Serum lactoferrin concentration of primiparous sow during gestation and lactation, and comparison between sow-fed and formula-fed piglets[1]

Marefa Jahan,[*,†] Nidhish Francis,[†] and Bing Wang[*,†,2]

*Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW 2650, Australia; and †School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2650, Australia

ABSTRACT: Lactoferrin (LF) is a sialylated iron-binding glycoprotein, occurring in several biological secretions like milk, saliva, and seminal fluids and is a major component of a mammalian innate immune system. It plays multiple protective roles against large group of microorganisms and performs anti-inflammatory and anti-cancer activities. The concentration of serum LF in gilt (primiparous sow) and their piglets remains unknown. We determined serum LF concentration in gilts during gestation and lactation to that of 19-d-old piglets, including sow-fed and formula-fed piglets using enzyme-linked immunosorbent assay (ELISA). We found that the concentration of serum LF in gilts varied during gestation (0.77 ± 0.10 µg/mL) and lactation (0.62 ± 0.11 µg/mL). The mean concentration of serum LF in gilts (0.72 ± 0.06 µg/mL) was significantly higher than that of piglets (0.42 ± 0.07 µg/mL, P = 0.004). Additionally, a marginal significant difference (P =0.06) was observed for serum LF concentration in sow-fed piglets (0.42 ± 0.03 µg/mL) at 19 d old compared to that of formula-fed piglets (0.33 ± 0.04 µg/mL) at 37 d old. This study provides noble information regarding the serum LF concentration in the healthy gilts and piglets and thereby the data can be used as a standard reference point for future studies on the role of LF in pig reproduction.

Key words: lactating sow, piglets, pregnant gilt, serum lactoferrin

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INTRODUCTION

Lactoferrin (LF) is an 80 kDa non-heme iron-binding glycoprotein and consists of multiple sialic acid (Sia) residues attached to the N-linked glycan chains. Sias are nine-carbon acidic monosaccharides present usually at the end of sugar chains (glycans) anchored to proteins and lipids on cell surfaces and also on to most of the secreted proteins (Wang and Brand-Miller, 2003; Varki, 2008). Sias have versatile beneficial functions such as promoting neurodevelopment, immune function, and gut maturation in the newborn (Wang, 2009).

Although initially LF was isolated from milk, the subsequent identification and distribution of LF in various tissues suggest that LF is provided with multiple physiological functions. LF is involved in the modulation of immune function by acting as the first line of host's defense response against bacteria, viruses, fungi (Orsi, 2004; Legrand, 2012), and parasites (Yamauchi et al., 2006). Furthermore, LF is also involved in modulation of inflammation (Tsuda et al., 2010),

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[2]Corresponding author: biwang@csu.edu.au

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regulation of iron absorption in the bowel, promoting bone growth, and inhibiting the growth of some type of human cancers (Ward and Conneely, 2004; Naot et al., 2005; Akiyama et al., 2013). It has been demonstrated that the specific structural characteristics of LF allows it to respond to a variety of physiological and environmental cues (González-Chávez et al., 2009). Recently, we demonstrated that milk LF promotes early neurodevelopment and cognition in postnatal piglets by upregulating the brain-derived neurotrophic factor (BDNF) signaling pathway and polysialylation (Chen et al., 2015) and alleviates early weaning diarrhea by upregulating intestinal gene expression of BDNF, UCHL1, and alkaline phosphatase activity (Yang et al., 2014). We also documented that supplementing bovine milk LF in gilts from early gestation until weaning tended to increase number of piglets born alive and increased growth rate of piglets (Jahan et al., 2017). Earlier studies have also demonstrated that supplementation of LF in pre-weaned calves (Joslin et al., 2002; Habing et al., 2017) improved survival rate and growth performance.

Sia residues have a generalized role in determining the survival of glycoproteins in the circulation (Morell et al., 1971). Sias are able to modulate the half-life of glycoproteins in circulation (Ashwell and Harford, 1982; Weigel and Yik, 2002). Thus, if Sia component is missing from the glycoprotein, the underlying sugar gets exposed and triggers signals for this naked glycoprotein to be cleared away from the circulation via endocytosis (Weigel and Yik, 2002). It has previously been shown that dietary supplemented Sia is absorbed from the gastrointestinal tract and 23% of the labeled Sia can cross the blood–brain barrier and taken up by the brain (Wang et al., 2007).

There is little information on normal concentration of LF in pig blood. Farnaud and Evans (2003) reported that LF concentration in human blood is around 1 µg/mL (0.001 g/L), although they can reach as high as 200 µg/mL (0.2 g/L) during an inflammatory response. However, what is the normal serum LF concentration in primiparous sows before their farrowing and weaning, and whether sow milk rich in sialylated oligosaccharides (conjugated Sia) can influence the synthesis and survival of LF protein in the serum is still unknown. Therefore, in the present study, we determined serum LF concentration in primiparous sows collected 4 to 5 d before farrowing and at 19th day of lactation (before weaning), and also in piglets at weaning and at 37 d old which were fed by mother and milk replacer, respectively.

MATERIALS AND METHODS

The experimental protocol was performed in accordance with guidelines established by the National Health and Medical Research Council of Australia and the experimental protocol was approved by the Animal Care and Ethics Committee, Charles Sturt University (protocol number A19034).

Animals and Housing

Fifteen pregnant domestic gilts (*Sus scrofa*) of approximately 220 d old with an average weight of 151.8 ± 15.1 kg were randomly selected from five different breeding lines at the Pig Improvement Company (PIC), Grong Grong Piggery, Grong Grong, NSW, Australia. Breed line 2 were pure bred Large Whites, breed line 3 were pure bred Landrace and breed lines 4, 7, and 9 were pure bred Duroc pigs. Gilts were housed in individual stalls in gestation shed for about 114 ± 1 d and then were transferred to farrowing sheds and kept there until the piglets were weaned (21 d). Pigs were fed a commercial gestation diet of 2.4 kg/d from mating to the end of gestation, and then a lactation diet of 5.8 kg/d over lactation until weaning. All animals were provided with water ad libitum. Sow-fed piglets (n = 20) were randomly selected from the litters of these experimental gilts.

Fourteen male domestic piglets (*S. scrofa*, Landrace and Large White cross) were collected from the same source at 3 d of age, weighing approximately 2 kg, from different litters. Piglets were housed in pairs in a home pen at a temperature-controlled and humidity monitored room until postnatal day 37. The piglets were fed a commercial piglet milk replacer (Wombaroo Food Products, Glen Osmond, Australia) from 3 to 37 d of age.

Blood Sample Collection

About 2–3 mL of blood samples were collected from the jugular vein of gilts at 5 to 7 d pre-labor and at 19th day of lactation before weaning by restraining the gilts using a nose noose. Blood samples from piglets were also collected from jugular vein at the end of feeding trial at 37 d of age. Each blood sample was collected in a serum clot activator tube (Greiner Bio-One, Australia) and centrifuged at 3,000 × g for 10 min at 4 °C. The serum samples were collected and stored at −80 °C pending analysis.

Enzyme-Linked Immunosorbent Assay

The serum LF concentration was measured using pig-specific LF enzyme-linked immunosorbent

assay (ELISA) kit according to manufacturer's instructions (Cusabio Technology LLC, Cat No. CSB-E16230p). The detection range of the kit was 0.156 to 10 µg/mL. An automated plate washer (ELx50 Washer, Biotek) was used to wash the plates during the procedure. The optical density of the samples was measured at 450 nm using a microplate spectrophotometer (SpectraMax, Bio-Strategy). Results were calculated following the manufacturer's instructions. All serum samples used in the study were confirmed to be free from any hemolysis.

Statistical Analysis

Linear mixed models were used to determine the differences in the LF concentration between gilts and piglets; prior farrowing and prior weaning gilts; and formula-fed and sow-fed piglets. Gilts/piglets, time point (prior farrowing and prior weaning), and group (formula fed, sow fed) were specified as the fixed effects, respectively, and animal ID was specified as a random effect. Age was used as a covariate while comparing the serum LF concentration between sow-fed and formula-fed piglets. Residual plots were used to ensure that the model assumptions were met for all models. The differences were considered significant when a P value was less than 0.05. Data were expressed as means ± SEM. The analysis was conducted using GenStat statistical software (17th edition).

RESULTS

Serum Lactoferrin Concentration in Gilts and Piglets

The mean concentration of serum LF in gilts was 0.77 ± 0.10 µg/mL at 5 to 7 d before farrowing and then decreased to 0.62 ± 0.11 µg/mL at 2 d before weaning, respectively (Fig. 1). The concentration of serum LF in the prior farrowing gilts was about 11% higher than that of prior weaning gilts.

The comparison of overall serum LF concentration in gilts prior to farrowing and weaning with that of sow-fed piglets at postnatal day 19 are shown in Fig. 2. The average serum LF concentration in gilts (0.72 ± 0.06 µg/mL) was about 26% higher than the piglets (0.42 ± 0.07 µg/mL) and the difference between the two groups was statistically significant (P = 0.004).

Comparison of Serum Lactoferrin Concentration Between Sow-Fed and Formula-Fed Piglets

The concentration of serum LF between the sow-fed and formula-fed piglets was shown in Fig. 3. Although there was no significant difference between these two groups (P = 0.06), the sow-fed piglets had 12% higher concentration of LF than that of the formula-fed piglets.

The percentage of serum LF in sow-fed and formula-fed piglets relative to prior farrowing gilts was observed to be 54.80% and 53.52%, respectively (Fig. 4A). Furthermore, in comparison to the prior weaning gilts, the percentage of serum LF in sow-fed and formula-fed piglets was 68% and 54%, respectively (Fig. 4B).

DISCUSSION

LF is widely present in various secretory fluids including blood. This study initially aimed to evaluate the average concentration of LF in the serum of gilts and piglets. Bovine milk LF as a functional dietary supplement in food products has been approved by FDA (US) and European Food Safety Authority (Gaynor and Gaynor, 2015). Our early study showed that dietary LF supplementation to gilts during gestation and lactation improves

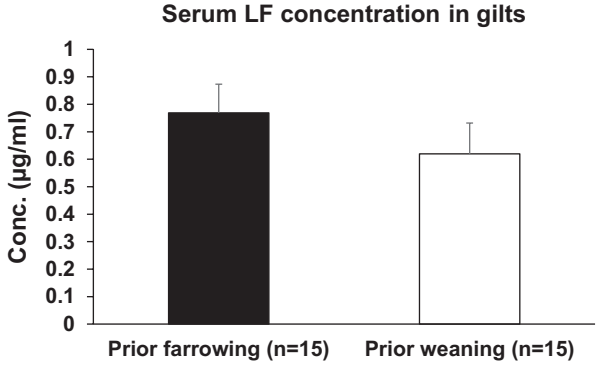

Figure 1. Serum lactoferrin concentration of gilts during gestation (5–7 d prior farrowing) (n = 15) and lactation (2 d prior weaning/19th day of lactation) (n = 15). Data are presented as mean ± SEM.

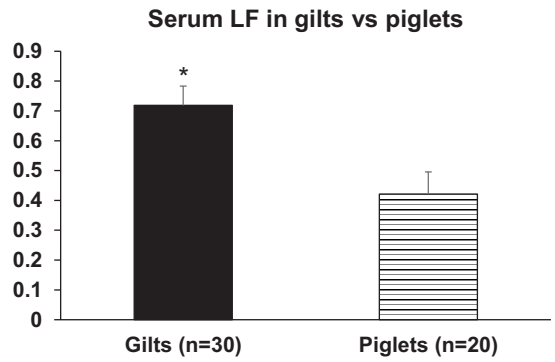

Figure 2. Comparison of mean serum lactoferrin concentration of gilts (n = 30) during gestation (5–7 d prior farrowing) and lactation (2 d prior weaning/19th day of lactation) with that of sow fed piglets (n = 20) at 19 d old prior to weaning. *P-value <0.05 between gilts and piglets. Data are presented as mean ± SEM.

pig production and immunity. However, the normal concentration of LF in serum for the healthy gilts during gestation and lactation and their piglets remained unknown. In the present study, we demonstrated that the overall mean concentration of serum LF in the gilts (primiparous sows) was 26% higher than that of piglets. In humans, the concentrations of serum LF increase during some inflammatory or infectious diseases (Scott, 1989a; Thomas et al., 2002; Legrand et al., 2004). In the current study, all pregnant gilts and piglets were healthy. However, it is not surprising that serum LF concentration in piglet was slightly lower than the pregnant gilts, as relatively lower serum LF in the piglets might contribute to a higher incidence of infectious diseases during early life observed in the pig industry. Many studies have already been reported that patients with LF deficiency have a significantly higher incidence of bacterial infections as compared to individuals with adequate LF concentrations (Barton et al., 2006). The concentration of serum LF between gilts and piglets was not significantly different similar to the studies on serum LF in other species. A study by Barton et al. (2006) reported that the serum LF concentration in 1- to 3-d-old healthy foals (0.249 ± 156 µg/mL) were not significantly different from healthy adult horses (0.559 ± 230 µg/mL). Investigations on 3-mo-old calves estimated the serum LF concentration to be 1.168 ± 0.181 µg/mL (Tóthová et al., 2016). The serum LF concentrations in normal healthy children (up to 8 yr of age) and adults (21 to 63 yr of age) were 0.08 to 0.57 and 0.19 to 0.81 µg/mL, respectively (Venge et al., 1984). Another study on infants from 24 to 36 wk of age reported that serum LF concentration ranged from 0.2 to 0.8 µg/mL (Scott, 1989b). Our results imply that serum LF is an essential glycoprotein for both gilts and piglets. To our knowledge, the findings of serum LF concentrations in healthy gilts and piglets have not been previously reported.

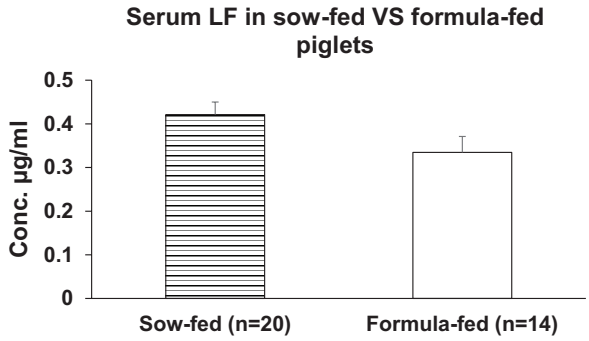

Figure 3. Comparison of serum lactoferrin concentration between sow-fed (n = 20) piglets at 19 d old and formula-fed (n = 14) piglets at 37 d old. Age used as a covariate during statistical analysis. Data are presented as mean ± SEM.

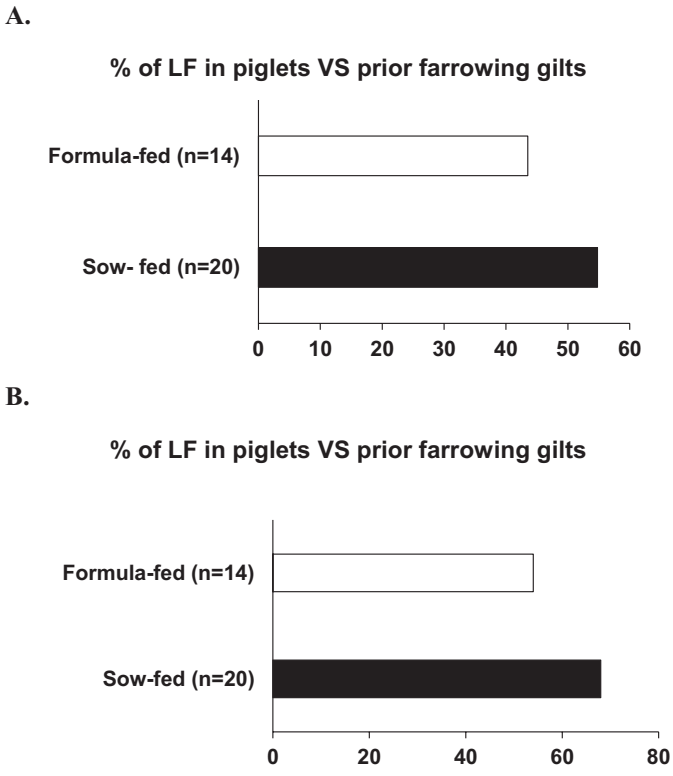

Figure 4. (A) Comparison of the percentage of lactoferrin in sow-fed (n = 20) and formula-fed (n = 14) piglets in relation to the lactoferrin concentration of the prior farrowing gilts (n = 15). (B) Comparison of the percentage of LF in lactoferrin in sow-fed (n = 20) and formula-fed (n = 14) piglets in relation to the lactoferrin concentration of the prior weaning gilts (n = 15).

Yang et al. (2000) determined the serum LF concentration on multiparous sows (parity 3–4) before farrowing and during 28 d of lactation. The mean serum LF concentrations of multiparous sows before farrowing and before weaning reported in that study were 19 and 22 µg/mL, respectively. These data differ from what we observed in this study with mean serum LF concentration of 0.769 ± 0.104 µg/mL for gilts before farrowing and 0.620 ± 0.112 µg/mL for gilts before weaning. Although not directly comparable due to differences in the parity of sows in these two investigations and different methods for the serum LF determination, this is the only available literature to date to make a comparison within species. It is worth mentioning that the serum LF concentration determined in our study is in line with the mean LF values determined in other species such as foals, adult horses, calves, infants, and adult humans (Venge et al., 1984; Barton et al., 2006; Tóthová et al., 2016). Besides parity, there are various other factors such as age, breed, environmental factors, management practices, and sampling method that can heavily influence clinical blood analysis in pigs (Wilson et al., 1972). In addition, the study undertaken by Yang et al. (2000) also acknowledged that they obtained relatively higher values for LF concentration in all their milk samples when compared to the previously published data on milk LF levels in pigs (Elliot et al., 1984). With the same ELISA method used for both milk and serum LF analysis in their study, it is highly likely that the LF concentration determined in the serum of these multiparous sows may also be an overestimate.

We further analyzed if the concentration of serum LF has physiological influences in the outcome of farrowing and lactating gilt. The LF concentration in the serum samples of gilts prior farrowing was about 11% higher than that of before weaning group (Fig. 2). This is not surprising given the fact that LF plays multiple critical roles in modulating the immune defense mechanisms as reported previously (Levay and Viljoen, 1995; Adlerova et al., 2008; Rosa et al., 2017). Pregnancy brings in various physiological changes within an animal's body and as these pregnant animals prepare themselves for parturition, it is imperative that these animals will modulate their immune defense system to prevent any potential invading pathogens. Of the several ways to modulate the immune defense mechanisms prior to farrowing, increasing the LF concentration may just be one normal body's responses. With multiple roles for LF including antimicrobial, immune defense systems, anti-inflammatory, and neurotropic activity, it is important that an animal maintains normal levels of LF for a better reproductive performance.

Taken together, this study determined the serum LF concentration in piglets and gilts at two different physiological namely, 5–7 d prior farrowing and 2 d before weaning that is a critical stage for pig production. This is the first ever study that determined the values of serum LF concentration in healthy gilts and piglets and thereby results from this report will be used as a standard reference point for any future studies on the role of LF in pig reproduction.

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