Lactoferrin as a new feed additive improves sow milk production and pig production

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Executive Summary

Lactoferrin (LF), a 80 kD non-haem iron-binding glycoprotein, has multiple biological functions. Its potential role in material intervention improving milk production and reducing the incidence of low birth weight birth piglet remains unknown. The aim of study is to investigate the effect of maternal LF supplementation during gestation and lactation in gilts on milk production and growth and development of piglets. Gilts were fed with commercial pig feed supplemented with 1 g Lf /day/gilt for the treatment group and 1 g milk Casein/day/gilt for the control group from day 1 post mating throughout gestation and lactation. Gilt body weight, the total number of piglet born, total number of alive piglets, dead piglets and IUGR piglets were recorded. The weight of the whole litter at birth, individual weights of piglets at birth and on day 19 was measured. Milk production of the gilts was measured on day 1, day 3, day 7 and day 19 of lactation by weighing the piglets before and after suckling and weight gain of the litter was used as an indication of gilt milk production per suckling episode. Immunoglobulin concentrations were determined by ELISA in the serum and faecal samples. The results showed that maternal Lf supplementation of the gilts:

(1) significantly increased body weight gain of their piglets during the first 21 days of life compared to the control group ($P<0.05$);
(2) significantly increased milk production at different time points of lactation compared to the control ($P<0.001$);
(3) tended to increase pregnancy rate, litter size and birthweight, number of piglets born alive, piglet weaning weight and gilt bodyweight gain and decrease the number of dead and IUGR piglets. These differences were, however, not significant ($P>0.05$), because number of subjects in each group were relatively small.
(4) significantly increased the concentration of IgA in gilt serum and sIgA in piglet serum ($P<0.05$).

In summary, the data obtained from this study have demonstrated that maternal Lf intervention improved milk production, pig production and immunity; however, the underlying molecular mechanisms remain to be explored.

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1. Introduction

Lactoferrin (Lf) is an 80 kDa iron-binding glycoprotein consisting of 703 amino acids and multiple terminal sialic acid (Sia) residues on N-linked glycan chains (1). As a member of the transferrin family of iron-binding proteins, Lf shares more than 60% homology at the amino acid level with transferrin (2) and a 77% homology with human and bovine species (3). LF has multiple biological functions, including its ability to modulate immune function and its anti-microbial activity against viruses, bacteria, and fungi (4,5). It functions to facilitate iron metabolism, to promote bone growth and to inhibit the growth of some human cancers (6-8). In human clinical studies, Lf was shown to play a protective role in reducing the incidence of invasive fungal infections (9) and late-onset sepsis (3). In very low birth weight (VLBW) neonates, Lf can prevent the development of necrotizing enterocolitis (10). Lf has been reported to prevent biofilm formation by pathogenic microbes and effectively modulates immune responses and improves body weight gain in IUGR rat pups. Thus, Lf, as a bioactive molecule, plays a critical role in boosting the immune system to modulate inflammation (6).

Intrauterine growth restriction (IUGR) refers to a condition of the mammalian embryo/fetus in which it does not reach its growth potential during pregnancy. In both humans and animals, IUGR neonates are reported to have higher perinatal mortality and morbidity associated with low efficiency of food utilization, and permanent stunting effects on postnatal growth and development (15) and thus, is a major problem in human medicine (16) and animal production (15). In pigs, 15–25% of newborns weigh 1.1 kg or less compared with a normal birth weight of 1.4 kg (15,17,18): thus IUGR is a greater problem in pigs than in any other domestic mammal. Again, the pig has similar dental characteristics, renal morphology and physiology, eye structure and visual acuity, skin morphology and physiology, cardiovascular anatomy and physiology, and digestive anatomy and physiology to human (21). Moreover, the structure and function of their developing brain

resembles those of human infants (22).

In some previous studies it has been shown that, IUGR piglets had a longer and thinner small intestine (SI) with reduced villous height and modified mucosal immune response (23) (24). IUGR also decreased the levels of proteins (immunoglobulins and annexin A1) that regulate immune function and there was evidence of cellular signalling defects, redox imbalance, reduced protein synthesis, and enhanced proteolysis which were considered to be the key mechanisms underlying abnormal absorption and metabolism of nutrients, as well as reduced growth and impaired development of the small intestine, liver, and muscle in IUGR neonates (25). Recently, (26) has described that dietary LF (a sialylated, iron-binding, glycoprotein with antioxidant activity) supplementation has a beneficial effect on gut maturation and immunity. Although Lf has multifunctional health benefits, its potential role in maternal intervention through improving milk production and reducing the incidence of low birth weight birth piglet remains unknown. The use of LF as a dietary additive to minimise the high 20% mortality of piglets in gilt litters and improve milk production of sow and gilts, represents a major advance in promoting the welfare status of commercial pig production. This improvement in production efficiency means that current levels of pork production can be achieved with less gilts and sows in commercial breeding herds.

2. Methodology

Animals and diet

Sixty domestic gilts (Sus scrofa) of approximately 220 d of age were used. All field work was completed at the PIC Grong Grong Piggery, Grong Grong, NSW. Gilts chosen were of similar age, with slight variations in conformation and body weight (BW) (151.8 ± 1.95 kg). Sixty gilts were allocated randomly to a treatment group (n=30) and a control group (n=30). Gilts were housed in individual stalls once mated successfully. Maternal nutritional intervention of all 60 gilts began 1 d post mating and continued until the end of a 21 d

lactation period. Nutritional intervention involved top feeding 1 g/d of Lf (Tatura Milk Industries Limited) for treatment gilts and top feeding of 1 g/d of casein (Murray Goulburn Co-Operative Co. Limited) for control gilts. An AND HM-200 Electronic Analytical scale (AND, Australia) was used to weigh Lf and casein. Each gilt was feed 2.5 kg of standard dry sow ration once per day.

Monitoring gilt body weight gain

A baseline BW was recorded prior to mating. During the first 21 d of gestation there was limited sample collection and disturbance of the gilts to optimise embryo implantation and survival. Gilt BW was recorded once/month during the first two months of gestation (day 1, 30 and 60). For the following 1.5 months of gestation, BW was recorded every two weeks (day 74, 90 and 104) using a Slater Brecknell VD-1000 Vet Deck Veterinary Scale.

Monitor pregnancy rate

There were three stages in which pregnancy rate of the treatment and control groups was calculated. The initial pregnancy rate was calculated thirty days after the first mating.. Pregnancy was confirmed by ultrasound. The second pregnancy rate was calculated by the number of successful pregnancies confirmed from gilts that had an initial negative pregnancy test, therefore, returned to oestrus and were mated for a second time (unsuccessful pregnant gilts were mated again). The third pregnancy rate was generated from the number of weaned 1st parity sows in each group that had a confirmed first pregnancy. Weaned sows were mated after approximately 134 days of maternal nutritional intervention.

Monitoring the piglets

On farrowing day, the total number of piglets born, total number of piglets born alive, dead piglets and IUGR piglets were recorded. The weight of the whole litter at birth, individual weight of piglets at birth and on day 19 was measured using a digital weighing machine. (Slater Brecknell ElectroSamson

Digital Hand Held Scale) Also piglets were graded based on individual birth body weight into either >1000 g, 1000- 900 g, 900 – 800 g, 800 – 700 g and < 700 g to determine the incidence of IUGR piglets.

Measuring milk production of the gilts

Milk production was measured by isolating the piglets from the sow using a plastic board for 30 minutes after feeding. Piglets were then weighed individually and the plastic board was removed allowing the piglets to suckle. Piglets were allocated 15 minutes to suckle or until suckling was completed. After feeding was completed, piglets were weighed individually and the weight gain of the litter was used as an indication of gilt milk production per suckling episode. Milk production per piglet assessed through piglet live weight changes over a suckling bout (weigh-suckle-weigh) was determined on PND 1, 3, 7 and 21. This procedure was determined twice to allow for accurate determination of gilt milk production. Piglet BW also allowed for the determination of individual piglet and litter growth rate during lactation.

Blood sample collection form the gilts and piglets

Blood samples from all the gilts and 3 piglets from each litter were collected directly from the jugular vein on day 19 of lactation. Each blood sample was collected in serum clot activator tube and EDTA Vacuette (Greiner bio-one, Australia) for serum and plasma, respectively. Separation of serum and plasma was conducted after centrifugation of the blood sample at 3000g for 10 minutes at 40C. Finally, the serum and plasma samples were stored at -800 C until analysis.

ELISA for immune markers

Pig IgM ELISA Kit (Life Diagnostics, Inc. USA), Pig IgG and IgA ELISA Kit (Bethyl Laboratories, Inc. USA) and Pig sIgA ELISA Kit (My Biosource, USA) were used to determine the concentration of different immune markers like Immunoglobulin M (IgM), Immunoglobulin G (IgG), Immunoglobulin A (IgA) and secretory immunoglobulin A (sIgA) in blood serum and fecal samples. Standard, diluents and wash solution was prepared according to the

instruction of the specific ELISA kit. The steps in assay were also performed as per the instruction. Each sample was duplicated in the plate wells. Any sample that had a duplicated absorbance value varying by 10% or greater was repeated. An automated plate washer (ELx50 Washer, BioTek,) was used to wash the plates. A microplate spectrophotometer (SpectraMax, Bio-strategy) was used to measure the optical density of serum and plasma samples at a selected wavelength of 450 nm.

Only those serum samples, which were devoid of any haemolysis were used for analysis. Fecal sIgA concentration of gilts was determined in the samples that were collected in the last month of gestation.

Statistical analysis

Linear mixed models (and binomial generalized mixed models where appropriate) were used to evaluate the effect of Lactoferrin on gilt weight gain during gestation, milk production, piglet growth and development and immunity during lactation. Line/gilt was used as the random effect and group (or group and days where appropriate) were used as a fixed effect. As the data were not balanced, final models were the reduced models (i.e. non-significant terms were removed from final models). Residual plots were used to ensure that the model assumptions were met for all models. The differences between lactoferrin and control groups were considered significant when p value was equal or less than 0.05. The analysis was conducted using GenStat statistical software (17th Edition).

3. Outcomes

1. Gilt body weight during gestation

There was no significant difference ($P>0.05$) in gilt BW gain between the two groups, treatment and control (Figure 1).

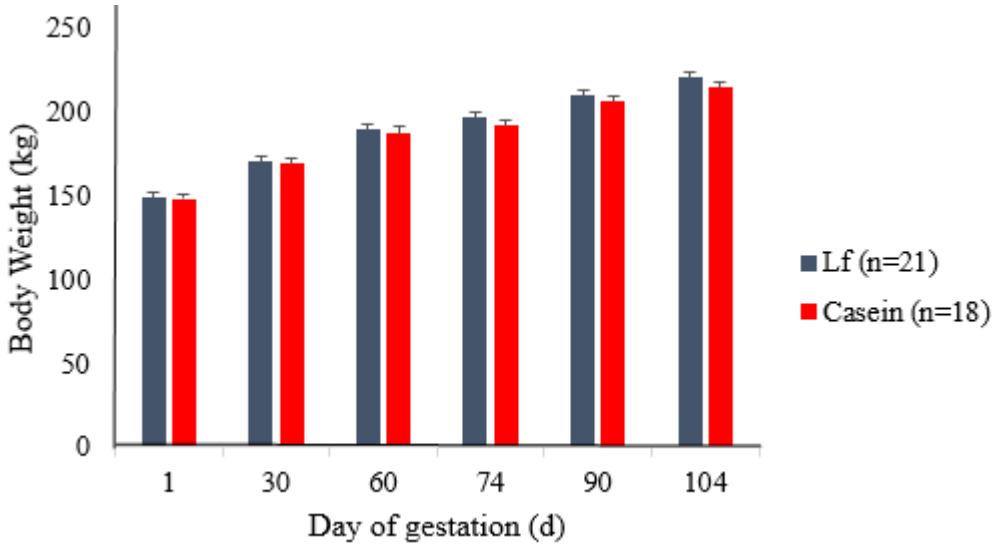

Figure 1. Mean live weight of gilts during Day1, Day 30, Day 60, Day 74 and Day 104 of gestation. Values are mean ± SEM. (LF group n = 21, Control group n = 18).

2. Pregnancy rate

Pregnancy rates were calculated at three stages. The initial pregnancy rate was calculated 30 d after the first mating attempt; therefore at approximately 30 d of gestation. Pregnancy was confirmed by ultrasound. The second pregnancy rate was determined by the percentage of successful pregnancies confirmed from gilts that had returned an initial negative pregnancy test, therefore returned to oestrus and being mated for a second attempt at pregnancy. The final pregnancy rate was generated from the percentage of weaned treatment and control first parity sows that had a confirmed second pregnancy. The results are presented in Table 1

Table 1. Incidence of pregnant rate between treatment and control group.

	Treatment group (Lf)			Control group (casein)		
Time point mating	Total number	Success (no.)	Pregnancy rate	Total number	Success (no.)	Pregnancy rate
First mating attempt	30	21	73%	30	18	60%

Return gilts 2nd mating	9	6	67%	12	7	58%
Weaned treatment sows	21	18	86%	18	14	78%

There was no difference (P>0.05) in pregnancy rates between the two groups, treatment and control.

3. Litter size

There was no difference (P>0.05) in the litter size, the number of born alive, stillborn, total dead and intrauterine growth retardation (IUGR) piglets between the maternal lactoferrin treatment and control groups (Figure 2).

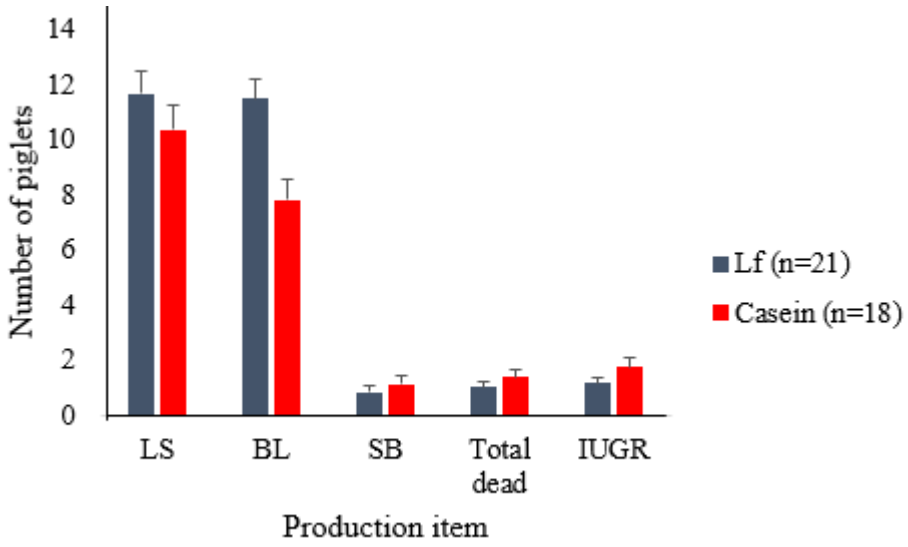

Figure 2. Mean production items during lactation between the two groups, treatment and control. LS: litter size; BL: born alive, SB: stillborn.

4. Incidence of IUGR

Maternal lactoferrin supplementation had a trend reduced the incidence of intrauterine growth restriction (IUGR) piglets, however the different between treatment and control group did not reach statistic significant (P =

0.094, Figure 3), as the number of gilts in each group is relatively small. Further study is clearly need to increase sample size of gilts in each group.

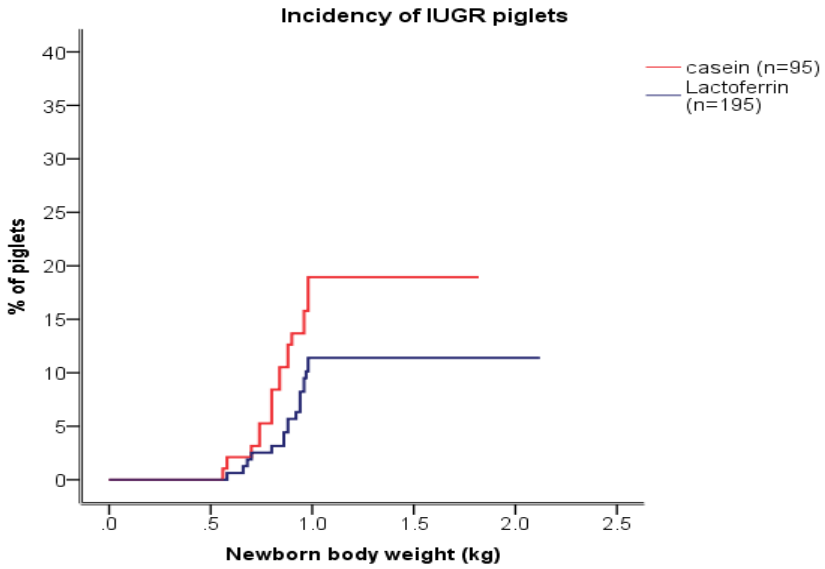

Figure 3. Incidence of intrauterine growth restricted piglets.

5. Piglet body weight

There were no significant difference in body weight of newborn piglets and weaning piglets between their mother gilts, who was supplemented with or without milk throughout gestation and lactation. However, the piglet's body weight gain per day of treatment group (n= 102) was significantly larger than that of the control group (n=73) and marked with * $P<0.05$ (Figure 4).

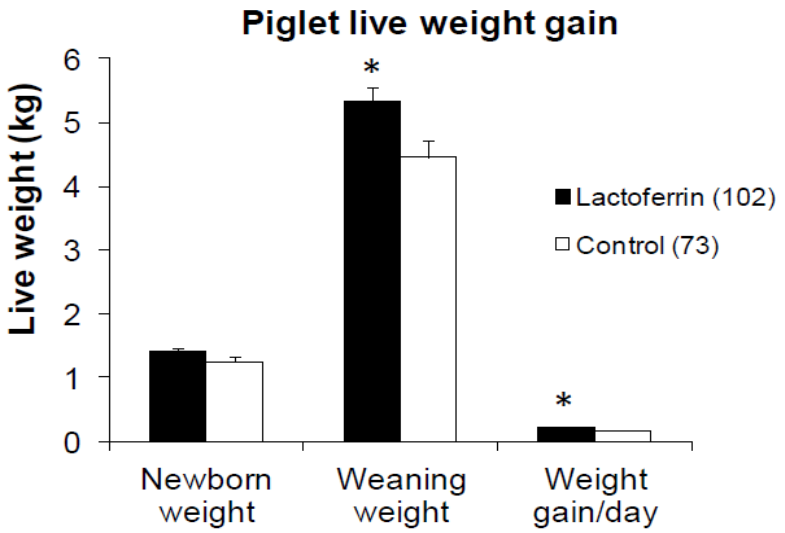

Figure 4. Newborn live weight, weaning weight and weight gain of piglets per day * Significant difference from Control Group ($P < 0.05$). Values are mean ± SEM. (LF group n = 102, Control group n = 73).

6. Milk production

Maternal lactoferrin supplementation in gilts throughout gestation and lactation significantly increased milk production compared with the control at three time points of lactation and marked with *P<0.001 (Figure 5).

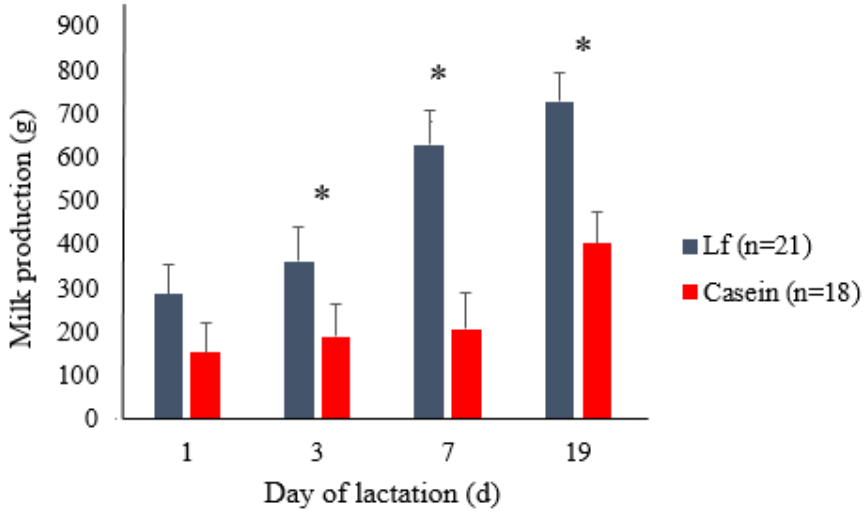

Figure 5. Mean gilt milk production during lactation (Mean +/- SE, $P < 0.001$).

7. Serum immunoglobulin concentration of gilts

Circulating concentrations of IgM, IgG, IgA and sIgA are given in Figure 6. The concentrations of IgM, IgG and sIgA were not significantly different between the treatment and control groups ($p>0.05$). However, a significantly ($p= 0.031$) higher concentration of IgA was found in the circulation of LF treated gilts compared to the control group (Figure 6).

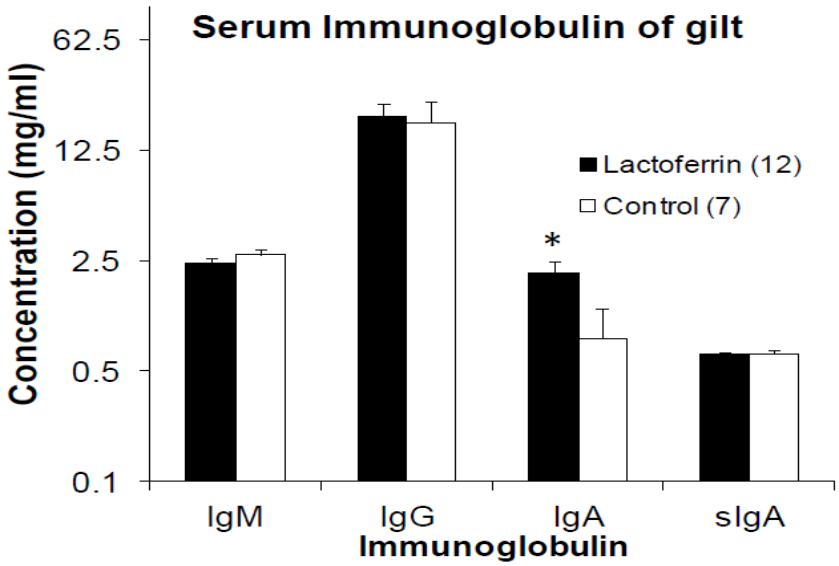

Figure 6: Concentrations of IgM, IgG, IgA and sIgA in the serum sample of gilts collected before weaning * Significant difference with Control Group ($P < 0.05$). Values are mean ± SE. (LF group n = 12, Control group n = 7).

8. Fecal sIgA concentration of gilts

The results of faecal sIgA concentration of gilts were shown in Figure 7. There were not significant different between the two groups ($P>0.05$), (Figure 7).

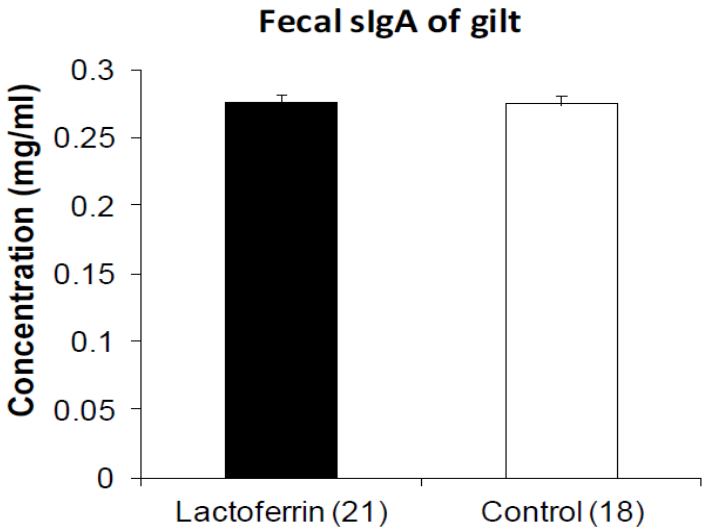

Figure 7. Concentration of Immunoglobulin sIgA in the fecal sample of gilts collected before farrowing. Values are mean ± SEM. (LF group n = 21, Control group n = 18).

9. Serum Immunoglobulin concentration of piglets.

The results of serum immunoglobulin concentration of piglets were shown in Figure 8. A significantly ($p = 0.001$) higher concentration of sIgA was detected in the serum of piglets from the LF treated group relative to the control group By contrast there was no significant difference between the two groups for the concentrations of IgM, IgG, IgA ($p>0.05$) (Figure 8).

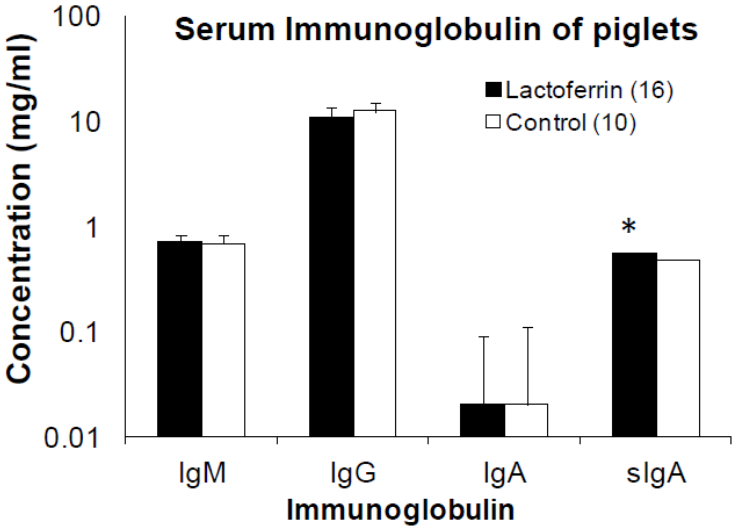

Figure 8. Concentration of Immunoglobulin IgM, IgG, IgA and sIgA in the

serum sample of piglets collected at weaning. * Significant difference with Control Group ($P < 0.05$). Values are mean ± SEM. (LF group n = 16, Control group n = 10).

4. Application of Research

Current study demonstrated that the potential for lactoferrin supplementation to the gilts improve the efficiency of commercial pig production.

Commercialization/Adoption Strategies

Development a functional feeds for pregnant and lactation gilts and sow through the personalized nutrition approaches.

5. Conclusion

Maternal nutritional intervention of lactoferrin in gilts during gestation and lactation:

1. tended to improve piglet body weight gain and therefore helps to optimise piglet growth.
2. significantly increased gilt milk production.
3. tended to increase litter size (through lower incidences of stillborn and mummified piglets) and piglet weaning weight.
4. significantly increased the concentration of IgA in gilt serum and sIgA in piglet serum ($P<0.05$).

In summary, the data obtained from this study have demonstrated that maternal lactoferrin intervention improved milk production, pig production and immunity; however, the underlying molecular mechanisms remained unknown. The results show the potential for lactoferrin supplementation to the gilts improve the efficiency of commercial pig production.

6. Limitations/Risks

This is pilot study of lactofferin intervention trial to improve the concept of the role of lactoferrin improves gilt milk production and pig production. The number of gilt per group is relative small. Further study is need to increase number of gilts or sow per group to confirm this pilot study findings.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. To file in the patent application on maternal lactoferrin supplementation improves milk production.
2. To support a large sample size trial to confirm and refine the pilot study findings
3. To develop a lactoferrin rich feeds for pregnant and lactation sow and gilts.

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Appendix 1 - Notes

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Appendix 1: