglet birth bodyweight (kg) for all piglets, piglets derived from sows and gilts, piglets derived from induced and non-induced sows and gilts, and for piglets allocated to the no supplementation (None), rennet supplementation (Rennet) and saline supplementation (Saline) treatment groups (n= number of piglets)

All piglets (n=310)											
1.60 ± 0.02											
Sows (n=152) [a]						**Gilts (n=158) [a]**					
1.74 ± 0.03						1.46 ± 0.02					
Induced (n=65) [b]			**Non-induced (n=87) [b]**			**Induced (n=84) [c]**			**Non-induced (n=74) [c]**		
1.82 ± 0.04			1.68 ± 0.03			1.45 ± 0.03			1.49 ± 0.03		
None[ǂ] (n=22)	**Rennet[ǂ] (n=22)**	**Saline[ǂ] (n=21)**	**None[ǂ] (n=29)**	**Rennet[ǂ] (n=29)**	**Saline[ǂ] (n=29)**	**None[ǂ] (n=28)**	**Rennet[ǂ] (n=28)**	**Saline[ǂ] (n=28)**	**None[ǂ] (n=25)**	**Rennet[ǂ] (n=25)**	**Saline[ǂ] (n=24)**
1.84 ± 0.06	1.74 ± 0.06	1.87 ± 0.07	1.68 ± 0.06	1.65 ± 0.04	1.70 ± 0.06	1.41 ±0.05	1.44 ± 0.03	1.49 ± 0.05	1.48 ± 0.05	1.44 ± 0.04	1.53 ± 0.06

ǂ No treatment groups were statistically significant.
* Same superscript letters denote statistical differences ($P<0.05$)

Piglets derived from sows had a statistically different and higher mean weaning bodyweight (8.06 ± SE 0.17 kg) than piglets derived from gilts (6.27 ± SE 0.16 kg) and piglets derived from induced sows had a statistically significant and higher mean weaning bodyweight (8.54 ± SE 0.30 kg) than piglets derived from non-induced sows (7.68 ± SE 0.18 kg) ($P<0.05$) (Table 3). All remaining comparisons were not significant ($P>0.05$).

Table 3 - Mean ± SE piglet weaning bodyweight (kg) for all piglets, piglets derived from sows and gilts, piglets derived from induced and non-induced sows and gilts, and for piglets allocated to the no supplementation (None), rennet supplementation (Rennet) and saline supplementation (Saline) treatment groups (n=number of piglets)

All piglets (n=291)											
7.16 ± 0.13											
Sows (n=145) [a]						**Gilts (n=146) [a]**					
8.06 ± 0.17						6.27 ± 0.18					
Induced (n=63) [b]			**Non-induced (n=82) [b]**			**Induced (n=78) [ǂ]**			**Non-induced (n=68) [ǂ]**		
8.54 ± 0.30			7.68 ± 0.18			6.19 ± 0.25			6.37 ± 0.25		
None[ǂ] (n=21)	**Rennet[ǂ] (n=22)**	**Saline[ǂ] (n=20)**	**None[ǂ] (n=27)**	**Rennet[ǂ] (n=27)**	**Saline[ǂ] (n=28)**	**None[ǂ] (n=24)**	**Rennet[ǂ] (n=26)**	**Saline[ǂ] (n=28)**	**None[ǂ] (n=24)**	**Rennet[ǂ] (n=22)**	**Saline[ǂ] (n=22)**
8.47 ± 0.52	8.52 ± 0.48	8.65 ± 0.56	7.97 ± 0.27	7.39 ± 0.33	7.69 ± 0.35	6.27 ± 0.46	6.06 ±0.41	6.25 ± 0.45	5.98 ± 0.49	6.57 ± 0.40	6.60 ± 0.41

ǂ No treatment groups were statistically significant.
* Same superscript letters denote statistical differences ($P<0.05$).

Piglets derived from sows had a statistically significant and higher mean growth per day (0.28 ± SE 0.006 kg) than piglets derived from gilts (0.23 ± SE 0.009 kg). Piglets derived from induced sows had a statistically significant and higher mean growth per day (0.30 ± SE 0.009 kg) than piglets derived from non-induced sows (0.26 ± SE 0.006 kg), and a significantly different and higher mean growth per day compared to piglets from induced and non-induced gilts. Piglets from non-induced sows had a significantly different and higher mean growth per day compared to piglets from induced and non-induced gilts ($P<0.05$) (Table 4). All remaining comparisons were not significant ($P>0.05$)

Table 4 - Mean ± SE piglet growth rate per day (kg) for all piglets, piglets derived from sows and gilts, piglets derived from induced and non-induced sows and gilts, and for piglets allocated to the no supplementation (None), rennet supplementation (Rennet) and saline supplementation (Saline) treatment groups (n=number of piglets)

All piglets (n=286)											
0.25 ± 0.004											
Sows (n=142) [a]						Gilts (n=144) [a]					
0.28 ± 0.006						0.23 ± 0.009					
Induced (n=60) [b,c]			Non-induced (n=82) [b,d,e]			Induced (n=78) [b,d]			Non-induced (n=66) [c,e]		
0.30 ± 0.009			0.26 ± 0.006			0.22 ± 0.006			0.24 ± 0.006		
None[ǂ] (n=19)	Rennet[ǂ] (n=21)	Saline[ǂ] (n=20)	None[ǂ] (n=27)	Rennet[ǂ] (n=27)	Saline[ǂ] (n=28)	None[ǂ] (n=24)	Rennet[ǂ] (n=26)	Saline[ǂ] (n=28)	None[ǂ] (n=22)	Rennet[ǂ] (n=22)	Saline[ǂ] (n=22)
0.30 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.22 ± 0.01	0.22 ± 0.009	0.23 ± 0.01	0.21 ± 0.01	0.25 ± 0.008	0.25 ±0.007

ǂ No treatment groups were statistically significant.
* Same superscript letters denote statistical differences ($P<0.05$).

For all piglets, piglet birth bodyweight and weaning bodyweight showed a low, but statistically significant positive correlation ($r=0.27$, $P<0.01$) (Figure 1a).

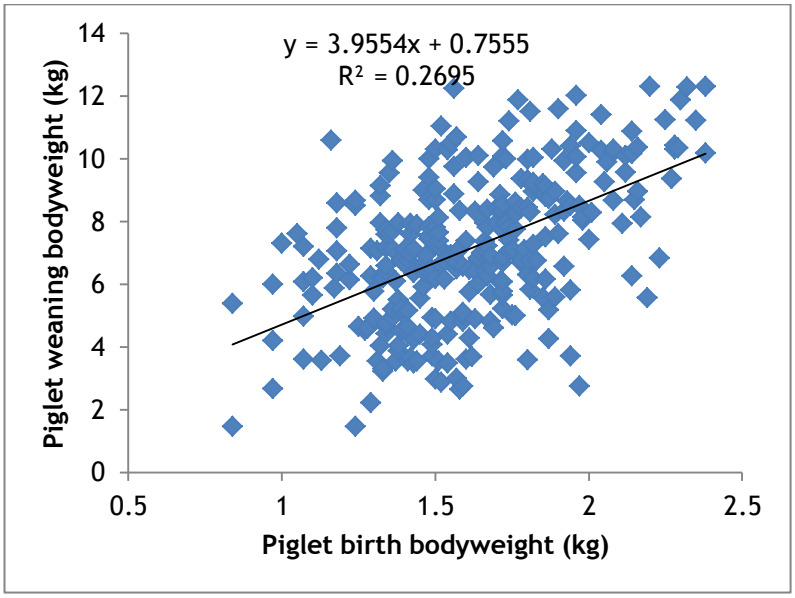

Figure 1a - Correlation between piglet bodyweight (kg) at birth and at weaning (21 days post farrowing);

Piglet serum globulin concentration and dam parity

Piglets derived from sows had a significantly different and higher serum globulin concentration (42.15 ± SE 0.94 g/L) 48-72 hours post farrowing compared to piglets from gilts (35.78 ± SE 0.70 g/L) (P<0.05) (Table 1).

Piglets receiving the 'None' treatment, which were derived from induced sows, had a significantly different and higher mean serum globulin concentration (39.55 ± SE1.62 g/L) than the 'None' treatment piglets derived from induced gilts (34.52 ± SE 1.48 g/L) (Figure 2). Piglets receiving the 'None' treatment which were derived from non-induced sows had a significantly different and higher mean serum globulin concentration (44.56 ± SE 1.82 g/L), than the 'None' treatment piglets derived from non-induced gilts (35 ± SE 1.73 g/L) (P<0.05) (Figure 2).

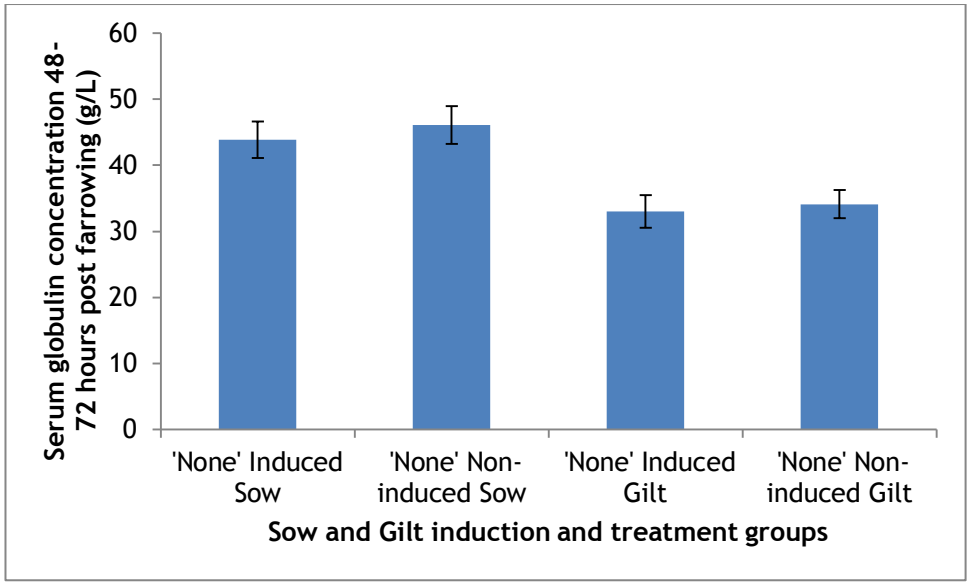

Figure 2 - Mean ± 95% Confidence Intervals for piglet serum globulin concentrations (g/L) 48-72 hours post farrowing for piglets randomly placed in the 'None' treatment groups for induced and non-induced sows and gilts.

Irrespective of treatments, piglets derived from induced sows had a significantly different and higher mean serum globulin concentration (40.80 ± SE 0.94 g/L) than piglets derived from either induced (36.22 ± SE 0.93 g/L) or non-induced gilts (35.27 ± SE 1.06 g/L). Piglets from non-induced sows had a significantly different and higher mean serum globulin concentration (43.19 ± SE 0.88 g/L) than piglets derived from either induced (36.22 ± SE 0.93 g/L) or non-induced gilts (35.27 ± SE 1.06 g/L) (P<0.05) (Table 1).

For all piglets, serum globulin concentration and piglet birth bodyweight showed a low, but statistically significant correlation (r=0.07, P<0.01) (Figure 1b).

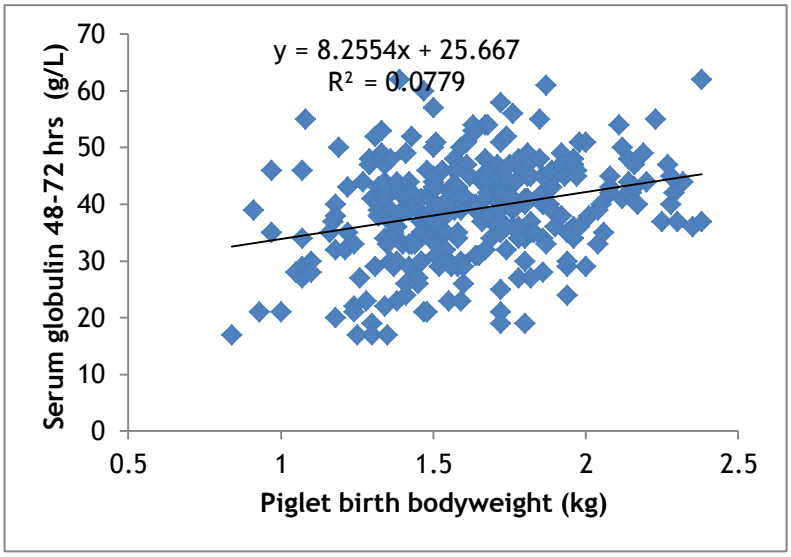

Figure1b - Correlation between piglet bodyweight at birth (kg) and piglet serum globulin concentrations (g/L) at 48-72 hours post farrowing.

Litter size (born alive), piglet serum globulin concentration and mortality rate

Mean litter size for all piglets was 10.3 ± SE 0.13 piglets per litter (Table 5). All litter size comparisons were not significant (P>0.05).

Table 5 - Mean ± SE of the litter size (born alive) for all piglets, piglets derived from sows and gilts and piglets derived from induced and non-induced sows and gilts (n=number of piglets).

All Piglets (n= 310) 10.3 ± 0.1			
Sows (n= 152) ǂ 10.3 ± 0.2		**Gilts (n= 158)** ǂ 10.4 ± 0.2	
Induced (n= 65) ǂ	**Non-induced (n= 87)** ǂ	**Induced (n= 84)** ǂ	**Non-induced (n= 74)** ǂ
10.7 ± 0.3	9.7 ± 0.2	10.4 ± 0.2	10.3 ± 0.3

ǂ No treatment groups were statistically significant.

Litter size (born alive) for all piglets, was negatively associated with both piglet birth body weight (r=0.13, P<0.01) and piglet serum globulin concentration 48-72 hours post farrowing (r=0.04, P<0.01) (Figure 3a and b).

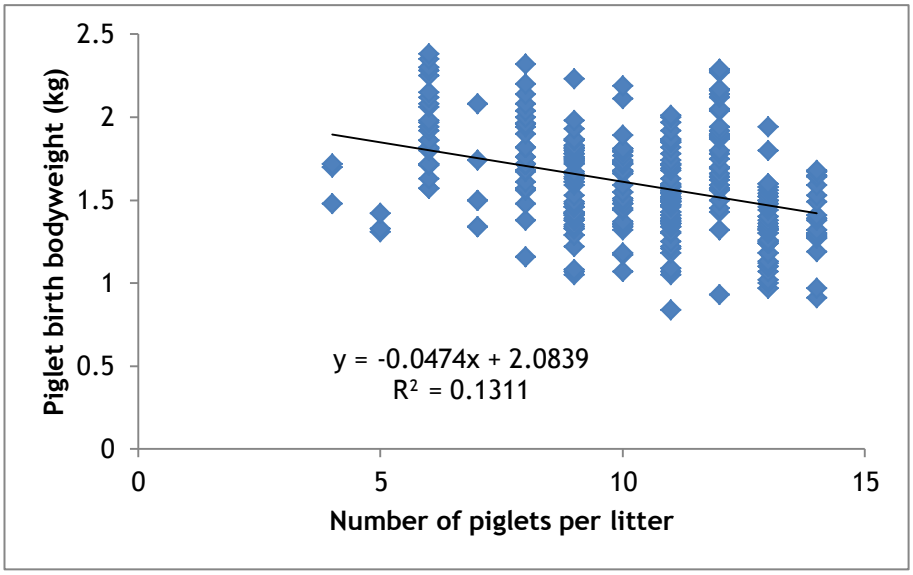

Figure 3a - Correlation between number of piglets born alive per litter and piglet bodyweight at birth (kg)

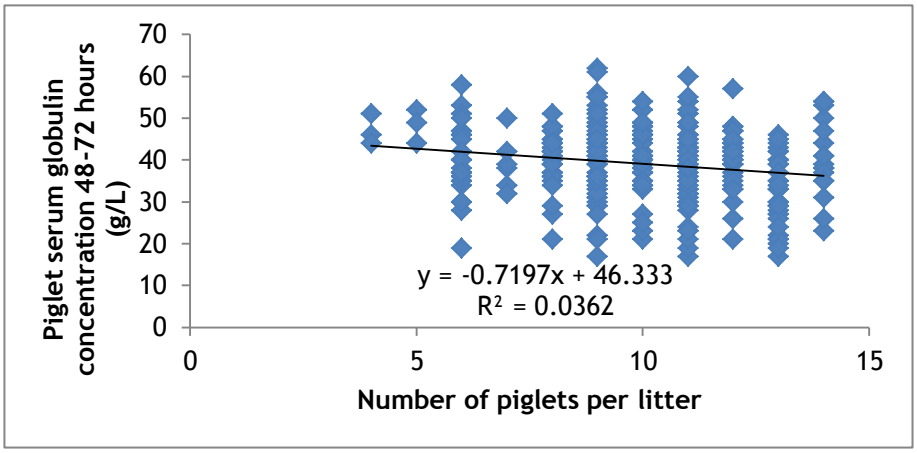

Figure 3b - Correlation between number of piglets born alive per litter and piglet serum globulin concentration (g/L) at 48-72 hours post farrowing.

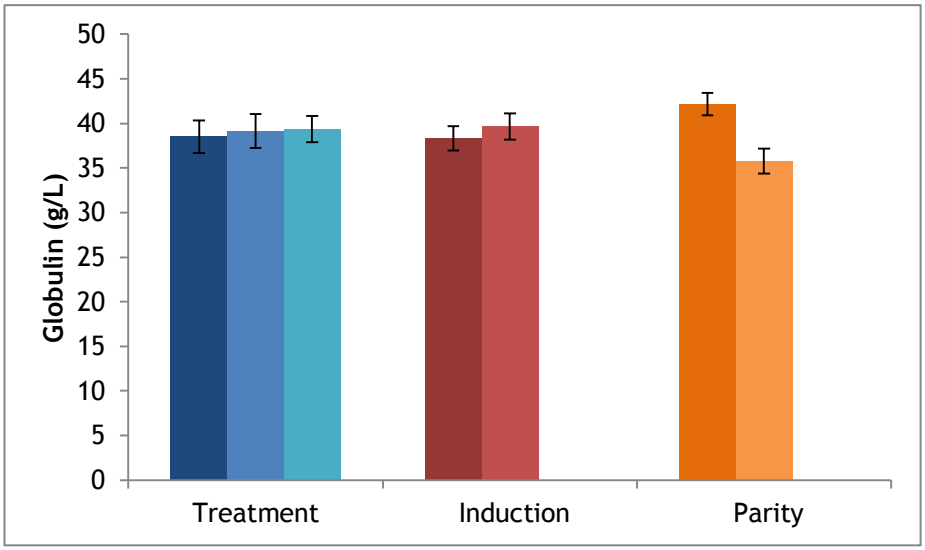

Figure 4 - Mean ± CI piglet serum globulin concentrations (g/L) at 48-72 hours post farrowing for piglets in each treatment group; no supplementation (dark blue), rennet supplementation (medium blue), saline supplementation (light blue), piglets derived from induced (dark red) and non-induced dams (light red), and piglets derived from sows (dark orange) and gilts (light orange).

Piglets supplemented with rennet had a mortality rate from birth to weaning of 7.5%, which was higher than the mortality rate recorded for piglets in the no supplementation treatment group (6.7%) and the saline supplementation group (4.0%). These mortality rates were not significant (P>0.05) (Figure 5).

Piglets derived from induced sows and gilts had a combined mortality rate of 5.4%, while piglets derived from non-induced sows and gilts had a combined mortality rate of 6.8%, which were also not significant (P>0.05) (Figure 5). Piglets derived from sows however had a significantly lower mortality rate (7%) than those derived from gilts (12%) (P<0.05) (Figure 5).

The mortality rate was also found to be negatively associated with litter size (r= 0.12, P<0.05) and piglet serum globulin concentration (r=-0.247, P<0.01).

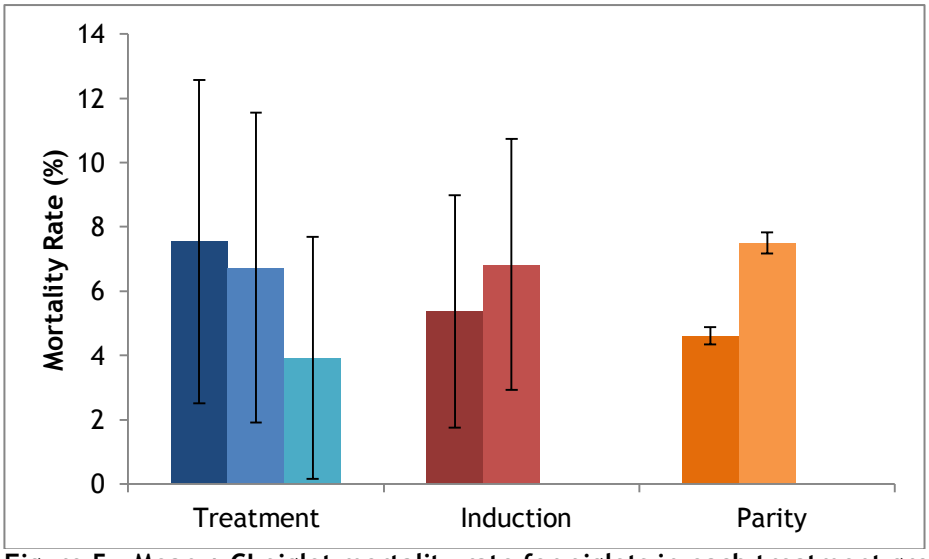

Figure 5 - Mean ± CI piglet mortality rate for piglets in each treatment group; no supplementation (dark blue), rennet supplementation (medium blue), saline supplementation (light blue), piglets derived from induced (dark red) and non-induced dams (light red), and piglets derived from sows (dark orange) and gilts (light orange).

4. Application of Research

Oral rennet supplementation of piglets in the first 12 hours following farrowing did not improve piglet serum globulin concentrations, piglet body weight (at weaning), growth rate or mortality rate of piglets. A study by Gregory (2003) showed improved GGT levels in dairy calves when rennet was added to colostrum. The study found 20% fewer calves with low GGT activity (<500 U/L), and the proportion of calves with low serum GGT activity fell by 60% (Gregory 2003). Other studies have also shown that GGT activity in both dairy and beef calves is correlated with serum IgG1 concentrations and with passive transfer of immunity in young calves (Parish, Tyler et al. 1997; Wilson, Tyler et al. 1999). It is possible that the dose of rennet that was administered to piglets assigned to the oral rennet supplementation group was insufficient to promote increased gastric curd formation. Immunoglobulin absorption has shown to be reduced when curd formation is inhibited in calves (Cruywagen 1990).

In this study, an increase in litter size was associated with a reduction in birth weight. These piglets tended to have reduced serum globulin concentrations when compared with larger piglets, in the same litter and overall. Additionally, these piglets also experienced higher mortality rates. Piglets with a low birth body weight tend to be the most at risk of mortality post farrowing. Spicer, Driesen et al. (1986) identified lower birth bodyweight with increasing litter size, especially in litters with more than 11 piglets, while a study by Glastonbury (1976) identified an increase in mortality rate of 9.8% to 34.5% as litter size increased from 7 piglets to 15 piglets and over. The relationship between piglets with low birth bodyweight and increasing litter size has been shown to be the result of reduced uterine blood flow available to each foetus during gestation (Pere and Etienne 2000). Small piglets, calculated as 75-80% of mean birth bodyweight of the litter, have a delayed first suckle and are not able to suckle effectively (Spicer, Driesen et al. 1986; Quiniou, Dagorn et al. 2002). Spicer, Driesen et al. (1986) also discovered that the majority of deaths in their study occurred in piglets with a birth bodyweight below 800g. Low birth weight was also found to be correlated with most causes of death (Spicer, Driesen et al. 1986).

The positive correlation between mean piglet birth bodyweight and serum globulin concentration in this study also illustrates the benefits of heavier piglets. Piglets with a higher birth bodyweight will tend to be able to compete more readily for teats, and thus consume more colostrum than those piglets that are born smaller (Quiniou, Dagorn et al. 2002). This will subsequently influence individual growth per day. However, there was a noticeable and significant difference in these results between piglets derived from sows and those derived from gilts. Piglets from sows with four parities and over have previously been found to have higher IgG colostrum concentrations than piglets from lower parity sows (Klobasa, Werhahn et al. 1987), while sows with two parities and over tend to have a greater colostrum yield (Devillers, Farmer et al. 2007). A study by Devillers, Farmer et al. (2007) found a positive correlation between colostrum yield and IgG colostrum concentration, while Quesnel (2011) found that colostrum intake was positively correlated with mean piglet birth bodyweight. Therefore, heavier piglets derived from sows will consume larger amounts of colostrum that contains higher concentrations of IgG.

5. Conclusion

Oral rennet supplementation of piglets in the first 12 hours post farrowing did not increase serum globulin concentrations in piglets at 48-72 hours, or influence piglet bodyweight at weaning, growth rate or mortality rate. However, parity of the dam, as well as a reduced litter size, had a significant and positive influence

on piglet bodyweight at birth and weaning, serum globulin concentrations, growth rate and mortality rate. Improvement of piglet mortality rates at commercial piggeries may benefit from increased monitoring of gilts to assist piglets who are born significantly smaller than their littermates.

6. Limitations/Risks

Oral rennet supplementation of piglets derived from induced or non-induced dams did not influence serum globulin concentrations in piglets at 48-72 hours, piglet bodyweight (at birth and weaning), growth rate or mortality rate using the study dose rates. The impact of higher dose rates of oral rennin on neonatal serum immunoglobulin concentrations has not been determined.

7. Recommendations

Oral rennin supplementation to the neonatal piglet is not recommended as no additional benefit could be determined.

Improvement of piglet mortality rates at commercial piggeries may benefit from increased monitoring of gilts to assist piglets who are born significantly smaller than their littermates. Dam parity (sow or gilt) and litter size had a significant correlation with piglet bodyweight at birth and weaning, serum globulin concentrations, growth rate and mortality rate.

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