The Pennsylvania State University

The Graduate School

Department of Dairy and Animal Science

SOW AND PIGLET RESPONSES TO ENDOTOXIN-

INDUCED MASTITIS

A Thesis in

Animal Science

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2004

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ABSTRACT

Mastitis is a common contributing factor to the relatively high pre-weaning

mortality rate and suboptimal sow-reared piglet growth performance experienced in pork

production. An endotoxin challenge model of mastitis was used to determine its effects

on sow milk composition, milk yield, and the growth of nursing piglets. Caseins (β-

casein in particular), lactoferrin, and immunoglobulins, as determined by SDS-PAGE and

ELISA, were high in colostrum, and levels decreased to mature levels by about day four

of a normal lactation. As mammary secretions changed from colostrum to milk,

decreasing levels of chloride and albumin provided evidence for the closure of tight

junctions by about day four of a normal lactation. Following endotoxin infusion to

induce mammary inflammation, sow rectal temperatures peaked at 1.1-1.7° C above

baseline by five hours post-infusion, and gradually recovered within 12-24 h. Milk TNF-

α was increased 30-fold by five hours post-infusion (serum TNF-α appeared to increase);

both recovered by 12 h. Milk total protein (20 %), albumin (44 %), and chloride (two-

fold) were increased, and milk α_s- and β-casein (45 %) were decreased post-infusion;

milk protein composition was altered for at least 48-60 h. It did not appear that

lactoferrin in milk responded to intramammary endotoxin infusion. Hourly milk yields of

infused mammary glands were decreased 16-31 % relative to control glands and the ADG

of piglets nursing infused glands were decreased 44-74 % relative to littermates nursing

control glands on the day of endotoxin infusion. The ADG of piglets nursing infused

glands remained decreased relative to littermates nursing control glands for at least five

days post-infusion. The apparent conversion of milk to live weight gain of pigs nursing infused glands was decreased relative to those nursing control glands. Intramammary endotoxin infusion in the sow alters milk composition and depresses piglet growth performance for at least three to five days after the resolution of clinical signs of mammary inflammation. This research demonstrates the impact of a mild inflammatory episode on pig production, and highlights the need for supplemental feeding of piglets at risk.

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LIST OF ABBREVIATIONS AND SYMBOLS

~ .... about
° .... degree
> .... greater than
≥ .... greater than or equal to
< .... less than
≤ .... less than or equal to
' .... minutes
% .... percent
± .... plus or minus
APCs .... antigen presenting cells
APP .... agalactia post-partum
APS .... ammonium persulfate
A.U. .... absorbance unit
BPI .... bactericidal/permeability-increasing protein
BSA .... bovine serum albumin
BW .... body weight
Ca^{2+} .... ionic calcium
C .... Celsius
CD .... cluster of differentiation
CFU .... colony forming units
Cl^- .... ionic chloride
$CuSO_4$ .... copper sulfate
c.v. .... coefficient of variation
d. .... day
DAB .... 3,3'-diaminobenzidine
DHIA .... Dairy Herd Improvement Association
DNA .... deoxyribonucleic acid
DWG .... daily weight gains
EGF .... epidermal growth factor
ELISA .... enzyme-linked immunosorbent assay
g. .... grams
ga. .... gauge
h. .... hours
HCl .... hydrochloric acid
HDL .... high-density lipoprotein
HRP .... horseradish peroxidase
H_2SO_4 .... sulfuric acid
IgA .... immunoglobulin A
IGF-I .... insulin-like growth factor I
IGF-II .... insulin-like growth factor II
IgG .... immunoglobulin G
IgG1 .... immunoglobulin G class 1

IgG2 .... immunoglobulin G class 2
IgM .... immunoglobulin M
IL-1 .... interleukin-1
IL-6 .... interleukin-6
IL-8 .... interleukin-8
i.m. .... intramuscular
i.p. .... intraperitoneal
I.U. .... international units
i.v. .... intraveneously
k. .... kilo or thousand
K^+ .... ionic potassium
KCl .... potassium chloride
kDa .... kilodaltons
kg .... kilogram
KH_2PO_4 .... potassium phosphate
$KMnO_4$ .... potassium permanganate
l .... liter
LBP .... lipopolysaccharide binding protein
LCT .... lower critical temperature
LDL .... low-density lipoprotein
Lf .... lactoferrin
LPS .... lipopolysaccharide
M .... molar
mA .... milliamps
mg .... milligrams
Mg^{2+} .... ionic magnesium
MHC .... major histocompatibility complex
ml .... milliliter
mM .... millimolar
MMA .... mastitis-metritis-agalactia
ms .... milliseconds
MW .... molecular weight
n. .... number
N. .... normal
Na^+ .... ionic sodium
NaCl .... sodium chloride
Na_2CO_3 .... sodium carbonate
Na_2HPO_4 .... sodium phosphate, dibasic
NaN_3 .... sodium azide
NaOH .... sodium hydroxide
ng .... nanogram
NH_4HCO_3 ....ammonium carbonate
nm .... nanometer
NRC .... United States National Research Council

P .... probability
pBC .... porcine β-casein
PBS .... phosphate buffered saline
pg.. picograms
PGE$_2$.. prostaglandin E$_2$
PHS .... porcine hypogalactia syndrome
pIgG .... porcine immunoglobulin G
pLf.. porcine lactoferrin
PMN... polymorphonuclear
pSA .... porcine serum albumin
PVC.. polyvinylchloride
PVDF .... polyvinylidene difluoride
pWC .... porcine whole casein
rBST... recombinant bovine somatotropin
RIA... radioimmunoassay
RNA .... ribonucleic acid
rPST .... recombinant porcine somatotropin
s.. seconds
SAS .... Statistical Analysis Software^{TM}
SCC .... somatic cell count
s.d. .... standard deviation
SDS .... sodium dodecyl sulfate
SDS-PAGE .... sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SNF .... solids not fat
SPF.. specific pathogen free
SPI.. sow productivity index
TCA.. trichloroacetate
TEMED...N,N,N',N'-tetramethylethylenediamine
TGF-α .... transforming growth factor α
TM.. trademark
TMB... 3,3,5,5'-tetramethylethylenediamine
TNF-α .... tumor necrosis factor α
TPB .... tryptose-phosphate-broth
TTBS...tween-tris buffered saline
Tween-20 .... polyoxyethylene (20) sorbitan monolaurate
TZ..thermoneutral zone
μg .... microgram
μl .... microliter
U.S. .... United States
USDA... United States Department of Agriculture
V.. volts
vs. .... versus
WSW.. weigh-suckle-weigh
X... times

ACKNOWLEDGEMENTS

with our "hobby farm" and in 4-H with our cattle and sheep under their guidance impacted me deeply, and have played a large role in making me the person that I am. In that area I also need to thank the members of the Clarke County and Virginia agricultural communities that helped to mold me growing up. I hope to be able to maintain our family's tradition of raising livestock, and am glad that I have had the opportunity to make my career in animal agriculture.

And he gave it for his opinion,
that whoever could make two ears of corn,
or two blades of grass,
to grow upon a spot of ground where only one grew before,
would deserve better of mankind,
and do more essential service to his country,
than the whole race of politicians put together.

-Jonathan Swift

Chapter I

INTRODUCTION

One of the biological limits to maximal pork production is sow milk production.
Even in the case of an average, healthy lactation, milk nutrient delivery is inadequate to
support the genetic growth potential of the modern piglet (Boyd et al., 1985; Harrell et al.,
1993). As a result, creep feeding programs have been developed in the modern pork
production setting to supplement nutrient delivery to the suckling litter in order to
improve growth performance from birth to weaning. In turn, this improved preweaning
growth performance reduces the time required during the postweaning phase to reach
slaughter weight, and therefore improves the profitability of pork production (Mahan and
Lepine, 1991; Bruininx et al., 2002).

Lactation failure is common in lactating sows, and undermines this effort by
reducing nutrient delivery from sow to piglet through milk during the preweaning phase
(Persson et al., 1989; 1996). It is unknown if piglets compensate for reduced nutrient
delivery by the sow's milk during lactation failure through increasing their consumption
of creep feed. Mastitis is one factor implicated in lactation failure, a condition ranging
from inadequate to a complete lack of milk production that severely compromises the
survivability and growth performance of the suckling piglet. By better understanding
mastitis in the sow, animal scientists may be able to reduce its prevalence and/or impact
upon the sow and suckling litter, thereby improving both animal welfare and the

profitability of pork production. Improved milk production by the sow could conceivably reduce or obviate the need for nutritional supplementation of the suckling litter, and eliminate the cost associated with creep feeding. Alternatively, improved milk production by the sow along with creep feeding could synergistically improve preweaning piglet growth performance by increasing the size of the piglet first consuming feed, and therefore having a greater feed intake.

Preweaning growth of suckling piglets is significantly reduced by mastitis in the lactating sow, thereby reducing post-weaning piglet growth performance and in turn, the overall profitability of pork production (Curtis, 1974; Dyck et al., 1987). The mechanisms of this reduced preweaning growth performance are not well-defined in pigs, but are likely related to reduced milk yield and milk nutritional value to the suckling piglet. Milk yield is known to be reduced, and milk composition altered in dairy cattle in response to mastitis (Carrol and Jain, 1969; Shuster et al., 1991), but less is known about the effects of mastitis on milk composition and yield in the sow. Milk yield and composition is routinely determined in dairy cattle through Dairy Herd Improvement Association (DHIA) programs, and can in turn be correlated to mastitis incidence and severity; such is not the case in pork production. Instead, the effects of mastitis in the sow on milk yield and composition are reflected in the growth performance of the suckling piglet from birth to weaning. The analysis of piglet preweaning growth performance is complicated by the health status of the piglet, controlled not only by the amount and nutritional value of the milk a suckling piglet consumes, but also by the sum total of environmental challenges the piglet faces. Milk is but one route by which the

piglet is protected against these challenges, and suckling piglets are commonly

supplemented with feed during the nursing period to improve preweaning growth

performance, thereby complicating the determination of the effects of mastitis on pork

production. The studies presented here were designed to determine the direct effects of

mastitis in the sow on the growth performance of the suckling litter, as influenced by sow

milk yield and composition. An intramammary endotoxin challenge model of mastitis

(Kensinger et al., 1999) was utilized at differing time points of a typical sow lactation

(with no supplemental feed to the piglets) to accomplish this goal. The resultant changes

in milk composition and yield were determined and related to piglet growth performance.

The overall objectives were:

1) To develop an ELISA method for quantifying β-casein in sow's milk.

2) To describe changes in total protein, β-casein, albumin, free chloride, and
lactoferrin in normal sow's colostrum and milk over the course of a typical
three week lactation.

3) To describe changes in sow rectal temperature and plasma TNF-α, and
changes in total protein, β-casein, albumin, free chloride, lactoferrin, and
TNF-α in milk from inflamed sow mammary glands following endotoxin
challenge.

4) To determine the effects of endotoxin-induced intramammary inflammation
    on hourly milk yield and the resultant growth performance of the suckling
    piglets.

The main hypotheses tested were:

1) Intramammary endotoxin challenge provokes localized mammary
    inflammation and systemic responses as measured by sow disposition, feed
    and water intake, plasma TNF-α, and rectal temperature in first-parity sows.

2) Porcine milk total protein, β-casein, albumin, chloride, lactoferrin, and TNF-α
    concentrations are significantly changed by intramammary inflammation.

3) Mean hourly milk yield is significantly reduced in inflamed mammary glands.

4) The growth rate of piglets suckling endotoxin treated glands is reduced
    relative to littermates suckling control glands.

Chapter II

LITERATURE REVIEW

Domestication of the pig

Wild pigs are typically organized socially into groups of sows and solitary boars that only intermingle for breeding purposes (Mauget, 1981). Groups generally consist of four or fewer sows with that year's litter, and yearling animals loosely associated with the group (Mauget, 1981). Individual piglets are cared for by all sows in the group, and cross-suckling is common (Mauget, 1981). Group membership is fluid, with new members joining the group easily, and multiple litters are farrowed and reared together (Mauget, 1981).

When domestic pigs are allowed a group housing environment, sows will still demonstrate the behaviors of separation from the group approximately one to two days prior to farrowing and nest building (Jensen 1986; Jensen et al., 1987). The sow and litter will stay in the farrowing nest for about ten days before rejoining the group (Jensen, 1986; Jensen and Redbo, 1987; Stangel and Jensen, 1991), and lactation will last 14-17 weeks if weaning is not imposed at an earlier age (Jensen, 1986).

Domestication began sometime during the Neolithic period (~ 8,000 years ago), but the Greeks and the Romans were the first societies to begin to purposely breed pigs. Two types of pig were developed for either the purpose of sport (wild type) or for food

production (domestic type) (Baxter, 1984). One of the first descriptions of organized pig production came from the Roman Varro, reporting a common herd size of 100-150 head, farrowing an ideal litter of less than eight piglets per sow, with weaning at eight weeks. The pig sty was described as an enclosure three feet square and three feet tall with a four-inch threshold to keep the young piglets in (Ash, 1934, as cited by Baxter, 1984).

Evolution of the U.S. pork industry

The current United States hog inventory is approximately 60 million head, with approximately six million head being kept for breeding, and 54 million intended for market (USDA, 2003b), with an estimated value of $4.2 billion (USDA, 2003a). These numbers are a substantial decrease below the record inventory of 83.7 million head seen in 1944 (USDA, 2003c). The United States ranks third for world hog population numbers behind China (336 million) and the European Union (123 million) (USDA, 2003a). The United States hog industry is centered in the Midwestern grain belt; the top ten states for hog numbers are, in order, Iowa, North Carolina, Minnesota, Illinois, Indiana, Missouri, Nebraska, Oklahoma, Kansas, and Ohio (USDA, 2003c). These ten states produce 84 % of the hogs and 83 % of the income from hog operations (USDA, 2003c). There are approximately 75,000 hog operations nationwide, but production is biased toward the larger operations; approximately 94 % of hogs are located on operations with > 500 head, a number that encompasses only 25 % of operations (USDA, 2003a). A more dramatic indication of the current concentration of ownership is revealed

by observing operations that house > 5,000 head; comprising just three percent of operations, these operations control 53 % of U.S. hogs (USDA, 2003a). Approximately 100 million hogs were slaughtered in the U.S. in 2002, at an average live weight of 265 pounds, and a dressed weight of 197 pounds. This resulted in 19.7 billion pounds of pork, for which producers received approximately $11.4 billion (USDA, 2003a, c).

It is commonly believed that small scale pig production on general purpose farms has been the norm, and that no other form occurred until the "industrialization" of pork production over the last 20-30 years, as demonstrated by the large commercial operations typical of the current pork industry. While it is true that the modern-style of hog farming is largely a recent development, Middleton reported versions of intensive pig feeding buildings in England during the late 18th century (as cited by Baxter, 1984).

Modern hog farming has changed and progressed tremendously in recent decades. Industrialization of the process has led to a concentration of ownership, an increase in operation size, and productivity. The pace of change in the United States has been particularly dramatic over the last decade. From 1994-2001 the number of operations with > 5,000 head increased from just fewer than 1,000 to 2,200 and the share of the pig crop represented by these farms increased from 27 to 75 % (USDA, 2002). During the same time, the number of operations with < 5,000 head declined by two-thirds, from 217,000 to 79,000; a major factor in this reduction was a period of historically low hog prices that forced less economically efficient operations out of business (USDA, 2002). The larger operations were able to minimize their per unit cost of production and thereby be more efficient, allowing themselves to remain in business while smaller, higher cost

per unit operations were unable to. An additional factor was the ability of larger farms to more effectively manage risk by the advance contracting of market prices and feed costs to ensure a target income.

Swine production has become much more efficient in recent years. For the time period 1979-2001, the number of pigs/year/breeding herd animal has increased by 57 % from 10.3 to 16.2. This increase was due in part to increased litter size (29 % of the increase), but mainly to increased litters/sow/year (71 % of the increase) (USDA 2002). Larger operations (> 5,000 head) were generally more efficient than smaller (< 5,000 head), as indicated by differences in pigs/litter (8.94 vs. 8.49) and pigs/year/breeding herd animal (16.59 vs. 15.05) (USDA, 2002). The smallest operations were the least efficient, with farms of 1-99 head having a pigs/litter size of just over 7.5 (USDA, 2002).

Increased hog operation sizes and "industrialization" of hog farming have led critics to question the effects of these changes on animal welfare. To attempt to address these concerns, group housing situations have been developed in Sweden: the sow and litter are housed as one unit from shortly before farrowing until the second week of lactation, at which point the sow and litter are returned to a group of sows and litters until weaning (Hultén et al., 1995). This comes with certain decreases in economic efficiency, as overall productivity and reproductive performance are decreased, a situation that becomes worse as sows become older (Hultén et al., 1997, 1998). Group housing has been shown to reduce teat and under-skin wounds at weaning (5-6 weeks), but there is an increase in the number of non-productive glands at the time of weaning, despite no difference in mastitis rates (Hultén et al., 1995). There is also an increase in skin wounds

on the rest of the body, due to fighting while establishing dominance hierarchies (Hultén et al., 1995). Group housed sows have smaller litters, and a lower percentage mated at ten days post-weaning; the difference is greatest for older sows (Hultén et al., 1998). Repeat breeding is similar between group and intensively housed sows for primiparous animals, but the frequency increases for older sows (Hultén et al., 1998). Additionally, pre-weaning mortality in the litters of older sows is increased, though not in the litters of primiparous sows (Hultén et al., 1997). There is also greater variation in pre-weaning mortality for litters of sows maintained in a group-housing situation as compared to conventional housing (Hultén et al., 1997).

Other researchers in western Texas have experimented with outdoor housing and deep-bedding systems as alternatives to confinement housing. Pigs in these production settings had higher growth rates and carcass weights than did conventionally housed pigs, with no difference in carcass quality (Gentry et al., 2002). Litters housed outdoors with their sow showed increases in behavioral activities such as time walking, time in play, and amount of nursing behavior, but production measures such as farrowing rate and litter size did not differ (Johnson et al., 2001; McGlone and Fullwood, 2001). These alternative systems of pork production show some promise for improving animal welfare, but may not be economically viable under current conditions.

Behavior of the sow and litter

Although nursing is a cooperative effort between the sow and the litter, nursing behavior is largely dictated by the sow. Using the findings of Barber et al. (1955), Gill and Thompson (1956), and Whittemore and Fraser (1974), the typical nursing episode can be described as follows. An initial phase involves the gathering of the litter around the sow, often preceded by the sow calling the litter to her by a series of grunts, during which the sow lies on her side. Assuming that nursing order has already been established through assertion of individual dominance, the piglets settle at their preferred teat(s). Otherwise, there will be an active fight for position, after which piglets begin a vigorous massage and slow sucking of their preferred teat for a period lasting approximately 85 s (Barber et al., 1955). Active milk ejection follows for a very short period (10-20 s on average), and is usually associated with an increase in sow grunting frequency to twice per second, a reliable indicator as to whether or not milk ejection has occurred (Whittemore and Fraser, 1974). Lastly, piglets continue to nuzzle and massage the teat until falling asleep, leaving the sow, or until the sow ends the nursing episode by rolling over and/or rising. Milk yield tends to be correlated to the total time spent in massage, and non-nutritive sucklings are common (Braude, 1954; Gill and Thompson, 1956; Whittemore and Fraser, 1974; personal observation).

It has long been known that piglets select a preferred teat relatively early in lactation, and aggressively defend the teat from littermates (McBride et al., 1965, Hartsock and Graves, 1976). In 1578, Heresbach (as cited by Baxter, 1984) observed that

a piglet "knows the teat that it was born to and it will suck no other, so much so, that if you remove the piglet the teat will go dry." Schmidt and Lauprecht (1926), Ohligmacher (1928), Hempel (1928), Bonsma and Oosthuizen (1935), Donald (1937) and Albig (1939) all agreed that "litter order" was established within the first week of lactation (all cited by Barber et al., 1955).

Pre-weaning mortality

Pre-weaning mortality is high in the pig as compared to other livestock species, with between 8-20 % of live births not surviving to weaning (Straw et al., 1998; Cutler et al., 1999). The most recent PigChamp Breeding Herd summary for 2002 indicated that the national herd average preweaning mortality was 13.12 % for the U.S. and 11.57 % for Canada (PigChamp, 2002). The majority of piglet deaths occur in the first four days following parturition, with a high proportion of these deaths (up to 60 % or more) directly attributable to the sow (Fahmy and Bernard, 1971; English and Morrison, 1984; Cutler et al., 1999). The sow is often responsible through either failing to provide an adequate milk supply, or through direct actions such as crushing and/or savaging of the piglet (English et al., 1977; Dyck and Swierstra, 1987; Prime et al., 1987). Fahmy and Bernard (1971) estimated that 19.2, 14.2, and 10.2 % of deaths were attributable to the sow: through crushing or savaging, scours, and paralysis, respectively.   English and Morrison (1984) estimated in a review of the literature that 73.7-79 % of postnatal mortality was from starvation (hypoglycemia being the ultimate cause of death) or crushing by the sow.

The mortality of an individual piglet often results from failure of the teat (and it attendant glands) it is nursing to provide an adequate supply of milk to facilitate its survival. The individually affected piglet in a large litter is faced with the proposition of its teat not providing milk, and having no other teat available due to competition from littermates. The piglet becomes weaker and less able to fight for a functional teat as a result, and can die through starvation if intervention is not quickly taken by the farrowing room manager to provide milk replacer, or to move it another sow with an available, functional teat.

The anatomy of the sow's udder also plays a role in piglet survival, with at least seven pairs of functional teats being considered ideal. Each teat contains two separate teat ducts and related mammary glands, an observation first made by Cooper in 1840 (Hartmann et al., 1984). The average litter consists of 10-12 piglets, and 14 teats are typically adequate, unless multiple glands fail to provide milk. The more anterior teats are preferable in terms of milk yield (Dyck et al., 1987), and are usually claimed by the largest piglets in the litter. Citing Donald (1937), Barber (1955) suggested that anterior teats are more preferable than the posterior teats to piglets because this is a "safer" zone for the piglet to be in, where they are less likely to be kicked away or stepped on when the sow rises from lying. By being in a safer position, the piglet nursing a more anterior gland could theoretically spend more time in massaging and nursing the gland, and therefore stimulating greater milk production.

Larger piglets often claim multiple teats (personal observation), further challenging the smaller piglet that is less able to compete for a highly productive teat,

leaving it more vulnerable to malnourishment, and potentially starvation. Cross-fostering has been developed as a management tool in response to minimize the relative disadvantage of smaller piglets through equalizing individual weights across the litter. This allows all piglets in a litter to have an equal opportunity to fight for productive teats, and can reduce piglet mortality by as much as 40 % (English et al., 1977). This theory was recently questioned by Milligan et al. (2001), who showed that survival rates of piglets tended to be reduced only in litters of variable birth weights; piglet weight gains were not affected, although later weight variation was reduced. The authors acknowledged that the study was restricted to litters of healthy, average sized (no less than 600 g) piglets, and litters of < 12 piglets on healthy sows (Milligan et al., 2001).

Like all mammals, the pig is a homeotherm that needs to maintain a stable core body temperature within a relatively narrow range, regardless of climate or activity. This ability is affected by environmental factors, primarily ambient temperature. The range of ambient temperatures in which the animal has the least metabolic demand is known as the thermoneutral zone (TZ). The lower limit of the TZ is referred to as the lower critical temperature (LCT) of the animal, and when the ambient temperature is below this point, body heat must be generated (Mount, 1979). The LCT of the neonatal piglet is a relatively high 34° C, meaning that the neonatal piglet requires high levels of heat generation for survival (Mount, 1959, 1966).

Chilling is likely the most important contributing factor to piglet mortality, through either direct hypothermia, or energy reserve depletion (Curtis, 1974), and results from the piglet failing to maintain body temperature at or above its LCT. When

compared to other newborn mammals, the neonatal piglet is physiologically weak, with low bodily energy reserves and high heat loss to the environment from a sparse pelage and minimal body fat for insulation (Curtis, 1974; Mount, 1979). Not only are bodily energy reserves low, but the neonatal piglet depends on carbohydrate oxidation for heat generation (Goodwin, 1957; McCance and Widdowson, 1959). At the same time, its ability to regulate carbohydrate metabolism is low due to low enzymatic activity for gluconeogenesis, a problem that resolves as the pig ages (Curtis et al., 1966).

Smaller piglets are the most vulnerable to chilling, as they have a higher surface area to body weight ratio than do their larger littermates, resulting in a greater rate of heat loss to the environment (Mount, 1968; Parker et al., 1980). The smallest pigs in the litter tend to be born later, and have a higher mortality rate than do the larger piglets (Hartsock and Graves, 1976). Their higher mortality may be related to greater stress from anoxia during the farrowing process and a lesser availability of immunoglobulin-rich colostrum as compared to their earlier-born littermates (Hartsock and Graves, 1976).

Once smaller piglets become chilled, they spend more time near the sow in a quest for additional nourishment and warmth to compensate for inadequate body heat production. This increases the risk of crushing by the sow because of the chilled piglet's lethargy, as it is less able to remove itself from danger as the sow rolls over and/or lowers herself from a standing position. During its search for warmth and nutrition, the chilled piglet does not take advantage of huddling with its littermates and thereby receiving body heat from them. Doing so could reduce the need to generate body heat by as much as

40 % (Mount, 1960). As a result, the chilled piglet has a higher heat production demand at the exact time in which it is least capable of increasing heat production.

In order to fend off chilling, the neonatal piglet must immediately consume adequate energy substrates (Friend, 1974), or it must attempt to conserve energy by lethargy or by staying close to the sow. This leaves the piglet vulnerable to crushing or savaging by the sow as described previously. Cold tolerance improves with age as a result of increased metabolic rate, particularly after the first day of life (Mount, 1959, 1968). When a newborn piglet was exposed to a 5° C environment for 90 minutes, there was resultant 4° C decrease in rectal temperature; the corresponding decrease for a 30 h old piglet was less than 1° C (Curtis et al., 1967). Additionally, body fat increases from < 1 % to 10 % by seven days of age (Curtis, 1970). Body fat continues to accumulate with age, and results in a decrease in LCT from 34° C to 19° C by the time the piglet attains a body weight of 10 kg (Bianca and Blaxter, 1961).

Pathogens are a greater concern during periods of cold stress, and lead to greater morbidity and mortality. When exposed to a 10 ° C environment shortly after birth, the acquisition of passive immunity in piglets was significantly depressed, mainly through decreased colostrum intake (Blecha and Kelley, 1981; Le Dividich and Noblet, 1981). Higher intakes of colostrum are associated with higher metabolic and survival rates (Noblet and Le Dividich, 1981), and it is therefore most critical for the herdsman to maintain a warm environment for piglets during the immediate postpartum period.

Pre-weaning growth

Pre-weaning piglet growth is dictated by the available milk supply, and the choice of teat is critical, for anterior teats tend to produce more milk than do posterior teats (Kim et al., 2000). Anterior teats are also more likely to be utilized by piglets; 60 % for the first four anterior pairs versus 40 % for the last four posterior pairs (Kim et al., 2000). Piglets nursing anterior teats gain weight faster than do those nursing posterior teats due to a greater milk supply which results from the mammary glands supplying those teats being heavier, and having more protein and DNA when measured at the end of lactation (Kim et al., 2000). By d 22 of lactation, piglets nursing anterior teats received as much as 15.3 % more milk than their littermates nursing posterior teats (Gill and Thompson, 1956).

It is likely that piglets play a role in the greater milk production of anterior teats. In a study by Auldist and King (1995), larger piglets tended to prefer the anterior teats, and were capable of more effective milk removal than smaller littermates (Auldist and King, 1995). Heavier piglets also provided a more intensive suckling experience, and therefore stimulated greater milk production (Auldist and King, 1995; King et al., 1997). This was confirmed by Pluske and Williams (1996), who showed that larger piglets obtained 8.5, 30, and 30 % more milk than smaller piglets on d 17, 19, and 24 of lactation, respectively. The increased milk production of anterior teats results in higher 21 d litter weights (Dyck et al., 1987), and the difference has been reported to be as much as 1.5-1.8 kg for piglets nursing the most extreme anterior versus posterior teats (Hoy et

al., 1991). Additionally, a deficit of 4.5 kg at weaning can result in as much as 15 additional days to reach a 105 kg slaughter weight (Mahan and Lepine, 1991), justifying the practice of creep feeding to nursing litters as is commonly practiced in industry. Beyond increasing weaning weight, providing creep feed positively affects feed intake during the early postweaning period, and results in a higher overall average daily gain through at least the first 34 d postweaning (Bruininx et al., 2002).

Colostrum and milk composition as it relates to survival and growth

The differences among mammalian species in milk composition have resulted from the differing evolutionary pressures faced by the individual species. Among those evolutionary pressures is the environment that the neonate faces. Arctic and marine species face a high rate of heat loss to the environment, necessitating the need to rapidly accumulate body fat to conserve body heat. Body fat is primarily derived from dietary fatty acids (Glass et al., 1967), and the milks of marine species therefore have relatively high levels of fat (Jenness and Sloan, 1970). Alternatively, water conservation by the dam is a very important factor in a desert environment. Therefore, the milk of desert animals is highly concentrated (Schmidt-Nielsen and Schmidt-Nielsen, 1952; Kooyman, 1963). Another evolutionary pressure is the nursing behavior of the young, which can be used to classify mammals into groups (Ben Shaul, 1962). Some mammals such as marsupials, primates, and pigs nurse on demand, while others such as rabbits, deer, and cats nurse on a scheduled interval (Jenness and Sloan, 1970). Demand nursing mammals

produce milk with a solids not fat (SNF) content composed of greater than 50 % sugars

and ash, with sugars providing over 25 % of calories (Jenness and Sloan, 1970).

Colostrum and milk serve many roles in piglet survival and growth. The most

immediate role is the support of thermoregulation. For a neonatal piglet to regain bodily

energy reserves and begin growth after farrowing, the composition of the secretion it is

consuming must quickly change from that of colostrum to that of milk to provide

sufficient carbohydrate, lipid, and protein (Close, 1992). Milk fat provides the most

energy to the neonatal piglet, followed by lactose (Hartman et al., 1984). Next in

importance is the provision of passive immunity. There is little or no placental transfer of

immunoglobulins in the pig, as demonstrated by an absence of serum immunoglobulins

in colostrum-deprived germ-free piglets (Kim et al., 1966). This leaves the neonatal

piglet completely dependent on across-gut transfer to serum of antibodies from colostrum

for protection from pathogens such as bacteria, viruses, and protozoal agents (Butler,

1971). This must occur soon after birth, for the gut of the neonatal piglet is only able to

absorb antibodies into the circulation for a short time period, 24-36 h at most (Brambell,

1958). Indeed, the majority (62-69 %) of first day immunoglobulin G (IgG) intake

occurs during the first 12 h of postnatal life (Milon et al., 1983). Peak piglet serum levels

of IgG, immunoglobulin A (IgA), and immunoglobulin M (IgM) are obtained about 12 h

following birth (Klobasa et al., 1981), and lower serum immunoglobulin concentrations

at 12-36 h after birth are associated with higher preweaning piglet mortality (Hendrix et

al., 1978; Blecha and Kelley, 1981; Klobasa et al., 1981). Additionally, piglets with high

serum IgG concentrations on day seven also have higher serum levels at 28 d post-

farrowing (Edwards and Rooke, 1999). This indicates that these piglets have a stronger immune status at weaning, and likely a greater rate of survival in the immediate postweaning period.

Beyond survival, milk provides for the nutritional needs of the piglet, as piglet growth rate and development are related to the yield and composition of the milk consumed (Fahmy, 1972; Lewis et al., 1978). Piglets are capable of a tremendous rate of gain, between 230-250 g/d to 21 d post-farrowing for sow-reared piglets (Harrell et al., 1993; Boyd and Touchette, 1998). This means that piglets weighing one kilogram at birth will double their weight in four to five days. The feed conversion of piglets consuming milk is very high, with piglets converting an average of approximately four grams of milk to one gram of total weight gain over the typical three week lactation (Lucas and Lodge, 1961; Salmon-Legagneur and Aumaître, 1962; White and Campbell, 1984; Noblet and Etienne, 1989). The conversion of milk to piglet gain is most efficient during early lactation, and decreases during lactation to about 4.5:1 in the third and fourth weeks (Lewis et al., 1978; Van Kempen et al., 1985). Additionally, litter weight gains over the first three days of lactation are highly correlated to litter weight gains over the first two weeks (Thompson and Fraser, 1988).

Piglet growth potential exceeds that supported by milk energy provision from milk from about day eight of lactation onward (Boyd et al., 1985; Harrell et al., 1993). This finding was supported by the work of Hodge (1974) and Tritton et al. (1993), who indicated that piglets fed milk replacer ad libitum grew at a faster rate than their sow-raised counterparts. Le Dividich (1999) showed that the growth rate of nursing litters

could be increased by 10-38 % through milk supplementation. The fact that milk supply

is limiting for piglet growth was most dramatically demonstrated by Harrell et al. (1993)

through ad libitum provision of milk replacer to hand-fed piglets. The authors

demonstrated that the genetic potential for growth to 21 d post-farrowing in the modern

piglet is at least 400 g/d. To support this rate of growth, the milk supply from sows

would have to reach 18 kg/d by d 12-14 of lactation for a litter of 10 pigs (Harrell et al.,

1993). These piglets maintained their increased weight per day of age relative to sow-

reared piglets to a finishing weight of 110 kg, as they reached this weight 10.4 d earlier,

and without adverse effects on carcass composition (Harrell et al., 1993).

Lastly, milk stimulates the growth and development of the gastrointestinal tract.

Piglets consuming only water lacked intestinal growth, whereas suckled piglets had

tremendous gastrointestinal growth during the first 24 h post-farrowing (Widdowson et

al., 1976). Colostrum and milk are equally able to promote perinatal gut development

(Simmen et al., 1990). Not only are receptors for insulin-like growth factor –I and –II

(IGF-I and –II) present on the enterocytes (Schober et al., 1990); IGF-I and -II are present

in milk, with IGF-II concentrations being twice as high as IGF-I in prepartum and

colostrum secretions (Donovan et al., 1994). IGF-II may therefore be more important

than IGF-I in early life. Other growth factors present in milk include insulin, epidermal

growth factor (EGF), and transforming growth factor-α (TGF-α), which are likely

involved in growth of the small intestine and recovery from injury such as infection or

ulceration (Donovan and Odle, 1994). There are likely many other hormones and growth

factors in milk whose functions have yet to be determined (see Grosvenor et al., 1993).

<u>Sow milk composition</u>

Among the first investigators to study the normal composition of sow milk were Scheven in approximately 1855, Von Gohren in 1865, and Lintner in 1866 (Jylling and Sørensen, 1960), but their methods are unknown, and these investigations usually involved only one or two sows at unknown stages of lactation. Therefore, more recent studies are presented.

The concentration of protein in sow colostrum is high shortly after farrowing, approximately 15-18 %, decreasing to a mature milk level of about 5-7 % over the first five days of lactation, followed by a slight increase to 6-7 % in later lactation (Perrin, 1954, 1955; Leskova and Onderscheka, 1968; Brent et al., 1973; Klobasa et al., 1987). Milk lactose concentrations increase from about 2.5 % shortly after farrowing to about 4-5 % within three days, and remain stable at 5-6 % in mature milk (Perrin, 1954, 1955; Klobasa et al., 1987; Jackson et al., 1995). Milk fat concentrations increase rapidly after farrowing to about 7-12 %, then stabilize at 9-12 % between the first and third weeks of lactation, and finally decline to about 5-8 % by the eighth week (Perrin 1954, 1955; Klobasa et al., 1987; Jackson et al., 1995). Milk composition in the sow does not appear to differ according to lactation number (Klobasa and Werhahn, 1996).

The following conclusions can be made based on the above reports. Total milk energy is high, being provided from high levels of lactose (3.1-5.9 %) and fat (5-12.5 %). Protein is also high (5.3-18 %), with concentrations being highest when the piglet is the youngest. Additionally, mature milk composition in the domestic sow is not very

different from that reported for the wild sow- 5-6 % protein, about 5 % lactose, and 7-9 % fat (Walkiewicz et al., 1997). Klobasa et al. (1987) argued that the change from colostrum to mature milk in the sow is signaled by a decrease in total protein and whey content (immunoglobulins in particular) and an increase in fat and lactose content. Perrin (1955) indicated that this is completed in roughly four to five days post-farrowing.

Specific proteins in mammary secretions

The two major protein fractions of milk are the casein (those proteins precipitated by pH 4-5; including α_{s1}-, α_{s2}-, β-, and κ-casein) and the whey fractions. The caseins are hydrophobic, phosphorylated at multiple serine and other residues, and also glycosylated (Swaisgood, 1982, 1992). Caseins in milk form themselves into a colloidal dispersion called micelles, which average 20-600 nm in diameter (Schmidt, 1982). The four caseins make up 93 % of the miceller dry matter, with the remaining 7 % composed of calcium (Ca^{2+}), phosphate (PO_4^{2-}), magnesium (Mg^{2+}), and citrate; the complex is referred to as colloidal calcium phosphate (Schmidt, 1982). The micelle forms around free calcium, entrapping approximately 27 % of the free calcium ion concentration in bovine milk (Swaisgood, 1982), and may be responsible for the delivery of calcium to the neonate to support growth. In sow milk, caseins comprise about 50 % of total milk protein, with β-casein being the predominant form (Aimutis et al., 1982; Klobasa et al., 1987). Upon chemical precipitation, porcine casein has a very fine texture as compared to bovine casein (Hartmann et al., 1984). This may result in a softer curd forming in the piglet's

stomach than the calf's, and may also partially explain the relatively short nursing interval of 40-60 minutes through a resultantly higher rate of passage (Hartmann et al., 1984).

The whey fraction includes the immunoglobulins, providing passive immunity to the neonate as well as some level of protection from infection to the mammary gland. Accordingly, concentrations are highest in colostrum when the neonate and gland are most vulnerable to infectious challenge. Immunoglobulin accounts for 14.3 % of sow colostral whey protein, or roughly four times the concentration of mature milk (Klobasa et al., 1987). Whereas milk IgG concentrations are reduced with advanced lactation, milk IgA concentrations increase dramatically (Karlsson, 1966; Curtis and Bourne, 1971; Klobasa and Butler, 1987; Klobasa et al., 1987). The reason is most likely for the protection of the neonate from enteric disease (Wilson, 1974). IgM and IgG are likely selectively transported from serum, and IgA is > 90 % made locally in the mammary glands (Bourne and Curtis, 1973). Older sows tend to have higher IgA concentrations in their milk, probably from greater cumulative immune stimulation over their lifespan (Klobasa and Butler, 1987). Theoretically, this allows older sows to transmit a greater variety of specific antibodies against enteric pathogens to the suckling piglet than do younger sows, and better provide immunity in the intestinal tract (Klobasa and Butler, 1987). This idea of enteric protection against pathogenic challenge is supported by the data of Curtis and Bourne (1971), who indicated that immunoglobulins absorbed from colostrum into piglet circulation have very short half-lives of 3.5 and 4.5 d, for IgA and IgM, respectively.

Albumin is also found in the whey fraction of sow milk, and originates from the serum (Karlsson, 1966; Carlsson et al., 1977). The appearance of albumin in milk is thought to be due to a combination of passive transport and/or diffusion; therefore the concentration of this protein in milk can be used indirectly to indicate the integrity of the blood-milk barrier (Klobasa and Butler, 1987). Accordingly, as colostrum changes to milk, albumin levels decrease as a proportion of total milk protein (Finkelstein and Hurley, 1987), as does total milk albumin concentration (Klobasa and Butler, 1987). Immunoglobulin levels in milk vary widely from animal to animal throughout lactation, but the variation in albumin stabilizes by day five of lactation, about the time of completion of the transformation from a colostral to a mature milk secretion (Klobasa and Butler, 1987). Lastly, the whey protein also contains a variety of growth factors, including insulin-like growth factors -I and -II (IGF-I and IGF-II).

Another protein found in the whey fraction is lactoferrin, the milk concentrations of which vary widely from species to species; human levels are approximately 2 g/l, murine levels about 0.28 mg/l, and bovine levels about 0.01 mg/l (Neville and Zhang, 2000). Lactoferrin has been reported in sow colostrum and milk (Masson and Heremans, 1971; Roberts and Boursnell, 1975; Jenness, 1982; Elliot et al., 1984; Hutchens et al., 1989; Magnuson et al., 1990; Chu et al., 1993). Chu et al. (1993) purified porcine lactoferrin, produced polyclonal antibodies against it, and developed an enzyme-linked immunosorbent assay (ELISA) method for measuring lactoferrin in sow's milk. This ELISA was later used by Yang et al. (2000) to report that lactoferrin in sow milk was approximately 1.6 mg/ml on day one of lactation, remained at that level for three days,

then gradually decreased over the course of lactation to between 0.4-0.5 mg/ml around

d 21-28. These values were slightly higher and persisted longer than those reported by

Elliot et al. (1984), who reported approximately 1.2 mg/ml around farrowing, quickly

declining to 0.3 mg/ml over the first week of lactation, and then to about 0.1 mg/ml

during the third week. Yang et al. (2000) attributed the difference to the ELISA method

being more accurate than the immunodiffusion method of Mancini et al. (1965) utilized

by Elliot et al. (1984).

Milk production in the sow

One of the biological limits constraining maximal pork production is sow milk

production, as current levels may not be capable of fully supporting the genetic potential

for lean tissue growth possessed by the modern-type piglet. Milk yield in the sow has

almost doubled in the last 30 years, both on a whole litter and per-piglet basis (Étienne et

al., 2000), such that milk production on a metabolic body size basis is now similar to that

of dairy cattle. The average modern sow produces about 10.8 kg milk/d over 21 d of

lactation, with the top 10 % of sows producing about 13.6 kg milk/d (Boyd and

Touchette, 1998). If the average lactating sow weighs 160 kg (Boyd et al., 1985), then

sow milk production is approximately 0.24-0.30 kg milk/kg of metabolic body size. For

comparison, the average Holstein cow weighs 666 kg, and produces 36.4 kg milk/d over

305 d of lactation (USDA, 2004); the corresponding milk production level is 0.27

kg/milk/kg of metabolic body size.

The disparity between sow milk supported piglet growth and the genetic capacity for growth has driven the development of creep feeding programs to make up the difference between milk supplied nutrients and that needed for optimal growth of the suckling piglet. This is an added expense to the pork production system, which may also help the transition to a solid diet in the post-weaning phase. As an alternative for increasing pre-weaning growth, pork producers could try to increase sow milk production further. Among the potential methods to do so is selection for genotypes associated with greater milk yield and/or improved milk composition, which has been heavily studied in the cow, due to the direct payment for milk yield and composition received by the dairy farmer. It has only been indirectly addressed in the sow through the development and use of the Sow Productivity Index (SPI).

In order to increase milk production, an understanding of the processes of mammogenesis and lactogenesis is necessary; both must occur in the sow's udder before lactation can commence. As evidenced by invasion of the mammary fat pad by the epithelial ductal tree and a tripling of mammary deoxyribonucleic acid (DNA) content, DNA proliferation is maximal between approximately d 75-90 of gestation in the sow (Kensinger et al., 1982). Lactogenesis is well underway by d 112 of gestation, as evidenced by the formation of functional lobuloalveolar structures (Kensinger et al., 1982). Full differentiation of secretory alveoli occurs over the last 12 d of gestation and the first four days of lactation, as shown by significant increases in the ribonucleic acid (RNA) to DNA ratio and the presence of colostrum (Kensinger et al., 1982). Mammary epithelial cell polarity, expanded endoplasmic reticulum cisternae, developed secretory

vesicles, and numerous microvilli are all in place by d 112 of gestation (Kensinger et al., 1986). Milk production continues to increases after farrowing, as reflected by increased glucose oxidation and lipogenesis by the mammary gland over the first four days of lactation (Kensinger et al., 1982). Lactose, which is produced in the mammary gland from glucose, is one of the major controlling factors for milk yield as it is the major osmole found in milk (Morrissey, 1985; Noble et al., 2002).

Lactation in the sow can be described as having a colostral, an ascending, a plateau, and a descending phase. The colostral phase lasts for 18-24 h post-farrowing, the ascending phase up until about d 14 of lactation, the plateau up until about d 28, and the descending phase comprising the remainder of the lactation (Klopfenstein et al., 1999). Therefore, sows in the modern production setting, with an 18-21 d lactation, rarely if ever, reach the descending phase (Klopfenstein et al., 1999).

Factors affecting milk yield

The genetic lineage of the sow, as reflected in the differences between maternal and terminal breeding lines, is the largest contributing factor to milk yield in the sow, and likely has its effect through milk protein expression. For example, α-lactalbumin is a milk protein that is closely associated with milk yield, and Illinois researchers have produced transgenic pigs incorporating bovine α-lactalbumin (Noble et al., 2002). Transgenic sows having the bovine α-lactalbumin transgene have increased milk production, and piglets reared by these gilts have an increased growth rate compared to

those raised by non-transgenic sows (Noble et al., 2002). The increase in milk production was largely confined to early lactation (day nine or before), but the resultant growth of piglets carried over to the full lactation (Noble et al., 2002). While transgenics will not likely be adopted as a means to increase milk production in the sow due to societal resistance, this data indicates that selection for increased (or differing genotypes of) α-lactalbumin in the sow could potentially be one manner by which to increase sow milk production and resultant preweaning piglet growth performance.

Similarly, milk protein variants in the cow have been shown to have effects on milk yield and composition (reviewed by Ng-Kwai-Hang, 1997, 1998). Of the known casein genotypes, the B variant of α_{s1}-casein and the A^2 variant of β-casein were associated with higher milk yield. Higher fat, protein, and total casein yields were associated with the C variants of α_{s1}-casein, the B variant of β-casein, and the B variant of κ-casein. The B variants of κ-casein and β-lactoglobulin led to shorter coagulation time, faster firming rates and firmer curds during the cheese making process. This could be expected to result in faster, stronger curd formation in the stomach of the young, and therefore slowed rate of passage, allowing for greater digestibility through prolonged enzymatic action. Additionally, the A variant of β-lactoglobulin was associated with higher total protein and β-lactoglobulin contents, offset by lower casein and fat levels. These data allow for selection for specifically preferred manufacturing characteristics for bovine milk. There are homologues for each of these proteins in the sow, and there are likely similar polymorphisms to be discovered that could be manipulated to improve the nutritional quality of sow's milk for the neonatal piglet.

Photoperiod manipulation has been exploited in the sow to increase 21 d litter weight by 13 % (Mabry et al., 1982). It has also been used to improve milk production in the cow through extending the period of light from 8 to 16 h per day, increasing milk yield between 10-15 % (Peters et al., 1978 a, b). This manipulation of duration of exposure to light increases prolactin levels in sheep (Forbes et al, 1975), goats (Buttle, 1974), and cattle (Bourne and Tucker, 1975), and this is the likely mode of action.

Dietary manipulations have been tried extensively to improve milk yield and/or composition in the sow. The addition of fat to the ration increased total milk fat levels and changed the fatty acid profile (Jackson et al., 1995; Seerley et al., 1974), and increased milk yield (Friend, 1974; Seerley et al., 1974, 1978 a, b; Cast et al., 1977; Boyd et al., 1978, 1982; Pettigrew, 1981; Stahly et al., 1981; Jackson et al., 1995). The addition of fat to the gestation ration improved litter survival and growth in the immediate neonatal period. Seerley et al. (1974) reported higher piglet carcass lipids, improved thermostability at 54 h of age, and improved small pig (< 1000 g) survival. The last results should be viewed with caution, as there is significant mammary growth from d 70-100 of gestation (Kensinger et al., 1982, 1986). Weldon et al. (1991) showed that increased dietary energy above U.S. National Research Council (NRC) levels from d 75-105 of gestation decreased mammary weight and DNA content. The mechanisms underlying the negative effects of increased energy during the gestational period upon mammary development have not yet been defined. Averette et al. (1999) increased milk fat without negatively affecting milk yield by waiting until after d 90 of gestation to increase fat in the ration. Sinclair et al. (1999) showed that increased dietary protein

increased litter growth rate in the Meishan pig, and reduced tissue catabolism during lactation in the European White pig. Lastly, supplementation of the gestation and lactation diet with L-carnitine was shown to increase milk protein and lactose, and tended to increase total milk energy, resulting in piglets growing faster than piglets of non-supplemented sows (Ramanau, 2004).

Growth hormone therapy (recombinant bovine somatotropin (rBST) or Posilac[TM]) is common in the dairy industry, but may not be applicable to swine production. Harkins et al., (1989) reported a 22 % increase in milk production on d 28 of lactation following daily treatment from d 12-29 with recombinant porcine somatotropin (rPST), resulting in faster growing piglets and sows losing less weight and backfat. However, Smith et al. (1991) reported 90 % sow mortality from hemorrhagic ulceration of the esophagus when treating with rPST. This extreme mortality problem likely eliminates the potential of using rPST to improve litter performance through increased milk yield if it can not be overcome.

Determination of milk yield

Estimation of milk yield in the sow is substantially more difficult than in other livestock species, due to the number of teats and the nature of porcine lactation. For the most accurate estimation of milk production consumed by the litter, more than 24 milkings would be required per day, due to the short nursing interval (Hernandez et al., 1987, as cited by Klopfenstein et al., 1999). Thankfully, the work of Mahan et al. (1971)

suggested that seven or eight measurements can be used to accurately estimate daily milk yield.

One of the methods used to measure milk yield in the sow is machine milking (Fraser et al, 1985; Grün et al., 1993); unfortunately, the equipment used is limited and/or hand-made. Additionally, the use of oxytocin is often required, which may affect the accuracy of the estimation. For these reasons, the use of machine milking in the sow to measure milk yield is impractical. Another is to back-calculate milk yield from piglet growth. According to Lewis et al. (1978) and Noblet and Etienne (1989), approximately 4.5 g milk have to be consumed to result in one gram of piglet growth. This value is higher than the 3.5 g milk/g gain proposed by Barber et al. (1955), and the 4.31 kg milk/kg gain proposed by Hodbod and Zeman (2001). Therefore, a reasonable value to use is probably approximately 4.0 g milk/g gain.

The most accurate, though expensive and difficult, method to determine milk yield over a day and/or a week is to measure piglet body water turnover (MacFarlane et al., 1969; Yang et al., 1980; Pettigrew et al., 1987; Pluske et al., 1997). This method is based on the dilution of endogenous water caused by milk water intake as measured by the dilution of injected deuterium-labeled water. According to Prawirodidgo et al. (1987, as cited by Klopfenstein et al., 1999), the predicted value of milk intake from this method has a correlation of 0.96 with actual milk intake as determined by weigh-suckle-weigh procedure. Among the concerns with this technique are its sensitivity to small errors in dosing, loss of sample, problems with dosing (intraperitoneal (i.p.) vs. intramuscular

(i.m.)), as well as a requirement that the piglet get water only from milk or colostrum, and not from the sow's water source (Pettigrew et al., 1987).

Weigh-suckle-weigh procedure

Due to the shortcomings of other methods discussed previously, the weigh-suckle-weigh (WSW) technique has become the most widely utilized method for the estimation of milk yield in the sow. Though labor intensive, it is relatively easy to perform. Briefly summarized, piglets are generally kept apart from the sow for one hour, encouraged to urinate and defecate by placing on a cold surface and by disturbance, and then weighed before and after a suckling episode. This is repeated for 8-10 h over the course of a day. Great care is taken to adjust for losses due to urination, defecation, and metabolic water losses during the suckling episode.

WSW techniques in the sow have been published by many authors over the last 50 years or so (Barber et al., 1955; Mahan et al., 1971; Lewis et al., 1978; Noblet and Etienne 1989), and primitive forms of the technique were reported before that. The first comprehensive study on the technique was published by Barber et al. (1955), reviewing 23 previous studies in the literature attempting to measure the 24 h milk yield of sows by WSW procedure. Barber et al. (1955) found all previously reported methods lacking, as they were generally compromised by excessively long intervals between sucklings; between two to four hours (e.g., Von Goehren, 1865; Davies, 1904; Schneider, 1934). Barber et al. (1955) standardized the technique by limiting the between nursing interval

to one hour so as to more accurately mimic the natural pattern. Additionally, Barber et al. (1955) emphasized the need to standardize the size of the litter, finding that sows with more pigs produce more milk due to an increased suckling stimulus.

Among the concerns with the WSW procedure are that it likely artificially depresses milk yield through perturbation of the interaction among the sow and litter, as determined by direct comparison with the isotope dilution method (Pettigrew et al., 1985). This was first suggested by Barber et al. (1955), who performed WSW hourly for up to 48 h. In their study, milk yields were almost always lower during the second 24 h period than during the first. Barber et al. (1955) also noted that piglets lost approximately two to five grams of weight during sucklings in which it was clear that no milk was ejected, which they attributed to losses from respiration, saliva, mucus and other "body debris"; this loss is termed metabolic loss. Wohlbier (1928) confirmed a similar metabolic loss of four to six grams during a nursing episode (as cited by Barber et al., 1955). Pettigrew et al. (1985) also stated that milk yield must be corrected for metabolic and salivary losses, or milk yield will be underestimated. Noblet and Etienne (1986) definitively calculated insensible water loss in the nursing pig to be 0.21 g/kg $BW^{0.75}$/min.

The total duration of time spent imposing the WSW procedure upon the sow and litter is a concern for accuracy. Another is which nursing episodes during the WSW procedure should be used for milk yield estimation. Mahan et al. (1971) reported that the longer the procedure is performed, the lesser the variance in the milk yield estimate; variance as measured over four hours was higher than when measured for eight, 12, or

16 h. Speer and Cox (1984) asserted that the first two nursing episodes have great

variability, and suggested that the data be discarded from the calculation of mean hourly

milk yield. Therefore, it is necessary to find an optimum between enough measurements

to overcome variance in the hourly estimation, and too many measurements such that

milk yield is artificially depressed by the WSW procedure. A middle point is to use milk

yield estimates over six hours; as the estimated milk yield over six hours reported by

Speer and Cox (1984) was approximately ¼ the estimates of milk yield over 24 h

reported by Barber et al. (1955), and Mahan et al. (1971). While the six hour estimate

may likely be representative of 24 h WSW milk yield, care must be taken in extrapolating

results to an undisturbed 24 h nursing period. Since the natural suckling frequency of the

sow and litter is approximately 45 min during early lactation (Hartmann et al., 1984), a

one hour interval between nursings may result in undernourishment of piglets during the

experimental period which may be compensated for overnight when the sow and litter are

undisturbed. This would be less of a concern when estimating milk yield during later

lactation, when the nursing interval is closer to 60 min (Hartmann et al., 1984).

Milk quality and mastitis in swine

    Mastitis is one factor implicated in the porcine hypogalactia syndrome (PHS);

alternatively known as lactation failure or agalactia post-partum (APP), a condition

ranging from an inadequate to a complete lack of milk production that severely

compromises the survivability and growth performance of the suckling piglet. Reported

rates of APP, the Scandinavian term for lactation failure, suggest a prevalence of 5-10 %
of all herds being affected (Bäckström, 1973 and Jorsal, 1983; as cited by Persson et al.,
1989). On an individual sow basis, a close study of one group of 78 sows through six
lactations reported an incidence rate of 26.6 % in normally managed sows, which was
reduced to 14.4 % through feed restriction (Persson et al., 1989).

Mammary lesions are common in lactating sows, but it can be difficult to directly
attribute the lesions to mastitis resulting from intramammary infection, as opposed to
external injury. In England, Delgado and Jones (1981) reported that 18.8 % of 367 cull
sows (20.4 % of 49 culled lactating sows) showed mammary lesions, but the lesions were
not consistent with acute coliform mastitis. Bacteria isolated included *Corynebacterium
pyrogenes, Streptococcus spp., Staphlyococcus spp., Bacteroides spp., and clostridia spp.*
Gram negative bacteria (*Escherichia coli* and *Klebsiella spp.*) typically seen in mastitis
were isolated in only seven lesions. As a result, the researchers felt that it was more
appropriate to study sows directly affected by mastitis, rather than to sample sows at
random, in order to understand the changes resulting from mastitis in the sow.

Mastitis in the sows is often caused by coliform bacteria, presenting with fever
(> 40.3° C), warm and swollen mammary glands, and reduced feed intake (Ringarp,
1960; Klopfenstein et al., 1999). Mastitis leads to either insufficient or complete lack of
milk consumption by the piglets (Ross, 1983), and increased mortality and suboptimal
growth (Curtis, 1974; Dyck et al., 1987) in the preweaning period. The effects of
reduced milk intake on piglets carry over into the post-weaning period, with smaller
piglets at weaning requiring a longer time to attain slaughter weight. The difference

between piglets weighing four and 8.5 kg at 21 d post-farrowing can mean up to an additional 15 d in the grower/finisher phase in order to reach a 105 kg slaughter weight, and thereby increasing the cost of pork production (Mahan and Lepine, 1991).

Mastitis in the sow is established early in lactation, and potentially even before farrowing; sows that developed agalactia spontaneously were reported to have higher numbers of stillborn piglets (Persson et al., 1989). *E. coli* is the one of the most commonly isolated causative organisms found in the milk of sows diagnosed with APP (Ringarp, 1960; Persson et al., 1996). In one Swedish study, 64 % of sows diagnosed with APP, and 16 % of clinically healthy sows had pure cultures of *E. coli* present on the first day of lactation; these infections took on average three to eight days to be eliminated (Persson et al., 1996). Other less common causative organisms of mastitis in the sow include α-, β-, and non-haemolytic streptococci, *P. multocida, A. pyogenes,* and *S. aureus* (Hultén et al., 1995). The causative strains of *E. coli* vary widely, with 167 separate strains being found in the cultures from the animals in that study and others; most strains were capable of binding fibronectin (Mörner et al., 1998). Citing Bertschinger et al. (1990), who had previously shown that protecting the mammary gland from fecal contact reduced mastitis, Mörner et al. (1998) felt that their data on *E. coli* in milk cultures supported the theory of mastitis in the sow being primarily of mammary origin through fecal contamination. Mörner et al. (1998) agreed with the arguments of Middleton-Williams (1977) that mastitis in the sow is a unique and separate condition from metritis (the alternative was implied by the former term for lactation failure; mastitis-metritis-agalactia, or MMA) in stating that mastitis in the sow does not result from, though it may

be concurrent with, systemic illness. In support of this argument, both Middleton-Williams (1971) and Morkoc (1983) reported mastitic sows showing no signs of uterine infection.

It is particularly difficult to diagnose and study spontaneous mastitis in the sow. Middleton-Williams et al. (1977) slaughtered 23 sows with elevated body temperature (ranging from 39.7-41.5 ° C); along with three clinically normal sows within three days of parturition. In all of the affected sows, there was evidence of focal, acute, and occasionally purulent and partly necrotizing mastitis. Within a given sow, mastitis was found in 1-23 mammary glands (two glands/teat in the sow). Additionally, two of the three "normal" sows also had discernible, though not clinical, mastitis. In contrast, Persson et al. (1996), and Persson (1997), showed that when natural coliform mastitis is confirmed by bacteriology and cytology, less than half of affected glands will show clinical signs, and some non-infected glands will show signs of inflammation.

It is clear that some sows may naturally resist infection by coliform bacteria more easily than others. Ross et al (1983) showed that sows from specific-pathogen-free (SPF) herds were much less susceptible to infection with *E. coli* O6:K23:H1 than those from a conventional herd. Doses of 0.5 X 10^4-10^7 colony forming units (CFU) into each of 12 glands (six teats total) at eight hours post-farrowing resulted in a significant febrile response, increased piglet mortality, and decreased piglet performance only in the sows from conventional herds. Sows from SPF herds responded no differently to the *E. coli* infusion than to tryptose-phosphate-broth (TPB) solution. Pre-inoculation lactoferrin levels were no different between the two groups, but the sows from the conventional herd

had higher levels than the SPF sows two to three days post-infusion. Lactoferrin may therefore respond to coliform infection. Additionally, there is evidence that low polymorphonuclear cell (PMN) number and activity may play a role in increased susceptibility of sows to *E. coli* (Löfstedt et al., 1983).

The classical clinical signs of mastitis may be due, in large part, to the action of PMNs, and their release of cytokines in response to bacterial or endotoxin challenge. While PMNs are necessary for fighting bacterial infection (Müller-Eberhard, 1989); they may also cause tissue injury and thereby increase the severity of clinical symptoms (Leff and Repine, 1993, as cited by Magnusson et al., 2001). Tissue injury is thought to be primarily due to the release of reactive oxygen intermediates and the proteolytic enzyme elastase (Müller-Eberhard, 1989).

The phagocytic capacity of PMNs may also play a role in the development of mastitis in the sow. Sows are most susceptible during early lactation, and there is evidence that PMNs in sow colostrum have a lower phagocytic activity than those found in milk, leaving the gland more vulnerable to bacterial infection (Österlundh et al., 2001). There is no evidence that there was a difference in cluster of differentiation-4$^+$ (CD4$^+$), CD8$^+$, or major histocompatibility complex class II (MHC class II) cells in mammary gland biopsies taken after parturition versus mid lactation (Magnusson, 1999).

The typical causative organisms for mastitis in the cow often differ from those in the sow. Whereas mastitis in the sow is typically caused by coliforms, *E. coli* in particular, mastitis in the cow is often caused by other bacteria as well, including *Staphylococcus aureus*, *Streptococcus agalactia*, environmental coliforms, streptococci,

and enterococci (Harmon, 1994). The first two are contagious, spreading easily from cow to cow through contact with the milker, whether machine or by hand (Harmon, 1994).

A normal healthy somatic cell count (SCC) in cow milk is less than one million cells/ml, with preferred levels of < 500,000 cells/ml (Paape et al., 1963); values above that are considered indicative of mastitis, and a common goal for producers is to have a bulk tank SCC below 200,000 cells/ml. The equivalent value for sow milk is between one to four million cells/ml for the healthy sow (Schollenberger et al., 1986; Hurley and Grieve, 1988; Drendel and Wendt, 1993). Colostral levels can approach 10 million cells/ml, decreasing to one million cells/ml by day six of lactation, and PMNs are the dominant somatic cell in sow milk throughout lactation (Evans et al., 1982).

PMNs represent 56-65 % of cells in sow milk at farrowing (Schollenberger et al., 1986; Hurley and Grieve, 1988; Magnusson et al., 1991). According to Schollenberger et al. (1986), PMNs were the dominant somatic cell throughout lactation, remaining at 50 % on d 31 of lactation, with a steady increase in epithelial cells (19-30 %) and macrophages (7-11 %). Hurley and Grieve (1988) indicated that PMNs decreased to 12-14 % on d 14 and 21 of lactation, respectively. Hurley and Grieve (1988) also reported that macrophages were moderate at farrowing (35 %), increased to 77-80 % of cells in milk during later lactation, and these proportions reversed by d 28 (44 % PMN, 52 % macrophage). Magnusson et al. (1991) reported that epithelial cells dominated in mature milk (60-89 %), though PMNs were the predominant phagocyte in milk throughout lactation, reaching as high as 65.5 %. The high levels of epithelial cells in sow milk

reported by Magnusson et al. (1991) have not been found in other species such as the cow (Lee et al., 1980) or the human (Ho et al., 1979). Magnusson et al. (1991) explained this by suggesting that the mammary gland in the sow is less robust, which had been previously suggested by Lee et al. (1983).

Mastitis is a major concern for pork production because a piglet selects a preferred teat relatively early in lactation and aggressively defends it against littermates (McBride et al., 1965, Hartsock and Graves, 1976). Thereby, if the gland an individual piglet suckles undergoes a case of mastitis, that piglet is often faced with malnourishment, as it is unlikely to be able to change the gland it is nursing. If this piglet is one of the larger and stronger piglets in the litter, it may force a smaller and weaker littermate to exchange teats. This smaller piglet has a greater risk of mortality when nursing a mastitic gland than a larger piglet would have had, as its physiological state makes it more vulnerable to starvation and disease challenge. The danger of malnourishment can be due to inadequate milk production by the sow, inadequate nutrient delivery from altered milk composition, inadequate milk consumption resulting from the action of bacterial toxins in milk upon the neonatal gut, or a combination of all three factors. Whatever the situation, a piglet nursing a mastitic gland is facing a substantial challenge to its survival.

Gram negative bacteria, endotoxin, and inflammation

In Berlin in 1892, Richard Pfeiffer first determined that there were two different types of toxin secreted by *Vibrio cholera*: a heat labile component and a separate heat

resistant component released upon cellular degradation, which he termed endotoxin (Williams, 2001). At about the same time in Bologna, Italy, Eugenio Centanni reported the same type of heat labile component, this one being released from *Salmonella typhi* (Williams, 2001). Pfeiffer and Centanni's endotoxin was first purified through trichloroacetate (TCA) extraction by Boivin and Mesrobeanu at the Pasteur Institute in 1932, and was determined to be composed of lipopolysaccharide (LPS), lipid, and a small amount of protein (Williams, 2001). The most common method used for the purification of endotoxin is the hot phenol/hot water extraction method developed by Westphal and Ludentz at the Max Planck Institute in 1940, which produces a pure, protein-free (< 3 %) LPS (Williams, 2001).

It is clear that the pathogenesis of coliform mastitis is a result of the body's response to endotoxin being released by lysis of Gram negative bacteria within the milk space (Said, 1973). Endotoxin (or LPS) is found in the outer layer of Gram negative bacteria, whether infectious or non-infectious, and is one of the most potent known microbial pyrogens (Williams, 2001). Endotoxin is primarily limited to this class of bacteria, though there are some rare examples of LPS from Gram positive bacteria or of non-bacterial origin such as algae reported (Williams, 2001). Endotoxin is not only heat stable, it is viable after steam sterilization and normal desiccation, and is small enough to pass through bacterial filters (Williams, 2001). Endotoxin requires temperatures of up to 200° C for up to an hour to render inert, is capable of binding to glass, plastic, and charcoal, and readily forms a bilayer in the aqueous environment (Williams, 2001).

These extraordinary properties make LPS a particular concern for pharmaceutical manufactures trying to provide an endotoxin-free product.

LPS consists of a Lipid A fraction, an inner and outer core of polysaccharides, and an O-antigen. In 1954, the Lipid A fraction was purified by 1 N hydrochloric acid (HCl) and heating at 100° C for 30 min; and was determined in the 1960s to be the pyrogenic (and toxic) component (Williams, 2001). LPS activates the classical but antibody-independent complement pathway, and binds to serum proteins such as high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and an LPS binding protein named LBP (Williams, 2001). The Lipid A region is the portion recognized by the monocyte and macrophage, thereby provoking the inflammatory/pyrogenic cascade leading to clinical symptoms. The O-antigen determines serological specificity, and is identical to the O-antigen of the parental bacterial strain. It modulates the activation of the alternative complement pathway, inhibits attachment of c5b-9 to the bacteria, and is therefore important for bacterial virulence, through the prevention of antibody recognition (Williams, 2001).

LPS binds to the CD14 receptor on the monocyte following attachment to, and presentation by LBP, a 60 kDa serum protein of hepatic origin (Schumann et al., 1990; Wright et al., 1990). Following the recognition of the LPS/LBP complex by the CD14 receptor, peak tumor necrosis factor-α (TNF-α) and intrerleukin-1 (IL-1) levels are seen within 60-90 min following endotoxin administration, with peak body temperature response closely paralleling this timeframe (Williams, 2001). The febrile response is thought to be triggered by the expression of prostaglandin E_2 (PGE_2) in the hypothalamus,

provoked by IL-1 (Williams, 2001). TNF-α and IL-1, in turn, trigger the expression of interleukin-6 (IL-6), which leads to the expression of selectins on blood vessel endothelial surfaces (Williams, 2001). Selectin expression allows for leukocyte attachment and transmigration from the blood space to the site of insult (infection or injury) (Williams, 2001). Lastly, interleukin-8 (IL-8) is released in response to IL-6, to feed back to the monocyte and down regulate TNF-α expression (Williams, 2001).

Live bacteria can be cleared through phagocytosis without provoking the cytokine cascade by macrophages, as the CD18 pathway recognizes LPS on live bacteria (Williams, 2001). Thereby, it is the death and lysis of Gram-negative bacteria in the site of infection that provokes the inflammatory response. Bactericidal/permeability-increasing protein (BPI) can bind LPS within the membrane of Gram-negative bacteria, allowing for lethal changes in its integrity, while preventing the production of TNF-α through recognition of LPS by LBP (Marra et al., 1992; Weiss et al., 1992). It is therefore possible that BPI could possibly be used as a therapeutic agent to treat Gram-negative infections (Weiss et al., 1992).

Lactoferrin in milk, mastitis, and pork production

A goal of pork producers should be to reduce or eliminate the incidence of mastitis in order to improve pork production, animal welfare, and profitability. This could be accomplished through thorough cleaning of the farrowing environment before and during lactation as a short-term solution, and could include genetic selection for milk

components that may play a role in defending the mammary gland against mastitis as a long-term solution. Milk has been shown to inhibit the growth of both Gram-positive and Gram-negative bacteria; one potential explanation is the presence of lactoferrin, a compound able to bind iron and therefore render it unavailable to the bacteria (Nuijens et al., 1996).

Additionally, lactoferrin may be in milk to prevent or reduce the inflammatory response to bacterial infection following neutrophil activation, and the resultant damage to the mammary gland that is characteristic of mastitis. Indeed, milk lactoferrin is increased in cows with mastitis as compared to levels in unaffected cows, and returns to control levels with the resolution of the infection (Harmon et al., 1975). The prevention of neutrophil activation by lactoferrin may be modulated through the binding of bacterial outer membrane components such as LPS of Gram-negative bacteria (Nuijens et al., 1996). This would prevent the interaction of LPS with monocytes in the milk space, and therefore prevent the production of pro-inflammatory cytokines such as IL-1 and TNF-α, and the activation of neutrophils (Nuijens et al., 1996).

Milk lactoferrin may also serve a protective function in the gut of the suckling piglet, thereby protecting it from enteric infection. The digestion of lactoferrin in the gut of the suckling piglet is reduced when compared to that found in the adult pig, allowing it to remain intact and have biological action (Drescher et al., 1999). Lee et al. (1998) showed that the prefeeding of lactoferrin to piglets before an experimental intravenous endotoxin challenge reduced piglet mortality from 73.7 to 16.7 %, and that lactoferrin also decreased the binding of LPS to monocytes and macrophages, and in turn, reduced

cytokine production. Separately, it has also been shown through tracer studies that lactoferrin likely plays a role in the absorption of iron from the gut, helping to increase the low iron stores of the neonatal piglet (Fransson et al., 1983).

Effects of mastitis on milk yield and composition

A better understanding of the effects of mastitis on sow milk production and composition, as well as the sow's physiological responses to inflammation, is needed in order to meet the goal of reduced incidence and impact of mastitis in pork production. It is well known that mastitis reduces milk yield and changes milk composition in the cow (Carrol and Jain, 1969; Shuster et al., 1991), but these effects are not as well defined in the sow. Additionally, milk production (yield) and composition are routinely determined in dairy cattle through Dairy Herd Improvement Association (DHIA) programs, with approximately four million cows on test in 2004 (Wiggans, 2004). Similar milk yield and composition determinations are not routinely performed in swine production.

The effects of mastitis in the sow on milk yield and composition are reflected in the growth performance of the suckling litter from birth to weaning. However, the analysis of piglet preweaning growth performance is complicated by the health status of the piglet, controlled not only by the amount and nutritional value of the milk a suckling piglet consumes, but also by the sum total of pathogenic challenges the piglet faces. Milk is but one route by which the piglet is protected against these challenges, and suckling piglets are commonly supplemented with feed during the nursing period to improve

preweaning growth performance, thereby complicating the determination of the effects of

mastitis on pork production. Before progress can be made in reducing its impact, the

problem of mastitis in the sow needs to be better defined at the physiological level.

Experimental models of mastitis

Intramammary infusion of endotoxin (LPS) has been used extensively to produce

an experimental model of the inflammation and resultant milk composition changes

associated with mastitis in the cow (Lappalainen et al., 1998). Among the first

researchers to use this model were Carrol et al. (1964), who infused 200 µg-20 mg of

endotoxin from *Aerobacter aerogenes* into one quarter of the udder. This resulted in

localized inflammation within two hours of infusion, elevated rectal temperatures by

seven hours, and increased milk BSA and leukocytes by three and six hours, respectively.

Intramammary infusion at high doses (10 mg of *E. coli* endotoxin into two quarters)

resulted in low, though detectable levels of endotoxin in the serum six to eight hours

post-infusion; though it is likely that it is the localized response to endotoxin challenge

that provokes the systemic effects (Ziv et al., 1976). The ability of milk to inhibit the

growth of coliforms was increased by endotoxin challenge, potentially through increased

milk lactoferrin (Harmon and Newbould, 1977).

Guidry et al. (1980) utilized oyster glycogen to provoke intramammary

inflammation, resulting in increased albumin, IgA, IgG class 1 (IgG1), IgG class 2 (IgG2),

IgM, and Wisconsin Mastitis Test scores in milk at 10 h post-infusion. They also used 10

and 100 µ g of *E. coli* endotoxin to provoke inflammation (Guidry et al., 1983). Within two hours of administration, there were signs of clinical mastitis in all the quarters infused with the 100 µg dose; milk leukocytes, albumin, IgG1, and conductivity were all significantly increased (Guidry et al., 1983). Peak milk leukocytes were obtained 16 h post-infusion, albumin at four hours, and conductivity at 10 h (Guidry et al., 1983). As albumin in milk decreased, milk IgG1 remained elevated, and the authors proposed that there was active, and not simply passive, transport of IgG into the mammary gland during mastitis. Responses were similar when 10 µg of endotoxin were used, but the effects were of a lesser magnitude and more transient; IgG2, albumin, sodium, and potassium levels in milk returned to baseline within 48 h, indicating re-closure of tight junctions in the mammary epithelium (Guidry et al., 1983). Lactose in milk remained low, while chloride remained elevated at 48 h post-infusion; this was seen as evidence that synthesis and transport mechanisms for these components were slower to return to normal (Guidry et al., 1983). The authors preferred the lower dose for its more transient effects, citing that inflammation remained at four days post-infusion with the high dose.

Milk yield was reduced 14.5 %, cheese yield 5 %, and casein 9.2 % by intramammary infusion of *E. coli* endotoxin, and the effects lasted for approximately five days post-infusion (Leavitt et al., 1982). IgG1, IgG2, IgM, and IgA concentrations in milk were all significantly increased in glands infused with 10 µg of *E. coli* O26:B6 endotoxin, and the greatest response was seen in IgG2 (Anderson et al., 1986). Additionally, the ability of milk PMNs from infused glands to phagocytize *Staphylococci spp.* was increased (Anderson et al., 1986).

A thorough study of the effects of intramammary endotoxin challenge on milk composition in the cow was performed by Shuster et al. (1991). Following infusion of 10 μg of endotoxin, milk yield was maximally reduced by 24 h post-infusion and lactose between 12-24 h, with both returning to normal by 72 h (Shuster et al., 1991). Milk total protein, fat, and SCC were all maximally increased by 24 h post-infusion, returning to normal by 72 h (Shuster et al., 1991). Milk albumin and lactoferrin were maximally elevated by 12 h post-infusion; albumin returned to normal by 36-48 h, and lactoferrin remained elevated for 168 h (Shuster et al., 1991). The same research group reported that, on a systemic basis, cows became refractory to repeated endotoxin challenge; milk yield was maintained and/or recovered in non-infused quarters in spite of high SCC (Shuster and Harmon, 1991).

Clinical mastitis was seen within two hours of intramammary infusion of 50 μg of *Salmonella typhimurium* endotoxin; total leukocyte number and the proportion of neutrophils were increased, and neutrophils remained the dominant milk leukocyte for 59 h post-infusion (Östensson, 1993). Intramammary endotoxin challenge increased vascular permeability and milk neutrophils, and resulted in the appearance of TNF-α and high IL-1 activity in milk (Shuster and Kehrli, 1995). Additionally, increased blood cortisol and fever were seen, milk yield was decreased, and milk composition was altered (Shuster and Kehrli, 1995). Rectal temperature, heart rate, milk SCC, and IgG concentrations were increased within three to six hours following infusion of 100 μg *E. coli* 0111:B4 endotoxin in the cow (Perkins et al., 2002). Blood leukocyte count and rumen motility were decreased, and TNF-α concentrations in serum and milk were only

modestly increased (Perkins et al., 2002). Negative energy balance in the periparturient period is unlikely to be a contributing factor to coliform mastitis (Perkins et al., 2002). Intramammary endotoxin challenge resulted in detectable LBP in plasma by eight hours and in milk by 12 h post-infusion, with maximal levels seen by 24 h; the pattern of LPB response paralleled that of soluble CD14, giving further support to the theory of endotoxin being responsible for provoking the inflammatory response seen in mastitis (Bannerman et al., 2003).

Milk Cl^- was shown to be a potential marker of mastitis in the cow, with its concentration being increased 40-50 % in bacteriologically positive udder quarters as compared to uninfected control quarters (Huszenicza et al. 1997). Accordingly, milk ion concentrations are changed in response to endotoxin-induced mastitis; milk Na^+ and Cl^- are increased and lactose and K^+ are decreased following infusion (Lappalainen et al., 1998). Milk albumin, another serum component, was also increased approximately 130-fold following endotoxin infusion (Lappalainen et al., 1988).

Milk casein was maximally reduced by endotoxin infusion approximately four hours post-infusion, and did not return to baseline until 24 h, being followed by a slight rebound peak at 34 h (Lappalainen et al., 1988). Lappalainen et al. (1988) described a maximal decrease in casein at four hours post-infusion, followed by a secondary nadir between 10-20 h post-infusion. The research group ascribed the decline at four hours to suppressed epithelial cell synthesis/secretion, and the decline from 10-20 h to activated plasmin (Kaartinen et al., 1988). This theory was supported by the work of Schaar and Funke (1986) who showed increased plasmin in high SCC milk, and that of Ma et al.

(2000), who showed higher lipolysis and proteolysis in milk with a high SCC as compared to low SCC milk.

Endogenous proteolysis of casein following endotoxin infusion results in the creation of degradation products termed proteose peptones (Moussaoui et al., 2002). There are at least two enzymes responsible: plasmin from the blood, and elastase from PMNs; both are maximally active in milk approximately four to eight hours following endotoxin infusion, with the latter having a second activity peak approximately 25-36 h post-infusion (Moussaoui et al., 2003). Among the proteose peptone fraction are at least five peptides that are internal fragments of casein subspecies; additionally, elastase activity in milk PMNs is increased by mammary inflammation (Moussaoui et al., 2003)

While endotoxin infusion has been the most widely used model to study coliform mastitis in the cow, there are data from studies that used live bacterial infusion. Infusion of *E. coli* reduced milk synthesis and α-lactalbumin in milk by 48 h post-infusion (Harmon et al., 1976). Additionally, albumin and IgG in milk were increased to a peak level by 54 h; albumin returned to normal more quickly than did IgG (Harmon et al., 1976). Lactoferrin in milk was increased 30-fold by 90 h, and remained at a four-fold increase at 264 h (Harmon et al., 1976). Maximal bacterial cell counts in milk were seen 10-14 h post *E. coli* infusion, though signs of mastitis were not yet seen (Shuster et al., 1995). Clinical signs were observed approximately 12 h post-infusion; signs included udder swelling and increased rectal temperature (Shuster et al., 1995). Albumin in milk, milk SCC, and cortisol in serum cortisol were all increased by 12 h post-infusion (Shuster et al., 1995). Milk yield was decreased 76 % by 24 h in the infused glands and decreased

63 % in non-infused glands, with a portion of cows being agalactic (Shuster et al., 1995). Milk fat initially decreased, and then rebounded, milk protein increased, and lactose decreased, and the bacterial infection was cleared approximately six days post-infusion (Shuster et al., 1995). TNF-α and IL-1 in milk were higher post-infusion, and serum growth hormone was increased (Shuster et al., 1995).

As compared to live bacterial infection, endotoxin challenge results in a transient model of mastitis. Endotoxin from *E. coli* decreases milk yield in cows more rapidly than does the live bacterium, but the total magnitude of response is less severe (Hoeben et al., 2000). The systemic response to endotoxin is less pronounced, as milk production was not decreased in non-infused quarters, though it was for cows that were infused with a live culture (Hoeben et al., 2000). Both types of infusion (endotoxin and live bacteria) increased milk TNF-α, but the absorption into serum was greater when bacteria were infused (Hoeben et al., 2000). It is also likely that it is TNF-α and other resultant cytokines that are responsible for most of the systemic effects of mastitis, and not the endotoxin itself (Hoeben et al., 2000).

Experimental models of mastitis have also been developed in the goat. Mammary arterial blood flow increased 100 % by two hours post endotoxin infusion, resolved by four hours, and peaked again at 30 % above the two hour increase by nine hours post-infusion (Dhondt et al., 1977). Body temperature peaked at five hours post-infusion, and resolved by 12-13 h; milk chloride increased by two hours, peaked at five hours, and resolved by 18 h post-infusion (Dhondt et al., 1977). When endotoxin was infused in the cow, mammary arterial blood flow increased 200 % by three hours post-infusion,

resolved by six hours, and peaked again at 70 % above the three hour increase by 10-11 h

post-infusion (Dhondt et al., 1977). Body temperature peaked at six hours post-infusion,

and resolved by nine hours; milk chloride peaked at 12 h, and was resolved by 24 h

(Dhondt et al., 1977). The researchers suggested that the apparent decrease in mammary

blood flow between peaks was due to shock resulting from the absorbed endotoxin

(Dhondt et al., 1977).

Other researchers reported that endotoxin challenge increased milk pH, SCC, and

Na^+, and decreased milk K^+ and lactose; additionally radio-labeled albumin and lactose

were more easily transferred across the blood-milk barrier (Lengemann and Pitzrick,

1986). Low doses of endotoxin (0.1 μg/gland) were able to provoke responses in milk

Na^+ and SCC, with maximal responses seen using only one microgram (Lengemann and

Pitzrick, 1986). Later work confirmed the trans-migration of milk ions (Na^+ and K^+)

across the blood-milk barrier in response to endotoxin infusion, and further noted that

changes in the permeability of the blood-milk barrier were primarily limited to the

alveolar space, as opposed to the larger ducts (Lengemann and Pitzrick, 1987). Body

temperature, milk sodium and chloride were maximally increased and potassium

maximally decreased by five hours post-infusion; milk SCC peaked approximately eight

hours post-infusion (Burvenich et al., 1989).

The development of experimental models of mastitis in the sow has not been as

extensive as in the cow. The use of live bacterial challenge to induce mastitis

experimentally in the sow has been difficult at best, with widely ranging rates of success.

Drendel and Wendt (1993) infused live mixtures of *Bacteroides spp., Peptococcus spp.,*

and *Streptococcus spp.* into pairs of sow mammary glands, and were unable to establish infection. However, they were able to detect increased cell counts and milk lysozyme concentrations; both factors were positively correlated to one another. Additionally, the greatest responses were seen when bacteria were infused in mixed cultures (Drendel and Wendt, 1993).

Among the factors affecting the establishment of experimental *E. coli* mastitis are time of infection relative to parturition and circulating neutrophil concentration (Magnusson et al., 2001); there is also some evidence that lactation failure may begin to develop prior to farrowing (Middleton-Williams et al., 1977). Sows that are inoculated closer to parturition (48 h vs. 96 h prior) had a tendency toward greater histological changes following inoculation; blood neutrophils were higher, and lymphocytes tended to be lower in this group prior to inoculation (Magnusson et al., 2001). Clinical signs and febrile response were seen only in the close-up sows, with wide variations in response. In those sows, there was a significant inflammatory response accompanied by moderate to heavy infiltration of neutrophils into the milk space (Magnusson et al., 2001). When sows were inoculated with *E. coli* 24 h prior to estimated parturition, there were significant increases in MHC class II, CD4$^+$ and CD8$^+$ cells in the gland, peaking at approximately 72 h post infusion (Löving and Magnusson, 2002). Sows that developed clinical signs had lower levels of MHC class II cells, and higher levels of CD4$^+$ and CD8$^+$ cells pre-infusion (Löving and Magnusson, 2002).

It is clear that mastitic symptoms following coliform infection in the sow are provoked by endotoxin (LPS) present in the cell wall (Morkoc et al., 1983; de Ruijter et

al., 1988). It is also likely that it is the local response of cells present in or migrating into the mammary gland following infection to produce inflammatory mediators, and not the absorption of LPS into circulation, that results in the febrile response to endotoxin (de Ruijter et al., 1988). Endotoxin triggers the release of inflammatory mediators such as cytokines, which in turn attract immunoactive cells (Janeway et al., 1999, as cited by Löving and Magnusson, 2002) and cause activation of antigen presenting cells (APCs) (Koerner et al., 1987). The severity of the mastitic episode is dependent on the magnitude of cytokine release in response to the presence of bacteria, rather than the rate at which the sow eliminates bacteria (Löving and Magnusson, 2002).

The clinical signs of mastitis seen in sows with naturally occurring low milk yield were also seen following infusion of 1.32 mg/kg BW of *E. coli* O111:B4 endotoxin into two glands on the day of farrowing (Nachreiner et al., 1972). As compared to control sows, endotoxin infusion increased rectal temperature, circulating non-segmented neutrophils, serum urea nitrogen, serum glutamic-oxalacetic transaminase, and plasma corticosteroid (Nachreiner et al., 1972). Circulating segmented neutrophils, serum free thyroxine and albumin were all significantly decreased (Nachreiner et al., 1972). Sow responses were similar if infusion was performed on the day of farrowing or on day seven of lactation (Nachreiner and Ginther, 1974). High doses of *E. coli* O55:B5 endotoxin (1.32, 0.66, and 0.33 mg/kg BW) resulted in measurable amounts of endotoxin in the plasma within 1.5-6 h of intramammary infusion of one gland (Elmore et al., 1978).

Endotoxemia, suppressed milk yield and reduced piglet growth were all provoked by high doses of *E. coli* O8:K87,88 endotoxin (3.3-6.6 mg/kg intravenously (i.v.), 44-48

mg/kg intramammarily; Tarasiuk and Pejsak, 1986). Additionally, approximately 32.5 %

of sows with naturally occurring coliform mastitis had detectable endotoxin in the serum

(Pejsak and Tarasiuk, 1989). Sows that had signs of endotoxemia following i.v.

endotoxin administration (VanderMeer et al., 1994), or i.v. administration of endotoxin

contaminated vaccine (Garcia et al., 1998) had detectable levels of TNF-α in the

circulation. Increased serum TNF-α has not been seen in sows given an intramammary *E.*

coli bacterial infusion (Magnusson et al., 2001).

Chapter III

TOTAL PROTEIN, β-CASEIN, ALBUMIN, CHLORIDE, AND LACTOFERRIN IN

NORMAL SOW COLOSTRUM AND MILK

A. Introduction

The modern piglet is capable of tremendous growth rates from birth to weaning, averaging between 230-250 g/d to 21 d post-farrowing for sow-reared piglets (Harrell et al., 1993; Boyd and Touchette, 1998). The magnitude of this growth performance is directly related to the composition and yield of milk received from the sow (Fahmy, 1972; Lewis et al, 1978). It is therefore important to describe what normal sow milk composition is in order to understand the effects of maternal mastitis on piglet growth performance. Normal domestic sow colostrum and milk protein, fat, and lactose composition have been described (Perrin, 1954, 1955; Klobasa et al., 1987; Jackson et al., 1995), as have immunoglobulins (Karlsson, 1966; Curtis and Bourne, 1971; Klobasa et al., 1987), albumin (Karlsson, 1966; Carlsson et al., 1977); and lactoferrin in milk (Elliot et al., 1984; Yang et al., 2000). From the literature, it appears that changes in the pattern of milk proteins during lactogenesis as described by SDS-PAGE have been reported in only one other study (Zou et al., 1992). Additionally, there are only limited data for β-casein (Kauf and Kensinger, 2002) and chloride (Seynaeve et al., 1996) concentrations in sow milk. Consequently, normal colostrum and milk samples were collected at various

time points from immediately after farrowing until d 20 of lactation. The objective for

this study was to describe the normal pattern of total protein, β-casein, albumin,

lactoferrin, and chloride in sow colostrum and milk.

B. Materials and Methods

Animals used for sample collection

Sixteen parity-one Yorkshire or Yorkshire crossbred sows were farrowed in

groups of four, due to facility constraints, from May 2002 to January 2003. Sows were

allowed to farrow spontaneously, and piglets were allowed to suckle birth sows for at

least one day to allow for maximal colostral intake.   On approximately day three of

lactation after all sows had farrowed, piglets were cross-fostered and litters standardized

to 9 (± 1) piglets for each of three sows per group, and the remaining piglets assigned to

the fourth sow, which was not used. Cross-fostering was performed to equalize piglet

weights and source litter. Milk samples were collected at 1, 6, 12, and 24 h following

farrowing, and on days 3, 5, 7, 13, and 20 of lactation. Milk samples (3-5 ml) were

collected by manual expression during nursing or following the administration of 1 ml

(10 I.U.) of oxytocin intramuscularly (i.m.) if needed. Samples were used only if the sow

remained healthy throughout the three week lactation. Therefore, samples from a total of

eleven sows were used for the analysis of milk proteins.

Electrophoresis techniques

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under

reducing conditions was used for analysis of samples using a Mini-Protean 3 gel

electrophoresis system (Bio-Rad). The method was a modification of Laemmli (1970).

Separating gels were composed of 13 % acrylamide with 4 M urea, 0.01 % sodium

dodecyl sulfate (SDS), and 0.375 M tris at a final pH of 8.8. Stacking gels were

composed of 4 % acrylamide with 4 M urea, 0.01 % SDS, and 0.125 M tris at a final pH

of 6.8. Gels were polymerized using 0.01 % ammonium persulfate (APS) and 0.001 %

N,N,N',N'-tetramethylethylenediamine (TEMED). A tris-glycine-SDS buffer (0.049 M

tris, 0.366 M glycine, and 0.1 % SDS, pH 8.3) was used for electrophoresis. Molecular

weight standards were included in all gels. SDS-PAGE Standards (Bio-Rad) were used

in gels analyzing milk proteins. Pre-stained SDS-PAGE Standards (Bio-Rad) were used

in gels destined for electrophoretic transfer and Western blotting. Samples were added to

10 μl (or 20 μl for Western blotting) of sample buffer (0.12 M tris, 3.84 % SDS, 19.2 %

glycerol, 9.6 % β-mercaptoethanol, and 0.024 % bromophenol blue, pH 6.8) prior to

loading, brought to a final volume of 25 μl (or 30 μl for Western blotting), and heated for

eight minutes at 100° C on a dry bath incubator (Boekel Grant Model 241000). Current

was then applied at a constant voltage of 58 V until samples had entered the separating

gel; voltage was then increased to 190 V until completion. Gels were stained for 10 min

with 0.1 % R250 coomassie brilliant blue in 10 % acetic acid and 40 % methanol. Gels

were destained overnight in 10 % acetic acid and 7 % methanol with two buffer changes.

Images of gels were captured using the Eagle Eye[®] still video imaging system

(Stratagene). Further details on electrophoresis methods are in Appendix A.

Lowry assay

Milk samples were saponified in 1 M NaOH, and analyzed for total protein by a

modification of the Lowry assay (Lowry et al., 1951), using a 750 nm wavelength for

absorbance determination. The Lowry procedure was selected for milk samples due to its

ability to completely dissolve proteins in membranes, such as those found in the cell and

milk fat droplet, allowing for a more accurate determination of total milk protein content.

Further details on protein determination methods are in Appendix B.

β-casein ELISA

A competitive ELISA assay for porcine β-casein was developed using purified β-

casein and rabbit anti-β-casein sera (Kauf and Kensinger, 2002) by adapting methods for

bovine β-casein (Pizanno et al., 1998; 2000) as follows. One hundred microliters/well of

porcine β-casein (2 μg/ml in 50 mM NH_4HCO_3, pH 8.5) were added to a PVC u-bottom

microtiter plate (Falcon, catalog # 353911), and allowed to incubate overnight at 4° C.

Washing steps (5 X each) were performed with 200 μl/well of sterile wash buffer (PBS-

137 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) followed by

inversion and blotting on a paper towel; blocking was performed with 200 μl/well of

blocking solution (5 % horse serum (Sigma # H-1270, heat inactivated for 1 h at 65 ° C), 0.05 % tween-20, in PBS), and all following incubations were for 1 h at 37° C in a closed water bath. Fifty microliters/well of standards (0, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 μg/well of β-casein) or milk samples (1 μl/well of a 1:50 dilution at least one week prior in 4.71 M urea, 147 mM tris, 0.05 % tween-20, pH 6.8- a 1:2500 final dilution) in sample buffer (50 mM NH_4HCO_3, 0.05 % tween-20, pH 8.5) competed with plate bound β-casein for binding to 50 μl/well of rabbit anti-porcine β-casein (1:5 k dilution) in blocking solution. All standards and milk samples were run in triplicate, and also contained 1 μl/well of a 1:150 dilution of porcine whey (San Gabriel, 1994) in 4.71 M urea, 147 mM tris, 0.05% tween-20, pH 6.8; the whey was added to reduce the background. One hundred microliters/well of (goat anti-rabbit IgG)-horseradish peroxidase conjugate (1:30 k dilution, Jackson Immunoresearch Labs # 111-035-003) in blocking solution was used as the secondary antibody; one hundred microliters/well of TMB substrate (Bethyl Labs, catalog # E102) was used for detection by visually monitoring color development for 1-3 min. Development was ceased with 100 μl/well of 1 M H_2SO_4, and absorbance at 450 nm wavelength recorded using a microplate reader (Bio-Tek Instruments model # EL311). A standard curve was generated by plotting absorbance against mass in Microsoft Excel, and unknown values were calculated from an exponential regression curve. Plates were rerun if the value for a standard pool was ± 2 s.d. of the mean value for the pool. Samples were rerun if the c.v. for the triplicate was ≥ 8 %. Further details on β-casein determination methods are in Appendix C.

<u>Albumin ELISA</u>

A sandwich ELISA assay (Bethyl Labs, catalog # E100-110) for porcine albumin

was performed as follows. One hundred microliters/well of goat anti-porcine albumin

(10 μg/ml in 50 mM Na_2CO_3, pH 9.6) were added to a Nunc C bottom Immunoplate

(Bethyl Labs, catalog # 446612), and allowed to incubate for 60 min at room temperature.

Plates were aspirated, and then washed and aspirated twice with 200 μl/well of wash

buffer (50 mM tris, 140 mM NaCl, 0.05% tween-20, pH 8.0); then blocked with 200

μl/well of post coat solution (50 mM tris, 140 mM NaCl, 1% BSA, pH 8.0), and

incubated for 30 min at room temp. Solutions in wells were aspirated, and then wells

were washed and aspirated twice with 200 μl/well of wash buffer. One hundred

microliters/well of standards (two-fold serial dilutions; 250 to 7.8 ng/ml) and samples

(1:50 k, 1:100 k, or 1:200 k dilution) in sample buffer (50 mM tris, 140 mM NaCl, 1%

BSA, 0.05% tween-20, pH 8.0) were then added and allowed to incubate for 60 min at

room temperature. Samples and standards were run in triplicate. Plates were aspirated,

and then washed and aspirated five times with 200 μl/well of wash buffer. One hundred

microliters/well of goat anti-porcine albumin conjugated to horseradish peroxidase

(1:100 k dilution) in sample buffer were then added and allowed to incubate for 60 min at

room temperature. Plates were aspirated, and then washed and aspirated five times with

200 μl/well of wash buffer. One hundred microliters/well of TMB reagent was then

added and allowed to develop for 30 min. Development was ceased with 100 μl/well of

2 M H_2SO_4, and absorbance at 450 nm wavelength recorded using a microplate reader

(Bio-Tek Instruments model # EL311). During aspiration, the pipette tip was washed

with distilled water between triplicate samples/standards. A standard curve was

generated by plotting concentration against absorbance in Microsoft Excel, and unknown

values were calculated from a linear regression curve. Plates were rerun if the value for a

standard pool was ± 2 s.d. of the average value for the pool. Samples were rerun if the

c.v. for the triplicate was ≥ 8 %. Further details on albumin determination methods are in

Appendix D.

Chloride ion determination

Prior to analysis for chloride, milk samples were diluted 1:400 with Milli-Q water.

Samples were injected into an ion chromatograph (Dionex Model 2010i) for the

determination of chloride concentration, using a sodium carbonate/bicarbonate eluent and

an anion exchange column (Dionex Model AS-4A) for separation. Chloride detection

and quantification was accomplished by use of a conductivity meter and comparison to

NIST-traceable standards.

Electrophoretic transfer

Milk samples to be used for Western blotting were electroblotted onto

polyvinylidene difluoride (PVDF) membrane (Pro-Blott, Bio-Rad) using a Mini Trans-

Blot electrophoretic transfer cell (Bio-Rad). Samples were isolated by SDS-PAGE (13%

acrylamide with 4 M urea) under reducing conditions. PVDF membrane was wetted in 100 % methanol for 5 min. Gels, filter pads, membranes, and sponges were then equilibrated for 15 min in 4° C transfer buffer (0.425 M tris, 0.192 M glycine, pH 8.3). The transfer sandwich was assembled, an ice pack added, and current was applied at a constant 350 mA for 2 h. Following transfer, the membrane was trimmed with a razor to reduce the volume of solutions needed for Western blotting. Further details on transfer methods are in Appendix E.

Western blotting

Western blotting was performed at room temperature using the Vectastain ABC (# PK-4001) and DAB substrate kits (Vector Labs). The ABC kit instructions were modified to optimize Western blotting for porcine lactoferrin, using lactoferrin standard and polyclonal antibody kindly provided by Dr. Shih-Rong Wang of the Animal Technology Institute of Taiwan (Chu et al., 1993). Western blotting steps were performed in 15 or 50 ml conical tubes, depending on membrane size, utilizing gentle agitation on a LabQuake^{TM} shaker. PVDF membranes were first equilibrated in modified TTBS (0.5% tween-20, 0.1 M tris, 0.9% NaCl, pH 7.5) overnight following electrophoretic transfer. Membranes were then transferred to a solution of rabbit anti-porcine lactoferrin (1:2 X 10^6 dilution in modified TTBS) for 30 min, followed by washing in modified TTBS with four changes over a total of 25 min. Antisera dilutions were previously tested over a range from 1:1000 to 1:2 X 10^6. Membranes were then

transferred to a solution of biotinylated goat anti-rabbit antibody (0.5 % antibody, 1 %

normal goat sera, and 1 % normal gilt sera in modified TTBS, pre-incubated for one hour

prior to use) for 30 min, followed by washing in modified TTBS with four changes over a

total of 25 min. Membranes were then transferred to a solution of avidin-biotinylated

horseradish peroxidase (ABC reagent in modified TTBS) for 30 min, followed by

washing in modified TTBS with four changes over a total of 25 min. Finally, membranes

were transferred to a solution of DAB substrate in distilled water for 30 min, then

transferred to a tube of distilled water for five min and allowed to air-dry. Images of

Western blots were captured using the Eagle Eye® (Stratagene) still video imaging

system, and optical density analysis performed using ONE-Dscan (Scanalytics

corporation). Further details on Western blotting methods are in Appendix F.

Statistical analysis

A total of 11 sows remained healthy throughout the three weeks of lactation, and

were therefore used for statistical analysis of milk protein composition. One sow had an

elevated rectal temperature on d 13 and 20 of lactation, and was therefore eliminated

from the study.

Statistical Analysis Software[TM] (SAS) version 8.2 (SAS Institute Inc., Gary, NC)

was used for all statistical models. The statistical model (Proc Mixed) used for normal

colostrum and milk composition included sow, time relative to farrowing, and farrowing

group as class variables; and took the form of milk component concentration equals the

combined effects of farrowing group and time relative to farrowing. The model was a repeated measures design, utilizing a spatial power covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measure was time relative to farrowing; the subject was sow within farrowing group. Statistical models were initially performed using all independent variables; non-significant factors were removed from the models prior to final analysis and calculation of LSmeans. The kenwardroger method was used for the denominator degrees of freedom (as recommended by Dr. Peter Tozer), and significance was declared at $P < 0.05$.

C. Results

Pattern of milk proteins in colostrum and milk in the healthy sow

Pooled sow colostrum and milk contained a complex mixture of proteins; with lactoferrin, albumin, immunoglobulins, and caseins present at high levels in colostrum immediately after farrowing, and decreasing in mature milk (see Figure 1). Results for pooled samples were representative of individual animals (data not shown). While total caseins appeared to be higher in colostrum than in mature milk, the concentrations of β-casein decreased and those of α_s-casein increased as colostrum changed to milk.

Figure 1. SDS-PAGE analysis of pooled normal sow colostrum and milk samples during lactation in the sow. MW equals 2 μl molecular weight markers. Lanes 2-10 are 0.2 μl of normal sow colostrum or milk collected from clinically normal mammary glands at the indicated time points after farrowing. pLf equals 2 μg porcine lactoferrin. pSA equals 2 μg porcine serum albumin. pIgG equals 4 μg porcine immunoglobulin G. pWC equals 4 μg porcine whole casein. pBC equals 2 μg porcine β-casein. n = 11 sows and 22 glands total.

Total protein concentration in colostrum and milk in the healthy sow

The mean total protein concentrations in colostrum and milk from sows were 17.0 % at 1 h after farrowing, decreased over time to 7.2 % on d 5 of lactation, and remained at approximately the same level through d 20 of lactation (see Figure 2). The concentration of total protein in milk was significantly lower for each consecutive time point relative to the previous until d 5 of lactation (P < 0.01). By d 5 of lactation, the concentration of total protein had assumed that of mature milk, as it did not differ from that at any following time point. The concentration of total protein in milk differed among farrowing groups (P < 0.05), with values of 10.2, 9.6, 10.2, and 11.1 % for groups

1, 2, 3, and 4, respectively; the mean value for group 2 was modestly lower than for the

others (P < 0.01). The raw data for milk total protein concentration in the colostrum and

milk of healthy sows are in Appendix Q.

Figure 2. Total protein in colostrum and milk during lactation in the sow. Milk samples were collected from clinically normal mammary glands at the indicated time points after farrowing. n = 11 sows and 22 glands total. Error bars are ± 0.46 %.

β-casein concentration in colostrum and milk in the healthy sow

The mean β-casein concentrations of colostrum and milk from sows were 15.2

mg/ml 1 h after farrowing, decreased over time to 9.1 mg/ml at 24 h of lactation, and

remained at approximately the same level until d 20 of lactation, at which time they

increased again to 10.2 mg/ml (see Figure 3). The concentration of β-casein in milk was

significantly lower for each consecutive time point relative to the previous until 24 h of

lactation (P < 0.01). By 24 h, the concentration of β-casein had assumed that of mature

milk, as it did not differ from that at any following time point until d 13 of lactation.

There was a trend for the concentration of β-casein in milk to be higher on d 13 of

lactation than on d 7 (P = 0.06), and it was significantly higher on d 20 than on d 7 (P <

0.01). The concentration of β-casein in milk differed among farrowing groups (P < 0.01),

with values of 8.3, 9.1, 11.4, and 12.3 mg/ml for groups 1, 2, 3, and 4, respectively; the

mean values for groups 1 and 2 were modestly lower than for the others (P < 0.01). The

raw data for milk β-casein concentration in colostrum and milk of healthy sows are in

Appendix R.

Figure 3. β-casein in colostrum and milk during lactation in the sow. Milk samples were collected from clinically normal mammary glands at the indicated time points after farrowing. n = 11 sows and 22 glands total. Error bars are ± 0.75 mg/ml.

Albumin concentration in colostrum and milk in the healthy sow

The mean albumin concentrations of colostrum and milk from sows were 13.3 mg/ml at 1 h after farrowing, decreased over time to 3.4 mg/ml on d 5 of lactation, and remained at approximately that level through d 20 of lactation (see Figure 4). The concentration of albumin in milk was significantly lower for each consecutive time point relative to the previous until d 5 of lactation ($P < 0.01$). By d 5, the concentration of albumin had assumed that of mature milk as it did not differ from that at any following time point. The concentration of albumin in milk differed among farrowing groups ($P < 0.01$), with values of 6.2, 4.9, 7.9, and 6.2 mg/ml for groups 1, 2, 3, and 4, respectively. The mean value for group 2 tended to be lower, and the mean value for group 3 tended to be higher, than for the others ($P < 0.05$). The raw data for milk albumin concentration in colostrum and milk of healthy sows are in Appendix S.

Figure 4. Albumin in colostrum and milk during lactation in the sow. Milk samples were collected from clinically normal mammary glands at the indicated time points after farrowing. n = 11 sows and 22 glands total. Error bars are ± 0.61 mg/ml.

Chloride concentration in colostrum and milk in the healthy sow

The mean chloride concentrations for colostrum and milk from sows were 93.5 mg/100 ml at 1 h after farrowing, decreased over time to 52.0 mg/100 ml on d 13 of lactation, and remained at approximately that level through d 20 of lactation (see Figure 5). The concentration of chloride in milk was significantly lower for each consecutive time point relative to the previous until d 13 of lactation (P < 0.05). By d 13, the concentration of chloride had assumed that of mature milk, as it did not differ from that on d 20. The concentration of chloride in milk differed among farrowing groups (P < 0.05), with values of 81.0, 67.9, 64.6, and 71.5 mg/100 ml for groups 1, 2, 3, and 4,

respectively. The mean value for group 1 was significantly higher than for the others

(P < 0.05). The raw data for milk chloride concentration in colostrum and milk of

healthy sows are in Appendix T.

Figure 5. Chloride in colostrum and milk during lactation in the sow. Milk samples were collected from clinically normal mammary glands at the indicated time points after farrowing.
n = 11 sows and 22 glands total. Error bars are ± 4.06 mg/100 ml.

Lactoferrin concentration in colostrum and milk in the healthy sow

The optimal dilution of the rabbit anti-porcine lactoferrin serum used for Western

blotting was determined to be 1:2 X 10^6 (data not shown). Lactoferrin was apparent in all

samples collected between 1 h after farrowing until d 20 of lactation (see Figure 6).

Densitometric scans of lactoferrin in pooled colostrum and milk samples were compared

to a 2 μg lactoferrin standard, and the optical density of the standard was compared to

samples in order to convert optical density values to protein concentration. Lactoferrin

concentrations in pooled colostrum and milk were approximately 9.1 mg/ml at 1 h after

farrowing, decreased to 6.4 mg/ml at 24 h and 3.2 mg/ml on d 5 of lactation, and

stabilized at about 1.9 mg/ml by d 7 of lactation.

Figure 6. Western blot analysis of lactoferrin in pooled normal sow colostrum and milk samples during lactation in the sow. MW equals 5 μl prestained molecular weight markers. pLf equals 1 μg porcine lactoferrin standard. Lanes 3-11 are 0.3 μl of normal sow milk or colostrum collected from clinically normal mammary glands at the indicated time points after farrowing. n = 11 sows and 22 glands total.

D. <u>Discussion</u>

Protein composition in sow milk changed greatly from that seen at the time of

farrowing until the transition to mature milk was complete (Figure 1). Consistent with

the idea of "leaky" tight junctions in the mammary epithelium at farrowing closing as the

gland reaches mature lactation, milk concentrations of serum components such as

albumin ($\sim$ 13 to 3 mg/ml) and chloride ($\sim$ 93 to 53 mg/100 ml), decreased from colostral

to mature milk levels (Figures 4 and 5). The values reported in this study for the

concentration of albumin in sow milk are in agreement with that previously reported

(Klobasa and Butler, 1987); those for the concentration of chloride in sow milk are

somewhat higher than that previously reported, 48-69 mg/100 ml on d 1 and 38-47

mg/100 ml on d 13 and 27 of lactation (Seynaeve et al., 1996).

Immune related proteins such as immunoglobulins (Figure 1) and lactoferrin

(Figures 1 and 6) were present in higher concentrations in colostrum shortly after

farrowing than in milk during more advanced lactation. Milk concentrations of total

immunoglobulins decreased with advancing lactation, which is consistent with previous

reports (Curtis and Bourne, 1971; Klobasa and Butler, 1987; Klobasa et al., 1987). Milk

lactoferrin concentrations, as described by Western blot procedure (Figure 6), were

approximately five to six-fold higher than the values reported by Yang et al. (2000) using

ELISA. The high early concentrations of these proteins, 14.3 % of total protein for

immunoglobulins (Klobasa et al., 1987) and 9.1 mg/ml for lactoferrin, may serve two

separate, but equally important purposes: the first being protection of the mammary gland

from infection, the second the protection of the gut of the suckling piglet. It has long

been accepted that high levels of colostral immunoglobulins are important for systemic

immunity of the neonatal piglet (Brambell, 1958; Butler, 1971; Milon et al., 1983). It has

also been proposed that milk immunoglobulins protect the neonatal gut against pathogens,

particularly IgA (Wilson (1974), as milk IgA levels are higher in the milk of mature sows,

presumably from greater cumulative immune stimulation (Klobasa and Butler, 1987).

High levels of lactoferrin in milk may provide protection against infection of both

the mammary gland and the neonatal gut through the sequestration of iron, rendering it

unavailable to support bacterial growth (Nuijens et al., 1996). The digestion of

lactoferrin in the gut of the suckling piglet is reduced compared to the adult pig (Drescher

et al., 1999), and prefeeding of lactoferrin prior to intravenous endotoxin challenge in

piglets reduced mortality from 73.7 to 16.7 % (Lee et al., 1998). Lactoferrin also

decreased the binding of lipopolysaccharide (LPS) to monocytes/macrophages, and

reduced resultant cytokine production (Lee et al., 1998). Lastly, milk lactoferrin may

also play a role in the absorption of iron from the gut, as demonstrated through tracer

studies by Fransson et al. (1983).

Milk total protein concentration was high shortly after farrowing (~ 17 %) and

decreased to about 7 % on d 5 of lactation (Figure 2). The pattern of change in milk total

protein concentration is consistent with previous reports (Perrin, 1955; Klobasa et al.,

1987; Jackson et al., 1995; Mateo et al., 2004), though absolute values are approximately

1-2 % higher. The discrepancy may be an artifact of the Lowry procedure, which fully

accounts for all protein in the sample, including those in membranes. Milk β-casein

concentration was also high shortly after farrowing ($\sim$ 15 mg/ml) and decreased to about 9 mg/ml by 24 h post-farrowing (Figure 3). These values are a novel report for the pig, and are consistent with the pattern seen for casein in milk seen in the cow: reducing slightly from parturition, and increasing in the proportion of total protein (Parrish et al., 1948; 1950). Additionally, milk α_S- and β-caseins appeared to be regulated independently in the sow, with the former increasing while the latter decreased during the transition from colostrum to milk (Figure 1). Based on full genome sequence data from several species, it has been suggested that the α_{s1}-, α_{s2}-, and β-casein genes derive from a single gene cluster, and are likely regulated together (Jones et al., 1985). This may not be the case in the pig, or more likely, it may be that the post-transcriptional processing of β-casein differs from that of the α_s-caseins.

E. <u>Implications</u>

This study is the second to report the changes in the pattern of milk proteins from colostrum to mature milk as described by SDS-PAGE (Zou et al., 1992), and also for the values of chloride in colostrum and milk during the same time frame (Seynaeve et al., 1996). In whole, the data presented here suggest that the transition from colostrum to mature milk in the sow requires approximately 3-5 d to complete, and this finding is largely in agreement with previous reports (Perrin 1954; 1955; Klobasa et al., 1987; Jackson et al., 1995). This report presents a basis for comparing milk samples taken from mastitic glands to normal milk samples in order to understand the reduced milk

nutritional quality resulting from mastitis, and to partly explain reduced piglet growth

performance in response to mastitis.

Chapter IV

ACUTE RESPONSES OF PRIMIPAROUS SOWS AND LITTERS TO ENDOTOXIN-

INDUCED MASTITIS DURING WEEK ONE OF LACTATION

A. Introduction

Pre-weaning mortality is high in the pig as compared to other livestock species,

with between 8-20 % of live births not surviving to weaning (Straw et al., 1998, Cutler et

al., 1999). The most recent PigChamp Breeding Herd summary for 2002 indicates the

national herd average pre-weaning mortality was 13.12 % for the U.S. and 11.57 % for

Canada (PigChamp, 2002). The majority of piglet deaths occur in the first four days

following parturition, with a high proportion of these deaths (up to 60 % or more) directly

attributable to the sow (Fahmy and Bernard, 1971; English and Morrison, 1984; Cutler et

al., 1999). The sow is often responsible through either failure to provide an adequate

milk supply, or through direct actions such as crushing and/or savaging (English et al.,

1977; Dyck and Swierstra, 1987; Prime et al., 1987). While an inadequate milk supply

directly contributes to piglet mortality through starvation, it also makes crushing a more

likely possibility, by causing the piglet to spend more time close to the sow in an effort to

obtain adequate nutrition.

Mastitis is a common contributing factor in porcine hypogalactia syndrome (PHS,

or lactation failure), a condition affecting 5-10 % of all herds (Bäckström, 1973 and

Jorsal, 1983, as cited by Persson et al., 1989) and 14.4-26.6 % of all sows, dependent upon feed restriction (Persson et al., 1989). Mastitis in the sow is most often caused by coliform bacteria (Ringarp, 1960; Klopfenstein et al., 1999), resulting in an insufficient or complete lack of milk consumption by the piglets (Ross, 1983), and increased mortality and suboptimal growth (Curtis, 1974; Dyck et al., 1987).   Therefore, it is important to understand how milk composition and yield changes in response to mastitis in order to determine how the nutrition of the suckling piglet is compromised, and to devise interventions to address the shortfall(s).

The effects of mastitis on milk composition have been well described experimentally in the cow, both following bacterial challenge (Harmon et al., 1976; Shuster et al., 1995; Hoeben et al., 2000) and endotoxin challenge (Carrol et al., 1964; Guidry et al., 1983; Shuster et al., 1991; Lappalainen et al., 1998). The effects of mastitis on milk composition in the sow are less well described, though there has been some work with live cultures (Drendel and Wendt, 1983; Magnusson et al., 2001; Löving and Magnusson, 2002) and endotoxin (Kauf and Kensinger, 2002). Additionally, there have been reports on changes in serum components in the sow following endotoxin challenge (Nachreiner et al., 1972; Nachreiner and Ginther, 1974). Reports on the effects of mammary inflammation on suckling piglet growth performance are limited (Tarasiuk and Pejsak, 1986).

Therefore, the objective of this study was to determine the effects of an intramammary endotoxin challenge (Kensinger et al., 1999) during the first week of lactation on milk protein composition, milk yield, and piglet growth performance.

B. Materials and methods

Animals and experimental design

Twenty parity-one Yorkshire or Yorkshire crossbred sows were farrowed in groups of four, due to facility constraints, from February to September 2000. Sows were allowed to farrow spontaneously, and piglets were allowed to suckle birth sows for at least one day to allow for maximal colostral intake.   On approximately d 3 of lactation after all sows had farrowed, piglets were cross-fostered and litters standardized to 9 ($\pm$ 1) piglets for each of three sows per group, and the remaining piglets assigned to the fourth sow, which was not used. Cross-fostering was performed to equalize piglet weights and source litter. Milk samples were collected from infused glands and pairwise control glands at approximately 4-5 h post intramammary endotoxin infusion on approximately d 3, 5, and 7 of lactation. Milk samples (3-5 ml) were collected by manual expression during nursing, and sows received 1 ml (10 I.U.) of oxytocin administered i.m. 0, 3, or 6 X daily (see Kensinger et al., 2001). Samples and data from sows and litters were used only if they completed the study in the time frame, and piglets stayed with their teat for 24 h post-infusion. Therefore, milk samples from a total of seven sows were used for the analysis of milk proteins, and data from eight sows and litters were used for analysis of milk yield and 24 h piglet weight gain.

Endotoxin challenge model of mastitis

On alternating days during the first week of lactation, typically d 3, 5, and 7, sows received intramammary infusions of lipopolysaccharide (LPS) endotoxin (Sigma # L-2880, phenol extract of *E. coli* 055:B5; 1.5 µg/gland/kg BW) at 0800 after nursing (Kensinger et al., 1999). Individual sow endotoxin infusions were staggered by 20 min such that 1 h was required to perform infusions on all three gilts within a group. Endotoxin was infused using tuberculin syringes and 24 ga. tubing adaptors into the streak canal of one mammary gland each of two separate teats on a total of three days. Mammary glands were chosen for infusion based on being functional, apparently non-infected, and supporting a robust, healthy piglet on the day of the experiment, and the same teat was never infused twice. Teats were taped closed with Elastikon™ tape to prevent leakage of infused endotoxin, and the tape was removed at 0900 following that hour's nursing so that glands were protected from nursing for two hours. Sow rectal temperatures were recorded hourly during the experimental day. Piglet nursing order, or identification of piglets relative to teat suckled, along with visual observations of treated glands, was recorded every few days before and after endotoxin administration to determine whether the treated glands still produced milk. Further details on the endotoxin challenge model are in Appendix G.

Weigh-suckle-weigh procedure

Milk yield was estimated by weigh-suckle-weigh (WSW) procedure adapted from Lewis et al. (1978), Noblet and Etienne (1989), and Farmer et al. (1992). Litters were separated from their dams on experimental days between hourly measurements of milk yield from 0800-1700. At hourly intervals beginning at 0900, piglets were quickly weighed individually using a Sartorious EB15DCE-IOUR balance (15 kg cap., 0.5 g accuracy). Recorded piglet weights were an automatically generated average of 20 weights. Piglets were then allowed to nurse for approximately 9 ½ minutes, and weighed individually again. Hourly milk yield estimates were calculated from the difference of the pre- and post-nursing piglet weights, and adjusted for insensible water loss (min*(0.21 g/kg $BW^{0.75}$)) (Noblet and Etienne, 1986). Piglets were encouraged to urinate and defecate by placing them in a cold container prior to the pre-nursing weighing and also by agitation of the piglets in the container. Piglets were transported from the scale to the farrowing crate and back in containers containing pre-weighed absorbent bench paper between obtaining pre- and post-nursing weights. The bench paper was weighed after obtaining the post-nursing weights to determine the amount of waste captured in the transport containers. This weight was equally divided between piglets observed to have eliminated in the containers, and added to the hourly milk yield estimate calculated using those piglets. Milk yield estimates were discarded if piglets urinated or defecated in the farrowing crate during nursing. Milk yield estimates calculated to be negative were also discarded, as these milk yields could not be accounted for. Individual piglet weights

were obtained at 0800 the following day so that 24 h weight gains could be calculated.

No creep feed was available to the piglets during this study; therefore, milk intake alone

was responsible for providing the nutrients necessary for piglet weight gain. Further

details on the weigh-suckle-weigh procedure are in Appendix G.

Lowry assay and albumin ELISA

Milk total protein and albumin concentrations were determined as previously

described in Chapter III.

β-casein ELISA

Milk β-casein concentrations were determined similarly as previously described

in Chapter III, but using a different range of standards, a different secondary antibody,

and without whey. Fifty microliters/well of standards (0, 20, 40, 60, 80, and 100 μg/ml

of β-casein) or milk samples (1:625 final dilution, previously diluted 1:50 at least one

week prior in 4.71 M urea, 147 mM tris, 0.05 % tween-20, pH 6.8) in sample buffer (50

mM NH_4HCO_3, 0.05 % tween-20, pH 8.5) competed with plate bound β-casein for

binding to 50 μl/well of rabbit anti-porcine β-casein (1:20 k dilution) in blocking solution.

All standards and milk samples were run in triplicate. One hundred microliters/well of

(goat anti-rabbit IgG)-horseradish peroxidase conjugate (1:30 k dilution, Rockland Inc.

611-1322) in blocking solution was used as the secondary antibody; values for samples

were calculated from a polynomial regression curve. Further details on β-casein

determination methods are in Appendix C.

Electrophoresis and Western blotting

Electrophoresis and Western blotting were performed as previously described in

Chapter III.

Statistical analysis

A total of seven sows completed three experimental days within the first week of

lactation, and were therefore used for statistical analysis of milk protein composition.

Sows farrowed spontaneously; therefore, experimental days occurred from d 2-10 of

lactation depending on the sow. For the purposes of this experiment, experimental days

three and four were classified as day three, experimental days five and six were classified

as day five, and experimental days seven and eight were classified as day seven for

presentation and statistical analysis. For the analysis of milk yield and average daily gain,

data from a total of eight sows and litters were used because those data were complete for

teat infusion status and piglet gender. The statistical design was a randomized complete

block design utilizing repeated measures on each experimental day.

Statistical Analysis Software^{TM} (SAS) version 8.2 (SAS Institute Inc., Gary, NC)

was used for all statistical models. The statistical model (Proc Mixed) used for rectal

temperature data included sow, time relative to endotoxin infusion, farrowing group, and day of lactation as class variables; and took the form of rectal temperature equals the combined effects of farrowing group, hour relative to endotoxin infusion, day of lactation, and the hour relative to endotoxin infusion by day of lactation interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were time relative to endotoxin infusion and day of lactation; the subject was sow within group.

The statistical model (Proc Mixed) used for milk composition included sow, farrowing group, day of lactation, and endotoxin treatment as class variables; and took the form of component concentration equals the combined effects of farrowing group, day of lactation, endotoxin treatment, and the day of lactation by endotoxin treatment interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were endotoxin treatment and day of lactation; the subject was sow within group.

The statistical model (Proc Mixed) used for hourly milk yield included sow, farrowing group, piglet, piglet sex, endotoxin treatment, number of maternal oxytocin injections, day of lactation, and hour relative to endotoxin infusion as class variables; and took the form of milk yield equals the combined effects of sow, endotoxin treatment, piglet sex, number of maternal oxytocin injections, day of lactation, hour relative to endotoxin infusion, the piglet sex by endotoxin treatment interaction, the endotoxin

treatment by hour relative to endotoxin infusion interaction, and the endotoxin treatment by day of lactation interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were day of lactation and hour relative to endotoxin infusion; the subject was piglet within sow, endotoxin treatment, and piglet sex.

The statistical model (Proc Mixed) used for 24 h weight gains of suckling piglets included sow, farrowing group, piglet, piglet sex, endotoxin treatment, number of maternal oxytocin injections, day relative to endotoxin infusion, and day of lactation as class variables; and took the form of weight gain equals the combined effects of endotoxin treatment, piglet sex, day relative to endotoxin infusion, number of maternal oxytocin injections, day of lactation, the piglet sex by endotoxin treatment interaction, the endotoxin treatment by day relative to endotoxin infusion interaction, and the endotoxin treatment by day of lactation interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were day relative to endotoxin infusion and day of lactation; the subject was piglet within sow, endotoxin treatment, and piglet sex.

All statistical models were initially performed using all independent variables; non-significant factors were removed from the models prior to final analysis and calculation of LSmeans. The kenwardroger method was used for the denominator

degrees of freedom (as recommended by Dr. Peter Tozer), and significance was declared at $P \leq 0.05$.

C. Results

Sow rectal temperature following intramammary endotoxin infusion during week one of
  lactation

The mean rectal temperatures of sows subjected to intramammary endotoxin challenge during week one of lactation were 39.7 °C before infusion, peaked at 40.8 ° C at 4 h post-infusion, and declined over time to 40.1 °C at 10 h post-infusion (see Figure 7). Rectal temperature was significantly increased above baseline by 3 h following endotoxin infusion ($P < 0.01$) and reached a peak response of 1.1° C above baseline at 4 h post-infusion. Rectal temperatures declined over time to 0.4 °C above baseline at 10 h post infusion, which was significant ($P < 0.01$). Mean rectal temperature differed according to the day of lactation ($P < 0.05$), with mean values of 40.0, 40.1, and 40.6 ° C on d 3, 5, and 7 of lactation, respectively; the mean value on d 7 was significantly higher than on d 3 or 5 of lactation ($P < 0.05$). However, the patterns of rectal temperature response were similar for d 3, 5, and 7 of lactation. Mean rectal temperature also differed among farrowing groups ($P < 0.05$), with mean values of 40.1, 40.6 and 40.0 for groups 3, 4, and 5, respectively; the mean value for group 4 was significantly higher than for group 5, but not higher than for group 3 ($P < 0.05$). The raw

data for the rectal temperatures following intramammary endotoxin infusion during week

one of lactation in the sow are in Appendix I.

Figure 7. Sow rectal temperatures following intramammary endotoxin infusion on d 3, 5, & 7 of lactation. Rectal temperatures were recorded at the indicated time points relative to endotoxin infusion. n = 21 sow days. Error bars average ± 0.14 °C.

Pattern of milk proteins in sow colostrum and milk following intramammary endotoxin
  infusion

The casein content of pooled colostrum and milk samples obtained from infused

glands relative to control glands was reduced by intramammary endotoxin infusion by 5 h

post-infusion (see Figure 8). Results for pooled samples were representative of

individual animals (data not shown). The density of the α_s-caseins bands changed more

than that of β-casein. It appeared that there was proteolytic cleavage of the caseins, as

multiple molecular weight fragments smaller than 21 kDa in molecular weight appeared in samples from endotoxin-infused glands. This proposed proteolytic degradation of casein may have been greater on d 3 than on d 5 or 7 of lactation. Also interesting was the appearance of an unidentified protein migrating at approximately 45 kDa in samples from endotoxin-infused glands. It was not clear if there was a significant increase in lactoferrin or immunoglobulins in sow milk at 5 h after endotoxin-infusion.

Figure 8. SDS-PAGE analysis of pooled sow colostrum and milk samples following endotoxin infusion. MW equals 2 μl molecular weigh markers. Lanes 2-7 are 0.2 μl of sow colostrum or milk collected from control (c) and endotoxin-infused (e) mammary glands approximately 5 h post-infusion on d 3, 5, & 7 of lactation. pLf equals 2 μg porcine lactoferrin. pSA equals 2 μg porcine serum albumin. pIgG equals 4 μg porcine immunoglobulin G. pWC equals 4 μg porcine whole casein. pBC equals 2 μg porcine β-casein. A equals α_s-casein. n = 7 sows and 14 glands total.

Total protein concentration in sow colostrum and milk following intramammary
    endotoxin infusion

The mean total protein concentration in normal colostrum and milk averaged 7.4,

5.4, and 6.6 % on d 3, 5, and 7 of lactation, respectively. Whereas there was a trend for

increased concentrations of total protein in milk 5 h after endotoxin infusion on d 5 and 7,

this trend was not evident on d 3. The raw data for milk total protein concentration in

colostrum and milk following endotoxin infusion are in Appendix J.

β-casein concentration in sow colostrum and milk following intramammary endotoxin
    infusion

β-casein concentrations of colostrum and milk from sows subjected to

intramammary endotoxin challenge during week one of lactation averaged 26.8 and 14.1

mg/ml at 5 h post-infusion for control and LPS-infused glands, respectively (see

Figure 9). The concentration of β-casein in milk was reduced by approximately 47 % at

5 h post-infusion in milk from infused glands when compared to milk from control glands

($P < 0.01$). The concentration of β-casein in milk did not differ among farrowing groups,

or among days of lactation, and there was no day by treatment interaction. The raw data

for milk β-casein concentration in colostrum and milk following endotoxin infusion are

in Appendix K.

Figure 9. β-casein in sow colostrum and milk following endotoxin infusion. Milk samples were collected from control and endotoxin-infused mammary glands approximately 5 h post-infusion on days 3, 5, & 7 of lactation. n = 7 sows and 42 glands total. Error bars are ± 1.81 and 2.28 mg/ml for control and LPS-infused glands, respectively.

Albumin concentration in sow colostrum and milk following intramammary endotoxin
    infusion

Albumin concentrations of colostrum and milk from sows subjected to

intramammary endotoxin challenge during week one of lactation averaged 1.4 and 1.9

mg/ml at 5 h post-infusion for control and LPS-infused glands, respectively (see

Figure 10). While not significant, there may be a trend for the concentration of albumin

in milk to increase 5 h following endotoxin infusion, particularly on d 5 and 7 of lactation.

The concentration of albumin in milk did not differ among farrowing groups, day of

lactation, or by LPS treatment, and there was no day by treatment interaction. The raw

data for milk albumin concentration in colostrum and milk following endotoxin infusion

are in Appendix L.

Figure 10. Albumin in sow colostrum and milk following endotoxin infusion. Milk samples were collected from control and endotoxin-infused glands approximately 5 h post-infusion. n = 7 sows and 42 glands total. Error bars are ± 0.24 and ± 0.33 mg/ml for control and LPS-infused glands, respectively.

Hourly sow colostrum and milk yields following intramammary endotoxin infusion

Hourly milk yields over 10 h, as determined by weigh-suckle-weigh procedure, of

sows subjected to intramammary endotoxin challenge during week one of lactation

averaged 14.9 and 12.5 g/h for control and LPS-infused glands, respectively (see

Figure 11). Hourly milk yields were lower (16 %) in infused glands when compared to

control glands on experimental days during the first week of lactation (P < 0.01). Hourly

milk yields differed among sows, and increased with the day of lactation (P < 0.01), as

mean values were 11.0, 15.0, and 15.2 g/h for d 3, 5, and 7 of lactation, respectively.

Hourly milk yields were significantly higher on d 5 and 7 than on d 3 of lactation

(P < 0.01). Hourly milk yields were different according to the hour relative to endotoxin

infusion, due to when sows received injections of oxytocin (P < 0.01). Hourly milk

yields did not differ according to sex of the suckling piglet, the sex by treatment

interaction, the treatment by hour interaction, or the treatment by day interaction. The

raw data for sow hourly milk yields following endotoxin infusion during week one of

lactation are in Appendix M.

Figure 11. Hourly milk yields over 10 h of control and LPS-infused sow mammary glands following endotoxin infusion on d 3, 5, & 7 of lactation. n = 213 teat days. Error bars are ± 0.36 and ± 0.68 g/h for control and LPS-infused glands, respectively.

24 h weight gains of suckling piglets following intramammary endotoxin infusion during
    week one of lactation

    Daily weight gains (DWG) differed according to the day relative to infusion by

treatment interaction ($P < 0.01$) (see Figure 12). Prior to infusion, DWG did not differ

for piglets nursing control and LPS-infused glands, but the DWG of piglets nursing LPS-

infused glands was approximately 44 % less than that for piglets nursing control glands

for the 24 h period following infusion (90.2 vs. 160.9 g/d; $P < 0.01$). DWG differed

according to day of lactation ($P < 0.01$), with mean values of 97.8, 140.1, and 142.0

g/24 h for d 3, 5, and 7 of lactation, respectively. Overall DWG were significantly higher

on d 5 and 7 than on d 3 of lactation ($P < 0.01$). DWG was not affected by piglet sex, day

relative to endotoxin infusion, number of oxytocin injections received by the sow, the sex

by treatment interaction, or the treatment by day of lactation interaction. The raw data

for piglet DWG following endotoxin infusion during week one of lactation are in

Appendix N.

Figure 12. 24 h weight gains of suckling piglets following maternal endotoxin infusion on d 3, 5, & 7 of lactation. n = 426 piglet days. Error bars average ± 6.66 g/24 h and ± 10.94 g/24 h for piglets nursing control and LPS-infused glands, respectively.

D. Discussion

Endotoxin challenge provoked an inflammatory response in all experimental sows, with infused mammary glands being firm, reddened, and warm to the touch within two hours of infusion. Significant increases in rectal temperature trailed by only an hour, with a peak response of 1.1° C above baseline by 5 h post-infusion (Figure 7). The rectal temperature response observed in these sows was approximately 0.3° C less and 1 h later than that reported for intramammary infusion of 0.5 µg/kg BW of endotoxin by de Ruijter et al. (1988). In all sows, clinical signs of anorexia, depression, and water refusal were seen by the time of peak rectal temperature response, though the extent of symptoms

varied from sow to sow. All sows were observed to be eating and drinking by the end of

the experimental day (10 h post infusion), and appeared clinically normal on the next day.

While the model utilized in this experiment results in mild and transient inflammation, it

is an accurate reflection of that seen in coliform mastitis. It has been previously shown in

the cow that intramammary endotoxin challenge is a transient model of mastitis; the

inflammatory response is more rapid and there is a less severe systemic response than in

a live bacterial challenge (Hoeben et al., 2000).

All sows showed a response to intramammary endotoxin, which differs from the

results seen by researchers using live cultures of *Bacteroides spp., Peptococcus spp., and*

Streptococcus spp. (Drendel and Wendt, 1993) and of *E. coli* (Magnusson et al., 2001;

Löving and Magnusson, 2002). The difficulty of establishing a consistent response to

bacterial challenge encountered by other researchers was the main factor behind the

development of the endotoxin challenge model (Kensinger et al., 1999) used in this study.

The inconsistency in provoking mastitis through bacterial challenge is likely due to the

fact that it is generally the endotoxin present in the cell wall of Gram negative bacteria

that provokes the inflammatory response, and not the whole bacterium (Morkoc et al.,

1983; de Ruijter et al., 1988). More specifically, lipopolysaccharide binding protein

(LBP) binds LPS and presents it to the CD14 receptor on the monocytes to provoke the

cytokine cascade (Schumann et al., 1990; Wright et al., 1990). Cytokines are released in

response to attract immunoactive cells (Janeway et al., 1999, as cited by Löving and

Magnusson, 2002) and activate antigen presenting cells (APCs) (Koerner et al., 1987).

When using bacterial challenge to model mastitis, the severity of the mastitic episode is dependent upon the magnitude of that cytokine release, rather than the rate of bacterial elimination (Löving and Magnusson, 2002). The amount and types of immune cells in the gland play a role in the response of the sow to bacteria, as Magnusson et al. (2001) reported that sows developing clinical signs following intramammary bacterial challenge had lower levels of MHC class II cells, and higher levels of $CD4^+$ and $CD8^+$ cells pre-infusion. Sows that fail to respond to live bacterial challenge may have greater concentrations of lactoferrin in the milk pre-infusion, which binds LPS and prevents its interaction with LBP and monocytes in the milk space (Nuijens et al., 1996). Additionally, bactericidal/permeability-increasing protein (BPI) can bind LPS within the membrane of the live bacteria, causing lethal changes in its integrity, and preventing the recognition of LPS by LBP (Marra et al., 1992; Weiss et al., 1992). By directly infusing endotoxin in this study, the inflammatory process was directly provoked while likely overwhelming any potential means of preventing cytokine release.

Inflammation in the mammary gland is often likened to returning the gland to a more immature state of lactation. Specifically, inflammation either provokes or results from an opening of tight junctions in the mammary epithelium, and is evidenced by the transmigration of serum components into the milk space. Additionally, it requires 3-5 d of lactation for colostrum to change over to mature milk in the sow (Chapter III). Not surprisingly, the responses of mammary glands that are producing a more colostral-like secretion differ from those producing a more milk-like secretion. Samples collected from infused glands on the first experimental day (d 3 of lactation) showed a decrease in total

protein, albumin (Figure 10), and possibly immunoglobulins (Figure 8) relative to samples from control glands. Once the mammary secretion reached a more mature milk-like composition (d 5 and 7 of lactation), milk compositional changes after inflammation more closely reflected those expected. Total protein appeared to be increased in samples from infused glands on d 5 and 7 of lactation, which may reflect either a concentration of the milk secretion, a massive influx of serum proteins, or a combination of the two. Serum components in milk such as albumin (Figure 10) and immunoglobulins (Figure 8) appeared to be higher in samples from LPS-infused glands than from control glands at 5 h post-infusion. Additionally, the concentrations of milk specific proteins such as total caseins were decreased (Figure 8); specifically, β-casein was decreased by 47 % (Figure 9).

An unexpected result was an apparently greater decrease in the α_s-caseins relative to β-casein in samples from infused glands (Figure 8). This trend has also been seen in spontaneous cases of mastitis in the cow (Matson et al., 2004). The infusion of *Streptococcus agalactiae* or endotoxin from *E. coli* O55:B5 the cow has been reported to have the opposite effect, greater degradation of β- than α_{S1}-casein (Anderson and Andrews, 1977). A potential explanation for the decrease in caseins is proteolytic degradation, as evidenced by the appearance of smaller molecular weight fragments (less than 21 kDa) in samples from inflamed glands, particularly as seen at the migration front (Figure 8). Decreases in total casein have been seen following endotoxin infusion in the cow (Lappalainen et al., 1988; Kaartinen et al., 1998), and the degradation products of casein seen in mastitic milk are termed proteose peptones (Moussaoui et al., 2002). Cow

milk with high somatic cell counts (SCC), one indicator of mastitis, has been shown to have increased plasmin (Schaar and Funke, 1986) and increased rates of lipolysis and proteolysis (Ma et al., 2000). Plasmin and elastase have been detected in milk following endotoxin infusion in the cow, and maximal activity for both is seen 4-8 h post-infusion (Moussaoui et al., 2003), similar to the timing of the proposed proteolytic degradation in this study.

Also of interest was the appearance of an unidentified protein in milk from inflamed glands migrating at approximately 45 kDa (Figure 8). Prior to inflammation, this band was not seen, but clearly increased in response to inflammation. Since milk samples were collected only 5 h post-infusion, it is likely that this protein was previously present at some level prior to mammary inflammation before increasing following infusion. This probably occurred either through either through release from a mammary cell (epithelial or immune), or by migration from the serum in response to the opening of tight junctions, much as is seen in the increase of albumin in milk from inflamed glands.

The effects of intramammary inflammation on milk yield and piglet weight gain were dramatic. Mean hourly milk yield on the day of infusion was reduced by 16 % in infused versus control glands (Figure 11); piglets nursing those glands gained approximately 44 % less weight over the 24 h period post-infusion when compared to littermates nursing control glands (Figure 12). The disparity between milk yield and piglet growth reduction from endotoxin infusion suggests that milk from inflamed glands was less nutritious to the suckling piglet, and it was clear that milk composition was altered, apparently for the worse. Assuming that the milk yield recorded during weigh-

suckle-weigh procedure was similar to that not recorded during the overnight period, a theoretical feed conversion for piglets consuming milk could be calculated by converting hourly milk yield to 24 h and dividing by piglet weight gain. Using this assumption, piglets nursing control glands consumed 357.6 g of milk in the 24 h following endotoxin infusion, resulting in 160.9 g gain, for a theoretical feed conversion of 2.22 g milk/g piglet gain. For piglets nursing inflamed glands, the values were 300.0 g milk and 90.2 g gain; a theoretical feed conversion of 3.33 g milk/g piglet gain. Therefore, during the first week of lactation, a piglet nursing an inflamed gland would have to consume approximately 55 % more milk than a control littermate to support an equivalent rate of growth.

While the WSW procedure is the simplest method, though a labor-intensive one, to estimate milk yield in the sow, it is clear that it disturbs the natural suckling pattern of sow and litter, and likely artificially depresses milk yield (Barber et al., 1955; Pettigrew et al., 1985). Since the calculated value for feed conversion for piglets nursing control glands in this study was significantly less than the approximately 4.0 g milk/g gain calculated by multiple researchers for piglets nursing normal glands (Barber et al., 1955; Lewis et al., 1978; Noblet and Etienne, 1989; Hodbod and Zeman, 2001), it was possible that the milk yields recorded in this study here may not have been completely representative of lactation in a naturally occurring case of mastitis. However, any errors in milk yield estimation likely affected all piglets equally, as approximately 89 % of possible individual milk yields were captured by the data. Some piglets that had no gain or lost weight during the experimental day had gained weight by the next morning.

Therefore, there was likely some compensatory milk yield in the overnight hours. Lastly, the "nursing order" or the fidelity of one piglet to one teat remained fluid during the first week of lactation, particularly so on d 3 and 5. Approximately 11 % (8/71) of piglets nursing infused glands early in lactation switched teats, and were exposed to endotoxin infusion at least a second time. There may therefore have been some carryover effects of consuming milk from LPS-infused glands in these pigs that could not be accounted for.

E. Implications

This experiment was designed to mimic the natural course of coliform mastitis in the sow by use of an endotoxin challenge model of mastitis performed at various time points during the first week of lactation. It is clear that mammary inflammation altered milk composition and that the resultant milk was less capable of supporting the growth rate of piglets. It should be noted that the model of mastitis used in this study results in a mild and transient period of inflammation, and the effects reported here likely represent the minimum that could be expected in naturally occurring coliform mastitis. Additionally, milk samples collected from an individual teat were a composite, in that only one gland per teat was infused, and each teat was served by two mammary glands. Determining the precise magnitude of the effect of mammary inflammation on milk composition in the sow and suckling piglet performance was complicated during the first week of lactation by the facts that the changeover of colostrum to milk had not yet been

completed (see Chapter III), and that the "nursing order" of the piglets was not fully

established.

Chapter V

CHRONIC RESPONSES OF PRIMIPAROUS SOWS AND LITTERS TO
ENDOTOXIN-INDUCED MASTITIS DURING WEEKS TWO AND THREE OF
LACTATION

A. <u>Introduction</u>

As previously described (Chapter IV), preweaning mortality is a significant
problem in the pork industry, and mastitis provoked by coliform bacteria is a common
contributing factor. From the study in Chapter IV, it was clear that mammary
inflammation changes milk composition in the sow by 5 h post-endotoxin infusion during
the first week of lactation, but the effects were inconsistent. This was likely due to the
fact that it required between 3 and 5 d for colostrum to change over to milk in the sow
(Chapter III). Other possibilities such as genetic differences between sows and previous
exposure to endotoxin may also explain some of the inconsistency in response to
endotoxin. Additionally, assessing the effects of mammary inflammation on piglet
growth performance were complicated by the fact that the "nursing order" was not yet
stable until d 5-7 of lactation (Chapter IV). Therefore, the objective of this study was to
repeat the intramammary endotoxin challenge model of mastitis (Kensinger et al., 1999)
during the second and third weeks of lactation, and determine the effects on milk protein
composition, yield, and piglet growth performance. The nursing order was more stable at

this point in time, and most piglets were strong and healthy, and therefore better able to defend their teat and its milk supply from littermates. Additionally, milk samples were more easily collected from the sow during milk ejection, as nursing behavior was well established. A second objective was to elucidate further the physiological changes resulting from intramammary endotoxin challenge, by measuring TNF-α concentrations in milk and serum, as well as to determine if milk lactoferrin and chloride levels changed in response to mammary inflammation in the sow. A last objective was to determine for how long milk composition was altered, and piglet growth compromised by intramammary inflammation. Milk samples were therefore collected for 60 h post-infusion, and daily piglet weights were recorded throughout lactation.

B. <u>Materials and methods</u>

<u>Animals and experimental design</u>

Sixteen parity-one Yorkshire and Yorkshire crossbred sows were farrowed in groups of four, due to facility constraints, from May 2002 to February 2003, and were the same sows as used in Chapter III. Sows were allowed to farrow spontaneously, and piglets were allowed to suckle birth sows for at least one day to allow for maximal colostral intake. On approximately d 3 of lactation after all sows had farrowed, piglets were cross-fostered and litters standardized to 9 ($\pm$ 1) piglets for each of three sows per group, and the remaining piglets assigned to the fourth sow, which was not used. Cross-

fostering was performed such as to equalize piglet weights and source litter. Milk

samples were collected from infused glands prior to infusion (control) and at 5, 12, 24, 36,

48, and 60 h post intramammary endotoxin infusion on approximately d 13 and 20 of

lactation. Milk samples (3-5 ml) were collected by manual expression following the

injection of 0.5 ml (5 I.U.) of oxytocin administered i.v. (at 5 h post-infusion) or 1.0 ml

(10 I.U.) administered i.m., as needed after 5 h. All sows in this study were catheterized

via the anterior vena cava to allow for blood sampling on experimental days, and blood

samples were collected at -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 h relative to endotoxin

infusion. One sow had an elevated rectal temperature on the morning of both

experimental days, and was not infused with endotoxin. Therefore, a total of 11 sows

were used for the experiment.

Endotoxin challenge model of mastitis

On approximately d 13 and 20 of lactation, sows received intramammary

infusions of lipopolysaccharide (LPS) endotoxin (Sigma # L-2880, phenol extract of *E.*

coli 055:B5; 1.5 μg/gland/kg BW) at 0900 after nursing (Kensinger et al., 1999).

Individual sow endotoxin infusions were staggered by 20 min such that 1 h was required

to perform infusions on all three gilts within a group. Endotoxin was infused using

tuberculin syringes and 24 ga. tubing adaptors into the streak canal of one mammary

gland each of two separate teats on a total of two days. Mammary glands were chosen

for infusion based on being functional, apparently non-infected, and supporting a robust,

healthy piglet on the day of the experiment, and the same teat was never infused twice. Teats were taped closed with Elastikon[TM] tape to prevent leakage of the infused endotoxin, and the tape was removed at 1000 following that hour's nursing so that glands were protected from nursing for two hours. Sow rectal temperatures were recorded hourly during the experimental day and at 0900 the day following challenge. Piglet nursing order, or identification of piglets relative to teat suckled, along with visual observations of treated glands, was recorded every few days before and after endotoxin administration to determine whether the treated glands still produced milk. Further details on the endotoxin challenge model are in Appendix G.

Weigh-suckle-weigh procedure

Weigh-suckle-weigh (WSW) procedure was performed as previously described in Chapter IV, but with the following changes. Litters were separated from their dams between hourly measurements (0900-1600). At hourly intervals beginning at 1000, piglets were quickly weighed individually using a Sartorious EB15DCE-IOUR balance (15 kg cap., 0.5 g accuracy). Individual piglet weights were also recorded at 0800 from the day of cross-fostering until two days following the final endotoxin challenge so that 24 h weight gains could be calculated. No creep feed was available to the piglets during this study; therefore, milk intake alone was responsible for providing the nutrients necessary for piglet weight gain. Further details on the weigh-suckle-weigh procedure are in Appendix G.

<u>Lowry assay, β-casein ELISA, albumin ELISA, and chloride ion chromatography</u>

Milk total protein, β-casein, albumin, and chloride ion concentrations were determined as previously described in Chapter III.

<u>TNF-α ELISA</u>

A direct ELISA assay (Pierce-Endogen # EP2TNFA) for porcine TNF-α was performed as follows. Fifty microliters of sample diluent and 50 μl of standards (two-fold serial dilutions; 2000 to 31.3 pg/ml) or samples were added to the included microplate, covered, and incubated at room temperature for 2 h. All standards and milk samples were run in duplicate. Plates were inverted and blotted on a paper towel, and then washed 3 X with wash solution from a water bottle. All wash steps were performed the same way. One hundred microliters/well of biotinylated antibody reagent were added to the plate, the plate covered, and then incubated at room temperature for 1 h. Plates were washed, 100 μl/well of prepared streptavidin-HRP solution were added to the plate, the plate covered, and then incubated at room temperature for 30 min. Plates were washed, 100 μl/well of TMB substrate solution added to the plate, the plate covered, and then incubated at room temp for 30 min. Development was ceased with 100 μl/well of stop solution, and absorbance read at 450-550 nm wavelength using a microplate reader (Bio-Tek Instruments model #EL311). A standard curve was generated by plotting concentration against absorbance in Microsoft Excel, and unknown values were

calculated from a linear regression curve. Further details on TNF-α determination methods are in Appendix H.

Electrophoresis and Western blotting

Electrophoresis and Western blotting were performed as previously described in Chapter III.

Statistical analysis

A total of eleven sows completed both experimental days in the second and third weeks of lactation, and were therefore used for statistical analysis. The statistical design was a randomized complete block design utilizing repeated measures on each experimental day.

Statistical Analysis Software[TM] (SAS) version 8.2 (SAS Institute Inc., Gary, NC) was used for all statistical models. The statistical model (Proc Mixed) used for rectal temperature and milk composition included sow, time relative to endotoxin infusion, farrowing group, and week of lactation as class variables; and took the form of rectal temperature or component concentration equals the combined effects of farrowing group, time relative to endotoxin infusion, week of lactation, and the time relative to endotoxin infusion by week of lactation interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as

determined by preliminary analysis with Proc Mixed. The repeated measures were time relative to endotoxin infusion and week of lactation; the subject was sow within group.

The statistical model (Proc GLM) used for plasma TNF-α included sow, time relative to endotoxin infusion, farrowing group, and week of lactation as class variables; and took the form of TNF-α equals the combined effects of farrowing group, sow within group, week of lactation, time relative to endotoxin infusion, and the time relative to endotoxin infusion by week of lactation interaction.

The statistical model (Proc Mixed) used for 24 h weight gains of suckling piglets during week two and three of lactation included sow, farrowing group, piglet, piglet sex, endotoxin treatment, day relative to endotoxin infusion, and week of lactation as class variables; and took the form of weight gain equals the combined effects of endotoxin treatment, piglet sex, day relative to endotoxin infusion, week of lactation, the piglet sex by endotoxin treatment interaction, and the endotoxin treatment by day relative to endotoxin infusion interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were week relative to endotoxin infusion and day relative to endotoxin infusion; the subject was piglet within sow, endotoxin treatment, and piglet sex.

The statistical model (Proc Mixed) used for 24 h weight gains of suckling piglets during week two of lactation included sow, farrowing group, piglet, piglet sex, endotoxin treatment, and day relative to endotoxin infusion as class variables; and took the form of weight gain equals the combined effects of endotoxin treatment, piglet sex, day relative

to endotoxin infusion, the piglet sex by endotoxin treatment interaction, and the endotoxin treatment by day relative to endotoxin infusion interaction. The model was a repeated measures design, utilizing a spatial power covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measure was day relative to endotoxin infusion; the subject was piglet within sow, endotoxin treatment, and piglet sex.

The statistical model (Proc Mixed) used for hourly milk yield included sow, farrowing group, piglet, piglet sex, endotoxin treatment, week of lactation, and hour relative to endotoxin infusion as class variables; and took the form of milk yield equals the combined effects of sow, endotoxin treatment, piglet sex, week of lactation, hour relative to endotoxin infusion, the piglet sex by endotoxin treatment interaction, the endotoxin treatment by hour relative to endotoxin treatment interaction, and the endotoxin treatment by week of lactation interaction. The model was a doubly repeated measures design, utilizing an unstructured by compound symmetry covariance structure, as determined by preliminary analysis with Proc Mixed. The repeated measures were week of lactation and hour relative to endotoxin infusion; the subject was piglet within sow, endotoxin treatment, and piglet sex.

All statistical models were initially performed using all independent variables; non-significant factors were removed from the models prior to final analysis and calculation of LSmeans. For Proc Mixed models, the kenwardroger method was used for the denominator degrees of freedom (as recommended by Dr. Peter Tozer). Significance was declared at $P \leq 0.05$.

C. <u>Results</u>

<u>Sow rectal temperature following intramammary endotoxin infusion during weeks two and three of lactation</u>

The mean rectal temperatures of sows subjected to intramammary endotoxin challenge during the second and third weeks of lactation were approximately 39.1 °C before infusion, peaked at 40.8 °C at 5 h post-infusion, and declined over time to 39.2 °C at 24 h post-infusion (see Figure 13). Rectal temperature was significantly increased above baseline by 1 h following endotoxin infusion ($P < 0.01$) and reached a peak response of 1.7° C above baseline at 5 h post-infusion. Rectal temperatures declined over time to 0.9 °C above baseline at 12 h post-infusion, which was significant ($P < 0.01$), then continued to decline until 24 h post infusion, at which point they were not significantly different than prior to infusion. Mean rectal temperature differed according to the day of lactation ($P < 0.01$), with mean values of 40.4 and 39.4 ° C on d 13 and 20 of lactation, respectively; the mean value on d 13 was significantly higher than on d 20 of lactation ($P < 0.01$). However, the pattern of rectal temperature response was similar for d 13 and 20 of lactation. Mean rectal temperature also differed among farrowing groups ($P < 0.05$); with mean values of 40.4, 40.0, 39.7, and 39.5 ° C for groups 1, 2, 3, and 4, respectively; the mean values for groups 1 and 2 were significantly higher than for groups 3 and 4 ($P < 0.05$). The raw data for rectal temperatures following intramammary endotoxin infusion during weeks two and three of lactation in the sow are in Appendix O.

Figure 13. Sow rectal temperatures following intramammary endotoxin infusion on d 13 & 20 of lactation. Rectal temperatures were recorded at the indicated time points relative to endotoxin infusion. n = 22 sow days. Error bars average ± 0.14° C. n = 11 sows.

TNF-α concentration in sow plasma following intramammary endotoxin infusion during weeks two and three of lactation

The mean TNF-α concentrations in plasma collected from sows subjected to intramammary endotoxin challenge during the second and third weeks of lactation were approximately 19.5 pg/ml before infusion, peaked at 29.5 pg/ml at 1.5 h post-infusion, and declined over time to 20.5 pg/ml at 7 h post-infusion (see Figure 14). There was a trend for the concentration of TNF-α in plasma to be increased following endotoxin infusion ($P < 0.09$). The concentration of TNF-α in plasma differed among sows ($P < 0.01$), and there was a large variation in response among sows (data not shown). The concentration of TNF-α in plasma did not differ according to the week by time

interaction. The raw data for plasma TNF-α concentration following intramammary

endotoxin infusion during weeks two and three of lactation are in Appendix P.

Figure 14. TNF-α in plasma following endotoxin infusion. Plasma samples were collected at the indicated time points relative to endotoxin infusion on days 13 & 20 of lactation. n = 11 sows. Error bars average ± 2.83 pg/ml.

Pattern of milk proteins in mature sow milk following intramammary endotoxin infusion

The casein contents of pooled milk samples obtained from infused glands

following endotoxin infusion on d 13 and 20 of lactation were reduced within 5 h, and

remained so for up to 60 h post-infusion (see Figures 15 and 16). Results for pooled

samples were representative of individual animals (data not shown). The density of the

α_s-caseins bands changed more than that of β-casein. It appears that there was

proteolytic cleavage of the caseins, as multiple molecular weight fragments smaller than 21 kDa in molecular weight appeared in samples collected post-infusion. This proposed proteolytic degradation of casein may have been greater after infusion on d 13 than on d 20 of lactation. Also interesting was the appearance of an unidentified protein migrating at approximately 45 kDa that was apparent in samples taken after infusion. The density of this protein appeared to be greater at 5 h post-infusion on d 20 than on d 13 of lactation, but may have been visible for a longer period of time after infusion on d13 of lactation. The density of the heavy chain immunoglobulin bands may have been greater following endotoxin infusion, but this was not clear.

Figure 15. SDS-PAGE analysis of pooled milk samples following endotoxin infusion on approximately d 13 of lactation. MW equals 2 μl molecular weight markers. Lanes 2-8 are 0.2 μl of sow milk collected infused glands prior to infusion (con) and at the indicated time points post infusion. pLf equals 2 μg porcine lactoferrin. pSA equals 2 μg porcine serum albumin. pIgG equals 4 μg porcine immunoglobulin G. pWC equals 4 μg porcine whole casein. pBC equals 2 μg porcine β-casein. A equals α_s-casein. n = 11 sows and 22 glands total.

Figure 16. SDS-PAGE analysis of pooled milk samples following endotoxin infusion on approximately d 20 of lactation. MW equals 2 μl molecular weight markers. Lanes 2-8 are 0.2 μl of sow milk collected infused glands prior to infusion (con) and at the indicated time points post infusion. pLf equals 2 μg porcine lactoferrin. pSA equals 2 μg porcine serum albumin. pIgG equals 4 μg porcine immunoglobulin G. pWC equals 4 μg porcine whole casein. pBC equals 2 μg porcine β-casein. A equals α_s-casein. n = 11 sows and 22 glands total.

Total protein concentration in mature sow milk following intramammary endotoxin infusion

The mean total protein concentrations in milk from sows subjected to

intramammary endotoxin challenge during weeks two and three of lactation were 6.9 %

prior to infusion, increased to 8.3 % by 12 h post-infusion, and remained at 8.1 % at 60 h

post-infusion (see Figure 17). The concentration of total protein in milk was increased by

approximately 9 % at 5 h post infusion ($P < 0.01$), and remained increased further to

approximately 20 % above baseline at 60 h post-infusion (P < 0.01). The concentration

of total protein in milk was higher on d 20 than on d 13 of lactation (8.3 vs. 7.5 %;

P < 0.01). The concentration of total protein in milk did not differ among farrowing

groups, and there was no day by time interaction. The raw data for total protein

concentration in milk following endotoxin infusion during weeks two and three of

lactation are in Appendix Q.

Figure 17. Total protein in mature sow milk following endotoxin infusion. Milk samples were collected from endotoxin-infused mammary glands at the indicated time points relative to infusion on d 13 & 20 of lactation. n = 11 sows and 44 glands total. Error bars average ± 0.20 %.

β-casein concentration in mature sow milk following intramammary endotoxin infusion

The mean β-casein concentrations of milk from sows subjected to intramammary endotoxin challenge during weeks two and three of lactation were 9.9 mg/ml prior to infusion, decreased to 7.0 mg/ml by 5 h post infusion, rebounded slightly at 12 h post-infusion, and remained decreased at 7.2 mg/ml at 60 h post-infusion (see Figure 18). The concentration of β-casein in milk was decreased by approximately 30 % at 5 h post-infusion, and remained lower at approximately 31 % below baseline at 60 h post infusion, following a slight rebound at 12 h post-infusion ($P < 0.01$). The concentration of β-casein in milk was higher on d 20 than on d 13 of lactation (8.4 vs. 7.4 mg/ml; $P < 0.05$). The concentration of β-casein in milk did not differ among farrowing groups, and there was no day by time interaction. The raw data for β-casein concentration in milk following endotoxin infusion during weeks two and three of lactation are in Appendix R.

Figure 18. β-casein in mature sow milk following endotoxin infusion. Milk samples were collected from endotoxin-infused mammary glands at the indicated time points relative to infusion on d 13 & 20 of lactation. n = 11 sows and 44 glands total. Error bars average ± 0.42 mg/ml.

Albumin concentration in mature sow milk following intramammary endotoxin infusion

The mean albumin concentrations in milk from sows subjected to intramammary endotoxin challenge during weeks two and three of lactation were 3.3 mg/ml prior to infusion, increased to 4.8 mg/ml by 12 h post-infusion, and returned to 3.6 mg/ml by 60 h post-infusion (see Figure 19). The concentration of albumin in milk was increased by approximately 44 % at 5 h post-infusion (P < 0.01), and remained higher at 21 % above baseline at 48 h post-infusion (P < 0.01). The concentration of albumin in milk at 60 h post-infusion was not significantly different from that prior to infusion. The concentration of albumin in milk differed among farrowing groups (P < 0.01), with mean

values of 4.0, 3.0, 5.6, and 3.9 mg/ml for groups 1, 2, 3, and 4, respectively; the mean value for group 3 was higher than for the others ($P < 0.05$). The concentration of albumin in milk did not differ according to the day of lactation, and there was no day by time interaction. The raw data for albumin concentrations in milk following endotoxin infusion during weeks two and three of lactation are in Appendix S.

Figure 19. Albumin in mature sow milk following endotoxin infusion. Milk samples were collected from endotoxin-infused mammary glands at the indicated time points relative to infusion on d 13 & 20 of lactation. n = 11 sows and 44 glands total. Error bars average ± 0.23 mg/ml.

Chloride concentration in mature sow milk following intramammary endotoxin infusion

The mean chloride concentrations in milk from sows subjected to intramammary endotoxin challenge during weeks two and three of lactation were 53.5 mg/100 ml prior

to infusion, increased to 171.4 mg/100 ml by 12 h post-infusion, and remained at 76.6

mg/100 ml at 24 h post-infusion (see Figure 20). The concentration of chloride in milk

was increased by approximately 221 % at 5 h post-infusion ($P < 0.01$), and remained

higher by approximately 43 % above baseline at 24 h post-infusion ($P < 0.01$). The

concentration of chloride in milk differed among farrowing groups ($P < 0.05$), with mean

values of 110.4, 96.7, 92.2, and 102.7 mg/100 ml for groups 1, 2, 3, and 4, respectively;

the mean values for groups 1 and 4 were higher than for groups 2 and 3 ($P < 0.05$). The

concentration of chloride in milk did not differ according to the day of lactation, and

there was no day by time interaction. The raw data for chloride concentrations in milk

following endotoxin infusion during weeks two and three of lactation are in Appendix T.

Figure 20. Chloride in mature sow milk following endotoxin infusion. Milk samples were collected from endotoxin-infused mammary glands at the indicated time points relative to infusion on d 13 & 20 of lactation. n = 11 sows and 44 glands total. Error bars average ± 5.30 mg/100 ml.

<u>TNF-α concentration in mature sow milk following intramammary endotoxin infusion</u>

The mean TNF-α concentrations in milk from sows subjected to intramammary endotoxin challenge during weeks two and three of lactation were 17.4 pg/ml prior to infusion, increased to 570.8 pg/ml by 5 h post-infusion, and returned to 25.8 pg/ml by 24 h post-infusion (see Figure 21). Due to non-normality of the data, a $\log_{10}$ transformation of values for the concentration of TNF-α in milk was performed prior to statistical analysis. The concentration of TNF-α in milk was increased by approximately 33-fold at 5 h post-infusion ($P < 0.01$). The concentration of TNF-α in milk at 12 h post-infusion was not significantly different from that prior to infusion. The concentration of TNF-α in milk did not differ among farrowing groups, or among day of lactation, and there was no day by treatment interaction. The raw data for TNF-α concentrations in milk following endotoxin infusion during weeks two and three of lactation are in Appendix U.

Figure 21. TNF-α in mature sow milk following endotoxin infusion. Milk samples were collected from endotoxin-infused mammary glands at the indicated time points relative to infusion on d 13 & 20 of lactation. n = 11 sows and 44 glands total. Error bars are ± 54.00 pg/ml.

Lactoferrin concentration in mature sow milk following intramammary endotoxin
  infusion

Rabbit anti-porcine lactoferrin serum was used for Western blotting of pooled

milk samples at a dilution of $1:2 \times 10^6$ (data not shown). Lactoferrin was present, though

at low concentrations, in all samples collected prior to and up to 60 h post-infusion on d

13 and 20 of lactation (see Figures 22 and 23). Results for pooled samples were

representative of individual animals (data not shown). There was little evidence that the

concentration of lactoferrin in milk changed in response to endotoxin infusion. The

concentration of lactoferrin in sow milk appeared to be higher on d 13 than on d 20 of

lactation.

Figure 22. Western blot analysis of lactoferrin in pooled milk samples following endotoxin infusion on approximately d 13 of lactation. MW equals 5 μl prestained molecular weight markers. pLf equals 2 μg porcine lactoferrin standard. Lanes 3-9 are 1.2 μl of sow milk collected from infused glands prior to (d 13) and at the indicated time points post-infusion. n = 11 sows and 22 glands total.

Figure 23. Western blot analysis of lactoferrin in pooled milk samples following endotoxin infusion on approximately d 20 of lactation. MW equals 5 µl prestained molecular weight markers. pLf equals 2 µg porcine lactoferrin standard. Lanes 3-9 are 1.2 µl of sow milk collected from infused glands prior to (d 20) and at the indicated time points post-infusion. n = 11 sows and 22 glands total.

Hourly sow milk yields following intramammary endotoxin infusion

Hourly milk yields over 8 h, as determined by WSW procedure, of sows subjected to intramammary endotoxin challenge during weeks two and three of lactation averaged 25.9 and 17.8 g/h for control and LPS-infused glands, respectively (see Figure 24). Hourly milk yields were lower (31 %) in infused glands when compared to control glands on experimental days during the second and third weeks of lactation ($P < 0.01$). Hourly milk yields differed among sows, and were different according to the hour relative to

endotoxin infusion and the treatment by hour interaction (P < 0.01). Milk yield did not

differ according to sex of the suckling piglet, week of lactation, or the sex by treatment

interaction. The raw data for sow hourly milk yields following endotoxin infusion during

the second and third weeks of lactation are in Appendix V.

Figure 24. Hourly milk yields over 8 h of control and LPS-infused sow mammary glands following endotoxin infusion on d 13 & 20 of lactation. n = 200 teat days. Error bars are ± 0.81 and ± 1.45 g/h for control and LPS-infused glands, respectively.

<u>24 h weight gains of suckling piglets following intramammary endotoxin infusion during weeks two and three of lactation</u>

Daily weight gains (DWG) differed according to the day relative to infusion by

treatment interaction (P < 0.01) (see Figure 25). Prior to infusion, DWG did not differ

for piglets nursing control and LPS-infused glands (d -1), but the DWG of piglets nursing

LPS-infused glands was approximately 74, 55, and 25 % less than that for piglets nursing control glands for each of the three consecutive 24 h periods following endotoxin infusion (39.3 vs. 153.5, 78.6 vs. 174.2, and 127.9 vs. 196.5 g/d for d 0, 1, and 2 relative to endotoxin infusion, respectively; P < 0.01). Mean DWG were decreased by approximately 45 and 28 % for each of the two consecutive 24 h periods following endotoxin infusion relative to the 24 h period prior to endotoxin infusion (96.4 and 126.4 vs. 175.4 g/d; P < 0.01). DWG did not differ among days of lactation, or according to piglet sex, or the sex by treatment interaction.

Figure 25. 24 h weight gains of suckling piglets following maternal intramammary endotoxin infusion on d 13 & 20 of lactation. n = 800 piglet days. Error bars average ± 6.99 and are ± 12.64 g/24 h for piglets nursing control and LPS-infused glands, respectively.

In order to determine for how long piglets nursing LPS-infused glands were at a disadvantage compared to their littermates nursing control glands, the data set for DWG was restricted to week two of lactation. This allowed for an additional two consecutive 24 h periods following endotoxin infusion to be analyzed. For the restricted data set, DWG differed according to the day relative to infusion by treatment interaction ($P < 0.01$) (see Figure 26). Prior to infusion, DWG did not differ for piglets nursing control and LPS-infused glands (d -1), but the DWG of piglets nursing LPS-infused glands was approximately 64, 56, 25, 34, and 21 % less than that for piglets nursing control glands for each of the five consecutive 24 h periods following endotoxin infusion (54.0 vs. 150.7, 74.4 vs. 168.8, 147.1 vs. 195.6, 141.6 vs. 213.1, and 152.6 vs. 193.9 g/d for d 0, 1, 2, 3, and 4 relative to endotoxin infusion, respectively; $P < 0.01$). Mean DWG were decreased by approximately 43 and 32 % for each of the two consecutive 24 h periods following endotoxin infusion relative to the 24 h period preceding endotoxin infusion (102.4 and 121.6 vs. 178.4 g/d; $P < 0.01$). DWG was not affected by piglet sex or the sex by treatment interaction. The raw data for piglet DWG following endotoxin infusion during week two and three of lactation are in Appendix W.

Figure 26. 24 h weight gains of suckling piglets following maternal endotoxin infusion on d 13 of lactation. n = 600 piglet days. Error bars are ± 9.16 and ± 17.24 g/24 h for piglets nursing control and LPS-infused glands, respectively.

D. Discussion

Endotoxin challenge provoked an inflammatory response in all experimental sows, with infused mammary glands being firm, reddened, and warm to the touch within 2 h of infusion. Increases in rectal temperature were significant within 1 h of infusion, and a peak response of 1.7° C above baseline was seen at 5 h post-infusion (Figure 13). The rectal temperature response observed in these sows was similar to that observed in the sows from Chapter IV, and was approximately 0.3° C greater and 1 h later than reported for intramammary infusion of 0.5 μg/kg BW of endotoxin by de Ruijter et al. (1988). Rectal temperatures were no different than baseline at 24 h post-infusion. In all sows,

clinical signs of anorexia, depression, and water refusal were seen by the time of peak

rectal temperature response; though the extent of symptoms seen varied from sow to sow.

All sows were observed to be eating and drinking by the end of the experimental day (8 h

post infusion), and appeared clinically normal on the next day. While the model utilized

in this experiment results in mild and transient inflammation, it is an accurate reflection

of that seen in coliform mastitis.

Milk lactoferrin concentrations did not appear to change in response to endotoxin

infusion at any time up to 60 h (Figures 22 and 23), or 2 ½ d post-infusion. This

contrasts with the results of Ross et al. (1983) using live bacteria, who showed an

increase in milk lactoferrin in non-SPF sows 2-3 d following infusion of 0.5×10^4-10^7

CFU of *E. coli* O6:K23:H1. While changes in plasma TNF-α concentrations did not

reach significance (Figure 14), the increase was nearly 50 % 1 ½ h following infusion,

which was attributed to wide variation in TNF-α values among sows (data not shown).

Additionally, this closely paralleled the timing of the rectal temperature response, which

was significant at 1 h post-infusion (Figure 13). Milk TNF-α concentrations were

significantly increased by 5 h post-infusion (Figure 21), and returned to baseline by 12 h,

as was consistent with a transient role for cytokines.

Inflammation in the mammary gland is often likened to returning the gland to a

more immature state of lactation. Specifically, inflammation provokes or results from an

opening of tight junctions in the mammary epithelium, and is evidenced by the

transmigration of serum components into the milk space. Following intramammary

endotoxin infusion, milk total protein concentrations (Figure 17) increased approximately

20 % within 5 h of infusion, which may reflect either a concentration of the milk secretion, a massive influx of serum proteins, or a combination of the two. Serum components in milk such as albumin (44 %, Figure 19), chloride (221 %, Figure 20), and potentially immunoglobulins (Figures 15 and 16) were increased in samples collected within 5 h post-infusion. Additionally, the concentrations of milk specific proteins such as total caseins were decreased (Figures 15 and 16); specifically, milk β-casein was decreased by 30 % (Figure 18) at 5 h post-infusion. The effects of mammary inflammation on milk component concentrations were long-lasting; milk total protein was increased (Figure 17) and milk β-casein was significantly decreased (Figure 18) for 60 h post-infusion. The concentrations of serum components in milk were also increased for extended time periods; milk chloride was increased for at least 24 h (Figure 20) and milk albumin was increased (Figure 19) for 48 h post-infusion.

As was seen following endotoxin infusion during the first week of lactation (Chapter IV), there was an apparently greater decrease in the α_s-caseins relative to β-casein (Figures 15 and 16). This trend has also been seen in spontaneous cases of mastitis in the cow (Matson et al., 2004). The opposite effect has been reported following infusion of *Streptococcus agalactiae* or endotoxin from *E. coli* O55:B5 in the cow (Anderson and Andrews, 1977). A potential explanation for the decrease in caseins is proteolytic degradation, as evidenced by the appearance of smaller molecular weight fragments (less than 21 kDa) in samples collected following endotoxin infusion, particularly as seen at the migration front (Figures 15 and 16). Decreases in total casein have been seen following endotoxin infusion in the cow (Lappalainen et al., 1988;

Kaartinen et al., 1998), and the degradation products of casein seen in mastitic milk are termed proteose peptones (Moussaoui et al., 2002). Cow milk with high somatic cell counts (SCC), one indicator of mastitis, has been shown to have increased plasmin (Schaar and Funke, 1986) and increased rates of lipolysis and proteolysis (Ma et al., 2000). Plasmin and elastase have been detected in cow milk following endotoxin infusion in the cow, and maximal activity for both is seen 4-8 h post-infusion (Moussaoui et al., 2003), similar to the timing of the proposed proteolytic degradation in this study. Later occurring changes may be the result of changes in protein expression as well as from proteolytic degradation.

Also of interest was the appearance of an unidentified protein migrating at approximately 45 kDa in post-infusion samples (Figures 15 and 16), and the same unidentified protein was seen after infusion in Chapter IV. Prior to inflammation, this band was not seen, but clearly increased in response to inflammation. The concentration of this protein also appeared to be higher at 5 h post-infusion on d 20 than on d 13, and the band remained apparent for a longer period of time after infusion on d 13 than on d 20 of lactation (Figures 15 and 16). Since the greatest concentrations of this protein were seen as soon as 5 h post-infusion, it is likely that this protein was previously present at some level prior to mammary inflammation before increasing following infusion. This probably occurred either through release from a mammary cell (epithelial or immune), or by migration from the serum in response to the opening of tight junctions, much as is seen in the increase of albumin and chloride in milk post-infusion.

The effects of intramammary inflammation on milk yield and piglet weight gain were dramatic. Mean hourly milk yield on the day of infusion was reduced by 31 % in infused versus control glands (Figure 24); piglets nursing those glands gained approximately 74, 55, and 25 % less weight over the three 24 h periods post-infusion when compared to control littermates (Figure 25). When the piglet weight gain data was restricted to week two of lactation, piglets nursing infused glands gained approximately 64, 56, 25, 34, and 21 % less weight over the five 24 h periods post-infusion when compared to control littermates (Figure 26). It is clear that the effects of altering milk yield and composition by mammary inflammation are long-lasting. The disparity between milk yield and piglet growth reduction from endotoxin infusion suggests that milk from inflamed glands was less nutritious to the suckling piglet, and it was clear that milk composition was altered, apparently for the worse. Assuming that the milk yield recorded during the weigh-suckle-weigh procedure was similar to that not recorded during the overnight period, a theoretical feed conversion for piglets consuming milk could be calculated by converting hourly milk yield to 24 h and dividing by piglet weight gain. Using this assumption, piglets nursing control glands consumed 621.6 g milk in the 24 h following endotoxin infusion, resulting in 153.5 g gain, for a theoretical feed conversion of 4.05 g milk/g gain. For piglets nursing inflamed glands, the values were 427.2 g milk and 39.3 g gain; a theoretical feed conversion of 10.87 g milk/g piglet gain. Therefore, during the second and third weeks of lactation, a piglet nursing an inflamed gland would have to consume approximately 168 % more milk than a control littermate to support an equivalent rate of growth.

While the WSW procedure is the simplest method, though a labor-intensive one, to estimate milk yield in the sow, it is clear that it disturbs the natural suckling pattern of sow and litter, and likely artificially depresses milk yield (Barber et al., 1955; Pettigrew et al., 1985). The calculated value for feed conversion for piglets nursing control glands in this study was similar to the approximately 4.0 g milk/g gain calculated by multiple researchers for normal milk (Barber et al., 1955; Lewis et al., 1978; Noblet and Etienne, 1989; Hodbod and Zeman, 2001). Additionally, any errors in milk yield estimation likely affected all piglets equally, as approximately 87 % of possible individual teat milk yields were captured by the data. Some piglets that had no gain or lost weight during the experimental day had gained weight by the next morning. Therefore, there was likely some compensatory milk yield in the overnight hours, though not as much as seen during endotoxin challenge during the first week of lactation (Chapter IV). Additionally, only 3 % (3/100) of piglets nursing experimental glands during later lactation switched teats, and were exposed to endotoxin infusion a second time. This was less than seen following endotoxin infusion during early lactation (Chapter IV), approximately 11 % (8/71) of piglets.

E. Implications

This experiment differed from that in Chapter IV in that endotoxin challenge was performed during the second and third weeks of lactation, as opposed to the first week of lactation, which is when mastitis typically occurs naturally in pork production. By doing

so, the changes in milk composition that result from mammary inflammation could be better differentiated from those caused by the transition from colostrum to mature milk. Additionally, the more stable "nursing order" during advanced lactation allowed for greater accuracy in determining the effects of mammary inflammation on piglet growth, as piglets were less likely to change glands after "their" gland had been infused with endotoxin. This was reflected in the calculation of feed conversion for piglets nursing control glands, which was more similar to those calculated by previous researchers than that calculated in the previous study (Chapter IV). It was clear that mammary inflammation dramatically altered milk composition in the immediate (5 h post-infusion) and long term (48-60 h post-infusion) period, and suppressed piglet weight gain for at least 5 d post-infusion. Importantly, these effects were apparent long after clinical signs had subsided, and the sow and litter appeared "normal." It should be noted that the model of mastitis used in this study results in a mild and transient period of inflammation, and the effects reported here likely represent the minimum that could be expected in naturally occurring coliform mastitis. Additionally, milk samples collected from an individual teat were a composite, in that only one gland per teat was infused, and each teat was served by two mammary glands.

Given that the endotoxin challenge model is mild and transient, the effects seen in naturally occurring mastitis are likely more severe and potentially longer-lasting. Using the weight gain data from week two, and making the assumption that a single episode of intramammary inflammation from coliform infection is cleared within five days, a piglet nursing an infected gland would weigh a minimum of 350 g less at weaning when

compared to littermates nursing control glands. Mahan and Lepine (1991) estimated that it requires an additional three days to reach market weight for each kilogram less weight at weaning. Therefore, piglets in this study nursing an inflamed gland would require at least an additional day to reach market weight than piglets nursing non-inflamed glands. Clinical signs during coliform mastitis last significantly longer than the 8-10 h febrile response reported here following endotoxin infusion, and typically occur during the first week of lactation when piglets are physiologically more vulnerable and more likely to die than piglets from the current study. The question remains as to whether or not subclinical mastitis is an under recognized source of economic loss in the swine industry, and the results of this study further underscore the need for good observation in farrowing room management to quickly diagnose mastitis in sows, and take appropriate action to save piglets.

Chapter VI

OVERALL DISCUSSION

The endotoxin challenge model utilized in these studies (1.5 μg/kg BW into two glands, 3.0 μg/kg BW total; approximately 500 μg endotoxin/sow), provokes a mild inflammatory episode in the sow (Kensinger et al., 1999). This dosage was higher than that used by some researchers (0.5 μg/kg BW, one gland, de Ruijter et al., 1988), and lower than that used by others (1.32 mg/kg BW, two glands, Nachreiner et al., 1972, 1974; 1.32, 0.66, or 0.33 mg/kg BW, one gland, Elmore et al., 1978). The clinical effects (anorexia, fever, lethargy, warm and swollen glands, etc.) appeared quickly (generally within 2-5 h post-infusion) and were transient, having generally resolved within 24 h of administration. For comparison, the endotoxin challenge model utilized by Shuster et al. (1991) in the dairy cow required only 10 μg endotoxin infused into one teat. Assuming a dairy cow averages 1500 lb, or about 670 kg, the dose per animal was approximately 0.015 μg/kg BW. Therefore, the dosage used in the sow for these studies was approximately 100 X on a gland basis and 200 X on a whole animal basis of the dosage of endotoxin as Shuster et al. (1991) used in the cow to provoke a similar inflammatory response. Even the lowest dosage for the sow found in the literature (0.5 μg/kg BW, 1 gland; de Ruijter et al., 1988) was approximately 30 X times the typical cow dosage.

The reasons why sows require so much more endotoxin to provoke the clinical signs of mastitis than do cows are likely evolutionary. As the cow has the capability of

sweating, it has evolved in a cleaner environment, largely on open grassland. Even in

modern milk production, keeping the udder clean and disinfected is a primary concern in

housing and parlor design, as well as proper milking technique. In contrast to the cow,

the sow is incapable of sweating, and must rely on external cooling; be that water, dirt, or

mud. Therefore, in the natural environment, the mammary glands of the sow spend a far

greater portion of time on the ground, often on bare dirt or in mud, as the animal attempts

to cool off. Coliforms are present in large numbers in soil, making it much more likely

that the porcine mammary gland will face infection than will the bovine mammary gland.

Additionally, piglets are naturally curious creatures and will root in the dirt if available,

or play with the dung eliminated by the mother. This leaves bacteria on and around the

mouth of the piglet that is then transferred to the mammary gland during the act of

suckling. As a result, the sow has likely developed either a more robust mammary

immune defense system, or a mammary gland that is more tolerant of infection than seen

in the cow.

Milk yield estimation in the sow is a difficult proposition at best. The WSW

procedure has been developed by multiple researchers (Barber et al., 1955; Lewis et al.,

1978; and Noblet and Etienne 1989, and others) as the best compromise between

practicality and accuracy. The procedure requires separating piglets from the sow

between nursings, weighing the piglets before and after nursing, and adjusting the

difference for metabolic losses and waste elimination. The resultant value is considered

to be the milk yield produced by the mammary gland. If urine or feces are not captured

for a piglet, the milk yield can not be calculated for the gland that piglet nursed. After

taking great care to encourage piglets to eliminate waste before the initial weight, to capture waste during the nursing episode, and to prevent the intake of water by piglets during nursing, approximately 89 and 87 % of all possible hourly milk yields were captured for the first and second studies (Chapters IV and V), respectively. This likely represents the upper end of the range of accuracy for milk yield estimation by weigh-suckle-weigh procedure. Furthermore, both the milk yield estimations and 24 h weight gains were more accurately calculated in the second study (Chapter V) than in the first (Chapter IV). The second study (Chapter V) took place during the second and third weeks of lactation, and the "nursing order" was more stable; piglets were much less likely to switch teats following intramammary infusion. During the first study (Chapter IV), approximately 11 % (8/71) of piglets switched teats; this rate was reduced to 3 % (3/100) piglets during the second study (Chapter V). When piglets switched teats during the first study (Chapter IV), piglets initially nursing infused glands occasionally forced piglets initially nursing control glands to switch places. By doing so, piglets identified as nursing infused glands on d 3 or 5 of lactation occasionally were forced to nurse infused glands again on d 5 or 7 of lactation, thereby complicating the analysis of milk yield and piglet growth through unaccounted for carryover effects of nursing inflamed glands prior to infusion.

The theoretical feed conversion value calculated for piglets nursing control glands during the first week of lactation (2.22 g milk/g gain; Chapter IV) was significantly greater than the approximately 4.0 g milk/g gain calculated by multiple researchers (Barber et al., 1955; Lewis et al., 1978; Noblet and Etienne, 1989; Hodbod and Zeman,

2001). There were two possible reasons for this; first was the carryover effect discussed previously, wherein piglets nursing so-called "control" glands on d 5 and 7 of lactation were nursing glands that had been previously infused on d 3 or 5, and were likely still affected by the prior infusion of the gland. It was demonstrated in the second study (Chapter V) that the effects of intramammary endotoxin infusion on the average daily gain of suckling piglets were evident for at least 5 d post-infusion. Secondly, a portion of piglets identified as nursing inflamed glands switched teats following endotoxin infusion, forcing their control littermates to nurse the infused gland instead. This occasionally resulted in individual piglets nursing a gland that was infused a second, or even a third time. These factors could significantly affect the calculations of milk yield and piglet weight gain.

The theoretical feed conversion value calculated for piglets nursing control glands during the second and third weeks of lactation (4.05 g milk/g gain; Chapter V) was similar to that calculated by multiple researchers (~ 4.0 g milk/g gain; Barber et al., 1955; Lewis et al., 1978; Noblet and Etienne, 1989; Hodbod and Zeman, 2001). Regardless of how accurately the feed conversion values were calculated, it is likely that any inaccuracies in milk yield and piglet gain data affected each value equally. Using the feed conversion values calculated for piglets nursing endotoxin-infused glands (3.33 g milk/g gain, Chapter IV; 10.87 g milk/g gain, Chapter V), it was clear that the milk from these glands was substantially less nutritious. It would require approximately 55 % more milk from the inflamed gland during week one of lactation (Chapter IV) and 168 % more

milk during weeks two and three of lactation (Chapter V) to support equivalent rates of growth as supported by milk from control glands.

Since the endotoxin challenge model of mastitis is mild and transient, it is likely that the effects of spontaneous coliform mastitis are even more dramatic. The data presented here clearly demonstrated significant alterations in milk composition as a result of mammary inflammation in the sow. The concentrations of serum components in milk (albumin, chloride, immunoglobulins) all increased in response to intramammary inflammation. The concentrations of milk-specific proteins (caseins, specifically β-casein) decreased in response to intramammary inflammation, and there was significant proteolytic degradation of milk proteins. The differential degradation of casein subspecies could affect the curdling properties of the milk, potentially increasing the rate of passage in the piglet's stomach and decreasing digestion of the milk. When coupled with a significantly reduced milk yield, the piglet nursing inflamed glands received fewer total nutrients to support its growth. Additionally, the piglet nursing a gland undergoing a naturally occurring mastitis episode may receive bacteria and/or endotoxin in the milk. However, there is little or no literature published that describe the effects of oral endotoxin on the baby pig. The piglet whose mother is undergoing an episode of coliform mastitis is clearly facing grave challenge to its survival and thriftiness.

The study in Chapter IV was developed with the intention of faithfully reproducing naturally occurring coliform mastitis. Therefore, sows were challenged on three alternating days of lactation during the first week of lactation. As discussed previously, this led to difficulties in accurately estimating milk yield. Conclusions on the

effect of mammary inflammation on milk composition were also complicated by the fact that samples were taken from separate control and endotoxin-infused glands once at 5 h post-infusion. Therefore, differences between milk samples partly resulted from being from different glands, with the added complication of being from glands whose secretion was still changing from colostrum to mature milk. Additionally, glands designated as controls on d 5 and 7 of lactation had occasionally been infused on d 3 and 5 of lactation. The milk composition results following endotoxin infusion on d 13 and 20 of lactation (Chapter V) clearly showed that changes were evident for at least 2 ½ d post-infusion. Piglet weights were also recorded for only 24 h following endotoxin infusion in the first study (Chapter IV), making it impossible to determine for how long piglet growth was compromised by endotoxin infusion of the gland it was nursing.

The second study (Chapter V) addressed these issues by changing the timing of endotoxin challenge to more advanced lactation wherein the secretion is that of mature milk, and nursing order is more stable. Additionally, samples were taken within the same glands prior to and following endotoxin infusion. Samples were taken over a longer period of time (up to 60 h post-infusion), and piglet weights were recorded daily for at least three days following the second endotoxin challenge. Experimental days were also at least five days apart, allowing for analysis of piglet growth rate following the first endotoxin challenge for a longer period of time before a second challenge of the sow. The milk yield estimations calculated in the second study (Chapter V) were likely more accurate, as piglets were more robust and the researchers were more skilled in the WSW. From the experiences encountered in these studies, it is clear that practice in the

technique allows for greater accuracy. The inflammatory process was more closely studied in Chapter V, through monitoring the responses in the concentrations of the cytokine TNF-α in plasma and blood, as well as the integrity of the blood-milk barrier by monitoring the concentrations of chloride in milk in addition to those of albumin.

The effects of maternal mastitis on the profitability and efficiency of hog production may be underappreciated by the hog industry, as they are largely reflected in mortality measures. Specifically, this includes direct losses of sows to extreme coliform mastitis and lost piglets due to starvation. Piglet malnutrition as a result of maternal mastitis may be under-recognized as a cause of loss, as losses are due not just to the direct effects of starvation, but also to the indirect effect of crushing. A malnourished piglet will spend more time near the sow in a futile search for milk and warmth, making it more likely to be injured by the sow's movements. This is due to both dangerous proximity to the sow and to piglet lethargy, such that the piglet can not get out of the sow's way in time to avoid injury.

A less appreciated area of lost efficiency is that from reduced growth performance of the suckling piglet that nurses a mastitic (clinical or subclinical) gland. Our data clearly showed that milk composition is altered for several days (at least 2 ½) following an episode of intramammary inflammation; long after clinical signs (fever, anorexia, and inflammation) have subsided. Milk yield was significantly suppressed by intramammary inflammation in the short term (8-10 h), but was also likely reduced for a longer period of time. The growth rate of piglets nursing control glands was significantly reduced in the short term; that of piglets nursing inflamed glands was significantly reduced for at least

five days post-infusion. It is also not clear that the growth rate of piglets challenged by maternal mammary inflammation ever catches up to that of control littermates, whose growth rate was also temporarily suppressed. These effects likely carry over into the post-weaning phase, as piglets that are lighter at weaning take longer to reach market weight.

As discussed previously, the endotoxin challenge model of mastitis is both mild and transient. In the cow, naturally occurring mastitis is both more severe and longer lasting (Hoeben et al., 2000); this likely holds true for the sow, as well. There are a range of potential options to deal with mastitis in the farrowing room to decrease its impact upon hog production. One is the use of antibacterial agents, be that through aggressive treatment of the underlying bacterial infection upon the diagnosis of mastitis, and/or the prophylactic administration of antibiotics in the lactating ration. Considering growing concerns over the routine use of anti-bacterial agents in food animal production and their elimination in the EU for hog production, the latter is not a promising avenue. The former is an option, assuming that the infection is detected early enough for antibiotic treatment to have an effect. Another strategy is the development of vaccines against bacterial organisms known to cause mastitis, a technique used with varying success in the dairy industry with the J-5 vaccine. Though mastitis in the sow is typically caused by coliforms, particularly *E. coli* (Ringarp, 1960; Persson et al., 1996), the wide variety of causative strains (as many as 167; Mörner et al., 1998) may make this task nearly impossible in the sow.

One of the more common strategies utilized currently is aggressive cross-fostering in the farrowing room; removing piglets to another sow within the farrowing room at the first sign of mastitis. This presumes excess "teat capacity" within the farrowing room, or enough productive teats currently unoccupied to move the piglets to. Commercial production units typically farrow greater numbers of sows in a group than there are farrowing crates, such that once all the sows have farrowed, piglets are cross-fostered onto the number of sows that the farrowing room can hold. This allows for greatest efficiency of use of the higher energy (and therefore cost) lactating ration by feeding the fewest number of sows needed to nurse the number of piglets born to a farrowing group. This also allows for an earlier return of sows not kept for lactation to the breeding herd earlier, thereby reducing the farrowing interval. The options for transferring piglets from a mastitic gland on one sow, to a non-infected gland on another, may very well be limited.

One last, and potentially the most promising, strategy, is the provision of supplemental nutrition in the form of milk replacer to all the piglets in the farrowing room. This would be provided within a day of farrowing, after allowing for adequate absorption of colostrum before milk replacer was fed. Typically, a creep ration is provided late in the first week of lactation to improve piglet growth performance. This works because the growth ability of the piglet begins to exceed the capacity of the sow to provide adequate nutrition for maximal growth by about the fifth to seventh day of lactation (Boyd et al., 1985; Harrell et al., 1993). Creep feeding also improves the transition to the post-weaning production phase, by acclimatizing piglets to a solid feedstuff. One problem with creep feeding is feed wastage while the piglets are starting

to consume feed; this could potentially be decreased by providing a milk replacer instead, at least during the first week of lactation when mastitis is most frequent. This allows for a feedstuff that the piglet is used to consuming (in a liquid formulation), and is closer nutritionally to milk than are the typical creep rations. Additionally, this option provides support for the weaker piglets in the litter, which may not be as able to fight for a high-producing milk gland as their larger, stronger littermates. Room-wide delivery systems for milk replacer have been developed (e.g., Supp-le-Mate^{TM}), and show promise for this avenue.

As prevention is the most effective strategy for dealing with disease in animal production, from both an economic and animal welfare perspective, vaccination would be the ideal option for reducing the impact of mastitis on hog production. However, this strategy presumes the availability of effective vaccines, and it may be some time before hog producers have this option available to them on a widespread basis. Under current conditions, my preferred option would be to provide supplemental milk replacer ad libitum to the piglets within 24 h of farrowing. The time delay would allow for adequate consumption of colostrum and the acquisition of passive immunity that it provides. After that point, piglets that are receiving less than maximal milk (from maternal mastitis, competition with littermates for high-producing glands, or simply an inability to effectively stimulate the mammary gland during nursing) can receive maximal nutrition, and be able to fully express their growth potential. On average, this would result in piglets having a higher plane of growth during the suckling phase, particularly so for the smaller piglets. In turn, piglets would be weaned at heavier weights, thereby

transitioning into the nursery/grower phase more quickly, and with fewer health complications. Lastly, piglets entering the finishing phase will be larger and healthier, allowing them to grow faster and shorten the time required to reach market weight and composition. Further research can determine whether this would be an economically viable option.

To recap the main hypotheses of this dissertation:

1) Intramammary endotoxin challenge provoked localized mammary inflammation, depressed sow disposition, feed and water intake, may have increased plasma TNF-α, and increased rectal temperature in first-parity sows.

2) Porcine milk total protein, albumin, chloride, and TNF-α were increased, β-casein was decreased, and there was no evidence for a change in lactoferrin following intramammary inflammation.

3) Mean hourly milk yield was decreased in inflamed mammary glands.

4) The growth rate of piglets suckling endotoxin treated glands was decreased for up to five days relative to control littermates.

LITERATURE CITED

Aimutis, W. R., E. T. Kornegay, and W.N. Eigel. 1982. Electrophoretic and biochemical comparison of casein and whey protein from porcine colostrum and milk. J. Dairy Sci. 65:1874-1881.

Albig, A. Z. 1939. Z. Schweinez. 46:626.

Anderson, M., and A. T. Andrews. 1977. Progressive changes in individual milk protein concentrations associated with high somatic cell counts. J. Dairy Res. 44:223-235.

Anderson, K. L., A. R. Smith, R. D. Shanks, H. L. Whitmore, L. E. Davis, and B. K. Gustafsson. 1986. Endotoxin-induced bovine mastitis: immunoglobulins, phagocytosis, and effect of flunixin meglumine. Am. J. Vet. Res. 47:2405-2410.

Ash, H. B. 1934. De Re Rustica. revision of W. D. Hooper. Loeb Classical Library, Wm Heinemann, Ltd, London, England.

Auldist, D. E., and R. H. King. 1995. Piglet's role in determining milk production in the sow. In: D. P. Hennesy and P. D. Cranwell (ed.) Manipulating Pig Production. p 114-118. Australasian Pig Science Association, Werribe.

Averette, L. A., J. Odle, M. H. Monaco, and S. M. Donovan. 1999. Dietary fat during pregnancy and lactation increases milk fat and insulin-like growth factor I concentrations and improves neonatal growth rates in swine. J. Nutr. 129:2123-2129.

Bäckström, L. 1973. Environment and animal health in piglet production. A field study of incidence and correlations. Acta Vet. Scand. Suppl. 41.

Bannerman, D. D., M. J. Paape, W. R. Hare, and E. J. Sohn. 2003. Increased levels of LPS-binding protein in bovine blood and milk following bacterial lipopolysaccharide challenge. J. Dairy Sci. 86:3128-3137.

Barber, R. S., R. Braude, and K. G. Mitchell. 1955. Studies on the milk production of Large White pigs. J. Agric. Sci. 46:97-118.

Baxter, S. 1984. Intensive Pig Production: Environmental Management and Design. Granada Publishers, London, England.

Ben Shaul, D. M. 1962. The composition of the milk of wild animals. Int. Zoo. Ybk. 4:333.

Bertschinger, H. U., E. Bürgi, V. Eng, and P. Wegmann. 1990. Senkung der inzidenz von puerperaler mastitis bei der sau durch schutz des gesäuges vor verschmutzung. (Reduction of the incidence of puerperal mastitis in the sow by protection of the mammary gland against fecal contamination.) Schweizer Arch. Tierheilk. 132:557-566.

Bianca, W., and K. L. Blaxter. 1961. The influence of the environment on animal production and health under housing conditions. In: Proc. VIIIth Int. Cong. on Anim. Prod. I:113.

Blecha, F., and K. W. Kelley. 1981. Cold stress reduces the acquisition of colostral immunoglobulin in piglets. J. Anim. Sci. 52:594-600.

Bourne, F. J., and J. Curtis. 1973. The transfer of immunoglobulins IgG, IgA, and IgM from serum to colostrums and milk in the sow. Immunology. 24:157-162.

Bourne, R. A., and H. A. Tucker. 1975. Serum prolactin and LH response to photoperiod in bull calves. Endocrinology. 97:473-475.

Bonsma, F. N., and P. M. Oosthuizen. 1935. S. Afr. J. Sci. 32:360.

Boyd, R. D., B. D. Moser, E. R. Peo, Jr., and P. J. Cunningham. 1978. Effect of energy source prior to parturition and during lactation on piglet survival and growth and on milk lipids. J. Anim. Sci. 47:883.

Boyd, R. D., B. D. Moser, E. R. Peo, Jr., A. J. Lewis, and R. K. Johnson. 1982. Effect of tallow and choline chloride addition to the diet of sows on milk composition, milk yield, and preweaning pig performance. J. Anim. Sci. 54:1-7.

Boyd, R. D., M. Harkins, D. E. Bauman, and W. R. Butler. 1985. The effect and practical implications of recombinant porcine growth hormone on lactation performance of sows. In: Proc. 1985 Corn. Nutr. Conf. p 10.

Boyd, R. D., and K. J. Touchette. 1998. Milk production curves for Camborough 22 sows estimated using piglet growth rate. Tech. Memo No. 171, PIC USA. as cited in: Boyd, R. D., and R. S. Kensinger. 1998. Metabolic precursors for milk synthesis. In: Verstegen et al. (ed.) The Lactating Sow. Wageningen Pers., The Netherlands.

Brambell, F. W. R. 1958. The passive immunity of the young mammal. Biol. Rev. Camb. Philos. Soc. 33:488-531.

Braude, R. 1954. Pig nutrition. Prog. Physiol. Farm Animals. 1:40-105.

Brent, B. E., E. R. Miller, D. E. Ullrey, and K. E. Kemp. 1973. Postpartum changes in the nitrogenous constituents of sow milk. J. Anim. Sci. 36:73-78.

Bruininx, E. M. A. M., G. P. Binnendijk, C. M. C. van der Peet-Schwering, J. W. Schrama, L. A. den Hartog, H. Everts, and A. C. Beynen. 2002. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. J. Anim. Sci. 80:1413-1418.

Burvenich, C., A. M. Massart-Leen, G. Vandeputte-van Messom, E. Roets, and G. Kiss. 1989. Effect of recombinant bovine somatotropin on endotoxin induced mastitis in lactating goats. Arch. Int. Physiol. Biochim. 97:P91-P95.

Butler, J. E. 1971. Transmission of immunity from mother to young. In: Proc. World Cong. on Fertil. and Steril. p 92.

Buttle, H. C. 1974. Seasonal variation of prolactin in plasma of male goats. J. Reprod. Fertil. 37:95-99.

Carlsson, R. N. K., B. I. Ingvarsson, and B. W. Karlsson. 1977. Isolation and characterization of albumin from porcine serum, colostrum, and urine. Int. J. Biochem. 8:285-294.

Carrol, E. J., O. W. Schalm, and J. Lasmanis. 1964. Experimental coliform (*Aerobacter aerogenes*) mastitis: characteristics of the endotoxin and its role in pathogenesis. Am. J. Vet. Res. 25:720-726

Carrol, E. J., and N. C. Jain. 1969. Observations on the bactericidal activity of normal milk, mastitic milk, and colostrums for Aerobacter aerogenes. Am. J. Vet. Res. 30:1123-1127.

Cast, W. R., B. D. Moser, E. R. Peo, Jr., and P. J. Cunningham. 1977. Fat, choline, and thyroprotein additions to the diet of lactating swine. J. Anim. Sci. 45(Suppl. 1):80. (Abstr.).

Chu, R. M., S. R. Wang, C. N. Weng, and V. G. Pursel. 1993. Isolation and characterization of porcine milk lactoferrin. Am. J. Vet. Res. 54:1154-1159.

Close, W. H. 1992. Thermoregulation in piglets: environmental and metabolic consequences. In: M. A. Varley, P. E. V. Williams, and T. L. J. (ed.) Occasional Publication No. 15-Neonatal Survival and Growth. British Soc. Anim. Prod. p 25.

Cooper, A. 1840. On the Anatomy of the Breast. Longman, Orme, Green, Brown, and Longman, London, England.

Curtis, S. E., C. J. Heidenrich, and C. W. Foley. 1966. Carbohydrate assimilation and utilization by newborn pigs. J. Anim. Sci. 25:655-662.

Curtis, S. E., C. J. Heidenrich, and R. B. Harrington. 1967. Age dependent changes of thermostability in neonatal piglets. Am. J. Vet. Res. 28:1887-1890.

Curtis, S. E. 1970. Environmental-thermoregulatory interactions and neonatal piglet survival. J. Anim. Sci. 31:576-580.

Curtis, J., and F. J. Bourne. 1971. Immunoglobulin quantitation in sow serum, colostrum, and milk and the serum of young pigs. Biochim. Biophys. Acta. 236:319-332.

Curtis, S. E. 1974. Responses of the piglet to perinatal stressors. J. Anim. Sci. 38:1031-1035.

Cutler, R. S., V. A. Fahy, E. M. Spicer, and G. M. Cronin. 1999. Preweaning mortality. In: Straw et al. (ed.) Diseases of Swine. 8th ed. p 985. Iowa State University Press, Ames.

Davies, L. R. 1904. 21st Ann. Rep. Wis. Agric. Exp. Sta.

Delgado, J. A., and J. E. T. Jones. 1981. An abbatoir survey of mamamry gland lesions in sows with special reference to the bacterial flora of mammary abscesses. Brit. Vet. J. 137:639-643.

De Ruijter, K., J. H. M. Verheijden, A. Pijpers, and J. Berends. 1988. The role of endotoxin in the pathogenesis of coliform mastitis in sows. Vet. Q. 10:186-190.

Dhondt, G., C. Burvenich, and G. Peeters. 1977. Mammary blood flow during experimental *Escherichia coli* endotoxin induced mastitis in goats and cows. J. Dairy Res. 44:433-440.

Donald, H. P. 1937. Emp. J. Exp. Agric. 5:361.

Donovan, S. M., L. K. McNeil, R. Jimenez-Flores, and J. Odle. 1994. Insulin-like growth factors and insulin-like growth factor binding proteins in porcine serum and milk throughout lactation. Pediatr. Res. 36:159-168.

Donovan, S. M., and J. Odle. 1994. Growth factors in milk as mediators of infant development. Annu. Rev. Nutr. 14:147-167.

Drendel, C., and K. Wendt. 1993. Reaktionen des laktierenden Schweinegesäuges auf infektiöse Belastungen. Monatshefte für Veterinarmed. 48:307-313.

Drescher, K., N. Roos, M. Pfeuffer, H. -M. Seyfert, J. Schrezenmeir, and H. Hagemeister. 1999. Recovery of ^{15}N-lactoferrin is higher that that of ^{15}N-casein in the small intestine of suckling, but not adult miniature pigs. J. Nutr. 129:1026-1030.

Dyck, G. W., and E. E. Swierstra. 1987. Causes of piglet death from birth to weaning. Can. J. Anim. Sci. 67:543-547.

Dyck, G. W., E. E. Swierstra, R. M. McKay, and K. Mount. 1987. Effect of location of the teat suckled, breed, and parity on piglet growth. Can. J. Anim. Sci. 67:929-939.

Edwards, S. A., and J. A. Rooke. 1999. Effects of management during the suckling period on post weaning performance of pigs. In: Proc. 50th Ann. Meet. Eur. Assoc. Anim. Prod., Zurich, Switzerland, p 8.

Elliot, J. I., B. Senft, G. Erhardt, and D. Fraser. 1984. Isolation of lactoferrin and its concentration in sow's colostrum and milk during a 21-day lactation. J. Anim. Sci. 59:1080-1084.

Elmore, R. G., C. E. Martin, and J. N. Berg. 1978. Absorption of *Escherichia coli* endotoxin from the mammary glands and uteri of early postpartum sows and gilts. Theriogenology. 10:439-446.

English, P. R., W. J. Smith, and A. MacLean. 1977. Management of the lactating sow and litter. In: The Sow-Improving Her Efficiency. p 170. Farming Press, Ltd, Suffolk, UK.

English, P. R., and V. Morrison. 1984. Causes and prevention of piglet mortality. Pig News Info. 5:369-376.

Étienne, M., C. Legault, J.-Y. Dourmad, and J. Noblet. 2000. Production laitière de la truie: Estimation, composition, facteurs de variation et evolution. Journées Rech. Porcine en France. 32:253-264.

Evans, P. A., T. J. Newby, C. R. Stokes, and F. J. Bourne. 1982. A study of cells in the mammary secretions of sows. Vet. Immunol. Immunopathol. 3:515-527.

Fahmy, M. H., and C. Bernard. 1971. Causes of mortality in Yorkshire pigs from birth to 20 weeks of age. Can. J. Anim. Sci. 51:251-359.

Fahmy, M. H. 1972. Comparative study of colostrum and milk composition of seven breeds of swine. Can. J. Anim. Sci. 52:621-627.

Farmer, C., D. Petticlerc, G. Pelletier, P. Brazeau. 1992. Lactation performance of sows injected with growth hormone-releasing factor during gestation and (or) lactation. J. Anim. Sci. 70:2636-2642.

Finkelstein, E., and W. L. Hurley. 1987. Characterisation of proteins in sow colostrum and milk. J. Dairy Sci. 70(Suppl. 1):149(Abstr.).

Forbes, J. M., P. M. Driver, A. A. El Shahat, T. G. Boaz, and C. G. Seanes. 1975. The effect of daylength and level of feeding on serum prolactin in growing lambs. Endocrinology. 64:549-554.

Fransson, G. -B., K. Thorén-Tolling, B. Jones, L. Hambraeus, and B. Lönnerdal. 1983. Absorption of lactoferrin-iron in suckling pigs. Nutr. Res. 3:373-384.

Fraser, D., C. Nicholls, and W. Fagan. 1985. A sow milking machine designed to compare the yield of different teats. J. Agric. Eng. Res. 31:371-376.

Friend, D. W. 1974. Effect on the performance of pigs from birth to market weight of adding fat to the lactation diet of their dams. J. Anim. Sci. 39:1073-1081.

Garcia, P., H. Holst, U. Magnusson, and H. Kindahl. 1998. Endotoxin-effects of vaccination with Escherichia coli vaccines in the pig. Acta. Vet. Scand. 39: 135-140.

Gentry, J. G., J. J. McGlone, J. R. Blanton, Jr., and M. F. Miller. 2002. Alternative housing systems for pigs: influences on growth, composition, and pork quality. J. Anim. Sci. 80:1781-1790.

Gill, J. C., and W. Thompson. 1956. Observations on the behaviour of suckling pigs. Br. J. Anim. Behav. 4:46-51.

Glass, R. L., H. A. Troolin, and R. Jenness. 1967. Comparative biochemical studies of milks-IV. Constituent fatty acids of milk fats. Comp. Biochem. Physiol. 22:415.

Goodwin, R. F. W. 1957. Relation between blood sugar concentration and some vital body functions in the newborn pig. J. Physiol. (Lond.). 136:208.

Grosvenor, C. E., M. F. Picciano, and C. R. Baumrucker. 1993. Hormones and growth factors in milk. Endocr. Rev. 14:710-728.

Grün, D., G. Reiner, and V. Dzapo. 1993. Investigations on breed differences in milk yield of swine. Part I. Methodology of mechanical milking and milk yield. Reprod. Dom. Anim. 28:14-21.

Guidry, A. J., M. J. Paape, and R. E. Pearson. 1980. Effect of udder inflammation on milk immunoglobulins and phagocytosis. Am. J. Vet. Res. 41:751-753.

Guidry, A. J., M. Ost, I. H. Mather, W. E. Shainline, and B. T. Weinland. 1983. Sequential response of milk leukocytes, albumin, immunoglobulins, monovalent ions, citrate, and lactose in cows given infusions of *Escherichia coli* endotoxin into the mammary gland. Am. J. Vet. Res. 44:2262-2267

Harkins, M., R. D. Boyd, and D. E. Bauman. 1989. Effect of recombinant porcine somatotropin on lactational performance and metabolite patterns in sows and growth of nursing pigs. J. Anim. Sci. 67:1997-2008.

Harmon, R. J., F. L. Schanbacher, L. C. Ferguson, and K. L. Smith. 1975. Concentrations of lactoferrin in milk of normal lactating cows and changes occurring during mastitis. Am. J. Vet. Res. 36:1001-1007.

Harmon, R. J., F. L. Schanbacher, L. C. Ferguson, and K. L. Smith. 1976. Changes in lactoferrin, immunoglobulin G, bovine serum albumin, and α-lactalbumin during acute experimental and natural coliform mastitis in cows. Infect. Immun. 13:533-542.

Harmon, R. J., and F. H. S. Newbould. 1977. The antibacterial activity of skim milk following intramammary endotoxin infusion. J. Dairy Sci. 60 (Suppl 1):136. (Abstr.).

Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77:2103-2112.

Harrell, R. J., M. J. Thomas, and R. D. Boyd. 1993. Limitations of sow milk yield on baby pig growth. In: Proc. Corn. Nutr. Conf. p156.

Hartmann, P. E., I. McCouley, A. D. Gooneratne, and J. L. Whitely. 1984. Inadequacies of sow lactation: survival of the fittest. Symp. Zool. Soc. London. 51:301-326.

Hartsock, T. G., and H. B. Graves. 1976. Neonatal behavior and nutrition-related mortality in domestic swine. J. Anim. Sci. 42:235-241.

Hempel, K. 1928. Arb. Dtsch. Ges. Zücht. 37:19.

Hendrix, W. F., K. W. Kelley, C. T. Gaskins, and D. J. Hinrichs. 1978. Porcine neonatal survival and serum gamma globulins. J. Anim. Sci. 47:1281-1286.

Heresbach, C. 1578. Foure Books of Husbandry. J. Wight Publishers, London, England.

Hernandez, A., J. Diaz, A. Avila, and M. Cama. 1987. A note on the natural suckling frequency of piglets. Cuban J. Agric. Sci. 21:292-294.

Ho, F. C. S., R. L. C. Wong, and J. W. M Lawton. 1979. Human colostral and breast milk cells. Acta Paediatr. Scand. 68:389-396.

Hodbod, P., and L. Zeman. 2001. Changes in milk composition of sows during 28 days of lactation. Czech. J. Anim. Sci. 46:509-513.

Hodge, R. W. 1974. Efficiency of food conversion and body composition of the preruminant lamb and the young pig. Br. J. Nutr. 32:113-126.

Hoeben, D., C. Burvenich, E. Trevis, G. Bertoni, J. Hamann, R. M. Bruckmaier, and J. W. Blum. 2000. Role of endotoxin and TNF-α in the pathogenesis of experimentally induced coliform mastitis in periparturient cows. J. Dairy Res. 67:503-514.

Hoy, S., B. Puppe, O. Tober, R. Lindemann, and P. Wolf. 1991. Zum einflub der saugordnung der fekel auf deren lebendmasseentwicklung und morbiditat von gerburt bis schlachtung (Suckling order of piglets- effects on live weight development and morbidity from birth to slaughter). Monatschefte Fur Veterinarmedizin. 46:477-482. as cited in: Hoy, S., and B. Puppe. 1992. Effects of teat order on performance and health in growing pigs. Pig News Info. 13:131N-136N.

Hultén, F., N. Lundeheim, A. -M. Dalin, and S. Einarsson. 1995. A field study on group housing of lactating sows with special reference to sow health at weaning. Acta. Vet. Scand. 36:201-212.

Hultén, F., N. Lundeheim, A. -M. Dalin, and S. Einarsson. 1997. Pre- and post-weaning piglet performance, sow food intake and change in backfat thickness in a group-housing system for lactating sows. Acta. Vet. Scand. 38:119-133.

Hultén, F., N. Lundeheim, A. -M. Dalin, and S. Einarsson. 1998. Reproductive performance among sows group-housed during late lactation. Acta. Vet. Scand. 39:237-250.

Hurley, W. L., and R. C. J. Grieve. 1988. Total and differential cell counts and N-acetyl-β-D-glucosaminidase activity in sows' milk during lactation. Vet. Res. Commun. 12:149-153.

Huszenicza, G., T. Kegl, M. Kulcsar, B. Olah, M. Gacs, K. Oppel, Z. Stollar, P. Jonsson, and S. Janosi. 1997. Diagnostic value of certain mastitis markers in following up the clinical and bacteriological changes in pharmacotherapeutic studies. Acta. Vet. Hung. 45:409-416.

Hutchens, T. W., J. S. Magnuson, and T. T. Yip. 1989. Rapid purification of porcine colostral whey lactoferrin by affinity chromatography on single-stranded DNA-agarose. Characterization, amino acid composition, and N-terminal amino acid sequence. Biochim. Biophys. Acta. 999:323-329.

Jackson, J. R., W. L. Hurley, R. A. Easter, A. H. Jensen, and J. Odle. 1995. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. J. Anim. Sci. 73:1906-1913.

Janeway, C. A., P. Travers, M. Walport, and J. D. Capra. 1999. Host defense against infection. in: Immunobiology: the immune system in health and disease, 4th ed. pp. 363-415. Current Biology Publications/Garland, London, UK/NY, USA.

Jenness, R., and R. E. Sloan. 1970. The composition of milks of various species: A review. Dairy Sci. Abstr. 32:599-612.

Jenness, R. 1982. Interspecies comparison of milk proteins. in Fox, P. F. (ed.). Developments in dairy chemistry-1. p. 87-114. Applied science publishers, London.

Jensen, P. 1986. Observations on the maternal behaviour of free-ranging domestic pigs. Appl. Anim. Behav. Sci. 16:131-142.

Jensen, P., and I. Redbo. 1987. Behaviour during nest leaving in free-ranging domestic pigs. Appl. Anim. Behav. Sci. 18:355-362.

Jensen, P., K. Florén, B. Hobroh. 1987. Peri-parturient changes in behaviour in free-ranging domestic pigs. Appl. Anim. Behav. Sci. 17:69-76.

Johnson, A. K., J. L. Morrow-Tesch, and J. J. McGlone. 2001. Behavior and performance of lactating sows and piglets reared indoors or outdoors. J. Anim. Sci. 79:2571-2579.

Jones, W. K., L. Y. Yu-Lee, S. M. Clift, T. L. Brown, and J. M. Rosen. 1985. The rat casein multigene family. Fine structure and evolution of the β-casein gene. J. Biol. Chem. 260:7042.

Jorsal, S. E. 1983. Morbiditet hos søer. Epidemiologiske undersøgelser i intensive sobesætninger med særligt henblik på farefebersyndromet. (Morbidity in sows). Licentiatafhandling. Institut for intern medicin. Den Kgl. Veterinær- og Landbohøjskole, København, Denmark.

Jylling, B., and P. H. Sørensen. 1960. Investigations on the composition of sow milk. Yearbook (1960) Copenhagen. p 20-36.

Kaartinen, L., K. Veijalainen, P. L. Kuosa, S. Pyörälä, and M. Sandholm. 1988. Endotoxin-induced mastitis. Inhibition of casein synthesis and activation of the caseinolytic system. J. Vet. Med. B. 35:353-360.

Karlsson, B. W. 1966. Immunoelectrophoretic studies on relationships between proteins of porcine colostrum, milk, and blood serum. Acta. Pathol. Microbiol. Scand. 67:83-101

Kauf, A. C. W., and R. S. Kensinger. 2002. Purification of porcine β-casein, N-terminal sequence, quantification in mastitic milk. J. Anim. Sci. 80:1863-1870.

Kensinger, R. S., R. J. Collier, F. W. Bazer, C. A. Ducsay, and H. N. Becker. 1982. Nucleic acid, metabolic, and histological changes in gilt mammary tissue during pregnancy and lactogenesis. J. Anim. Sci. 54:1297-1308.

Kensinger, R. S., R. J. Collier, and F. W. Bazer. 1986. Ultrastructural changes in porcine mammary tissue during lactogenesis. J. Anat. 145:49-59.

Kensinger, R. S., A. L. Magliaro, R. Grabowski, L. M. Sordillo, and L. C. Griel, Jr. 1999. Development of a mammary inflammation model of lactation failure in swine. J. Anim. Sci. 77(Suppl 1):218. (Abstr.).

Kensinger, R. S., D. M. Sanzotti, A. L. Magliaro, A. C. W. Kauf, and L. C. Griel, Jr. 2001. Effect of oxytocin (OT) on hourly milk secretion in gilts with mastitis. J. Anim. Sci. 79(Suppl 1): 428 (Abstr.).

Kim, Y. B., S. G. Bradley, and D. W. Watson. 1966. Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum deprived piglets. J. Immunol. 97:52-63.

Kim, S. W., W. L. Hurley, I. K. Han, and R. A. Easter. 1999. Growth of nursing pigs related to the characteristics of nursed mammary glands. J. Anim. Sci. 78:1313-1318.

King, R. H., B. P. Mullan, F. R. Dunshea, and H. Dove. 1997. The influence of piglet body weight on milk production of sows. Livest. Prod. Sci. 47:169-174.

Klobasa, F., E. Werhahn, and J. E. Butler. 1981. Regulation of humoral immunity in the piglet by immunoglobulins of maternal origin. Res. Vet. Sci. 31:195-206.

Klobasa, F. E., and J. E. Butler. 1987. Absolute and relative concentrations of immunoglobulins G, M, and A, and albumin in the lacteal secretion of sows of different lactation numbers. Am. J. Vet. Res. 48:176-182.

Klobasa, F., E. Werhahn, and J. E. Butler. 1987. Composition of sow milk during lactation. J. Anim. Sci. 64:1458-1466.

Klobasa, F., and E. Werhahn. 1996. The interdependence between parameters of milk composition and reproduction performance in relation to lactation number in sows. Züchtungskunde. 68:297-304.

Klopfenstein, C., C. Farmer, and G. P. Martineau. 1999. Diseases of the mammary glands and lactation problems. In: Straw et al. (ed.). Diseases of Swine. 8th ed. p. 833-860. Iowa State University Press, Ames, Iowa.

Koerner, T. J., T. A. Hamilton, and D. O. Adams. 1987. Suppressed expression of surface Ia on macrophages by lipopolysaccharide: evidence for regulation at the level of accumulation of mRNA. J. Immunol. 139:239-243.

Kooyman, G. L. 1963. Milk analysis of the kangaroo rat, dipodomys merriami. Science (New York). 142:1467-1468.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227:680-685.

Lappalainen, R., L. Kaartinen, K. Veijalainen, P. L. Kuosa, S. Sankari, S. Pyörälä, and M. Sandholm. 1988. Sequential changes of mineral and trace elements in milk during the course of endotoxin-induced mastitis as analyzed by particle induced -X-ray (PIXE), -γ-ray emission (PIGE) and ion selective electrodes. J. Vet. Med. B. 35:664-676.

Leavitt, B. E., J. O'Leary, R. J. Harmon, and C. L. Hicks. 1982. The effect of *Escherichia coli* endotoxin infusion on properties of milk and cheese. J. Dairy Sci. 65 (Suppl 1):70. (Abstr.)

Le Dividich, J., and J. Noblet. 1981. Colostrum intake and thermoregulation in the neonatal pig in relation to environmental temperature. Biol. Neonate. 40:167-174.

Le Dividich, J. 1999. A review- neonatal and weaner pig: management to reduce variation. In: Manipulating Pig Production VII. Proc. 7th Bienn. Conf. Australasian Pig Sci. Assoc., Adelaide, Australia. p 135.

Lee, C. S., F. B. P. Wooding, and P. Kemp. 1980. Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum, and milk from normal cows. J. Dairy Res. 47:39-50.

Lee, C. S., I. McCauley, and P. E. Hartman. 1983. Light and electron microscopy of cells in pig colostrum, milk, and involuntary secretion. Acta. Anat. (Basel). 117: 270-280.

Lee, W. J., J. L. Farmer, M. Hilty, and Y. B. Kim. 1998. The protective effects of lactoferrin feeding against lethal shock in germfree piglets. Infect. Immun. 66:1421-1426.

Leff, J. A., and J. E. Repine. 1993. Neutrophil-mediated tissue injury. in: Abramsson, J. S., and J. G. Wheeler. (eds.). The Neutrophil. pp. 229-262. IRL Press, Oxford.

Lengeman, F. W., and M. Pitzrick. 1986. Effects of endotoxin on mammary secretion of lactating cows. J. Dairy Sci. 69:1250-1258.

Lengemann, F. W., and M. Pitzrick. 1987. Endotoxin of *Escherichia coli* and permeability of the mammary gland of goats. J. Dairy Sci. 70:201-208.

Leskova, V. R., and K. Onderscheka. 1968. Elektrophoretische untersuchungen des kolostrums und der milch des schweines. Zeitschrift fur Tierphysiologie, Tierernahrund und Futtermittelkunde. 23:257-262.

Lewis, A. J., V. C. Speer, and D. G. Haught. 1978. Relationship between yield and composition of sow's milk and weight gain of nursing pigs. J. Anim. Sci. 47:634.

Lintner, V. 1866. Chemische analyse einer schweinemilch. Chem. Centralbl. 11:447. as cited in: Jylling, B., and P. H. Sørensen. 1960. Investigations on the composition of sow milk. Yearbook (1960) Copenhagen. pp. 20-36.

Löfstedt, J., J. A. Roth, R. F. Ross, and W. C. Wagner. 1983. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Escherichia coli* mastitis in sows. Am. J. Vet. Res. 44:1224-1228.

Löving, M., and U. Magnusson. 2002. Sows intramammarily inoculated with *Escherichia coli* at parturition. II. Effects on the densities of MHC class II$^+$, CD4$^+$ and CD8$^+$ cells in the mammary gland. Vet. Immunol. Immunopathol. 90:45-54.

Lowry, O. H., N. J. Rosbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-75.

Lucas, I. A. M., and G. A. Lodge. 1961. The nutrition of the young pig: A review. Tech. Comm. No. 22, Common. Bur. Anim. Nutr., Rowett Institute, Aberdeen, Scotland.

Ma, Y., C. Ryan, D. M. Barbano, D. M. Galton, M. A. Rudan, and K. J. Boor. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83:264-274.

Mabry, J. W., F. L. Cunningham, R. R. Kraeling, and G. B. Rampacek. 1982. The effect of artificially extended photoperiod during lactation on maternal performance of the sow. J. Anim. Sci. 54:918-921.

MacFarlane, W. V., B. Howard, and B. D. Siebert. 1969. Tritiated water in the measurement of milk intake and tissue growth of ruminants in the field. Nature (Lond.). 221:578-579.

Magnuson, J. S., J. F. Henry, T. T. Yip, and T. W. Hutchens. 1990. Structural homology of human, bovine, and porcine milk lactoferrins: evidence for shared antigenic determinants. Pediatr. Res. 28:176-181.

Magnusson, U., H. Rodriguez Martinez, and S. Einarsson. 1991. A simple, rapid method for differential cell counts in porcine mammary secretions. Vet. Rec. 129:485-490.

Magnussson, U. 1999. Longitudinal study of lymphocyte subsets and major histocompatibility complex-class II expressing cells in mammary glands of sows. Am. J. Vet. Res. 60:546-548.

Magnusson, U., A. Pedersen Mörner, A. Persson, E. Karlstam, S. Sternberg, and H. Kindahl. 2001. Sows intramammarily inoculated with *Escherichia coli*: influence of time of infection, hormone concentrations, and leucocyte numbers on development of disease. J. Vet. Med. B. 48:501-512.

Mahan, D. C., D. E. Becker, H. W. Norton, and A. H. Jensen. 1971. Milk production in lactating sows and time lengths used in evaluating milk production. J. Anim. Sci. 33:35-37.

Mahan, D. C., and A. J. Lepine. 1991. Effect of pig weaning weight and associated nursery feeding programs on subsequent performance to 105 kilograms body weight. J. Anim. Sci. 69:1370-1378.

Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitations of antigene by single radial immunodiffusion. Immunochemistry. 2:235-254.

Marra, M. N., C. G. Wilde, M. S. Collins, J. L. Snable, M. B. Thornton, and R. W. Scott. 1992. The role of bactericidal/permeability-increasing protein as a natural inhibitor of bacterial endotoxin. J. Immunol. 148:532-537.

Masson, P. L., and J. F. Heremans. 1971. Lactoferrin in milk from different species. Comp. Biochem. Physiol. 39b:119-129.

Mateo, C. D., D. N. Peters, and H. H. Stein. 2004. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:1339-1342.

Matson, K. M., A. C. W. Kauf, A. L. Magliaro, and R. S. Kensinger. 2004. Evidence for differential degradation of α_s- and β-casein in milk from mastitis quarters. ADSA-ASAS-PSA joint meeting (accepted).

Mauget, R. 1981. Behavioural and reproductive strategies in wild forms of sus scrofa (European wild boar and feral pigs). Curr. Top. Vet. Med. Anim. Sci. 2:3-13.

McBride, G., J. W. James, and G. S. F. Wyeth. 1965. Social behaviour of domestic animals. VIII. Variations in the weaning weight in pigs. Anim. Prod. 7:67-71.

McCance, R. A., and E. M. Widdowson. 1959. The effect of lowering the ambient temperature on the metabolism of the newborn pig. J. Physiol. (Lond.). 147:124.

McGlone, J. J. and S. D. Fullwood. 2001. Behavior, reproduction, and immunity of crated pregnant gilts: effects of high dietary fiber and rearing environment. J. Anim. Sci. 79:1466-1474.

Middleton, J. 1798. View of the Agriculture of Middlesex. London. as cited in: Baxter, S. 1984. Intensive Pig Production: Environmental Management and Design. Granada Publishers, London, England.

Middleton-Williams, D. M., J. Pohlenz, G. Lott-Stolz, and H. U. Bertschinger. 1977. Untersuchungen über das mastitis-metritis-agalaktie-syndrom (milchfieber) der sau. I. Patologische befunde bei spontanfällem. Schweiz. Arch. Tierheilkd. 119:213-222.

Milligan, B. N., D. Fraser, and D. L. Kramer. 2001. Birth weight variation in the domestic pig: effects on offspring survival, weight gain and suckling behaviour. Appl Anim. Behav. Sci. 73:179-191.

Milon, A., A. Aumaître, J. Le Dividich, J. Franz, and J. J. Mitzger. 1983. Influence of birth prematurity on colostrum composition and subsequent immunity of piglets. Ann. Rech. Vet. 14:533-540.

Morkoc, A., L. Bäckström, L. Lund, and A. R. Smith. 1983. Bacterial endotoxin in blood of dysgalactic sows in relation to microbial status of uterus, milk, and intestine. J. Am. Vet. Med. Assoc. 183:786-789.

Mörner, A. P., A. Faris, and K. Krovacek. 1998. Virulence determinants of *Escherichia coli* isolated from the milk of sows with coliform mastitis. J. Vet. Med. B. 45:287-295.

Morrissey, P. A. 1985. Physical and chemical properties of lactose. in Fox, P. F. (ed.) Developments in dairy chemistry, vol 3. p. 1-34.

Mount, L. E. 1959. The metabolic rate of the newborn pig in relation to environmental temperature and to age. J. Physiol. (Lond). 147:333-345.

Mount, L. E. 1960. The influence of huddling and body size on the metabolic rate of the young pig. J. Agric. Sci. 55:101-105.

Mount, L. E. 1966. Thermal and metabolic considerations between the newborn pig and human infant. In: L. K. Bustad and R. O. McClellan (ed.) Swine in Biomedical Research. p 501. Battelle Memorial Institute.

Mount, L. E. 1968. The climatic physiology of the pig. Edward Arnold, London.

Mount, L. E. 1979. Adaptation to thermal environment: man and his productive animals. Edward Arnold, London.

Moussaoui, F., I. Michelutti, Y. Le Roux, and F. Laurent. 2002. Mechanisms involved in milk endogenous proteolysis induced by a lipopolysaccharide experimental mastitis. J. Dairy Sci. 85:2562-2570.

Moussaoui, F., F. Laurent, J. M. Girardet, G. Humbert, J-L. Gaillard, and Y. Le Roux. 2003. Characterization and proteolytic origins of specific peptides appearing during lipopolysaccharide experimental mastitis. J. Dairy Sci. 86:1163-1170.

Müller-Eberhard, H. J. 1989. Innate immunity. Curr. Opin. Immunol. 2:3-4.

Nachreiner, R. F., M. C. Garcia, and O. J. Ginther. 1972. Clinical, hematologic, and blood chemical changes in swine given endotoxin (*Escherichia coli*) during the immediate postpartum period. Am. J. Vet. Res. 33:2489-2499.

Nachreiner, R. F., and O. J. Ginther. 1974. Induction of agalactia by administration of endotoxin (*Escherichia coli*) in swine. Am. J. Vet. Res. 35:619-622.

Neville, M. C., and P. Zhang. 2000. Lactoferrin secretion into milk: comparison between ruminant, murine, and human milk. J. Anim. Sci. 78(Suppl. 3):26-35.

Ng-Kwai-Hang, K. F. 1997. A review of the relationship between milk protein polymorphism and milk composition/milk production. In: 1997. Proc. IDF Sem., Palmerston North, New Zealand, International Dairy Federation. p 22.

Ng-Kwai-Hang, K. F. 1998. Genetic polymorphism of milk proteins: relationships with production traits, milk composition, and technological properties. Can. J. Anim. Sci. 78(Suppl. 1):131-147.

Noble, M. S., S. Rodriguez-Zas, J. B. Cook, G. T. Bleck, W. L. Hurley, and M. B. Wheeler. 2002. Lactational performance of first-parity transgenic gilts expressing bovine α-lactalbumin in their milk. J. Anim. Sci. 80:1090-1096.

Noblet, J., and J. Le Dividich. 1981. Energy metabolism of the newborn pig during the first 24 h of life. Biol. Neonate. 40:175-182.

Noblet, J., and M. Etienne. 1986. Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. J. Anim. Sci. 63:1888-1896.

Noblet, J., and M. Etienne. 1989. Estimation of sow milk nutrient output. J. Anim. Sci. 67:3352-3359.

Nuijens, J. H., P. H. C. van Berkel, and F. L. Schanbacher. 1996. Structure and biological actions of lactoferrin. J. Mammary Gland Biol. Neoplasia. 1(3):285-295.

Ohligmacher, K. 1928. Arb. Dtsch. Ges. Zücht. 37:42.

Östensson, K. 1993. Total and differential leukocyte counts, N-acetyl-β-D-glucosaminidase activity, and serum albumin content in foremilk and residual milk during endotoxin-induced mastitis in cows. Am. J. Vet. Res. 54:231-238.

Österlundh, I., H. Holst, and U. Magnusson. 2001. Effect of mammary secretions on functions of polymorphonuclear leukocytes in pigs. Am. J. Vet. Res. 62:1250-1254.

Paape, M. J., H. D. Hafs, and W. W. Snyder. 1963. Variation of estimated numbers of milk somatic cells stained with Wright's stain or pyronin y-methyl green stain. J. Dairy Sci. 46:1211-1216.

Parker, R. O., P. E. V. Williams, F. X. Aherne, and B. A. Young. 1980. Serum concentration changes in protein, glucose, urea, thyroxine, triiodothyronine, and thermostability of neonatal pigs farrowed at 25 and 10 ° C. Can. J. Anim. Sci. 60:503-509.

Parrish, D. B., G. H. Wise, J. S. Hughes, and F. W. Atkeson. 1948. Properties of the colostrum of the dairy cow. II. Effect of prepartal rations upon the nitrogenous constituents. J. Dairy Sci. 31:889-895

Parrish, D. B., G. H. Wise, J. S. Hughes, and F. W. Atkeson. 1950. Properties of the colostrum of the dairy cow. V. Yield, specific gravity, and concentrations of total solids and its various components of colostrum and early milk. J. Dairy Sci. 33:457-465.

Pejsak, Z., and K. Tarasiuk. 1989. The occurrence of endotoxin in sows with coliform mastitis. Theriogenology. 32:335-341.

Perkins, K. H., M. J. VandeHaar, J. L. Burton, J. S. Liesman, R. J. Erskine, and T. H. Elsasser. 2002. Clinical responses to intramammary endotoxin infusion in dairy cows subjected to feed restriction. J. Dairy Sci. 85:1724-1731.

Perrin, D. R. 1954. The composition of sows milk during the course of lactation. J. Dairy Res. 21:55-62.

Perrin, D. R. 1955. The chemical composition of the colostrum and milk of the sow. J. Dairy Res. 22:103-107.

Persson, A., A. E. Pedersen, L. Göransson, and W. Kuhl. 1989. A long term study on the health status and performance of sows on different feed allowances during late pregnancy. I. Clinical observations, with special reference to agalactia post partum. Acta. Vet. Scand. 30:9-17.

Persson, A., A. P. Mörner, and W. Kuhl. 1996. A long term study on the health status and performance of sows on different feed allowances during late pregnancy. III. Escherichia coli and other bacteria, total cell content, polymorphonuclear leucocytes, and pH in colostrum and milk during the first three weeks of lactation. Acta. Vet. Scand. 37:293-313.

Perssson, A. 1997. Mastitis in sows. Thesis. Acta Universitatis Agriculturae Sueciae, Veterinaria 10. Uppsala, Sweeden.

Peters, R. R., L. T. Chapin, R. S. Emery, and H. A. Tucker. 1978 a. Effect of supplemental lighting on body growth and milk yield during fall and winter. J. Dairy Sci. 61 (Suppl. 1):162. (Abstr.).

Peters, R. R., L. T. Chapin, K. B. Leining, and H. A. Tucker. 1978 b. Supplemental lighting stimulates growth and lactation in cattle. Science (Wash DC). 199:911-912.

Pettigrew, J. E. 1981. Supplemental dietary fat for peripartal sows: A review. J. Anim. Sci. 53:107-117.

Pettigrew, J. E., A. F. Sower, S. G. Cornelius, and R. L. Moser. 1985. A comparison of isotope dilution and weigh-suckle-weigh methods for estimating milk intake by pigs. Can. J. Anim. Sci. 65:989-992.

Pettigrew, J. E., S. G. Cornelius, R. L. Moser, and A. F. Sower. 1987. A refinement and evaluation of the isotope dilution method for estimating milk intake by piglets. Livestock Prod. Sci. 16:163-174.

PigChamp. 2002. 2002 Breeding herd summary. Available at: http://www.pigchamp.com/2002Datashare.htm. Accessed December 11, 2003.

Pizzano, R., M. A. Nicolai, R. Siciliano, and F. Addeo. 1998. Specific detection of the Amadori compounds in milk by using polyclonal antibodies raised against a lactosylated peptide. J. Agric. Food Chem. 46:5373-5379.

Pizzano, R., M. A. Nicolai, P. Padovano, P. Ferranti, F. Barone, and F. Addeo. 2000. Immunochemical evaluation of bovine β-casein and its 1-28 phosphopeptide in cheese during ripening. J. Agric. Food Chem. 48:4555-4560.

Pluske, J. R., and I. H. Williams. 1996. Split weaning increases the growth of light piglets during lactation. Aust. J. Agric. Res. 47:513-523.

Pluske, J. R., T. W. Fenton, M. L. Lorschy, J. E. Pettigrew, A. F. Sower, and F. X. Aherne. 1997. A modification to the isotope-dilution technique for estimating milk intake of pigs using pig serum. J. Anim. Sci. 75:1279-1283.

Prawirodigdo, S., R. H. King, A. C. Dunkin, and H. Dove. 1987. Estimation of milk intake by pigs using deuterium oxide dilution. in Manipulating pig production I. Australasian Pig Science Association, Albury, Australia.

Prime, R. W., V. A. Fahy, W. Ray, R. S. Cutler, and E. G. Spicer. 1987. On farm validation of research-lowering preweaning mortality rates in pigs. Report to Pig Research Council, Department of Primary Industries and Energy, Canberra, Australia.

Ramanau, A., H. Kluge, J. Spilke, and K. Eder. 2004. Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. J. Nutr. 134:86-92.

Ringarp, N. 1960. Clinical and experimental investigations into a post-parturient syndrome with agalactia in sows. Acta. Agric. Scand. 7(Suppl).

Roberts, T. K., and J. C. Boursnell. 1975. The isolation and characterization of lactoferrin from sow milk and boar seminal plasma. J. Reprod. Fertil. 42:579-582.

Ross, R. F. 1983. Current ideas on MMA in sows. Anim. Nutr. Health. Nov-Dec:46.

Ross, R. F., R. L. Harmon, B. J. Zimmerman, and T. F. Young. 1983. Susceptibility of sows to experimentally induced *Escherichia coli* mastitis. Am. J. Vet. Res. 44:949-954.

Said, A. H. 1973. Experimental *Escherichia coli* mastitis in cattle, its pathogenesis and treatment. Tijdschr. Diergeneesskd. 98:387-395.

Salmon-Legagneur, E., and A. Aumaître. 1962. Influence de la quantité de lait et de sa composition sur la croissance de porcelet sous la mere. Ann. Zootech (Paris). 11:181-196.

San Gabriel, A. M. 1994. Assay development and effects of lactogenic hormones on casein synthesis in porcine mammary tissue. M. S. dissertation, The Pennsylvania State University, University Park.

Schaar, J., and H. Funke. 1986. Effect of subclinical mastitis on milk plasminogen and plasmin compared with that on sodium, antitrypsin, and N-acetyl-β-D-glucosaminidase. J. Dairy Res. 53:515-528.

Schmidt, D. G. 1982. Association of caseins and casein micelle structure. In: P. F. Fox (ed.) Developments in Dairy Chemistry-1. p 61. Applied Science Publishers, London and New York.

Schmidt, J., and E. Lauprecht. 1926. Züchtungskunde. 1:50.

Schmidt-Nielsen, K., and B. Schmidt-Nielsen. 1952. Water metabolism of desert mammals. Physiol. Rev. 32:135.

Schneider, K. T. 1934. Landw. Jb. Schweiz. 80:3.

Schober, D. A., F. A. Simmen, D. L. Hadsell, and C. R. Baumrucker. 1990. Perinatal expression of type-I IGF receptors in porcine small intestine. Endocrinology. 126:1125-1132.

Schollenberger, A., A. Degorski, T. Frymus, and A. D. Schollenberger. 1986. Cells of sow mammary secretions. I. Morphology and differential cell counts during lactation. J. Vet. Med. A. 33:31-38.

Schumann, R. R., S. R. Leong, G. W. Flaggs, P. W. Gray, S. D. Wright, J. C. Mathison, P. S. Tobias, and R. J. Ulevitch. 1990. Structure and function of lipopolysaccharide binding protein. Science. 249:1429-1431.

Seerley, R. W., T. A. Pace, C.W. Foley, and R. D. Scarth. 1974. Effect of energy intake prior to parturition on milk lipids and survival rate, thermostability, and carcass composition of piglets. J. Anim. Sci. 38:64.

Seerley, R. W., F. M. Griffin, and H. C. McCampbell. 1978 a. Effect of sow's dietary energy source on sow's milk and piglet carcass composition. J. Anim. Sci. 46:1009.

Seerley, R. W., J. S. Maxwell, and H. C. McCampbell. 1978 b. A comparison of energy sources for sows and subsequent effects on piglets. J. Anim. Sci. 47:1114.

Seynaeve, M., R. De Wilde, G. Janssens, and B. De Smet. 1996. The influence of dietary salt level on water consumption, farrowing, and reproductive of lactating sows. J. Anim. Sci. 74:1047-1055.

Shuster, D. E., and R. J. Harmon. 1991. Lactating cows become partially refractory to frequent intramammary endotoxin infusions: recovery of milk yield despite a persistently high somatic cell count. Res. Vet. Sci. 51:272-277.

Shuster, D. E., R. J. Harmon, J. A. Jackson, and R. A. Hemken. 1991. Suppression of milk production during endotoxin-induced mastitis. J. Dairy Sci. 74:3763-3774.

Shuster, D. E., and M. E. Kehrli Jr. 1995. Administration of recombinant human interleukin 1 receptor antagonist during endotoxin-induced mastitis in cows. Am. J. Vet. Res. 56:313-320.

Shuster, D. E., M. E. Kehrli Jr., and C. R. Baumrucker. 1995. Relationship of inflammatory cytokines, growth hormone, and insulin-like growth factor-I to reduced performance during infectious disease. Proc. Soc. Exp. Biol. Med. 210:140-149.

Simmen, F. A., K. R. Cera, and D. C. Mahan. 1990. Stimulation by colostrum of mature milk of gastrointestinal tissue development in newborn pigs. J. Anim. Sci. 68:3596-3603.

Sinclair, A. G., J. M. Shaw, S. A. Edwards, S. Hoste, and A. McCartney. 1999. The effect of dietary protein level on milk yield and composition and piglet growth and composition of the Meishan Synthetic and European White breeds of sow. J. Anim. Sci. 68:701-708.

Smith, V. G., A. D. Leman, W. J. Seaman, and F. VanRavenswaay. 1991. Pig weaning weight and changes in hematology and blood chemistry of sows injected with recombinant porcine somatotropin during lactation. J. Anim. Sci. 69:3501-3510.

Speer, V. C., and D. F. Cox. 1984. Estimating milk yield of sows. J. Anim. Sci. 59:1281-1285.

Stahly, T. S., C. M. Thompson, and G. L. Cromwell. 1981. Effect of sow milk composition on the rate, efficiency, and composition of gain in neonatal pigs. J. Anim. Sci. 53 (Suppl. 1):264-265. (Abstr.).

Stangel, G., and P. Jensen. 1991. Behaviour of semi-naturally kept sows and piglets (except suckling) during 10 days post-partum. Appl. Anim. Behav. Sci. 31:211-227.

Straw, B. E., C. E. Dewey, and E. J. Bürgi. 1998. Patterns of crossfostering and piglet mortality on commercial US and Canadian swine farms. Prev. Vet. Med. 33:83-89.

Swaisgood, H. E. 1982. Chemistry of the milk protein. In: P. F. Fox (ed.) Developments in Dairy Chemistry-1. p 1. Applied Science Publishers, London and New York.

Swaisgood, H. E. 1992. Chemistry of the caseins. In: P. F. Fox (ed.) Advanced Dairy Chemistry-Volume 1. p. 63. Elsevier Applied Science, London and New York.

Tarasiuk, K., and Z. Pejsak. 1986. Role of *Escherichia coli* endotoxin in the aetiology of post-partum agalactia in sows. Medycyna Weterynaryjna. 42:323-327.

Thompson, B. K., and D. Fraser. 1988. Variation in piglets weights: weight gains in the first days after birth and their relationship with later performance. Can. J. Anim. Sci. 68:581-590.

Tritton, S. M., R. H. King, R. G. Campbell, and A. C. Edwards. 1993. The effects of dietary protein manipulation on the lactation performance of first-litter sows. In E. S.

Batterham (ed.) Manipulating Pig Production IV. p. 265. Australian Pig Sci. Assoc., Atwood, Australia.

USDA. 2002. U.S. hog breeding herd structure, September 2002. Available: http://usda.mannlib.cornell.edu/reports/nassr/livestock/hog-herd/spehog02.pdf. Accessed Feb. 6, 2004.

USDA. 2003 a. Chapter 7, Statistics of cattle, hogs, and sheep in Agricultural statistics, 2003. Available: http://www.usda.gov/nass/pubs/agr03/03_ch7.pdf. Accessed Feb. 6, 2004.

USDA. 2003 b. Quarterly hogs and pigs; December 2003. Available: http://usda.mannlib.cornell.edu/reports/nassr/livestock/php-bb/2003/hgpg1203.pdf. Accessed Feb. 6, 2004.

USDA. 2003 c. Pages 35-48 in Statistical highlights of United States agriulture, 2002/2003. Available: http://www.usda.gov/nass/pubs/stathigh/2003/stathi03.pdf. Accessed Feb. 6, 2004.

USDA. 2004. Genetic and phenotypic trends for Holstein, calculated May 2004. Available: http://www.aipl.arsusda.gov/dynamic/trend/current/trndx.html. Accessed June 28, 2004.

VanderMeer, T. J., M. J. Menconi, B. P. O'Sullivan, V. A. Larkin, H. Wang, R. L. Kradin, and M. P. Fink. 1994. Bactericidal/permeability-increasing protein ameliorates acute lung injury in porcine endotoxemia. J. Appl. Physiol. 76:2006-2014.

Van Kempen, G. J. M., C. Geerse, M. W. A. Verstegen, and J. Mesu. 1985. Effect of feeding level on milk production of sows during four weeks of lactation. Neth. J. Agric. Sci. 33:23-34.

Von Gohren, T. 1865. Analyse der Schweinemilch. Landwirsch. Vers. Sta. Baden. 7:351.

Walkiewicz, A., E. Wielbo, and S. Matyka. 1997. Chemical content of milk from wild sows (sus scrofa ferus). Annal. Univ. Mariae Curie-Skodowska. Sectio EE. Zootech. 15:93-98.

Weiss, J., P. Elsbach, C. Shu, J. Castillo, L. Grinna, A. Horwitz, and G. Theofan. 1992. Human bactericidal/permeability-increasing protein and a recombinant NH_2-terminal fragment cause killing of serum-resistant Gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria. J. Clin. Invest. 90:1122-1130.

Weldon, W. C., A. J. Thulin, O. A. MacDougald, L. J. Johnston, E. R. Miller, and H. A. Tucker. 1991. Effects of increased dietary energy and protein during late gestation on mammary development in gilts. J. Anim. Sci. 69:194-200.

White, C. E., and D. R. Campbell. 1984. Milk yield from sows and growth of nursing pigs during a 28-d lactation study. Florida Animal Science Research Report SW-1984-3. p 47. University of Florida, Gainesville.

Whittemore, C. T., and D. Fraser. 1974. The nursing and suckling behaviour of pigs. II. Vocalization of the sow in relation to suckling behaviour and milk ejection. Br. Vet. J. 130:346-356.

Widdowson, E. M., V. E. Colombo, and C. A. Artavanis. 1976. Changes in the organs of pigs in response to feeding for the first 24 h after birth. II. The digestive tract. Biol. Neonate. 28:272-281.

Wiggans, G. R. 2004. USDA summary of DHI participation. Available at: http://www.aipl.arsusda.gov/publish/dhi/current/partx.html. Accessed June 28, 2004.

Williams, K. L. 2001. Endotoxins: Pyrogens, LAL testing, and depyrogenation. Marcel Dekker, Inc. New York. Basel.

Wilson, M. R. 1974. Immunological development of the neonatal pig. J. Anim. Sci. 38:1018-1021.

Wohlbier, W. 1928. Biochem. Z. 202:39. as cited in Barber, R. S., R. Braude, and K. G. Mitchell. 1955. Studies on the milk production of large white pigs. J. Agric. Sci. 46:97-118.

Wright, S. D., R. A. Ramos, P. S. Tobias, R. J. Ulevitch, J. C. Mathison. 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 249:1431-1433.

Yang, T. S., B. Howard, and W. V. Macfarlane. 1980. A note on milk intake of piglets measured by tritium dilution. Anim. Prod. 31:201-203.

Yang, T. S., S. C. Wu, and S. R. Wang. 2000. Serum and milk lactoferrin concentration and the correlation with some blood components in lactating sows. Res. Vet. Sci. 69:95-97.

Ziv, G., I. Hartman, E. Bogin, J. Abidar, and A. Saran. 1976. Endotoxin in blood and milk and enzymes in the milk of cows during experimental *Escherichia coli* endotoxin mastitis. Theriogenology. 6:343-352.

Zou, S., D. G. McLaren, and W. L. Hurley. 1992. Pig colostrum and milk composition: comparisons between Chinese Meishan and US breeds. Livest. Prod. Sci. 30:115-127.

Appendix A. ELECTROPHORESIS TECHNIQUES

Reagents

       30% acrylamide/bis (37.5:1)
       Ammonium persulfate (APS)
       Glycine
       Isobutanol
       Sodium dodecyl sulfate (SDS)
       TEMED
       Tris base
       Tris-HCl
       Urea

Equipment

       Bio-Rad Mini-Protean 3 gel electrophoresis system
       Bio-Rad Power Pack 300
       BTA dry bath incubator/tube warmer (Boekel Grant Model 241000)
       Beakers/bottles
       Dry bath incubator/tube warmer
       Pipettes/tips
       Staining trays
       Stir/heat plate
       15 ml, 50 ml, and microfuge tubes

Buffers

       7.18 M Urea, 0.673 M Tris, pH 8.8

          Weigh out 21.56 g urea, 0.827 g Tris-HCl, and 3.45 g Tris base, and add to empty beaker. Bring volume to approximately 40 ml with Milli-Q water. Stir until dissolved and solution reaches room temperature. Adjust pH to 8.8, and bring to 50 ml volume with Milli-Q water.

       4.71 M Urea, 0.147 M Tris, pH 6.8

          Weigh out 14.14 g urea, 1.16 g Tris-HCl, and add to empty beaker. Bring volume to approximately 40 ml with Milli-Q water. Stir until dissolved and solution reaches room temperature. Adjust pH to 6.8; bring to 50 ml with Milli-Q water.

10% SDS

Weigh out 1 g SDS, add to 15 ml tube, and thoroughly dissolve in 10 ml of Milli-Q water.

10% APS-

Weigh out 0.1 g APS, add to microfuge tube, and dissolve in 1 ml of Milli-Q water; APS should be made fresh.

Sample buffer

Thoroughly mix 1.2 ml of 0.5 M Tris-HCl (pH 6.8), 1.92 ml 10% SDS, 0.96 ml of glycerol, 0.48 ml of β-mercaptoethanol, and 0.24 ml of 0.5% bromophenol blue in a 15 ml tube.

Electrophoresis buffer

Weigh out 6 g Tris base, 27.5 g glycine, 1 g SDS, and add to 900 ml of Milli-Q water. Check that pH is 8.3, and bring to 1 l final volume with Milli-Q water. Do not adjust pH, remake buffer if it is not 8.3.

Gel stain (10% Acetic Acid, 40% Methanol, 1% Coomassie)

Mix 250 ml Milli-Q water, 50 ml glacial acetic acid, 200 ml Methanol, and 0.5 g R250 coomassie brilliant blue in dedicated brown bottle.

Gel destain (10% Acetic Acid, 7% Methanol)

Mix 830 ml Milli-Q water, 100 ml glacial acetic acid, and 70 ml methanol.

Water-saturated isobutanol

In small brown bottle, add equal volume of isobutanol to Milli-Q water. Mix several times and allow to separate. Repeat over a day or two until solutions quickly separate.

<u>Protocol</u>

13% Acrylamide/4 M Urea Separation Protocol (Single Gel Recipe)

1) Clean all glass plates with absolute ethanol, and assemble plates on pouring stand.

2) Insert comb, mark bottom of well on outside of plate using Sharpie marker, and make an additional mark about 1/2 cm below first mark.

3) In 15 ml tube, combine 5.57 ml 7.18 M Urea/0.673 M Tris (pH 8.8), 0.1 ml 10% SDS, and 4.33 ml 30% Acrylamide/bis (37.5:1). Close tube, gently rock 2-3 times.

4) Add 0.1 ml 10% APS, 0.01 ml TEMED to tube. Close tube, gently rock 2-3 times, and fill space between plates to lower mark.

5) Fill space between plates to upper mark with water-saturated isobutanol (top layer in the bottle), thereby covering lower solution. Allow polymerization to proceed for 25 minutes.

6) Pour off isobutanol, thoroughly rinse (5-6 times) top of polymerized gel with Milli-Q water.

7) In 15 ml tube, combine 4.25 ml 4.71 M Urea/0.147 M Tris (pH 6.8), 0.05 ml 10% SDS, and 0.625 ml 30% Acrylamide/bis (37.5:1). Close tube, gently rock 2-3 times.

8) Add 0.05 ml 10% APS, 0.005 ml TEMED to tube. Close tube, gently rock 2-3 times, and fill space between plates to approximately 2 mm below top of plates.

9) Carefully insert comb, taking care not to trap air bubbles, and allow polymerization to proceed for 25 minutes.

Sample Preparation (all samples)

1) Add 10 μl (20 μl for Lf Western blotting) sample buffer to microfuge tube, up to 15 μl of sample, and adequate 4.71 M Urea/0.147 M Tris (pH 6.8) to bring final volume to 25 μl (30 μl for Lf Western blotting).

2) Thoroughly vortex samples, and spin down in microfuge; Heat on tube warmer for 8 minutes, then spin down.

Electrophoresis

1) Disassemble pouring stand and assemble gel sandwich according to manual.

2) Fill upper chamber, unlock cams, and allow plates to wet, and then reassemble. Fill upper chamber, then fill lower chamber to about 1 inch.

Insert upper chamber into the lower chamber solution, being careful not to trap air bubbles below it, then fill lower chamber to about 4 inches; all above steps are performed with electrophoresis buffer.

3) Remove comb from gel, being careful not to tear the wells. Flush wells thoroughly with electrophoresis buffer from tank.

4) Load samples (25 µl or 30 µl) with P20 pipette, connect leads, and apply current at a constant 58 V until samples enter separating gel (about 30 minutes).

5) Apply current at a constant 190 V until dye front is approximately 2 mm from the bottom of the gel (about 45 minutes).

6) Turn power off, disconnect leads, disassemble gel apparatus, and carefully remove gel.

7) Either stain gel or transfer to membrane.

Staining and Image Capture

1) Stain 10 minutes in gel stain.

2) Destain overnight with two buffer changes in gel destain.

3) Capture gel images using Eagle Eye system using white light box.

Appendix B. LOWRY ASSAY

Reagents

      Bovine serum albumin (BSA)
      Cupric sulfate ($CuSO_4$)
      Folin-Ciocalteu reagent
      Sodium carbonate (Na_2CO_3)
      Sodium hydroxide (NaOH)
      Sodium tartate
      Tris base

Equipment

      Beakers/bottles
      13 X 100 mm borosilicate tubes
      Milton Roy Spec 20 Spectrophotometer
      5 ml, 1ml, 200 μl, 20 μl, and 10 μl pipettes and tips

Buffers

      10 mM Tris, pH 8.2

            Prepare as 100 mM Tris by weighing out 1.21 g of Tris base, and add to 90 ml Milli-Q water. Stir until dissolved, adjust pH to 8.2, and bring to 100 ml volume with Milli-Q water.   Dilute 1:10 with Milli-Q water as needed to make 10 mM Tris.

      BSA (5 mg/ml) in 10 mM Tris

            Weigh out 25 mg BSA and add to 15 ml tube. Bring volume to 5 ml with 10 mM Tris, pH 8.2. Allow to completely dissolve before use.

      1 M NaOH

            Weigh out 20 g NaOH and add to 450 ml Milli-Q water. Stir until dissolved and solution reaches room temperature. Bring volume to 500 ml with Milli-Q water.

      1 % $CuSO_4$

            Weigh out 1 g $CuSO_4$ and add to 90 ml Milli-Q water. Stir until dissolved and bring volume to 100 ml with Milli-Q water.

1 % Sodium tartate

Weigh out 1 g Sodium tartate and add to 90 ml Milli-Q water. Stir until dissolved and bring volume to 100 ml with Milli-Q water.

2 % Na_2CO_3

Weigh out 20 g Na_2CO_3 and add to 900 ml Milli-Q water. Stir until dissolved and bring volume to 1 l with Milli-Q water.

1 N Folin-Ciocalteu reagent

Dilute 1:2 with Milli-Q water from 2 N stock, store in a brown bottle at 4 ° C.

Protocol

*All samples/standards performed in triplicate.

1) Prepare standards and samples by adding 0, 5, 10, 15, 20, 30, and 40 μl of 5 mg/ml BSA in 10 mM Tris (pH 8.2) to standard tubes; and 15 μl of sample to unknown tubes.

2) Bring all tubes to 500 μl with 10 mM Tris (pH 8.2). Add 500 μl 1 M NaOH.

3) Heat samples for 10 minutes in boiling water bath, and cool for 10 minutes at room temperature.

4) Add 5 ml fresh alkaline copper reagent to each tube, prepared as follows:

Mix in order- 1 ml 1 % $CuSO_4$, 1 ml 1 % Sodium tartate, and 100 ml 2 % Na_2CO_3; preparing enough for all tubes.

5) Vortex tubes and incubate for 10 minutes at room temperature.

6) Add 500 μl 1 N Folin-Ciocalteau reagent (Phenol reagent) to each tube, vortex immediately, and incubate for 30-60 minutes at room temperature with additional mixing.

7) Read absorbance at 750 nm on Spec 20 (Remember to use "red" bulb and red filter).

8) Plot standard concentration (X) against absorbance (Y) in Excel, and calculate unknowns from linear regression curve.

Appendix C. β-CASEIN ELISA

Reagents

Ammonium carbonate (NH_4HCO_3)
Bovine serum albumin (BSA)
Horse Serum (Sigma #H-1270)
Milk samples diluted at least one week prior to 1:50 in 4.71 M Urea, 147 mM Tris, 0.05 % Tween-20, pH 6.8 (Store at 4 ° C)
Porcine whey diluted 1:150 in 4.71 M Urea, 147 mM Tris, 0.05 % Tween-20, pH 6.8
Potassium chloride (KCl)
Potassium phosphate (KH_2PO_4)
Primary Antisera: Rabbit anti-Porcine β-casein (rabbit 918, 5 Dec 00 bleed) diluted 1:10 in RIA buffer (25 mM Tris, 150 mM NaCl, 0.1 % BSA, 0.02 % NaN_3, pH 7.2)
Purified porcine β-casein (determine concentration by Lowry Assay)
Secondary antibody: (Goat anti-rabbit IgG)-horseradish peroxidase conjugate (Rockland # 611-1322, or Jackson Immunoresearch #111-035-003)
Sodium chloride (NaCl)
Sodium phosphate (Na_2HPO_4)
Sodium azide (NaN_3)
Sulfuric acid (H_2SO_4)
TMB Substrate (Bethyl Labs # E-102)
Tris base
Tris-HCl
Tween-20
Urea

Equipment

Beakers/bottles
Bio-Tek Instruments microplate reader (Model EL311)
Microfuge (1.5 ml), 15 ml, and 50 ml tubes
8-well multichannel pipette (250 μl)
Pipettes and tips
Plastic troughs
PVC U-bottom 96-well microtiter plate (Falcon # 353911)
Water bath

Buffers

4.71 M Urea, 0.147 M Tris, 0.05 % Tween-20, pH 6.8

Weigh out 14.14 g urea, 1.16 g Tris-HCl, and add to empty beaker. Bring volume to approximately 40 ml with Milli-Q water. Stir until dissolved and solution reaches room temperature, and add 50 µl Tween-20. Adjust pH to 6.8 and bring to 50 ml with Milli-Q water.

2 % NaN_3

Weigh out 2 g NaN_3, add to 100 ml Milli-Q water, and stir until dissolved.

RIA buffer- 25 mM Tris, 150 mM NaCl, 0.1 % BSA, 0.02 % NaN_3, pH 7.2

Weigh out 3.51 g Tris-HCl, 0.335 g Tris base, 8.766 g NaCl, 1 g BSA, and add to 900 ml Milli-Q water. Stir until dissolved and add 10 ml 2 % NaN_3. Adjust pH to 7.2 and bring to 1 l volume with Milli-Q water.

Coating buffer: 50 mM NH_4HCO_3, pH 8.5

Weigh out 395 mg NH_4HCO_3, and add to 80 ml Milli-Q water. Stir until dissolved, adjust pH to 8.5, and bring to 100 ml with Milli-Q water.

Wash buffer: PBS- 137 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4 (Sterilize by autoclaving)

Weigh out 8 g NaCl, 200 mg KCl, 1.44 g NaH_2PO_4, 240 mg KH_2PO_4, and add to 800 ml Milli-Q water. Stir until dissolved, adjust pH to 7.4, and bring to 1 l with Milli-Q water.

Blocking solution and antisera diluent (make fresh before each assay): 5 % heat inactivated (1 h at 65 ° C) horse serum (Sigma # H-1270), 0.05 % Tween-20, in PBS

Determine amount of blocking solution/diluent needed for the assay (200 µl/ well for blocking, 100 µl/ well for antisera; allow for about 5 % extra). For each 10 ml, add 500 µl of horse serum and 5 µl of Tween-20 to wash buffer.

Sample diluent: 50 mM NH_4HCO_3, 0.05 % Tween-20, pH 8.5

Add 50 µl of Tween-20 to 100 ml of coating buffer.

Enzyme substrate: TMB- 3, 3', 5, 5'-tetramethyl benzidine (Bethyl Labs # E102),
    reagents A and B

Stop solution: 1 M H_2SO_4

    Add 5.55 ml H_2SO_4 to 80 ml of Milli-Q water, and bring to 100 ml with
Milli-Q water.

Protocol

1) Dilute purified porcine β-casein to 2 μg/ml with coating buffer, coat plate
   with 100 μl/well, and incubate overnight at 4° C. Invert plate and blot on a
   paper towel, then wash 2 X with 200 μl/well of wash buffer.

2) Add 200 μl/well of blocking solution, and incubate for 1 h at 37° C in closed
   water bath. Invert plate and blot on a paper towel, then wash 2 X with
   200 μl/well of wash buffer.

3) Dilute standards and samples as follows: Dilute purified porcine β-casein to
   0, 1.25, 2.5, 5, 10, 20, 40, and 80 μg/ml (0.0625, 0.125, 0.25, 0.5, 1, 2, and
   4 μg/well) with sample diluent, including 1 μl of 1:150 porcine whey/50 μl of
   final sample dilution. Dilute 1:50 milk samples (working dilution) by adding
   1 μl/50 μl final sample dilution, including the same 1 μl of 1:150 porcine
   whey/50 μl final sample dilution as added to standards. Prepare enough of
   each sample and standard for 4 wells (200 μl). Prepare primary antisera (1:5 k
   final dilution) by adding 25 μl of 1:10 stock dilution to 225 μl antisera diluent.
   Add 150 μl of that to 1,350 μl of antisera diluent, then 5 μl of that to 45 μl of
   antisera diluent for every well, allowing extra for pipetting error(s). Add 50
   μl/well of β-casein standards or milk samples and 50 μl/well of primary
   antisera to plate in triplicate, including one constant sample between plates.
   Incubate for 1 h at 37° C in a closed water bath. Invert plate and blot on a
   paper towel, then wash 5X with 200 μl/well of wash buffer.

4) Prepare secondary antibody (1:30 k final dilution) by adding 15 μl stock to
   135 μl of antisera diluent. Add 100 μl of that to 900 μl of antisera diluent,
   repeat, and then add 3.33 μl of that to 96.77 μl of antisera diluent for every
   well, allowing extra for pipetting error(s). Add 100 μl/well of secondary
   antibody, and incubate 1 h at 37° C in a closed water bath. Invert plate and
   blot on a paper towel, then wash 5 X with 200 μl/well of wash buffer.

5) Mix the TMB reagent (equal parts of the two substrates), add 100 μl/well, and incubate approximately 1-5 minutes at room temperature (watch for color development to approximately 1 A.U./well for highest standard).

6) Add 100 μl/well of 1 M H_2SO_4 to stop reaction.

7) Read absorbance at 450 nm on a plate reader (requires 15 minutes to warm up).

8) Plot absorbance (X) against standard mass/well (Y) using Excel, calculate unknowns from second order polynomial regression curve.

9) Rerun samples if c.v. $\geq$ 8 % for the triplicate, rerun the plate if value for constant sample is $\pm$ 2 s.d. of the average value between plates.

Appendix D. ALBUMIN ELISA

<u>Reagents</u>

Bethyl Labs porcine albumin ELISA kit (Catalog # E100-110)
Bovine serum albumin (BSA)
Sodium carbonate (Na_2CO_3)
Sodium chloride (NaCl)
Sulfuric Acid (H_2SO_4)
TMB substrate (Bethyl Labs # E-102)
Tris base
Tris-HCl
Tween-20

<u>Equipment</u>

Beakers/bottles
Bio-Tek Instruments microplate reader (Model EL311)
Microfuge (1.5 ml), 15 ml, and 50 ml tubes
8-well multichannel pipette (250 μl)
Pipettes/tips
Plastic troughs
Sidearm flask and Tygon tubing (setup for aspiration)

<u>Buffers</u>

All buffers made with distilled, <u>not</u> Milli-Q water

Coating buffer: 50 mM Na_2CO_3, pH 9.6

Add 2.65 g Na_2CO_3 to 400 ml distilled water. Stir until dissolved, adjust pH to 9.6, and bring to 500 ml with distilled water.

Wash solution: 50 mM Tris, 140 mM NaCl, 0.05% Tween-20, pH 8.0

Add 4.44 g Tris-HCl, 2.65 Tris base, and 8.18 g NaCl to 800 ml distilled water. Stir until dissolved, add 500 μl Tween-20, adjust pH to 8.0, and bring to 1 l volume with distilled water.

Post coat solution: 50 mM Tris, 140 mM NaCl, 1% BSA, pH 8.0

Add 2.22 g Tris-HCl, 1.33 g Tris Base, 4.09 g NaCl, and 5 g BSA to 400 ml distilled water. Stir until dissolved, adjust pH to 8.0, and bring to 500 ml with distilled water.

Sample diluent: 50 mM Tris, 140 mM NaCl, 1% BSA, 0.05% Tween-20, pH 8.0

      Add 2.22 g Tris-HCl, 1.33 g Tris base, 4.09 g NaCl, and 5 g BSA to 400 ml distilled water. Stir until dissolved, add 250 μl Tween-20, adjust pH to 8.0, and bring to 500 ml with distilled water.

Enzyme substrate: TMB- 3, 3', 5, 5'-tetramethyl benzidine (Bethyl Labs #E102),
      reagents A and B

Stop solution: 2 M H_2SO_4

      Add 11.11 ml H_2SO_4 to 80 ml distilled water. Bring to 100 ml with distilled water.

Protocol

1) Dilute the affinity-purified antibody (stock dilution) 1:100 with coating buffer. Add 100 μl/well to the plate included in the kit, and incubate at room temp for 60 min. Remove by aspiration, then wash plate 2 X with 200 μl/well of wash solution. (Remove by aspiration, dipping pipette tip in distilled water between triplicate wells).

2) Add 200 μl/well of the post-coat solution, and incubate at room temp for 30 min. Remove the post-coat and wash 2 X with 200 μl/well of wash solution.

3) Dilute the standards and samples in sample/conjugate diluent as follows: Add 1 μl of albumin standard to 5 ml of diluent (10,000 ng/ml); then add 100 μl of that to 1.9 ml of diluent (500 ng/ml). Then make serial twofold dilutions (1 ml + 1 ml) of standards to a final range of 250, 125, 62.5, 31.25, 15.63, and 7.81 ng/ml. Dilute milk samples in diluent based on Lowry test results (1:50 k, 1:100 k, or 1:200 k final dilution). Add 100 μl/well of samples and standards in triplicate, including one constant sample between plates. Incubate at room temp for 60 min. Remove and wash 5 X with 200 μl/well of wash solution.

4) Dilute the antibody/HRP conjugate to 1:100 k as follows: Add 1 μl stock solution to 1 ml sample diluent; then add 1 μl of that to 100 μl of sample diluent for each well. Add 100 μl/well of antibody complex and incubate at room temp for 60 min. Remove and wash 5 X with 200 μ/well of wash solution.

5) Mix the TMB reagent (equal parts of the two substrates), add 100 μl/well, and incubate approximately 30 minutes at room temperature (watch for color development to approximately 1.0 A.U./well for highest standard).

6) Add 100 μl/well of 2M H_2SO_4 to stop reaction.

7) Read the absorbance at 450 nm on a plate reader (requires 15 minutes to warm up).

8) Plot standard concentration (X) against absorbance (Y) using Excel, and calculate unknowns from linear regression curve.

9) Rerun samples if c.v. $\geq$ 8 % for the triplicate, rerun the plate if value for constant sample is $\pm$ 2 s.d. of the average value between plates.

Appendix E. ELECTROPHORETIC TRANSFER

Reagents

Glycine
Sodium dodecyl sulfate (SDS)
Tris base

Equipment

Bio Rad Mini Trans-Blot Electrophoretic Transfer Cell
Bio Rad Pro-Blott PVDF Membrane
Bio Rad Power Pack 300
Bio Rad filter paper
Beakers/bottles
Razor
Staining trays

Buffers

Transfer Buffer

Weight out 3.03 g Tris base, 14.4 g glycine, 0.125 g SDS and add to 900 ml Milli-Q water. Adjust pH to 8.3 and bring to 1 l volume with Milli-Q water. Store at 4 ° C until use.

Protocol

1) Refrigerate transfer buffer and freeze ice pack overnight.

2) Isolate samples on 13% acrylamide, 4 M Urea SDS-PAGE gel under reducing conditions.

3) Wet PVDF membrane (cut to 10 cm X 8 cm) in 100% Methanol until saturated.

4) Equilibrate PVDF membrane, gel, filter papers, and sponges in transfer buffer for 15 minutes.

5) Assemble transfer sandwich and transfer apparatus according to instructions. Fill with transfer buffer, add ice pack, connect leads and apply current at a constant 350 mA for 2 h.

6) Disconnect leads and disassemble transfer apparatus. Remove membrane for Western blotting.

<u>Appendix F. WESTERN BLOTTING</u>

<u>Reagents</u>

DAB substrate kit
Normal gilt serum
Potassium permanganate ($KMnO_4$)
Sodium carbonate (Na_2CO_3)
Sodium chloride (NaCl)
Tris base
Tris-HCl
Tween-20
Vectastain ABC kit

<u>Equipment</u>

Beakers/bottles
LabQuake[TM] shaker
Pipettes/tips
Razor blade
50 ml tubes

<u>Buffers</u>

0.1 M Tris, 0.9% NaCl, 0.5% Tween-20, pH 7.5 (modified TTBS)

Weigh out 12.70 g Tris-HCl, 2.36 g Tris base, 9 g NaCl and add to 800 ml Milli-Q water. Add 5 ml Tween-20, and adjust pH to 7.5. Bring to 1 l final volume with Milli-Q water.

Vectastain ABC reagent

Add two drops reagent A and two drops reagent B to 10 ml modified TTBS and allow to stand at least 30 minutes before use.

Biotinylated secondary antibody

Add 2 drops normal goat serum, 50 μl normal gilt serum, and 1 drop biotinylated goat anti-rabbit antibody to 10 ml modified TTBS. Incubate at room temperature for 1 hour prior to use.

DAB substrate solution (from kit)

Do not make until immediately before use. Add 2 drops buffer stock solution to 15 ml distilled (not Milli-Q water) and mix well. Add 4 drops DAB stock solution and mix well. Add 2 drops hydrogen peroxide solution and mix well. Add 2 drops nickel solution and mix well.

DAB substrate neutralizing solution (3% potassium permanganate, 2% sodium carbonate

Weigh out 15 g potassium permanganate, 10 g sodium carbonate, and add to 500 ml Milli-Q water.

Protocol (note that this is a modification of kit instructions)

All incubations carried out at room temperature with gentle rocking on LabQuake shaker in 50 ml tubes.

Analysis of milk samples-

1) Following electrophoretic transfer, immerse membrane in 20 ml of modified TTBS in 50 ml tubes. Incubate for 30 min with gentle rocking.

2) Transfer membrane to appropriate dilution of primary antisera (rabbit polyclonal anti-porcine β-casein serum) in 20 ml of modified TTBS. Incubate for 30 min with gentle rocking.

3) Wash membrane 5 times in 20 ml of modified TTBS for 5 min each with gentle rocking.

4) Make Vectastain ABC reagent.

5) Transfer membrane to biotinylated secondary antibody (goat anti-rabbit IgG) in 20 ml modified TTBS. Incubate for 30 min with gentle rocking.

6) Wash membrane 5 times in 20 ml of modified TTBS for 5 min each with gentle rocking.

7) Transfer membrane to 20 ml of Vectastain ABC reagent. Incubate for 30 min with gentle rocking.

8) Wash membrane 5 times in 20 ml of modified TTBS for 5 min each with gentle rocking.

9) Transfer membrane to 20 ml of DAB substrate solution. Incubate up to 20 minutes with gentle rocking while monitoring color development.

10) Transfer membrane to 20 ml of distilled water. Incubate for 5 minutes with gentle rocking, then allow to air dry.

11) Capture images using Eagle Eye still video imaging system.

For titering of primary antibody-

1) When running the gel, load 1 μl of mature porcine milk per lane, leaving an empty lane between samples.

2) Cut membrane into strips using a razor through the corresponding unused lanes prior to Western blot procedure.

3) Western blots are performed in 15 ml tubes and 5 ml volumes according to previous instructions.

4) Each strip is incubated with separate test primary antisera (from different bleeds, different rabbits, or different dilutions).

Appendix G. ENDOTOXIN CHALLENGE/WEIGH-SUCKLE-WEIGH

Reagent

Lipopolysaccharide (endotoxin) phenol extract of *E. coli* O55:B5
(Sigma # L-2880)
Oxytocin

Equipment

Sartorius balance (model # EB15DCE-IOUR); 15 kg cap, ± 0.5 g accuracy, live
weight averaging capability
Bench paper
Board (large enough to provide flat surface for balance)
4 carts to move containers
Digital medical thermometer
Johnson & Johnson Elastikon[TM] tape
Plastic container (large enough to hold 1 piglet on balance)
8 Rubbermaid containers (large enough to hold ½ litter each)
Timer

Protocol

1) Farrow litters in groups of four sows each. Sows should be bred at the same
time, such that they should farrow within a few days of each other. Allow the
first two sows to farrow naturally, and then induce the remaining two sows to
farrow within 24 h. Cross-foster and standardize litters within one day of the
last sow farrowing to nine piglets (± one piglet) for each of three sows;
striving to balance litter weight and source litter among sows. Remaining
piglets are assigned to the fourth sow, which is not used for the experiment.
Piglets are weighed individually daily, from the day of cross-fostering, to the
end of the experiment.

2) On experimental day, separate piglets from sows at 0700, record baseline
rectal temperature, and record rectal temperatures throughout the day. Infuse
the streak canals of two clinically normal, productive mammary glands (one
canal of each of two separate teats) with 1.5 μg/kg BW of LPS endotoxin in
Milli-Q water at 0800, following nursing. Endotoxin binds plastic, rubber,
and glass, so LPS should be prepared in a glass vial several days in advance.
Vortex LPS solution thoroughly at 0615, and mix thoroughly by hand before
drawing into two tuberculin syringes with 24 ga. tubing adapters (not needles)
attached. Tape teat closed with Elastikon[TM] tape; do not remove tape from
teat until 0900, after that hour's nursing, such that infused glands are not

nursed until two hours after infusion. All times are for the first sow, infusions are staggered by 20 minutes for each subsequent sow, such that the procedure for all three sows requires approximately one hour to perform.

3) At hourly intervals (0900, 1000, etc.) allow piglets to nurse sow as follows. Fifteen minutes before nursing, remove piglets from heated creep area, and transfer to two "cold" containers. Encourage piglets to urinate/defecate by disturbance, making sure piglets are awake and moving about. Five minutes before nursing, weigh piglets individually (using 20 X live-weight averaging function), and transfer to two "clean" containers with pre-weighed pieces of bench paper inside. Weighing should be performed as quickly and quietly as possible. Return piglets to sow and allow to nurse for 9 ½ minutes. Take note of piglets that urinate/defecate in the "clean" box and farrowing crate, and try to prevent piglets from drinking from the sow's water source. Following nursing, remove piglets to "clean" containers with the pre-weighed bench paper. Weigh piglets again, and return to heated creep area. Weigh bench paper from "clean" containers, and repeat the process with the other two sows/litters, cleaning the containers and replacing the pre-weighed bench paper between litters. As for the infusion procedure, the weigh-suckle-weigh procedure is staggered by 20 minutes for each subsequent sow, such that the procedure for all three litters requires approximately one hour to complete. Repeat procedure for 8 or 10 nursings, as required for the experiment.

4) Hourly milk yield per teat/piglet is calculated as the difference between the pre and post nursing weight of each piglet. Yield is adjusted for metabolic weight loss (minutes*(0.21 g/kg $BW^{0.75}$)) of piglets (Noblet and Etienne, 1986). Additionally, weight loss due to urination and defecation within the "clean" containers is accounted for by adding back the difference between initial and final weights of the bench papers, divided equally between piglets observed to have eliminated in the "clean" containers. After adjustment for metabolic loss and elimination, negative values are treated as zero milk yields. Milk yield is treated as missing values for piglets that eliminated in the crate, or drank from the sow's water source, as milk yield cannot be accounted for by these piglets. Negative daily weight gains are not adjusted.

<u>Appendix H. TNF-α ELISA</u>

<u>Reagents</u>

Pierce-Endogen Porcine TNF-α ELISA Kit (catalog # EP2TNFA)

<u>Equipment</u>

Beakers/bottles
Bio-Tek Instruments microplate reader (Model EL311)
Microfuge (1.5 ml) tubes
8-well multichannel pipette (250 μl)
Pipettes/tips
Plastic troughs
Water bottle

<u>Buffers-</u> All provided in the kit

Wash solution (30X)- dilute to 1 X with Milli-Q water
Sample diluent, Biotinylated antibody reagent, Streptavidin-HRP reagent,
TMB substrate solution, and Stop solution all used "as-is"

<u>Protocol</u>

*All solutions/reagents should be at room temperature, thaw samples at room
temp; Standard must be used within 1 h of reconstitution.

1) Prepare standards by serial (1:2) dilution from stock; 200 μl/tube. Standards
are 2,000, 1,000, 500, 250, 125, 62.5, 31.3, and 0 pg/ml.

2) Add 50 μl of Standard diluent to each well, add 50 μl of standards or samples
to duplicate wells, cover plate, and incubate at room temperature (20-25 C) for
2 hours. Invert plate and blot on paper towel, wash plate 3 X with wash
solution in water bottle.

3) Add 100 μl of Biotinylated antibody reagent to each well, cover plate, and
incubate at room temperature (20-25 C) for 1 hour. Invert plate and blot on
paper towel, wash plate 3 X with wash solution in water bottle.

4) Add 100 μl of prepared Streptavidin-HRP solution to each well, cover plate,
and incubate at room temperature (20-25 C) for 30 minutes. Invert plate and
blot on paper towel, wash plate 3 X with wash solution in water bottle.

5) Add 100 µl of premixed TMB substrate solution to each well, cover plate, and develop at room temperature (20-25 C) for 30 minutes.

6) Stop reaction by adding 100 µl of the provided stop solution to each well.

7) Measure absorbance on a plate reader set at 450-550 nm.

8) Plot standard concentration (X) against absorbance (Y) in excel, calculate unknowns from linear regression curve.

Appendix I. RAW RECTAL TEMPERATURE DATA (EARLY LACTATION)

Table 1 represents the raw data for rectal temperature in degrees Celsius, as determined by digital thermometer, of gilts subjected to the endotoxin challenge mastitis model during week one of lactation, and used for subsequent statistical analysis.

Gilt	x1-1	20-14	3-1	27-4	35-7	42-7	42-1		
day 3 h								Ave	s.d.
0	38.0	39.9	39.8	38.6	40.2	39.9	39.6	39.41	0.82
1	38.0	40.2	39.7	38.7	40.0	39.6	38.9	39.30	0.78
2	38.1	40.6	39.8	38.5	40.9	39.6	39.4	39.56	1.04
3	39.2	40.7	40.2	39.6	40.6	40.5	39.9	40.09	0.56
4	39.9	40.9	40.9	40.9	41.3	40.3	40.8	40.72	0.46
5	40.0	40.4	40.5	41.1	40.6	40.3	40.8	40.52	0.33
6	39.7	40.0	40.1	40.6	40.2	40.1	40.7	40.17	0.34
7	39.2	39.8	39.8	39.6	40.2	40.1	40.5	39.87	0.42
8	39.4	39.6	39.6	39.8	40.4	40.1	40.2	39.87	0.36
9	38.8	39.6	39.7	39.8	40.3	40.0	40.2	39.79	0.51
10	38.6	39.8	39.7	39.3	40.1	40.1	40.1	39.67	0.54
day 5									
0	40.2	40.3	39.7	40.2	39.6	39.6	38.9	39.79	0.50
1	40.0	40.3	39.4	40.2	39.3	39.5	39.1	39.69	0.48
2	40.3	41.3	39.7	40.2	39.6	39.6	38.9	39.96	0.76
3	40.3	41.3	40.4	40.8	40.2	40.6	39.7	40.48	0.50
4	40.8	41.3	41.0	40.6	40.6	40.8	40.3	40.77	0.32
5	41.3	40.9	40.7	41.2	40.5	40.3	40.4	40.76	0.40
6	41.5	40.6	40.2	41.2	40.6	40.0	40.5	40.67	0.53
7	41.3	40.5	40.4	40.7	40.1	40.6	40.1	40.53	0.42
8	41.2	40.2	40.2	40.4	40.3	40.2	39.6	40.29	0.48
9	40.7	40.2	40.0	40.8	40.4	40.1	39.8	40.29	0.36
10	40.7	40.2	40.3	40.7	40.4	40.2	39.3	40.27	0.47
day 7									
0	40.2	40.6	39.9	40.1	40.1	39.7	39.5	40.00	0.35
1	39.9	40.1	39.7	40.3	40.0	39.6	39.2	39.83	0.36
2	40.0	40.1	39.8	40.2	40.6	39.7	39.2	39.94	0.44
3	40.9	41.0	40.7	40.9	40.5	40.4	39.6	40.59	0.49
4	41.2	41.1	41.2	41.9	40.5	40.9	40.4	41.02	0.48
5	41.2	41.3	40.8	41.9	40.9	41.4	40.8	41.19	0.42
6	41.3	41.0	41.6	41.7	40.7	41.3	41.0	41.23	0.34
7	41.4	40.3	41.5	41.6	40.4	40.7	40.4	40.90	0.56
8	40.8	40.2	42.7	41.2	40.4	40.7	40.4	40.91	0.84
9	40.6	40.1	40.8	40.8	40.4	40.4	40.2	40.47	0.27
10	40.6	40.1	40.7	40.5	40.2	40.4	39.8	40.34	0.30

Table 1. Rectal temperatures, in degrees Celsius, of gilts subjected to the endotoxin challenge mastitis model during week one of lactation.

Appendix J. RAW MILK PROTEIN DATA (EARLY LACTATION)

Table 2 represents the raw data for milk protein content in percent, as determined by Lowry assay, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation, and used for subsequent statistical analysis.

Gilt		x1-1	20-14	3-1	27-4	35-7	42-7	42-1	Ave	s.d.
lday 3	con	8.5500	9.0948	6.8601	8.1923	5.9380	5.7222	7.3472	7.3864	1.2954
	LPS	6.4071	6.2466	4.4269	6.1750	6.6241	4.0317	6.4525	5.7663	1.0660
lday 5	con	4.1119	6.1534	7.1236	3.9585	6.0332	6.0143	4.7309	5.4465	1.1893
	LPS	6.3427	5.5390	7.2554	5.4259	6.1809	5.8044	7.9668	6.3593	0.9373
lday 7	con	6.3282	6.1252	6.5966	6.2597	7.2285	5.6126	8.2377	6.6269	0.8624
	LPS	6.0370	6.2991	7.2115	6.8506	6.0135	6.0874	8.3027	6.6860	0.8464

Table 2. Milk protein contents, in percent, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation. LPS- milk from endotoxin-infused gland. Con- milk from non-infused gland. Lday- day of lactation.

Appendix K. RAW MILK β-CASEIN DATA (EARLY LACTATION)

Table 3 represents the raw data for milk β-casein content in mg/ml, as determined by ELISA, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation, and used for subsequent statistical analysis.

	Gilt x1-1	20-14	3-1	27-4	35-7	42-7	42-1	Ave	s.d.
lday 3 con	26.4982	25.4056	37.8711	36.3924	17.5043	19.3889	27.8484	27.2727	7.7189
LPS	11.6995	17.7585	9.8642	20.3855	9.5342	10.4863	6.0941	12.2603	5.0151
lday 5 con	20.9159	26.6537	38.7789	27.4518	25.8896	22.9424	25.3691	26.8573	5.7198
LPS	13.2470	16.3065	22.9042	6.5410	3.5123	16.6741	20.1523	14.1911	7.0184
lday 7 con	19.7128	25.4695	44.2285	24.1810	19.6334	26.5479	24.1210	26.2706	8.3591
LPS	4.2464	5.8288	28.8098	2.5983	13.6710	21.4512	14.9613	13.0810	9.6770

Table 3. β-casein contents, in mg/ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation. LPS- milk from endotoxin-infused gland. Con- milk from non-infused gland. Lday- day of lactation.

Appendix L. RAW MILK ALBUMIN DATA (EARLY LACTATION)

Table 4 represents the raw data for milk albumin content in mg/ml, as determined by ELISA, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation, and used for subsequent statistical analysis.

	Gilt	x1-1	20-14	3-1	27-4	35-7	42-7	42-1	Ave	s.d.
lday 3	con	2.00	3.56	1.75	2.55	1.87	0.87	1.71	2.0443	0.8329
	LPS	1.64	2.23	1.13	1.87	2.15	2.48	1.73	1.8900	0.4467
lday 5	con	1.69	1.43	1.27	1.79	0.94	1.40	0.17	1.2414	0.5481
	LPS	2.04	2.42	2.67	1.84	3.73	1.93	0.70	2.1900	0.9208
lday 7	con	0.31	2.05	1.89	1.24	1.12	0.63	0.21	1.0643	0.7274
	LPS	2.16	0.38	1.75	1.21	1.84	2.96	1.78	1.7257	0.7964

Table 4. Albumin contents, in mg/ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during week one of lactation. LPS- milk from endotoxin-infused gland. Con- milk from non-infused gland. Lday- day of lactation.

Appendix M. RAW SOW HOURLY MILK YIELD DATA (EARLY LACTATION)

Table 5 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 12-5, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 12-5

Lday	Piglet	trt	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	Ave	s.d.
3	1	con	.	.	.	27.2	10.5	9.7	8.8	6.0	14.9	8.5	12.23	7.13
3	2	con	.	.	.	62.9	0.0	4.6	29.0	0.0	4.2	23.2	17.70	23.03
3	3	con	.	.	.	0.0	11.3	12.2	28.2	4.7	6.9	9.0	10.33	8.90
3	4	con	.	.	.	18.7	7.7	11.0	7.6	15.4	12.5	10.1	11.86	4.06
3	5	con	.	.	.	13.1	0.0	8.3	5.8	3.2	5.6	5.2	5.89	4.09
3	6	con	.	.	.	3.3	17.6	1.7	13.4	10.1	7.8	0.0	7.70	6.47
3	7	LPS	.	.	.	27.4	14.1	17.6	7.5	7.4	8.3	7.9	12.89	7.52
3	8	LPS	.	.	.	13.6	6.9	2.9	12.9	8.4	3.4	1.2	7.04	4.89
3	9	con	.	.	.	14.4	0.0	.	13.0	11.8	6.3	12.8	9.72	5.53
5	1	LPS	.	3.7	15.7	13.6	13.6	7.7	1.6	10.6	29.6	8.6	11.63	8.19
5	2	con	.	15.7	24.7	20.7	30.6	4.7	14.7	19.7	45.7	16.7	21.47	11.55
5	3	con	.	8.1	29.1	4.0	25.0	.	15.0	21.0	16.0	11.0	16.15	8.53
5	4	LPS	.	18.7	22.7	2.4	26.6	12.7	12.6	16.6	33.6	0.0	16.21	10.83
5	5	con	.	3.0	9.0	10.9	26.8	12.9	10.8	9.8	10.8	7.8	11.31	6.44
5	6	con	.	23.6	25.1	7.5	.	46.1	13.5	.	40.5	12.5	24.11	14.59
5	7	con	.	18.8	18.8	14.7	29.7	4.8	12.7	.	29.7	11.7	17.61	8.67
5	8	con	.	9.6	15.6	15.5	14.5	5.6	15.5	19.5	25.5	8.5	14.42	6.00
5	9	con	.	12.9	16.9	2.4	32.3	3.4	10.4	10.4	17.3	10.3	12.92	8.88
7	1	con	.	16.6	79.5	6.5	11.5	8.5	32.5	11.6	11.5	10.5	20.97	23.24
7	2	con	.	1.6	81.5	12.5	25.4	.	51.4	16.5	17.4	15.4	27.71	26.07
7	3	con	.	18.8	81.2	19.7	2.7	23.7	.	15.7	16.6	22.7	25.14	23.56
7	4	con	.	16.2	.	18.2	0.0	19.2	22.2	20.4	22.9	21.1	17.11	7.90
7	5	con	.	10.5	59.9	8.4	22.3	22.3	43.3	14.2	8.3	12.3	23.08	17.61
7	6	LPS	.	7.2	77.6	6.1	20.1	17.1	19.5	14.2	5.0	15.1	20.21	22.26
7	7	con	.	14.3	59.3	14.3	0.0	24.2	19.6	23.3	14.2	18.2	20.82	16.08
7	8	LPS	.	10.8	94.7	7.1	12.6	12.1	9.6	14.2	9.9	14.1	20.57	27.89
7	9	con	.	10.1	.	0.0	24.5	.	51.4	.	13.9	15.0	19.15	17.68

Table 5. Hourly milk yields, in grams, by infusion status of teat, of gilt 12-5 on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 6 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 26-2, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 26-2	Piglet	trt	h 1	2	3	4	5	6	7	8	9	10	Ave	s.d.
Lday 3	1	con	.	.	.	9.8	1.0	1.0	10.3	0.0	0.0	0.0	3.16	4.73
	2	con	.	.	.	4.2	4.4	2.3	11.3	1.6	0.0	0.0	3.40	3.91
	3	con	.	.	.	.	7.6	0.0	15.7	0.0	0.0	0.0	3.88	6.54
	4	con	.	.	.	4.3	0.0	1.3	11.3	1.0	0.0	0.5	2.63	4.10
	5	con	.	.	.	0.3	6.3	0.0	30.9	0.5	0.2	0.7	5.46	11.45
	6	con	.	.	.	2.9	4.2	3.5	15.2	0.0	0.8	1.4	3.99	5.17
	7	LPS	.	.	.	2.4	3.2	0.0	9.2	0.0	0.0	0.0	2.23	3.33
	8	con	.	.	.	5.3	0.0	0.0	10.1	0.0	0.0	2.1	2.50	3.89
	9	con	.	.	.	4.2	6.1	2.1	.	0.6	0.0	4.0	2.83	2.34
Lday 5	1	con	.	9.0	15.8	5.0	8.0	5.0	6.1	8.0	7.0	10.1	8.22	3.32
	2	LPS	.	4.8	17.8	1.8	12.8	3.8	2.9	4.8	7.8	16.9	8.16	6.13
	3	con	.	11.3	16.3	13.2	7.8	8.2	16.4	11.2	12.2	14.3	12.32	3.10
	4	con	.	8.6	4.9	5.8	6.9	5.8	6.1	11.9	10.9	5.0	7.32	2.57
	5	con	.	23.5	12.5	9.4	13.5	7.4	17.6	10.5	17.4	17.5	14.37	5.07
	6	con	.	12.3	10.3	8.2	0.8	7.2	5.4	5.3	14.2	8.4	8.01	4.01
	7	con	.	14.1	15.0	11.9	9.5	10.9	8.1	9.0	9.9	12.1	11.17	2.33
	8	con	.	11.9	15.9	11.7	8.3	10.7	10.9	12.8	16.7	14.9	12.64	2.72
	9	con	.	14.6	14.6	20.4	9.5	6.4	10.6	9.5	12.4	2.6	11.18	5.15
Lday 7	1	LPS	.	14.0	3.9	0.0	45.9	0.9	4.0	0.0	2.9	1.9	8.17	14.78
	2	con	.	5.8	5.7	0.0	55.7	5.7	2.8	0.6	3.7	9.7	9.97	17.40
	3	LPS	.	8.1	0.0	0.0	21.1	1.1	3.1	10.0	0.0	0.0	4.82	7.18
	4	con	.	5.7	6.7	0.0	27.6	1.6	1.7	4.5	7.6	7.6	7.00	8.21
	5	con	.	15.2	23.2	0.1	66.1	10.1	0.0	8.0	18.1	15.1	17.32	19.87
	6	con	.	11.2	9.1	1.9	27.0	6.0	4.1	1.9	4.0	0.0	7.24	8.22
	7	con	.	15.8	13.7	0.6	30.7	2.7	10.8	3.6	5.7	5.7	9.92	9.33
	8	con	.	6.6	20.6	0.5	56.6	13.5	7.6	7.4	2.5	6.5	13.53	17.20
	9	con	.	8.1	10.0	0.9	38.9	0.0	13.0	0.8	9.1	9.0	9.98	11.82

Table 6. Hourly milk yields, in grams, by infusion status of teat, of gilt 26-2 on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 7 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 6-1, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 6-1

Lday	Piglet	trt	h 1	2	3	4	5	6	7	8	9	10	Ave	s.d.
3	1	con	.	0.0	6.4	5.4	23.5	2.6	17.1	2.6	21.4	5.4	9.38	8.83
	2	con	.	6.4	13.7	12.7	57.8	9.9	12.9	5.9	23.7	2.6	16.18	16.74
	3	con	.	5.9	9.4	13.4	42.4	3.6	7.5	8.5	30.4	0.3	13.49	13.82
	4	con	.	5.1	17.6	0.0	77.6	0.0	19.7	.	20.5	12.5	19.13	25.06
	5	con	.	7.8	0.1	16.0	61.0	5.2	7.2	9.2	43.9	7.9	17.59	20.64
	6	con	.	21.0	20.5	6.4	73.5	8.6	17.6	17.6	54.4	8.4	25.33	23.05
	7	con	.	5.9	15.1	8.0	43.1	1.3	5.3	7.3	32.0	16.9	14.99	13.94
	8	LPS	.	20.4	24.8	17.7	.	.	6.9	0.0	19.7	8.7	14.03	8.93
5	1	con	.	0.0	34.9	5.4	44.8	17.3	25.3	16.3	9.3	16.3	18.84	14.21
	2	con	.	0.0	29.6	15.6	19.6	27.5	14.5	15.6	29.5	18.5	18.93	9.35
	3	LPS	.	0.0	21.3	23.3	10.3	2.3	16.3	21.3	28.2	35.2	17.58	11.62
	4	con	.	0.0	54.5	1.5	44.5	18.4	24.4	20.5	17.4	9.4	21.18	18.24
	5	con	.	1.9	50.8	9.8	15.7	20.7	8.7	16.7	20.7	5.7	16.74	14.35
	6	con	.	0.0	54.4	20.3	27.3	27.3	18.3	21.3	23.3	33.3	25.06	14.37
	7	con	.	0.0	27.9	5.8	11.8	27.8	0.0	20.8	18.7	17.7	14.50	10.77
	8	LPS	.	6.8	55.7	21.7	17.7	11.6	7.6	0.0	10.6	15.6	16.37	16.09
7	1	LPS	.	23.2	23.3	7.2	8.3	13.1	3.2	18.0	5.2	14.2	12.86	7.49
	2	con	.	11.4	34.2	45.4	31.0	22.3	29.4	25.2	19.1	14.3	25.81	10.56
	3	LPS	.	12.2	10.2	6.2	16.3	11.1	8.2	17.1	15.2	14.1	12.29	3.72
	4	con	.	35.3	.	25.2	0.0	46.2	43.2	35.0	29.2	.	30.59	15.34
	5	con	.	14.5	.	21.4	32.6	35.4	26.5	12.4	31.4	18.4	24.08	8.68
	6	con	.	33.2	26.9	42.1	0.0	40.1	32.1	0.1	30.1	38.1	26.97	16.01
	7	con	.	7.6	14.7	1.6	24.7	24.5	21.6	24.3	17.5	17.5	17.11	8.07
	8	LPS	.	15.5	4.6	6.4	24.6	25.4	11.5	18.2	21.2	21.4	16.53	7.61

Table 7. Hourly milk yields, in grams, by infusion status of teat, of gilt 6-1 on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 8 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt x1-3, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt x1-3

Lday	Piglet	trt	h 1	2	3	4	5	6	7	8	9	10	Ave	s.d.
3	1	con	.	29.4	31.4	0.0	0.0	31.5	26.4	27.3	19.4	22.3	20.86	12.47
	2	con	.	29.3	19.3	0.0	4.3	9.5	0.0	0.0	0.0	12.3	8.30	10.43
	3	con	.	20.4	25.4	19.3	7.4	20.1	20.0	16.4	19.5	17.4	18.43	4.83
	4	con	.	28.6	20.6	9.5	7.6	0.0	0.0	0.0	16.6	28.5	12.38	11.72
	5	con	.	0.0	1.8	0.0	30.8	22.0	16.9	0.8	22.9	.	11.90	12.61
	6	con	.	.	35.4	0.0	18.4	17.6	14.5	5.4	5.5	4.4	12.65	11.40
	7	con	.	22.3	10.3	15.1	22.2	0.5	10.3	19.1	8.3	16.1	13.80	7.15
	8	LPS	.	14.1	12.1	0.0	0.2	15.4	11.2	15.1	11.2	16.1	10.60	6.22
5	1	LPS	.	5.2	2.5	.	0.0	4.3	3.2	8.3	1.3	.	3.54	2.73
	2	con	.	16.2	33.5	32.1	3.3	28.3	0.0	0.0	32.3	29.2	19.43	14.68
	3	con	.	17.3	7.6	24.2	25.8	27.4	0.0	12.3	20.3	21.3	17.36	9.12
	4	LPS	.	21.3	12.7	.	.	0.5	0.0	17.4	9.5	15.4	10.97	8.19
	5	con	.	24.5	.	.	0.0	15.7	0.0	21.6	.	10.5	12.05	10.51
	6	con	.	29.3	.	33.2	20.4	36.4	0.0	0.0	24.4	15.3	19.88	14.00
	7	con	.	12.9	19.4	15.9	5.1	20.2	0.0	8.1	2.1	22.0	11.74	8.23
	8	con	.	20.8	.	17.8	6.0	25.1	0.0	11.9	13.0	19.9	14.31	8.31
7	1	con	.	3.3	25.3	2.2	2.3	10.7	10.3	0.3	0.0	0.0	6.04	8.31
	2	LPS	.	6.1	31.2	0.1	0.0	10.1	24.2	.	14.1	0.0	10.73	11.81
	3	con	.	12.1	0.0	17.1	3.2	34.1	50.2	30.6	20.1	14.1	20.17	15.85
	4	con	.	14.3	5.4	11.2	12.4	25.2	34.3	17.3	17.3	15.3	16.97	8.41
	5	con	.	.	0.0	.	.	.	.	.	.	.	0.00	.
	6	LPS	.	7.2	4.2	3.1	2.3	9.1	27.2	10.2	20.2	6.2	9.97	8.39
	7	con	.	11.8	6.9	10.7	12.4	13.7	26.9	7.8	15.7	6.8	12.52	6.21
	8	con	.	8.7	3.8	18.6	4.8	26.6	34.7	0.0	16.6	8.2	13.56	11.51

Table 8. Hourly milk yields, in grams, by infusion status of teat, of gilt x1-3 on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 9 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt x1-1, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt x1-1

lday	Piglet	trt	h 1	2	3	4	5	6	7	8	9	10	Ave	s.d.
3	1	con	.	3.9	42.0	0.0	2.9	5.8	2.0	0.0	0.0	13.9	7.83	13.54
	2	con	.	4.6	16.7	0.0	5.6	6.5	3.7	0.0	0.8	13.7	5.73	5.91
	3	con	.	0.0	28.6	0.0	0.0	0.0	0.0	1.4	8.7	10.5	5.47	9.60
	4	con	.	11.6	46.6	0.6	6.6	3.5	12.6	0.0	16.7	9.6	11.98	14.15
	5	con	.	.	30.5	3.5	9.4	.	11.4	3.3	.	3.4	10.25	10.52
	6	LPS	.	7.5	85.6	3.6	8.5	13.4	6.5	1.4	1.7	12.5	15.63	26.58
	7	con	.	1.2	28.3	0.3	2.2	2.1	2.2	7.0	16.4	12.2	7.99	9.41
	8	con	.	0.0	37.1	3.0	2.0	3.8	1.0	0.0	8.1	13.0	7.56	11.86
5	1	LPS	.	28.3	8.5	.	29.8	10.7	.	29.8	.	20.5	21.27	9.70
	2	con	.	26.1	33.3	0.0	31.6	7.4	14.5	38.5	28.4	25.3	22.79	12.79
	3	LPS	.	16.0	27.2	0.0	23.5	1.3	.	23.4	9.3	.	14.39	11.07
	4	con	.	5.1	27.2	10.4	2.6	7.4	19.5	16.5	10.4	.	12.39	8.18
	5	LPS	.	16.0	.	31.3	17.4	23.2	28.3	4.3	27.2	.	21.10	9.30
	6	con	.	22.1	21.3	0.0	17.5	5.4	18.4	17.5	16.3	8.3	14.09	7.67
	7	con	.	.	26.8	7.0	21.2	4.0	.	18.1	.	15.8	15.48	8.62
	8	con	.	14.4	22.6	9.8	24.9	10.7	19.8	26.9	.	10.6	17.46	6.94
7	1	LPS	.	2.4	0.0	1.4	3.4	6.4	10.4	15.4	45.4	7.4	10.24	14.04
	2	LPS	.	2.2	0.2	0.2	0.0	.	8.3	19.2	52.3	10.3	11.59	17.77
	3	con	.	70.1	0.0	17.1	5.2	23.2	13.2	21.2	32.2	0.0	20.24	21.63
	4	con	.	0.0	1.3	12.2	8.3	12.3	8.3	11.3	31.3	0.0	9.44	9.64
	5	con	.	13.0	0.0	14.0	18.1	20.1	8.1	18.1	66.1	13.5	19.00	18.68
	6	con	.	3.2	.	0.0	28.2	14.2	9.2	10.2	30.2	0.0	11.90	11.80
	7	con	.	3.6	0.0	7.6	32.6	19.6	0.0	22.6	28.7	10.7	13.93	12.35
	8	con	.	13.4	0.0	1.4	37.5	15.5	17.5	17.5	41.5	.	18.04	14.93

Table 9. Hourly milk yields, in grams, by infusion status of teat, of gilt x1-1 on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 10 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 35-7, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 35-7

Lday	Piglet	trt	h=1	2	3	4	5	6	7	8	9	10	Ave	s.d.
3	1	con	.	8.6	12.3	23.3	24.4	.	15.4	18.3	34.3	26.3	20.36	8.34
	2	con	.	5.6	3.4	22.3	23.4	14.4	26.4	21.3	28.3	23.3	18.71	8.93
	3	LPS	.	7.4	21.1	.	3.1	1.2	2.2	.	2.1	20.1	8.17	8.73
	4	con	.	5.3	12.0	1.0	42.0	10.0	27.0	15.9	19.9	1.9	15.00	13.20
	5	LPS	.	2.3	18.0	0.0	16.0	.	0.1	26.9	26.9	16.9	13.01	10.57
	6	con	.	3.4	0.0	15.0	20.1	2.1	18.1	14.0	.	4.0	9.59	8.01
	7	LPS	.	0.0	20.0	6.0	10.0	2.0	2.1	17.0	22.0	8.9	9.78	8.19
	8	con	.	0.0	6.0	17.9	28.0	18.0	22.0	.	21.9	0.0	14.23	10.75
	9	con	.	0.0	19.4	.	32.4	.	.	14.3	.	.	16.53	13.40
	10	con	.	16.3	30.0	3.0	18.0	4.0	9.1	6.0	43.0	16.9	16.26	13.18
5	1	LPS	.	19.2	20.2	22.2	31.3	13.2	40.4	21.2	18.2	32.2	24.23	8.57
	2	con	.	24.2	38.3	20.3	.	2.2	36.5	0.0	33.2	35.3	23.75	15.29
	3	con	.	21.8	22.9	18.9	26.5	0.9	38.2	22.9	16.8	41.9	23.42	11.96
	4	con	.	32.8	35.8	1.8	.	4.8	32.1	.	21.8	30.8	22.84	14.06
	5	LPS	.	27.7	15.8	22.8	21.8	14.7	7.0	8.8	14.7	16.8	16.68	6.61
	6	con	.	10.7	25.8	19.8	.	2.8	23.1	.	12.8	12.8	15.40	7.97
	7	con	.	15.8	13.9	9.9	.	0.8	24.2	10.9	.	17.9	13.34	7.30
	8	con	.	0.7	0.0	0.0	25.8	2.7	41.0	0.0	1.7	26.8	10.97	15.78
	9	con	.	11.9	10.0	10.0	.	14.0	33.3	5.0	8.0	36.0	16.03	11.81
	10	con	.	17.7	16.8	.	17.8	3.8	44.0	18.8	43.0	16.8	19.39	12.03
7	1	con	.	32.1	23.1	4.1	19.1	17.1	14.1	21.1	19.1	19.2	18.78	7.43
	2	LPS	.	4.2	13.2	0.0	17.2	19.2	.	5.7	11.2	18.2	11.11	7.15
	3	con	.	26.6	25.6	24.6	18.7	15.6	17.6	24.5	22.7	26.7	22.26	4.38
	4	con	.	.	.	30.5	20.6	.	20.6	.	.	23.7	23.98	4.05
	5	LPS	.	9.6	11.6	8.6	9.6	7.6	7.6	11.6	9.7	14.7	10.07	2.27
	6	LPS	.	21.5	0.0	25.5	14.6	38.5	20.5	16.5	25.6	26.6	21.03	10.50
	7	con	.	.	16.7	.	11.7	.	9.6	14.6	.	17.8	14.08	3.42
	8	con	.	29.5	24.0	.	4.5	18.5	26.0	18.5	24.5	.	20.79	8.19
	9	con	.	30.7	17.7	.	49.2	22.7	11.7	23.7	.	26.8	26.07	11.91
	10	con	.	21.5	.	.	20.5	29.5	.	.	21.5	25.6	23.72	3.78

Table 10. Hourly milk yields, in grams, by infusion status of teat, of gilt 35-7, on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 11 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 42-7, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 42-7

Piglet	trt	1	2	3	4	5	6	7	8	9	10	Ave	s.d.
lday 3													
1	con	.	10.3	9.3	.	19.4	32.4	11.3	12.3	8.4	0.0	12.93	9.50
2	con	.	12.3	6.3	9.4	7.5	39.5	5.4	.	9.4	24.4	14.28	11.82
3	con	.	9.3	0.0	6.9	14.9	12.0	5.9	13.8	9.9	7.8	8.94	4.53
4	con	.	9.0	0.0	11.1	10.2	6.2	13.1	9.0	11.1	3.0	8.08	4.25
5	con	.	19.9	16.8	19.0	.	8.0	.	8.9	.	16.9	14.92	5.16
6	LPS	.	13.2	3.2	0.4	32.4	.	.	14.3	11.3	11.2	12.29	10.29
7	con	.	11.3	5.3	10.0	21.5	10.5	0.0	12.3	7.4	10.3	9.84	5.79
8	LPS	.	.	2.8	1.0	15.0	10.0	0.0	10.9	15.0	14.9	8.70	6.48
9	LPS	.	9.9	0.0	0.0	17.1	59.0	13.0	13.9	2.0	0.9	12.87	18.54
10	con	.	.	7.0	11.2	35.2	17.2	6.2	23.1	6.2	10.1	14.53	10.24
lday 5													
1	LPS	.	3.2	5.3	13.3	10.4	25.3	32.3	29.2	.	11.3	16.29	11.12
2	con	.	15.2	21.3	.	21.4	8.3	0.0	19.2	.	9.3	15.78	5.87
3	con	.	7.7	2.8	4.8	18.9	13.7	0.0	5.7	10.7	17.8	9.12	6.61
4	con	.	9.8	0.0	0.0	21.1	24.9	20.0	.	0.0	31.0	13.35	12.52
5	con	.	3.6	18.7	10.7	.	25.6	0.0	20.6	16.6	19.7	14.44	8.89
6	con	.	7.2	0.3	7.3	8.5	10.3	13.3	10.1	4.2	14.3	8.39	4.35
7	con	.	14.1	15.1	.	15.3	.	.	24.0	.	12.2	16.14	4.56
8	LPS	.	9.8	3.9	0.0	21.0	25.8	9.9	0.0	3.8	.	9.28	9.58
9	con	.	5.9	0.0	3.0	9.2	10.0	11.0	6.9	2.0	9.0	6.33	3.89
10	con	.	7.9	16.0	14.0	1.2	14.9	14.0	9.8	2.9	15.0	10.63	5.54
lday 7													
1	con	.	2.2	27.2	22.2	14.2	0.1	5.2	4.2	9.2	16.2	11.19	9.38
2	LPS	.	1.2	0.0	43.1	.	35.6	5.2	.	12.1	7.2	14.91	17.29
3	LPS	.	0.0	0.0	0.0	0.0	5.6	3.7	2.2	2.6	2.7	1.87	2.02
4	con	.	3.6	3.6	32.6	24.6	19.5	11.6	10.6	5.5	8.6	13.36	10.12
5	con	.	0.0	.	30.4	21.4	0.4	.	.	15.4	13.4	13.50	11.88
6	con	.	2.1	9.1	13.1	7.1	7.0	6.2	6.6	8.1	2.1	6.82	3.38
7	con	.	.	.	24.8	.	.	.	.	11.7	.	18.25	9.26
8	con	.	4.7	15.7	16.7	6.7	10.6	0.0	0.7	6.7	7.7	7.72	5.84
9	con	.	3.8	11.8	15.8	10.8	13.7	3.8	4.8	9.8	6.8	9.01	4.42
10	con	.	3.7	17.6	3.7	13.7	23.6	11.7	10.7	8.6	5.7	11.00	6.62

Table 11. Hourly milk yields, in grams, by infusion status of teat, of gilt 42-7, on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Table 12 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 42-1, on experimental days during week one of lactation, and used for subsequent statistical analysis.

Gilt 42-1

Lday	Piglet	trt	h 1	2	3	4	5	6	7	8	9	10	Ave	s.d.
Lday 3	1	con	.	19.4	33.4	15.4	16.4	13.5	14.4	14.4	37.5	21.4	20.64	8.83
	2	con	.	16.4	58.4	8.3	20.4	15.4	8.3	12.3	33.5	18.3	21.26	15.86
	3	LPS	.	26.7	43.7	12.7	14.7	18.8	8.7	16.6	3.9	4.7	16.72	12.38
	4	con	.	11.4	45.3	12.0	.	10.4	0.0	0.2	9.5	0.3	11.01	15.98
	5	con	.	0.0	27.1	.	12.3	15.2	.	13.0	.	0.0	11.22	10.23
	6	con	.	22.3	35.3	.	6.1	0.4	.	16.2	13.5	0.2	14.31	12.28
	7	con	.	17.2	26.1	0.0	17.1	13.2	10.1	5.1	13.3	13.1	11.58	7.57
	8	con	.	18.2	42.2	13.0	18.6	3.2	8.1	1.1	8.3	0.1	12.37	12.96
	9	con	.	.	18.7	.	.	.	6.6	7.6	.	0.6	10.42	7.98
	10	LPS	.	15.9	38.3	8.2	7.3	14.4	7.2	3.7	6.5	6.2	11.48	11.26
Lday 5	1	con	.	39.2	28.3	28.3	23.2	33.2	31.2	20.2	36.2	30.1	29.99	5.95
	2	con	.	42.2	36.2	27.2	31.2	31.2	.	30.2	42.1	36.0	34.54	5.55
	3	LPS	.	19.6	7.6	6.6	6.5	9.5	20.6	20.6	15.5	17.9	13.82	6.20
	4	con	.	16.2	16.2	6.0	17.1	18.1	15.1	10.1	19.1	10.9	14.31	4.35
	5	con	.	.	26.8	0.0	34.7	.	.	34.7	.	.	24.05	16.46
	6	con	.	22.0	0.1	2.1	38.0	.	28.0	21.0	.	23.7	19.27	13.66
	7	con	.	15.9	1.0	18.0	12.9	12.9	8.9	1.9	0.0	9.7	9.02	6.66
	8	con	.	17.0	15.1	13.1	17.0	15.0	9.0	12.0	.	14.8	14.13	2.69
	9	con	.	.	12.6	5.6	13.5	.	7.5	12.5	9.4	.	10.18	3.19
	10	LPS	.	15.9	4.0	9.0	11.9	7.4	13.9	17.9	.	17.7	12.21	5.07
Lday 7	1	con	.	28.1	15.1	30.2	28.1	14.1	26.1	20.2	87.1	5.2	28.24	23.56
	2	LPS	.	15.1	9.0	17.1	15.1	4.1	24.0	21.1	91.1	0.0	21.84	27.09
	3	con	.	8.5	6.4	9.5	0.0	9.5	9.5	9.6	16.5	13.6	9.23	4.56
	4	con	.	20.9	9.9	21.0	8.9	15.9	22.8	12.0	30.9	0.0	15.81	9.20
	5	con	.	22.5	18.5	31.6	18.5	16.5	20.5	22.6	24.5	0.6	19.53	8.36
	6	LPS	.	15.7	4.7	29.8	13.7	30.7	17.6	21.8	21.7	0.8	17.39	10.14
	7	LPS	.	1.9	0.0	1.9	2.8	1.8	6.8	7.0	13.9	2.0	4.23	4.32
	8	con	.	19.8	0.0	1.9	16.8	19.8	2.8	16.9	44.8	0.0	13.64	14.56
	9	con	.	5.3	12.2	11.3	.	18.2	.	8.4	24.2	.	13.27	6.87
	10	con	.	8.8	13.7	14.8	14.8	11.8	27.2	10.9	37.8	0.0	15.53	10.95

Table 12. Hourly milk yields, in grams, by infusion status of teat, of gilt 42-1, on experimental days during week one of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation.

Appendix N. RAW PIGLET 24 H WEIGHT GAIN DATA (EARLY LACTATION)

Table 13 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 12-5 & 26-2 at specified days relative to LPS infusion of mammary glands during week one of lactation, and used for subsequent statistical analysis.

Gilt 12-5

lday 7

Piglet	trt	day -1	day 0
1	con	108	169
2	con	217	299
3	con	208	336
4	con	161	209
5	con	158	239
6	LPS	180	144
7	con	158	233
8	LPS	153	135
9	con	161	211
Ave		167.11	219.44
s.d.		32.13	67.11

lday 5

Piglet	trt	day -1	day 0
1	LPS	173	77
2	con	218	239
3	con	285	251
4	LPS	157	196
5	con	136	174
6	con	174	233
7	con	127	200
8	con	144	175
9	con	172	206
Ave		176.22	194.56
s.d.		48.81	51.75

lday 3

Piglet	trt	day -1	day 0
1	con	232	153
2	con	165	125
3	con	96	82
4	con	33	153
5	con	-23	40
6	con	66	151
7	LPS	205	104
8	LPS	15	-56
9	con	121	156
Ave		101.11	100.89
s.d.		87.41	70.93

Gilt 26-2

lday 7

Piglet	trt	day -1	day 0
1	LPS	71	28
2	con	90	114
3	LPS	135	35
4	con	57	91
5	con	157	201
6	con	34	58
7	con	18	49
8	con	108	114
9	con	88	-5
Ave		84.22	76.11
s.d.		45.15	61.58

lday 5

Piglet	trt	day -1	day 0
1	con	43	42
2	LPS	171	54
3	con	102	115
4	con	98	93
5	con	132	163
6	con	17	42
7	con	95	104
8	con	93	99
9	con	44	151
Ave		88.33	95.89
s.d.		47.69	44.09

lday 3

Piglet	trt	day -1	day 0
1	con	-8	48
2	con	15	53
3	con	1	1
4	con	-21	-76
5	con	118	37
6	con	-44	-7
7	LPS	-3	-58
8	con	-8	-30
9	con	-68	-47
Ave		-2.00	-8.78
s.d.		51.54	47.55

Table 13. 24 h weight gains, in grams, of piglets nursing gilts 12-5 & 26-2 at specified days relative to LPS infusion of mammary glands during week one of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation. Day- day relative to endotoxin infusion.

Table 14 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 6-1 & x1-3 at specified days relative to LPS infusion of mammary glands during week one of lactation, and used for subsequent statistical analysis.

Gilt 6-1

Lday 7

Piglet	trt	day -1	day 0
1	con	168	228
2	LPS	154	100
3	LPS	149	137
4	con	335	427
5	con	209	266
6	con	306	369
7	con	161	229
8	LPS	166	201
Ave		206.00	244.63
s.d.		73.36	109.62

Lday 5

Piglet	trt	day -1	day 0
1	con	128	208
2	con	242	187
3	LPS	231	90
4	con	168	228
5	con	233	228
6	con	243	287
7	con	166	173
8	LPS	148	146
Ave		194.88	193.38
s.d.		47.09	59.48

Lday 3

Piglet	trt	day -1	day 0
1	con	26	202
2	con	129	96
3	con	84	190
4	con	209	134
5	con	128	164
6	con	142	230
7	con	111	191
8	LPS	197	111
Ave		128.25	164.75
s.d.		58.73	47.11

Gilt x1-3

Lday 7

Piglet	trt	day -1	day 0
1	con	-52	115
2	LPS	223	-9
3	con	281	222
4	con	117	175
5	LPS	-120	.
6	LPS	209	53
7	con	138	22
8	con	137	138
Ave		116.63	102.29
s.d.		137.41	83.98

Lday 5

Piglet	trt	day -1	day 0
1	LPS	210	-47
2	con	173	216
3	con	130	287
4	LPS	201	81
5	con	182	57
6	con	97	263
7	con	84	120
8	con	104	158
Ave		147.63	141.88
s.d.		49.84	112.47

Lday 3

Piglet	trt	day -1	day 0
1	con	254	280
2	con	276	108
3	con	134	258
4	con	200	249
5	con	146	225
6	con	203	197
7	con	18	142
8	LPS	100	113
Ave		166.38	196.50
s.d.		84.56	67.72

Table 14. 24 h weight gains, in grams, of piglets nursing gilts 6-1 & x1-3 at specified days relative to LPS infusion of mammary glands during week one of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation. Day- day relative to endotoxin infusion.

Table 15 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts x1-1 & 35-7 at specified days relative to LPS infusion of mammary glands during week one of lactation, and used for subsequent statistical analysis.

Gilt x1-1

lday 3

Piglet	trt	day -1	day 0
1	con	-31	213
2	con	172	179
3	con	146	220
4	con	82	229
5	con	172	233
6	LPS	197	150
7	con	100	141
8	con	41	142
Ave		109.88	188.38
s.d.		77.53	40.01

lday 5

Piglet	trt	day -1	day 0
1	LPS	108	215
2	con	92	207
3	LPS	105	152
4	con	95	82
5	LPS	126	218
6	con	173	138
7	con	24	147
8	con	91	170
Ave		101.75	166.13
s.d.		41.43	46.60

lday 7

Piglet	trt	day -1	day 0
1	LPS	186	45
2	LPS	168	56
3	con	162	227
4	con	61	144
5	con	187	210
6	con	119	156
7	con	160	166
8	con	142	201
Ave		148.13	150.63
s.d.		41.66	67.95

Gilt 35-7

lday 3

Piglet	trt	day -1	day 0
1	con	88	175
2	con	206	170
3	LPS	100	96
4	con	154	43
5	LPS	177	146
6	con	97	133
7	LPS	125	55
8	con	78	112
9	con	123	172
10	con	194	148
Ave		134.20	125.00
s.d.		45.96	47.61

lday 5

Piglet	trt	day -1	day 0
1	LPS	157	173
2	con	128	156
3	con	116	195
4	con	182	151
5	LPS	127	137
6	con	169	165
7	con	136	111
8	con	180	169
9	con	121	200
10	con	152	205
Ave		146.80	166.20
s.d.		24.59	29.38

lday 7

Piglet	trt	day -1	day 0
1	con	134	180
2	LPS	113	53
3	con	124	183
4	con	195	182
5	con	52	103
6	LPS	193	186
7	con	123	131
8	con	183	225
9	con	151	198
10	con	190	186
Ave		145.80	162.70
s.d.		45.97	51.53

Table 15. 24 h weight gains, in grams, of piglets nursing gilts x1-1 & 35-7 at specified days relative to LPS infusion of mammary glands during week one of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation. Day- day relative to endotoxin infusion.

Table 16 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 42-7 & 42-1 at specified days relative to LPS infusion of mammary glands during week one of lactation, and used for subsequent statistical analysis.

Gilt 42-7

lday 7

Piglet	trt	day -1	day 0
1	con	58	249
2	LPS	176	173
3	LPS	116	-25
4	con	240	152
5	con	137	236
6	con	55	66
7	con	206	218
8	con	103	125
9	con	93	106
10	con	182	133
Ave		136.60	143.30
s.d.		62.73	83.29

lday 5

Piglet	trt	day -1	day 0
1	LPS	113	133
2	con	161	323
3	con	61	123
4	con	111	228
5	con	231	246
6	con	19	63
7	con	104	207
8	LPS	63	82
9	con	39	82
10	con	69	150
Ave		97.10	163.70
s.d.		62.57	84.61

lday 3

Piglet	trt	day -1	day 0
1	con	-29	94
2	con	-64	127
3	con	67	82
4	con	41	97
5	con	103	216
6	LPS	118	27
7	con	9	104
8	LPS	139	45
9	LPS	102	-12
10	con	39	138
Ave		52.50	91.80
s.d.		66.17	63.46

Gilt 42-1

lday 7

Piglet	trt	day -1	day 0
1	con	131	275
2	LPS	193	151
3	con	59	84
4	con	101	144
5	con	167	226
6	LPS	158	172
7	LPS	51	-4
8	con	90	158
9	con	95	94
10	con	101	112
Ave		114.60	141.20
s.d.		46.60	77.36

lday 5

Piglet	trt	day -1	day 0
1	con	161	289
2	con	226	327
3	LPS	98	77
4	con	88	115
5	con	140	259
6	con	105	212
7	con	108	93
8	con	28	150
9	con	22	88
10	LPS	161	88
Ave		113.70	169.80
s.d.		61.96	94.25

lday 3

Piglet	trt	day -1	day 0
1	con	33	228
2	con	14	270
3	LPS	31	148
4	con	67	41
5	con	206	138
6	con	194	97
7	con	79	55
8	con	58	69
9	con	94	53
10	LPS	76	152
Ave		85.20	125.10
s.d.		65.35	77.55

Table 16. 24 h weight gains, in grams, of piglets nursing gilts 42-7 & 42-1 at specified days relative to LPS infusion of mammary glands during week one of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Lday- day of lactation. Day- day relative to endotoxin infusion.

Appendix O. RAW RECTAL TEMPERATURE DATA (ADVANCED LACTATION)

Table 17 represents the raw data for rectal temperature in degrees Celsius, as determined by digital thermometer, of gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

week 2

Gilt h	8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
-2	.	.	.	40.1	39.1	39.7	38.6	39.0	39.3	38.8	38.9	39.18	0.48
-1	38.7	39.3	40.6	39.4	38.4	39.3	38.7	39.3	39.6	39.0	39.1	39.20	0.57
0	38.8	38.6	40.0	39.4	39.1	39.4	38.4	39.3	39.4	39.3	38.7	39.14	0.47
1	38.8	39.1	40.3	40.5	39.3	39.6	39.4	39.3	39.9	39.6	38.7	39.51	0.57
2	39.7	39.8	41.0	41.2	40.4	39.9	39.8	39.6	39.9	39.9	38.8	40.01	0.65
3	39.9	40.1	41.5	40.8	40.7	40.5	40.9	39.8	40.5	40.4	39.3	40.40	0.61
4	40.1	39.6	41.6	41.2	41.2	41.1	40.4	40.3	40.8	40.8	40.3	40.67	0.59
5	40.5	40.8	41.9	41.6	41.3	41.3	41.1	40.7	40.8	40.8	40.4	41.02	0.45
6	40.7	40.1	41.7	41.8	41.1	41.8	41.3	40.6	41.1	40.6	40.8	41.04	0.56
7	40.6	39.7	41.2	41.1	40.7	41.6	40.6	40.8	41.3	40.4	40.3	40.76	0.53
12	39.7	39.7	40.8	40.0	39.6	39.9	39.6	39.8	40.9	40.4	39.5	39.99	0.50
24	39.3	39.1	40.6	39.0	38.8	39.3	38.6	39.7	39.8	38.9	39.6	39.32	0.58

week 3

Gilt h	8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
-2	38.6	38.6	40.2	39.3	39.2	39.3	38.1	38.7	39.8	38.6	38.9	39.03	0.63
-1	38.9	39.1	40.1	39.3	39.0	39.2	38.9	39.0	39.4	39.3	38.4	39.14	0.40
0	38.8	38.8	40.3	39.3	39.0	39.3	38.5	38.9	39.3	38.9	38.9	39.10	0.46
1	38.9	39.1	40.3	39.2	39.1	39.2	38.4	39.0	39.0	39.0	38.9	39.09	0.44
2	38.9	39.1	40.3	39.9	39.7	39.4	39.7	39.4	39.5	39.6	38.9	39.49	0.42
3	39.6	40.1	41.2	41.1	40.7	40.3	39.4	39.4	40.1	40.5	39.5	40.17	0.65
4	40.7	40.9	41.3	41.3	41.0	40.8	39.9	39.8	41.2	40.7	40.3	40.72	0.53
5	41.1	40.5	41.3	41.3	41.2	40.8	39.7	39.4	40.9	40.2	40.3	40.60	0.65
6	41.1	40.5	40.9	41.2	41.4	40.8	39.7	39.3	40.4	39.9	39.8	40.46	0.70
7	40.8	40.3	40.9	41.8	41.5	41.2	40.5	39.2	40.3	40.2	40.7	40.68	0.71
12	39.9	40.5	41.0	40.8	41.2	40.6	39.1	39.6	40.1	39.6	39.7	40.19	0.68
24	38.9	39.1	40.3	39.2	39.6	39.3	37.9	39.3	39.8	38.4	39.1	39.17	0.64

Table 17. Rectal temperatures, in degrees Celsius, of gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation.

Appendix P. RAW PLASMA TNF-α DATA (ADVANCED LACTATION)

Table 18 represents the raw data for plasma TNF-α content in pg/ml, as determined by ELISA, of plasma samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

week2

h	Gilt 8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
-0.5	46.84	37.99	45.88	14.93	17.07	4.30	5.57	5.97	.	.	0.00	19.84	18.71
0.0	41.86	38.60	43.52	17.31	15.40	11.46	0.94	10.34	0.00	.	0.00	17.94	17.30
0.5	46.66	38.03	44.29	12.18	17.55	7.60	0.00	9.54	0.00	.	0.00	17.59	18.55
1.0	60.70	38.91	43.45	40.64	27.46	7.60	34.75	5.58	0.00	.	0.00	25.91	21.28
1.5	50.39	39.86	47.30	30.77	122.45	4.51	30.10	11.54	0.00	.	0.00	33.69	36.54
2.0	46.49	38.64	44.45	22.41	65.79	13.06	16.80	18.84	0.00	.	0.00	26.65	21.52
3.0	53.43	38.60	46.21	17.55	45.63	22.42	6.76	20.08	0.00	.	0.00	25.07	19.83
4.0	57.24	38.73	46.39	17.06	37.84	19.23	16.82	10.75	0.00	.	0.00	24.41	19.62
5.0	.	.	.	21.91	38.30	13.76	15.17	15.99	0.00	.	0.00	15.02	13.19
6.0	.	.	.	19.47	27.37	21.18	11.94	17.21	0.00	.	0.00	13.88	10.54
7.0	.	.	.	16.60	23.65	15.88	5.58	19.66	0.00	.	.	13.56	8.96

week3

h	Gilt 8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
-0.5	44.46	38.55	19.88	0.00	54.88	0.00	20.49	13.97	0.00	18.56	0.00	19.16	19.47
0.0	44.84	37.91	23.25	0.00	67.66	0.00	25.47	9.95	0.00	20.26	0.00	20.85	22.23
0.5	48.85	38.03	23.28	0.00	51.29	0.00	22.14	7.94	0.00	14.61	0.00	18.74	19.77
1.0	49.05	37.92	15.71	0.00	28.39	0.00	28.00	3.60	0.00	48.18	17.49	20.76	18.92
1.5	41.97	38.55	15.74	0.00	34.40	0.00	20.90	0.00	0.00	110.52	11.97	24.91	32.63
2.0	51.32	38.64	25.80	0.00	41.40	0.00	30.08	4.00	0.00	75.80	11.80	25.35	24.99
3.0	53.17	38.43	30.15	0.00	49.46	0.00	31.33	0.00	0.00	71.08	20.26	26.72	25.00
4.0	63.16	38.34	35.56	0.00	51.71	0.00	30.07	0.00	0.00	41.24	22.52	25.69	22.91
5.0	.	.	.	3.44	50.74	0.00	18.85	0.00	0.00	44.26	21.23	17.32	20.54
6.0	.	.	.	0.25	50.77	0.00	22.16	0.00	1.32	51.79	21.23	18.44	22.31
7.0	.	.	.	1.43	44.53	0.00	22.14	0.00	0.00	44.26	0.00	14.05	20.20

Table 18. TNF-α contents, in pg/ml, of plasma samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation.

Appendix Q. RAW MILK PROTEIN DATA (ADVANCED LACTATION)

Table 19 represents the raw data for milk protein content in percent, as determined by Lowry assay, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

	h	Gilt 8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
week 1														
D1,1hr	1	12.2124	15.9212	16.2398	21.7337	16.2697	15.2515	20.5220	13.4521	21.7337	17.7294	16.1169	17.0166	3.1523
D1,6hr	6	14.5829	15.2329	15.6790	16.9641	13.3699	14.6485	18.7306	13.4521	18.7524	14.6938	15.0336	15.5582	1.8544
D1,12hr	12	12.9133	13.6271	12.7094	12.1949	11.5753	11.9569	16.4425	11.9598	15.8031	15.2016	12.6432	13.3661	1.6894
D1,24hr	24	9.8800	13.3212	9.2173	10.1821	6.8699	7.3890	8.8976	9.1741	10.0064	10.0830	12.2664	9.7534	1.8622
D3	72	7.7475	11.9249	8.6429	8.6000	5.5939	6.7874	7.3762	7.3099	9.8169	11.4278	8.6486	8.5342	1.9157
D5	120	7.9116	7.8542	7.5213	7.4462	6.1046	6.8544	6.6943	7.2215	7.3213	5.9161	8.8606	7.2460	0.8409
D7	168	5.9583	8.2973	7.3089	6.3863	6.9290	6.2662	7.6182	6.4256	8.6674	7.1173	7.6045	7.1435	0.8619
week 2	312													
con	0	6.3473	6.1378	5.7261	6.4446	6.2703	6.0014	6.3317	7.5073	6.5611	7.4545	6.7100	6.4993	0.5531
4/5hr	5	6.8113	6.6242	7.8816	7.1855	7.0540	6.3702	6.9080	8.6137	7.1120	7.4694	7.5960	7.2387	0.6294
12hr	12	9.0589	8.1882	6.8904	8.7026	6.7831	8.7949	9.5245	8.3417	7.9903	6.3571	9.8811	8.2284	1.1445
24hr	24	8.6641	8.0881	8.6675	9.0017	6.7247	7.5789	8.7180	8.5038	7.5690	6.3267	9.4729	8.1196	0.9731
36hr	36	7.6900	9.0065	8.2386	8.5103	7.5855	6.8567	7.5815	8.3638	7.1477	6.1138	9.4336	7.8662	0.9672
48hr	48	8.8573	8.6475	8.2211	8.0872	6.7278	7.6673	8.6227	8.8428	7.4788	7.8015	9.1353	8.1899	0.7240
60hr	60	7.8411	8.7978	7.5908	8.1983	7.6958	7.7042	7.5228	7.5784	8.1032	6.6308	8.6879	7.8501	0.5971
week 3	480													
con	0	6.9461	6.4746	6.9984	6.6242	7.2153	6.1627	7.5995	8.8134	7.0822	8.2363	8.1767	7.3027	0.8202
4/5hr	5	7.4699	6.4596	7.5074	8.2333	7.0770	6.4854	9.0285	7.7531	7.8268	9.0479	7.7523	7.6947	0.8590
12hr	12	8.9581	9.1651	7.5587	.	9.1328	8.4706	8.3880	7.3894	7.7910	7.5202	8.3983	8.2772	0.6801
24hr	24	10.3857	11.1100	7.1730	7.5199	8.7903	8.6254	8.7107	7.1020	7.4111	8.7974	7.6381	8.4785	1.3115
36hr	36	8.9413	9.0352	6.9596	7.0019	7.3756	8.3675	9.7640	7.8809	7.3885	9.4056	8.0258	8.1951	0.9808
48hr	48	9.8748	10.4797	6.8283	8.6944	7.2118	7.2768	9.4840	7.1247	7.4863	9.1667	7.5925	8.2927	1.2837
60hr	60	8.6224	8.9039	7.5833	8.8038	7.4054	8.3085	8.8870	7.6162	8.0656	9.3080	8.5960	8.3727	0.6284

Table 19. Milk protein contents, in percent, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation. Week 1-Normal milk samples collected at indicated times following farrowing. Con-milk sample collected from mammary glands prior to endotoxin infusion. 4/5-60 hr- milk sample collected at indicated times following endotoxin infusion.

Appendix R. RAW MILK β-CASEIN DATA (ADVANCED LACTATION)

Table 20 represents the raw data for milk β-casein content in mg/ml, as determined by ELISA, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt	h	8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
week 1														
D1, 1hr	1	12.6965	11.6466	11.8019	19.7433	10.9335	10.1429	13.2500	16.8559	30.0362	13.5911	14.9591	15.0597	5.6916
D1, 6hr	6	10.8734	10.3450	8.7773	15.7077	9.4960	8.3153	17.9185	15.2302	19.7968	11.3261	12.9847	12.7974	3.8558
D1, 12hr	12	8.2875	10.1406	8.3832	9.8143	7.9879	7.6879	18.8781	12.3049	11.2240	13.1817	11.9990	10.8990	3.2536
D1, 24hr	24	6.3087	10.7282	7.2666	7.9413	6.5698	7.0362	9.7371	9.5490	9.8125	12.9665	10.8346	8.9773	2.1146
D3	72	5.4567	6.7807	6.5697	7.5883	6.0327	8.0360	7.5670	8.4120	8.5572	14.5677	10.8199	8.2171	2.5550
D5	120	6.0333	6.5461	5.0638	8.5074	6.2806	6.3748	9.8344	8.8232	9.4529	10.3211	12.4143	8.1502	2.2636
D7	168	6.0109	7.6338	4.7085	7.4515	4.8570	8.8809	7.4736	7.3334	13.2773	8.5506	9.1023	7.7527	2.3515
week 2	312													
con	0	8.1752	9.6078	8.0374	10.1120	6.6796	9.4013	11.8338	12.2499	11.5069	11.2200	8.7263	9.7773	1.7886
4/5hr	5	5.9811	7.8243	6.2587	6.9557	5.1412	5.5746	6.2573	6.9087	5.9426	7.6287	5.3825	6.3505	0.8835
12hr	12	8.2567	8.3263	6.1244	7.1666	6.0113	7.1529	7.9130	7.0861	7.2159	11.4736	7.6470	7.6703	1.4666
24hr	24	5.9001	7.8426	6.3959	6.5521	5.6188	6.9213	8.5041	7.4770	7.1444	11.2200	8.3657	7.4493	1.5609
36hr	36	6.0557	5.7265	5.9472	6.3427	5.6155	5.8197	6.9454	6.8574	6.2226	9.8236	6.6196	6.5433	1.1754
48hr	48	5.4906	5.2688	4.7568	5.3640	6.3932	6.4315	9.6050	7.5555	5.5633	11.3394	5.9367	6.7004	2.0447
60hr	60	5.5111	6.4546	4.2787	6.1961	6.9377	6.4806	6.6997	5.4909	6.4193	8.8890	6.7012	6.3690	1.1324
week 3	480													
con	0	9.4810	8.5340	6.1793	13.5102	7.1377	11.1245	10.7011	12.4898	8.3833	12.1399	11.1033	10.0713	2.3203
4/5hr	5	7.0536	7.5803	6.4041	7.8388	5.7331	9.2831	8.3161	9.4640	7.1355	8.8161	6.3721	7.6361	1.2361
12hr	12	7.1327	8.9599	6.7740	.	7.9313	13.3143	13.6171	11.1513	7.5371	11.0888	6.9900	9.4497	2.6387
24hr	24	8.4013	10.2483	6.0006	6.8126	8.3854	10.1557	11.3304	10.4430	9.0163	12.4289	7.9766	9.1999	1.9311
36hr	36	6.4152	6.4093	5.0380	7.3457	5.3906	9.8769	12.5452	11.7840	7.5465	11.0888	7.9549	8.3087	2.6125
48hr	48	6.1580	6.6389	5.3837	6.7861	5.4002	9.0855	11.5291	8.3495	5.7463	11.9417	7.7941	7.7103	2.3198
60hr	60	5.8201	5.9178	4.8985	6.7949	4.1515	7.4768	10.8214	8.0800	5.9058	11.4736	7.3410	7.1529	2.2848

Table 20. β-casein contents in mg/ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation. Week 1-Normal milk samples collected at indicated times following farrowing. Con-milk sample collected from mammary glands prior to endotoxin infusion. 4/5-60 hr- milk sample collected at indicated times following endotoxin infusion.

Appendix S. RAW MILK ALBUMIN DATA (ADVANCED LACTATION)

Table 21 represents the raw data for milk albumin content in mg/ml, as determined by ELISA, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

	Gilt	h	8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
week 1	D1, 1hr	1	10.49	16.08	16.49	17.79	9.80	7.94	15.27	16.84	9.34	15.93	8.78	13.16	3.82
	D1, 6hr	6	7.95	15.00	14.67	11.51	8.26	6.43	12.55	17.27	7.51	10.08	9.74	11.00	3.52
	D1, 12hr	12	6.09	13.48	6.63	6.19	6.05	5.39	12.93	15.11	6.90	12.20	7.81	8.98	3.64
	D1, 24hr	24	4.55	9.19	4.01	4.34	2.10	1.85	4.15	8.70	4.26	6.85	6.90	5.17	2.43
	D3	72	3.00	7.83	2.94	3.99	2.57	2.30	3.67	5.06	4.61	7.52	4.38	4.35	1.86
	D5	120	1.98	3.17	2.95	2.34	3.60	2.73	2.99	5.32	2.70	3.78	4.34	3.26	0.95
	D7	168	2.06	3.84	2.63	2.57	3.47	2.14	3.96	5.09	3.21	3.51	3.33	3.25	0.89
week 2	312														
	con	0	2.85	3.33	2.69	2.05	2.39	1.89	3.16	6.12	2.71	2.99	2.72	2.99	1.12
	4/5hr	5	3.86	4.28	5.49	3.46	3.96	3.03	4.66	8.84	3.50	5.54	3.95	4.60	1.62
	12hr	12	4.50	4.36	4.62	3.26	3.43	3.74	5.51	8.89	4.09	4.48	4.53	4.67	1.53
	24hr	24	4.38	4.44	4.13	3.56	2.63	3.41	5.42	8.14	2.92	4.78	4.52	4.39	1.49
	36hr	36	3.03	4.71	3.78	2.80	3.32	1.88	3.72	7.77	2.59	3.11	4.00	3.70	1.55
	48hr	48	3.39	4.48	3.41	2.59	2.78	2.60	4.77	7.61	2.91	4.53	4.00	3.92	1.46
	60hr	60	3.06	3.54	3.67	2.34	3.53	1.95	3.60	6.93	3.40	3.36	3.81	3.56	1.26
week 3	480														
	con	0	2.98	2.74	3.36	1.94	3.07	1.32	3.79	7.55	2.47	4.29	3.17	3.34	1.62
	4/5hr	5	4.05	4.29	4.63	4.51	4.38	1.99	5.30	8.96	4.00	5.24	3.78	4.65	1.68
	12hr	12	3.81	5.06	3.54	.	5.26	3.70	4.08	7.90	2.87	4.40	4.03	4.46	1.40
	24hr	24	4.48	6.37	2.69	3.67	4.60	4.51	4.52	7.51	2.77	4.21	3.49	4.44	1.43
	36hr	36	3.92	4.58	2.51	2.97	3.60	3.73	5.30	7.11	2.45	4.26	3.06	3.95	1.36
	48hr	48	4.04	5.17	2.41	3.31	2.71	2.91	4.12	6.88	2.74	4.16	3.06	3.77	1.32
	60hr	60	3.27	3.86	2.90	2.80	3.12	2.84	3.55	6.64	2.84	2.52	2.89	3.39	1.15

Table 21. Albumin contents in mg/ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation. Week 1-Normal milk samples collected at indicated times following farrowing. Con-milk sample collected from mammary glands prior to endotoxin infusion. 4/5-60 hr- milk sample collected at indicated times following endotoxin infusion.

Appendix T. RAW MILK CHLORIDE DATA (ADVANCED LACTATION)

Table 22 represents the raw data for milk chloride content in mg/100 ml, as determined by anion-exchange chromatography, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

	Gilt	h	8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
week 1	D1, 1hr	1	100.4	104.4	113.2	73.6	76.4	134.0	79.2	88.0	77.6	92.8	95.2	94.07	18.36
	D1, 24hr	24	84.0	95.2	83.6	83.2	82.8	88.8	110.4	71.6	73.2	78.8	85.2	85.16	10.64
	D5	120	82.4	76.4	112.0	62.4	57.2	57.2	66.0	54.4	94.0	62.4	62.4	71.53	18.08
week 2	312														
	con	0	58.0	51.6	66.0	53.6	47.2	41.6	41.2	42.0	70.8	47.2	58.4	52.51	9.99
	4/5hr	5	154.8	137.2	215.6	127.2	155.2	147.2	171.2	137.2	184.0	154.0	153.2	157.89	24.81
	24hr	24	66.4	88.8	92.0	69.2	59.6	76.4	83.6	72.4	83.6	72.0	90.0	77.64	10.67
week 3	480														
	con	0	54.4	45.2	87.6	48.4	55.6	58.4	56.0	38.4	66.8	49.2	55.2	55.93	12.86
	4/5hr	5	300.4	190.0	209.6	215.2	186.0	134.8	212.8	150.4	150.8	154.4	146.8	186.47	47.85
	24hr	24	98.0	157.2	72.8	58.8	76.0	75.6	72.4	55.2	67.6	59.6	55.2	77.13	29.30

Table 22. Chloride contents, in mg/100 ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation.   Week 1-Normal milk samples collected at indicated times following farrowing. Con-milk sample collected from mammary glands prior to endotoxin infusion. 4/5-60 hr- milk sample collected at indicated times following endotoxin infusion.

Appendix U. RAW MILK TNF-α DATA (ADVANCED LACTATION)

Table 23 represents the raw data for milk TNF-α content in pg/ml, as determined by ELISA, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation, and used for subsequent statistical analysis.

	h	Gilt 8-10	8-6	X2-10	38-5	26-7	30-1	44-4	47-5	26-2	21-1	19-4	Ave	s.d.
week 2														
con	0	0.00	0.00	0.00	21.06	80.12	18.55	36.53	36.53	0.00	24.66	12.85	20.94	24.14
4/5hr	5	1889.38	294.73	606.23	878.72	813.61	900.91	828.27	451.44	94.89	309.97	85.91	650.37	511.80
12hr	12	0.00	0.00	.	27.98	155.73	7.93	36.53	49.72	0.00	10.37	18.43	30.67	47.08
week 3														
con	0	0.00	0.00	26.72	8.55	107.89	26.11	30.90	29.02	0.00	0.00	9.17	21.67	31.34
4/5hr	5	1304.18	448.09	360.06	420.36	674.09	1232.18	166.58	195.84	184.49	193.85	308.65	498.94	409.30
12hr	12	3.56	0.00	18.55	.	169.03	1.07	37.79	42.80	0.00	0.00	29.86	30.27	51.55

Table 23. TNF-α contents, in pg/ml, of milk samples from gilts subjected to the endotoxin challenge mastitis model during weeks two and three of lactation. Con-milk sample collected from mammary glands prior to endotoxin infusion. 4/5-60 hr- milk sample collected at indicated times following endotoxin infusion.

Appendix V. RAW SOW HOURLY MILK YIELD DATA
(ADVANCED LACTATION)

Table 24 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 8-6, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 8-6

week 2

Piglet	trt	1	2	3	4	5	6	7	8	Ave	s.d.
1	con	2.7	17.5	29.2	24.1	23.1	17.6	0.0	16.6	16.36	10.18
2	con	7.5	11.0	10.6	6.6	25.5	0.0	0.0	0.6	7.71	8.51
3	con	30.8	22.8	32.9	10.8	29.8	34.8	4.8	27.8	24.31	10.90
4	LPS	5.8	16.8	17.9	2.0	21.8	38.8	0.0	8.8	13.99	12.72
5	con	25.9	36.9	17.0	21.2	41.9	2.0	0.0	32.0	22.12	15.34
6	con	0.0	6.8	44.9	6.1	32.8	32.9	0.0	23.9	18.42	17.38
7	LPS	0.0	29.9	34.0	0.0	33.9	29.9	0.0	32.9	20.07	16.69
8	con	1.5	31.8	34.9	27.9	0.0	13.8	9.8	12.9	16.58	13.44

week 3

Piglet	trt	1	2	3	4	5	6	7	8	Ave	s.d.
1	con	7.9	12.8	39.0	0.0	5.6	17.8	15.4	0.1	12.34	12.62
2	con	16.3	13.1	28.1	0.0	31.9	10.3	0.0	8.2	13.48	11.73
3	LPS	0.0	0.0	16.6	0.0	1.7	46.6	6.6	3.6	9.38	16.03
4	con	24.7	.	17.8	2.6	42.7	43.6	28.7	43.6	29.11	15.57
5	con	41.8	22.7	15.9	.	53.5	1.0	16.8	14.0	23.67	17.97
6	con	.	22.7	0.0	21.7	39.7	37.6	19.7	19.7	23.00	13.20
7	con	18.8	1.7	20.7	.	46.8	65.7	0.2	0.7	22.08	25.40
8	LPS	0.0	0.6	0.6	0.0	3.7	49.6	37.7	10.6	12.85	19.59

Table 24. Hourly milk yields, in grams, by infusion status of teat, of gilt 8-6, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 25 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 8-10, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 8-10

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	con	.	1.1	0.0	0.0	1.9	4.8	18.7	5.2	4.51	6.58
2	con	2.4	0.0	1.8	.	.	18.1	.	1.1	4.68	7.57
3	con	.	.	0.9	.	.	176.2	0.0	0.0	44.29	87.96
4	LPS	.	.	.	.	2.9	90.2	1.0	0.0	23.52	44.48
5	con	2.4	0.0	4.8	.	0.8	126.1	.	.	26.84	55.53
6	con	.	0.3	.	0.7	.	93.4	.	1.0	23.84	46.37
7	LPS	.	.	.	0.0	.	62.4	.	.	20.80	36.03
8	con	0.0	.	2.9	3.5	.	5.2	0.0	4.9	2.77	2.31
9	con	.	.	10.8	.	.	41.8	.	0.3	17.63	21.56
week 3											
1	con	26.8	35.2	53.4	0.0	0.0	144.7	16.7	7.8	35.58	47.76
2	con	37.8	49.7	58.6	0.0	8.7	152.7	44.7	25.2	47.16	47.13
3	con	43.8	31.2	49.7	1.2	0.0	105.8	14.2	18.8	33.09	34.52
4	con	35.8	20.8	0.0	0.0	0.0	59.8	0.0	0.0	14.56	22.69
5	con	14.7	51.7	37.4	0.7	0.0	112.7	27.7	20.8	33.22	36.58
6	LPS	18.9	0.0	18.9	0.0	10.3	101.9	1.9	7.0	19.87	34.03
7	con	0.9	56.4	46.8	2.9	0.0	122.9	0.0	14.9	30.59	43.44
8	con	44.9	40.8	42.4	0.0	0.0	172.8	2.8	16.8	40.05	57.05
9	LPS	0.0	0.7	0.1	5.7	6.6	111.2	0.2	0.0	15.57	38.74

Table 25. Hourly milk yields, in grams, by infusion status of teat, of gilt 8-10, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con-piglet nursed a non-infused mammary gland.

Table 26 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt x2-10, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt x2-10

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	con	27.4	40.5	30.6	43.8	34.3	12.9	13.7	17.4	27.58	11.92
2	LPS	15.1	11.7	29.8	6.0	.	62.1	0.0	6.1	18.70	21.38
3	con	0.0	37.9	22.0	23.9	33.0	40.1	8.4	12.1	22.17	14.50
4	con	33.6	28.0	23.0	32.0	21.0	28.2	19.0	18.2	25.37	5.91
5	LPS	29.0	16.9	14.8	13.9	15.0	11.1	4.9	4.1	13.71	7.80
6	con	26.8	20.1	14.2	24.1	21.2	30.3	10.1	5.3	19.02	8.55
7	con	9.1	45.0	26.0	34.9	26.0	25.1	20.9	27.1	26.76	10.34
8	con	.	6.1	8.1	22.1	21.1	.	18.0	21.8	16.20	7.22
9	con	4.5	31.9	21.9	32.9	28.9	16.1	12.9	16.1	20.66	10.07
week 3											
1	con	55.6	42.6	49.3	48.6	48.6	57.1	14.3	42.9	44.87	13.41
2	con	0.0	25.8	34.5	23.9	0.0	58.8	0.8	23.1	20.86	20.48
3	con	23.6	27.7	24.7	26.8	51.8	22.7	18.7	37.7	29.22	10.66
4	con	15.8	14.7	35.8	38.8	38.9	19.8	0.0	20.8	23.07	13.78
5	con	30.8	35.7	42.7	29.8	25.2	12.7	0.0	13.7	23.86	14.03
6	con	22.8	26.8	18.9	24.5	27.0	58.9	0.0	11.9	23.83	16.83
7	con	56.7	42.7	53.7	44.7	50.8	54.7	0.0	33.7	42.12	18.64
8	LPS	.	0.0	0.0	7.9	36.3	0.0	.	6.8	8.50	14.09
9	LPS	0.0	4.7	33.7	21.8	0.0	29.7	5.7	17.7	14.18	13.40

Table 26. Hourly milk yields, in grams, by infusion status of teat, of gilt x2-10, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 27 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 26-7, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 26-7

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	con	29.1	32.1	31.0	30.0	0.0	67.1	1.0	2.3	24.08	22.72
2	LPS	0.0	0.0	29.0	23.0	0.0	62.1	0.0	4.0	14.78	22.36
3	con	22.9	23.9	19.9	32.9	14.0	73.0	0.0	6.9	24.20	22.25
4	con	.	29.0	29.0	27.7	17.0	88.5	.	.	38.24	28.57
5	LPS	1.0	0.0	42.9	33.0	0.0	81.0	0.0	0.0	19.73	30.19
6	con	24.3	35.8	40.8	44.8	0.0	90.9	11.6	4.8	31.65	29.18
7	con	25.9	35.9	35.9	.	0.0	97.9	0.0	0.0	27.94	35.05
8	con	0.0	40.1	39.1	31.1	.	70.2	0.0	25.1	29.37	24.57
9	con	20.0	35.0	29.9	31.7	18.0	59.0	19.8	2.2	26.94	16.56
week 3											
1	LPS	7.9	11.2	11.9	12.9	44.7	39.9	0.0	0.0	16.06	17.00
2	con	9.0	27.0	15.9	17.9	21.0	46.0	0.9	5.9	17.97	14.12
3	con	30.8	22.1	33.8	36.8	69.6	99.8	5.8	11.8	38.81	31.31
4	con	.	26.9	.	22.8	29.9	111.9	.	.	47.86	42.77
5	con	0.0	14.2	13.9	20.8	0.0	76.9	0.0	2.9	16.09	25.86
6	con	33.7	31.0	12.7	7.7	0.8	152.7	3.7	10.2	31.57	50.42
7	con	27.8	.	34.7	31.7	24.8	103.8	.	11.7	39.09	32.67
8	LPS	0.0	10.2	12.9	13.9	0.0	89.0	0.0	1.9	15.99	30.09
9	con	29.9	11.8	35.8	31.8	33.9	70.9	0.0	13.8	28.48	21.36

Table 27. Hourly milk yields, in grams, by infusion status of teat, of gilt 26-7, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 28 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 38-5, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 38-5

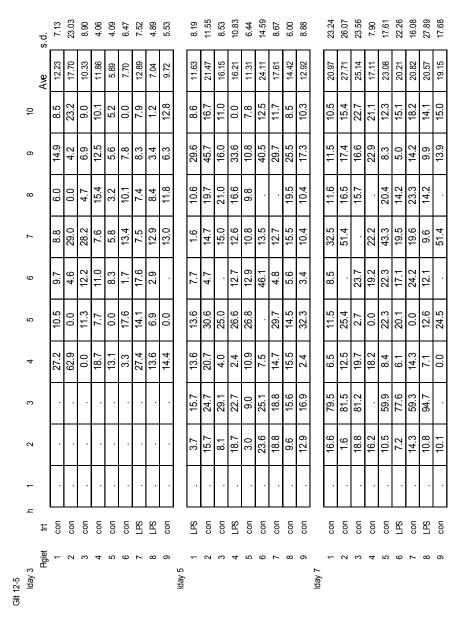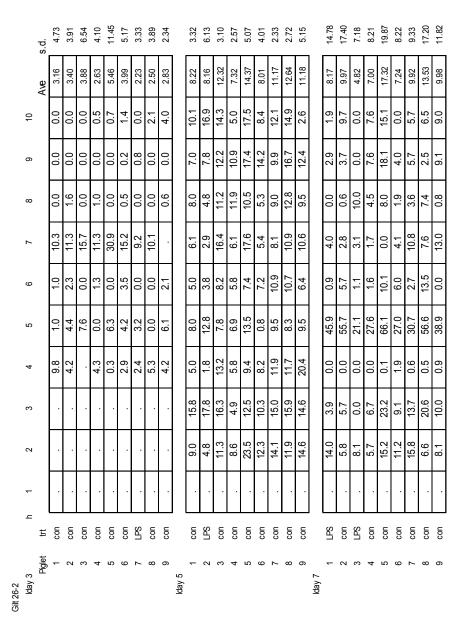| Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	con	.	0.0	32.1	.	30.1	91.0	0.0	.	30.65	37.18
2	con	30.9	0.0	44.9	35.9	11.0	94.9	12.9	.	32.94	31.53
3	con	0.0	0.0	44.9	37.9	36.9	168.8	6.9	6.4	37.71	56.09
4	con	28.0	0.0	35.0	32.0	12.0	63.0	0.0	.	24.29	22.42
5	LPS	0.5	0.0	33.0	24.0	0.0	90.0	9.0	2.0	19.83	30.95
6	con	.	0.0	11.3	2.3	3.3	14.3	1.3	.	5.41	5.88
7	LPS	1.9	0.0	40.9	39.9	0.0	150.9	.	7.9	34.49	54.41
8	con	24.2	0.0	34.2	33.2	21.2	27.6	0.0	10.2	18.81	13.82
9	con	19.1	0.0	9.1	4.1	7.1	138.1	.	12.6	27.17	49.28
week 3											
1	LPS	0.0	0.0	.	17.9	0.0	69.0	0.0	8.9	13.69	25.33
2	LPS	0.0	0.0	30.8	.	0.0	.	0.8	.	6.32	13.69
3	con	0.0	39.7	.	.	.	.	.	.	19.85	28.07
4	con	0.0	35.8	47.8	42.8	0.0	158.9	3.8	4.8	36.74	53.29
5	con	0.0	39.9	45.9	40.9	0.0	99.9	.	0.0	32.37	36.56
6	con	0.0	19.1	20.2	15.2	0.0	49.2	.	0.1	14.82	17.73
7	con	7.0	38.8	41.8	28.8	0.0	112.4	.	0.0	32.68	39.33
8	con	2.1	23.9	40.0	.	0.0	25.0	0.0	8.9	14.29	15.57
9	con	0.0	34.9	36.0	38.9	0.0	93.0	.	2.9	29.39	33.26

Table 28. Hourly milk yields, in grams, by infusion status of teat, of gilt 38-5, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con-piglet nursed a non-infused mammary gland.

Table 29 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 30-1, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.
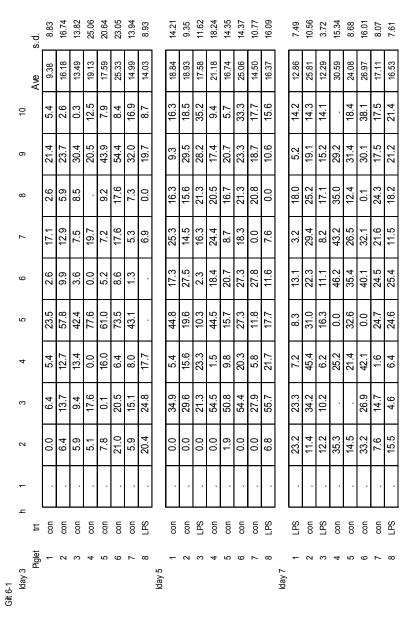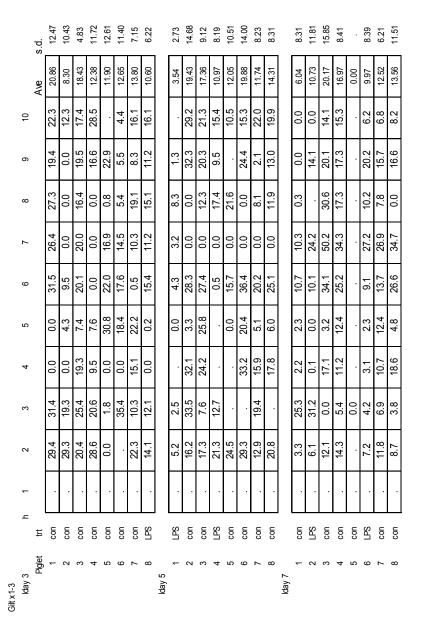
Gilt 30-1

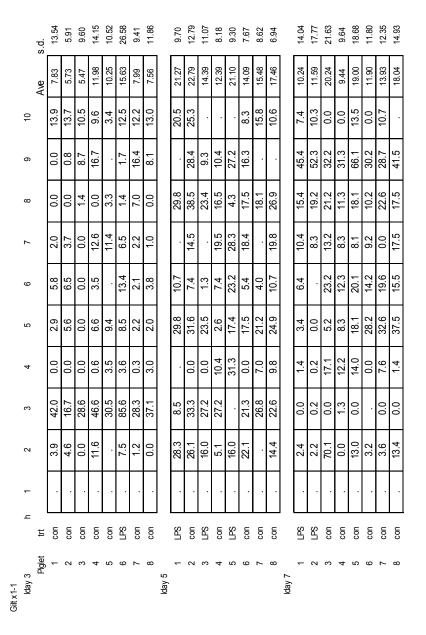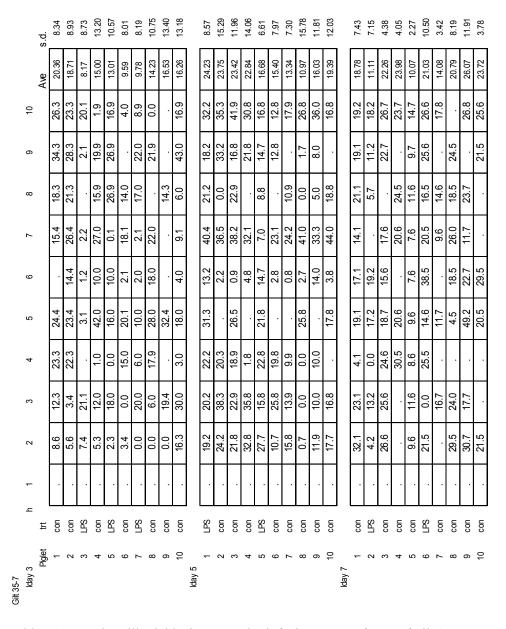| Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	con	30.9	15.6	35.0	37.9	61.1	134.1	0.0	.	44.94	43.67
2	LPS	0.0	0.0	.	22.9	0.0	181.9	2.8	44.0	35.93	66.46
3	con	52.8	40.5	.	16.8	53.8	.	.	.	41.01	17.21
4	con	38.8	29.8	49.8	61.8	12.8	.	0.0	0.0	27.56	24.28
5	con	21.0	35.7	26.1	31.0	55.2	0.0	.	0.0	24.15	19.69
6	LPS	0.0	45.8	3.9	28.8	7.8	208.8	.	0.0	42.17	75.46
7	con	.	0.0	.	40.9	39.9	.	0.0	33.0	22.75	20.99
8	con	44.8	39.8	28.8	48.8	51.0	107.0	12.4	29.7	45.28	27.97
week 3											
1	con	21.2	21.6	.	35.8	0.8	95.8	0.0	2.3	25.36	33.85
2	con	0.0	28.8	.	.	24.9	39.8	.	3.9	19.46	16.98
3	con	.	61.7	.	55.7	0.0	135.7	0.0	8.7	52.36	54.05
4	LPS	.	0.0	24.7	.	0.0	69.7	0.0	.	18.87	30.34
5	con	53.2	28.8	16.8	.	37.9	128.8	0.0	14.4	39.99	42.80
6	con	45.7	20.7	34.7	0.0	11.8	77.7	0.0	0.8	23.94	27.59
7	con	44.7	64.7	.	.	48.8	49.7	0.0	3.8	35.28	26.77
8	LPS	11.1	0.0	53.2	65.7	0.0	.	.	18.7	24.77	28.04

Table 29. Hourly milk yields, in grams, by infusion status of teat, of gilt 30-1, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 30 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 44-4, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 44-4

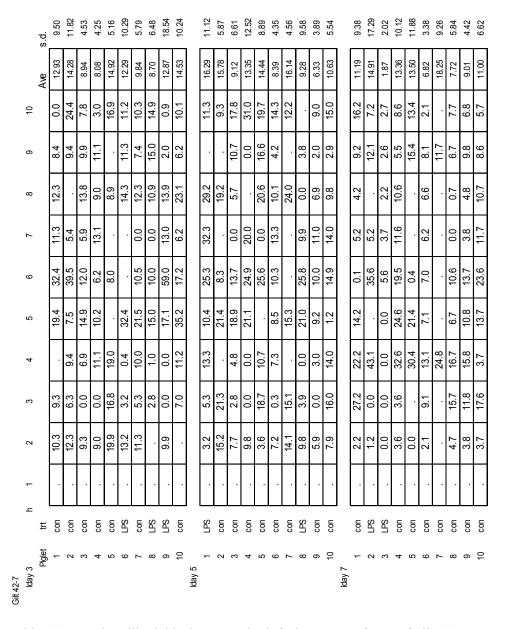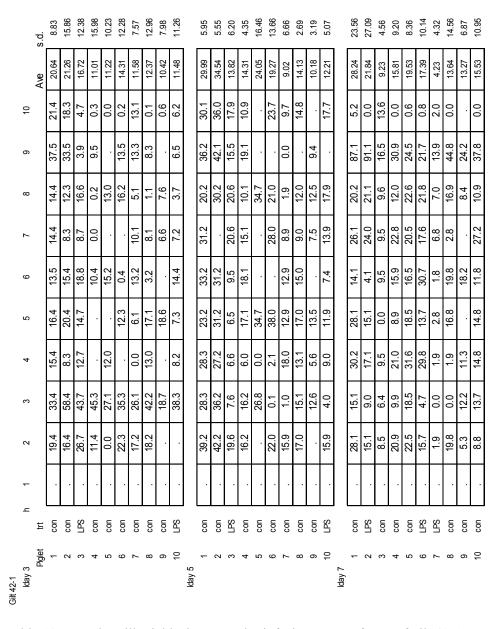|	Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2	1	con	2.8	40.9	54.9	22.5	48.9	69.5	0.0	0.0	29.93	27.38
	2	con	27.2	28.2	.	31.2	30.3	48.4	0.0	0.0	23.63	17.64
	3	LPS	0.0	3.4	8.2	17.1	0.0	83.2	5.1	1.1	14.76	28.24
	4	con	17.0	42.0	44.0	35.0	34.1	94.6	0.0	0.0	33.33	30.33
	5	con	12.9	33.0	37.0	31.0	28.0	138.1	2.6	7.9	36.32	43.01
	6	con	2.2	22.3	28.3	24.3	24.3	53.4	11.9	0.0	20.83	16.91
	7	LPS	1.1	0.0	15.2	23.7	0.2	95.7	1.1	2.1	17.38	32.83
	8	con	0.4	29.4	38.5	31.4	30.5	40.6	0.5	0.0	21.41	17.91
	9	con	0.0	31.1	37.1	57.7	.	54.7	0.0	5.0	26.51	25.05
week 3	1	con	14.5	43.7	21.0	64.7	0.0	.	4.7	0.0	21.22	24.55
	2	LPS	0.0	0.0	25.2	.	0.0	4.0	0.0	0.0	4.18	9.41
	3	LPS	0.9	0.0	0.0	10.9	0.0	89.9	0.0	21.7	15.44	31.10
	4	con	19.8	54.8	11.8	.	.	37.8	0.0	12.6	22.80	20.01
	5	con	31.8	19.8	23.8	30.8	27.5	95.0	5.8	9.8	30.53	27.70
	6	con	26.0	23.0	27.0	33.5	10.8	65.3	7.0	32.8	28.19	17.75
	7	con	15.8	20.5	16.0	15.0	0.0	118.0	2.0	.	26.77	40.96
	8	con	14.2	14.2	.	10.3	.	52.2	0.0	2.3	15.53	18.94
	9	con	39.8	.	.	15.8	.	62.8	0.0	.	29.62	27.53

Table 30. Hourly milk yields, in grams, by infusion status of teat, of gilt 44-4, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 31 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 47-5, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 47-5

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2											
1	LPS	0.0	0.0	3.6	10.1	9.7	64.1	17.3	3.0	13.47	21.28
2	con	40.0	36.0	39.6	.	53.7	71.0	17.2	17.9	39.35	19.01
3	con	0.9	0.0	50.9	51.0	51.0	94.9	11.1	.	37.10	34.83
4	con	51.0	21.6	40.0	40.1	4.1	99.0	5.0	23.9	35.57	30.61
5	LPS	1.0	0.0	25.5	0.0	0.0	47.0	0.0	4.0	9.68	17.42
6	con	8.1	19.1	25.7	38.7	55.8	45.1	10.3	18.2	27.64	17.18
7	con	.	3.2	44.1	0.0	.	71.2	30.2	24.2	28.82	26.62
8	con	37.1	29.1	38.1	41.2	31.2	42.1	8.3	27.2	31.78	10.97
9	con	.	21.1	57.7	48.7	46.2	51.2	6.4	21.1	36.07	19.53
week 3											
1	con	25.4	28.7	29.9	25.9	.	84.0	0.0	4.9	28.41	27.28
2	con	5.8	69.8	61.8	22.8	30.8	101.8	0.0	0.0	36.58	37.50
3	con	51.7	46.7	20.7	41.7	0.0	112.8	0.0	6.7	35.05	37.73
4	LPS	0.0	0.0	34.8	24.8	0.0	108.4	0.0	14.8	22.87	37.07
5	con	18.8	39.8	35.8	23.8	30.8	78.9	0.0	16.3	30.55	23.20
6	con	29.9	26.9	28.9	33.9	29.9	79.0	0.0	0.0	28.58	24.52
7	con	14.0	46.0	24.0	.	0.0	89.1	0.0	0.0	24.74	33.02
8	LPS	.	0.0	28.9	0.0	4.9	103.9	0.0	0.0	19.67	38.63
9	con	15.9	53.9	31.9	30.9	32.9	83.0	0.0	0.0	31.05	27.72

Table 31. Hourly milk yields, in grams, by infusion status of teat, of gilt 47-5, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 32 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 26-2, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 26-2

	Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2	1	con	.	.	0.5	14.6	12.5	60.8	2.5	3.6	15.76	22.79
	2	con	1.0	.	12.3	25.4	28.3	109.4	0.0	.	29.40	40.93
	3	con	0.0	5.7	26.5	26.5	0.0	96.5	.	0.5	22.24	34.84
	4	LPS	0.0	.	4.7	49.0	39.9	165.0	.	1.0	43.26	63.26
	5	LPS	.	.	11.2	8.7	8.2	128.4	.	.	39.11	59.54
	6	con	0.5	.	3.5	18.6	23.2	28.6	.	1.6	12.66	12.29
	7	con	.	.	2.3	23.4	26.3	106.6	.	0.0	31.73	43.51
	8	con	1.1	0.1	4.1	5.2	12.1	43.2	.	.	10.99	16.34
	9	con	0.0	0.0	17.8	18.1	32.8	137.3	.	17.1	31.87	47.88
	10	con	.	2.4	26.1	36.5	0.0	92.7	0.4	0.0	22.58	34.24
week 3	1	con	.	.	.	19.3	14.3	31.5	.	.	21.70	8.85
	2	con	4.0	0.1	0.0	21.0	13.1	8.2	4.0	.	8.40	7.61
	3	con	0.0	.	.	44.1	26.1	71.4	5.8	3.1	21.51	27.46
	4	LPS	.	.	0.0	10.8	1.8	143.0	0.0	.	31.12	62.72
	5	con	3.0	.	2.0	33.0	18.1	50.2	.	2.1	18.06	19.99
	6	LPS	.	.	.	8.2	7.3	49.5	2.2	0.3	13.50	20.39
	7	con	.	.	.	32.1	26.1	52.3	.	1.1	27.90	21.08
	8	con	1.9	.	0.9	7.9	6.0	26.3	1.9	4.0	7.01	8.88
	9	con	2.7	.	9.7	50.7	46.8	157.0	.	.	53.39	61.76
	10	con	.	.	.	29.2	18.2	53.5	3.0	.	25.97	21.28

Table 32. Hourly milk yields, in grams, by infusion status of teat, of gilt 26-2, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 33 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 21-1, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 21-1

	Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
week 2	1	con	19.2	9.2	6.2	12.2	13.2	28.3	12.1	3.2	12.94	7.85
	2	con	5.2	13.1	3.1	18.0	14.0	9.2	8.0	10.1	10.10	4.87
	3	con	17.2	26.2	24.2	24.1	26.1	33.2	0.0	25.1	22.02	9.90
	4	con	22.0	17.9	25.9	31.9	27.9	0.0	24.9	26.9	22.18	9.86
	5	LPS	0.3	.	8.3	3.3	3.3	4.4	.	0.3	3.31	2.96
	6	con	16.5	14.5	17.5	19.4	18.4	30.6	13.4	16.4	18.34	5.31
	7	LPS	.	0.0	2.4	1.3	12.3	.	.	13.4	5.88	6.43
	8	con	6.7	12.6	15.6	18.6	17.6	16.7	5.5	16.6	13.76	5.03
	9	con	12.3	18.2	7.2	19.2	19.2	50.3	.	15.2	20.23	13.95
	10	con	9.2	.	2.1	.	2.1	26.3	3.0	0.1	7.12	9.87
week 3	1	con	.	.	0.1	7.1	1.1	18.3	2.1	0.0	4.78	7.11
	2	con	4.5	12.4	0.0	18.5	15.5	32.7	.	2.5	12.29	11.36
	3	con	0.0	14.8	5.8	12.8	7.8	70.4	.	2.8	16.36	24.41
	4	LPS	0.0	0.0	0.7	10.7	.	7.8	3.7	.	3.82	4.52
	5	con	0.0	11.0	5.1	5.1	12.1	32.2	1.1	1.1	8.47	10.61
	6	con	3.0	9.0	1.1	18.1	19.0	41.2	.	2.0	13.35	14.34
	7	con	5.2	2.1	.	13.2	5.2	30.4	.	.	11.21	11.47
	8	con	5.1	6.1	0.2	22.2	12.1	49.3	.	.	15.82	18.05
	9	LPS	1.9	4.9	1.9	12.9	13.9	56.0	0.9	2.9	11.91	18.53
	10	con	.	7.9	0.0	2.0	0.0	6.3	1.0	0.0	2.46	3.28

Table 33. Hourly milk yields, in grams, by infusion status of teat, of gilt 21-1, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Table 34 represents the raw data for hourly milk yields in grams, by teat nursed as determined by weigh-suckle-weigh procedure of piglets nursing gilt 19-4, on experimental days during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 19-4

week 2

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
1	con	3.9	0.0	18.3	24.3	0.0	78.4	22.6	8.3	19.47	25.73
2	con	.	1.4	14.4	32.4	0.0	71.6	22.7	0.4	20.43	25.77
3	LPS	0.0	.	11.1	7.1	5.1	75.2	0.0	8.1	15.25	26.77
4	con	.	.	17.3	33.3	3.3	83.4	8.6	6.2	25.33	30.43
5	con	2.8	0.2	13.2	31.2	.	115.3	6.1	7.2	25.14	41.06
6	con	.	1.2	30.2	28.2	.	75.4	15.1	16.2	27.73	25.58
7	con	3.3	.	15.4	21.4	.	61.5	19.7	5.4	21.10	21.13
8	con	2.0	3.0	22.1	0.1	2.1	74.2	0.0	14.1	14.69	25.30
9	LPS	.	.	5.2	1.2	.	11.4	.	.	5.95	5.12
10	con	.	.	45.0	58.5	.	72.1	12.9	16.0	40.87	25.99

week 3

Piglet	trt	h 1	2	3	4	5	6	7	8	Ave	s.d.
1	con	.	35.8	0.0	35.9	0.0	67.3	10.8	24.0	24.82	24.06
2	LPS	0.0	1.0	0.1	15.0	16.1	52.5	.	.	14.13	20.24
3	con	.	28.8	21.9	28.9	26.9	168.3	.	15.0	48.30	59.01
4	con	.	16.9	21.0	32.9	45.0	86.3	0.9	15.0	31.13	28.05
5	con	.	40.8	27.9	30.3	0.0	75.2	.	0.0	29.04	28.14
6	con	.	36.8	30.9	39.8	51.9	111.2	6.8	28.9	43.76	32.74
7	LPS	.	9.0	6.1	15.5	5.1	27.5	.	5.1	11.38	8.82
8	con	.	35.7	32.8	50.8	48.8	118.1	.	17.9	50.67	35.13
9	con	.	6.1	6.2	7.2	14.3	36.7	.	31.3	16.97	13.66
10	con	0.0	31.7	29.7	28.7	38.8	110.0	9.7	.	35.51	35.56

Table 34. Hourly milk yields, in grams, by infusion status of teat, of gilt 19-4, on experimental days during weeks two and three of lactation. Teat infusion status is represented by piglet number and treatment. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland.

Appendix W. RAW PIGLET 24 H WEIGHT GAIN DATA
(ADVANCED LACTATION)

Table 35 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 8-6 & 8-10 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 8-6, week 2

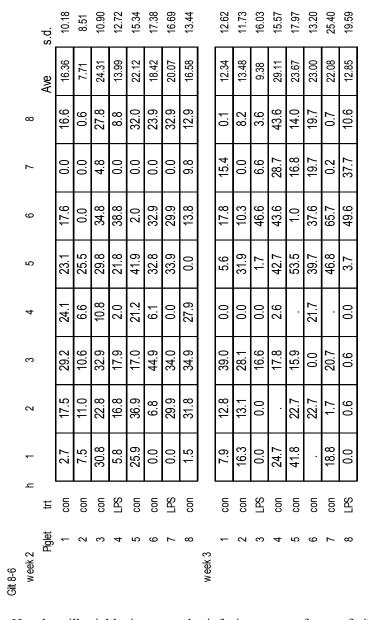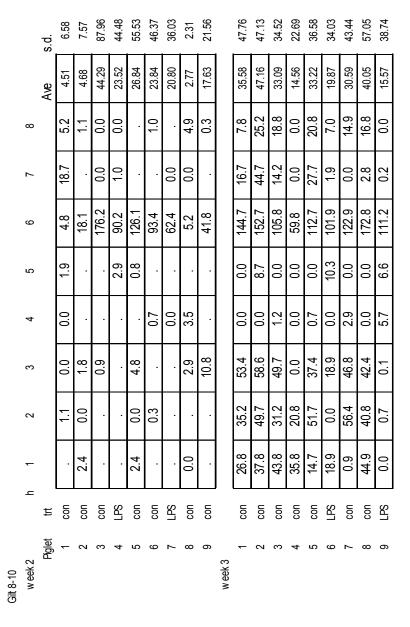| Piglet	trt	day -1	0	1	2	3	4
1	con	138	120	152	201	209	175
2	con	74	70	93	142	154	96
3	con	230	254	275	309	327	360
4	LPS	236	150	112	180	273	311
5	con	162	202	243	291	328	239
6	con	237	182	231	282	286	193
7	LPS	259	220	244	277	315	317
8	con	176	194	229	277	307	339
Ave		189.09	174.03	197.38	244.88	274.88	253.75
s.d.		63.06	58.64	68.30	61.41	62.40	93.22

Gilt 8-6, week 3

Piglet	trt	day -1	0	1	2
1	con	205	32	-9	157
2	con	154	126	158	161
3	LPS	272	138	159	233
4	con	230	337	235	428
5	con	128	150	124	184
6	con	110	93	83	175
7	con	-195	151	106	256
8	LPS	296	173	113	188
Ave		150.00	150.00	121.13	222.75
s.d.		154.34	87.34	70.15	89.81

Gilt 8-10, week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	-92	-43	203	285	228	299
2	con	43	24	32	218	325	324
3	con	66	88	128	200	253	330
4	LPS	228	-36	2	261	105	107
5	con	202	88	51	110	223	220
6	con	151	117	242	-154	142	169
7	LPS	196	63	124	234	159	109
8	con	-258	51	33	143	177	194
9	con	120	92	116	-76	74	134
Ave		72.82	49.33	103.44	135.67	187.33	209.56
s.d.		158.62	57.04	81.70	153.30	78.08	89.40

Gilt 8-10, week 3

Piglet	trt	day -1	0	1	2
1	con	293	216	286	305
2	con	359	227	312	355
3	con	172	207	289	322
4	LPS	159	32	90	85
5	con	240	196	272	270
6	con	141	97	107	159
7	con	207	193	171	196
8	con	314	257	309	310
9	LPS	4	-22	-27	15
Ave		209.89	155.89	201.00	224.11
s.d.		107.27	96.79	121.47	117.71

Table 35. 24 h weight gains, in grams, of piglets nursing gilts 8-6 & 8-10 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

Table 36 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts x2-10 & 26-7 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt x2-10

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	304	250	301	286	350	361
2	LPS	196	-21	78	215	140	189
3	con	138	112	296	73	233	274
4	con	190	182	158	202	220	200
5	LPS	233	74	132	176	158	204
6	con	149	137	170	198	166	189
7	con	236	222	230	299	335	312
8	con	180	128	205	222	207	235
9	con	209	138	188	211	221	228
Ave		203.81	135.78	195.33	209.11	225.56	243.56
s.d.		50.03	80.35	72.85	65.19	73.61	60.35

week 3

Piglet	trt	day -1	0	1	2
1	con	310	344	333	361
2	con	130	190	88	185
3	con	230	244	263	257
4	con	176	228	256	191
5	con	169	253	225	199
6	con	169	184	175	174
7	con	236	315	294	276
8	LPS	196	-119	46	144
9	LPS	118	58	93	76
Ave		192.67	188.56	197.00	207.00
s.d.		59.11	141.68	101.56	82.16

Gilt 26-7

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	165	185	195	209	176	216
2	LPS	142	30	91	116	116	150
3	con	176	150	250	205	240	211
4	con	147	141	215	150	232	170
5	LPS	178	30	141	122	110	181
6	con	221	205	261	246	284	290
7	con	200	195	235	207	297	232
8	con	170	177	211	193	238	182
9	con	174	191	166	214	253	206
Ave		174.78	144.89	196.11	184.67	216.22	204.22
s.d.		24.37	68.31	55.00	44.78	67.74	41.02

week 3

Piglet	trt	day -1	0	1	2
1	LPS	216.0	-34.0	11.0	91.0
2	con	150.0	160.0	213.0	247.0
3	con	211.0	253.0	296.0	262.0
4	con	170.0	116.0	242.0	180.0
5	con	181.0	109.0	233.0	180.0
6	con	290.0	242.0	280.0	259.0
7	con	232.0	192.0	290.0	253.0
8	LPS	182.0	28.0	116.0	138.0
9	con	206.0	146.0	267.0	271.0
Ave		204.22	134.67	216.44	209.00
s.d.		41.02	93.82	94.58	64.40

Table 36. 24 h weight gains, in grams, of piglets nursing gilts x2-10 & 26-7 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS-piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

Table 37 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 38-5 & 30-1 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 38-5

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	186	202	193	197	179	204
2	con	183	255	249	286	267	250
3	con	167	292	263	274	357	266
4	con	133	173	248	264	266	291
5	LPS	95	105	162	214	218	186
6	con	-16	42	103	144	112	135
7	LPS	166	112	154	187	187	190
8	con	136	164	200	198	211	215
9	con	5	157	156	160	231	248
Ave		117.22	166.89	192.00	213.78	225.33	220.56
s.d.		75.29	76.84	53.68	50.46	68.45	47.96

week 3

Piglet	trt	day -1	0	1	2
1	LPS	204	16	99	99
2	LPS	250	45	118	138
3	con	266	220	306	357
4	con	291	228	268	258
5	con	186	240	246	262
6	con	135	79	152	143
7	con	190	182	226	264
8	con	215	154	226	238
9	con	248	205	237	273
Ave		220.56	152.11	208.67	225.78
s.d.		47.96	84.51	70.04	82.19

Gilt 30-1

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	144	208	168	176	165	257
2	LPS	199	48	24	114	109	134
3	con	185	339	234	349	262	311
4	con	180	303	194	366	245	261
5	con	153	221	194	277	234	258
6	LPS	182	144	114	181	140	210
7	con	203	237	227	287	219	254
8	con	194	213	253	327	254	200
Ave		180.00	214.13	176.00	259.63	203.50	235.63
s.d.		21.18	89.93	75.17	92.02	57.71	53.28

week 3

Piglet	trt	day -1	0	1	2
1	con	257	127	204	245
2	con	134	60	162	223
3	con	311	247	198	230
4	LPS	261	-71	6	-10
5	con	258	203	220	255
6	con	210	151	210	223
7	con	254	121	168	249
8	LPS	200	67	187	275
Ave		235.63	113.13	169.38	211.25
s.d.		53.28	97.50	68.96	91.12

Table 37. 24 h weight gains, in grams, of piglets nursing gilts 38-5 & 30-1 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS-piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

Table 38 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 44-4 & 47-5 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.

Gilt 44-4

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	237	168	210	323	331	255
2	con	184	84	210	177	210	203
3	LPS	154	37	88	144	233	291
4	con	236	88	165	218	257	269
5	con	158	194	165	279	238	283
6	con	121	77	139	164	150	161
7	LPS	158	64	21	68	67	70
8	con	112	69	117	111	117	51
9	con	219	148	191	228	239	249
Ave		175.44	103.22	145.11	190.22	204.67	203.56
s.d.		46.75	53.42	62.06	80.56	80.19	90.74

week 3

Piglet	trt	day -1	0	1	2
1	con	138	223	220	235
2	LPS	147	-150	89	8
3	LPS	-8	-10	145	93
4	con	185	-88	-103	20
5	con	173	142	224	236
6	con	109	172	132	191
7	con	110	172	265	98
8	con	96	96	104	111
9	con	205	182	-101	-8
Ave		128.33	82.11	108.33	109.33
s.d.		63.11	132.79	132.84	94.15

Gilt 47-5

week 2

Piglet	trt	day -1	0	1	2	3	4
1	LPS	158	107	-15	90	98	123
2	con	224	266	204	229	314	280
3	con	229	220	171	205	207	244
4	con	222	156	163	129	136	124
5	LPS	168	50	30	130	136	116
6	con	207	152	133	168	150	143
7	con	182	107	8	64	141	117
8	con	211	201	206	196	232	167
9	con	204	230	134	193	190	197
Ave		200.56	165.44	114.89	156.00	178.22	167.89
s.d.		25.49	69.77	85.07	55.98	65.65	60.26

week 3

Piglet	trt	day -1	0	1	2
1	con	62	192	180	114
2	con	85	290	211	301
3	con	125	256	176	225
4	LPS	70	77	98	88
5	con	114	229	110	186
6	con	98	164	154	-30
7	con	120	207	128	201
8	LPS	107	85	16	92
9	con	124	254	166	189
Ave		100.56	194.89	137.67	151.78
s.d.		23.50	74.60	58.03	96.64

Table 38. 24 h weight gains, in grams, of piglets nursing gilts 44-4 & 47-5 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS-piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

Table 39 represents the raw data for 24 h weight gains in grams, of piglets nursing gilts 26-2 & 21-1 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.
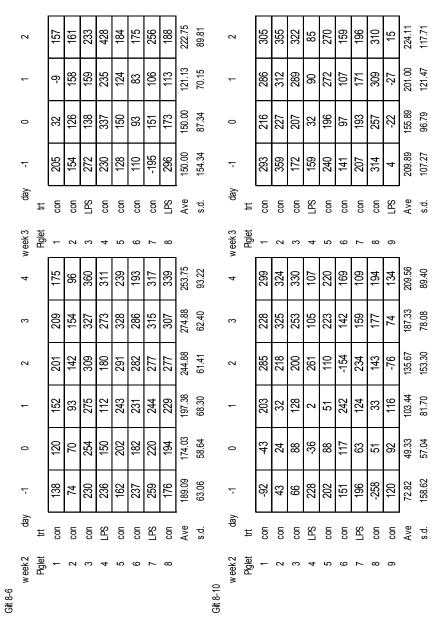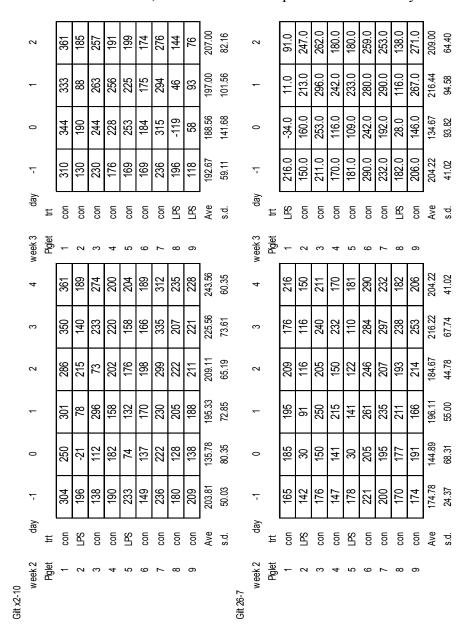
Gilt 26-2

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	152	25	31	124	72	30
2	con	253	113	171	100	189	59
3	con	252	113	114	157	210	109
4	LPS	330	32	23	168	191	143
5	LPS	241	-28	-25	82	60	20
6	con	220	17	40	91	124	25
7	con	207	99	67	99	125	29
8	con	85	8	20	52	17	-27
9	con	325	141	226	209	346	197
10	con	208	44	41	116	78	13
Ave		227.30	56.40	70.80	119.80	141.20	59.80
s.d.		73.26	55.91	77.08	46.40	95.92	68.74

week 3

Piglet	trt	day -1	0	1	2
1	con	90	-7	139	44
2	con	104	-18	64	71
3	LPS	46	6	161	155
4	con	205	-44	144	129
5	LPS	152	17	164	154
6	con	88	-10	66	52
7	con	128	-25	162	140
8	con	48	-18	4	.
9	con	369	-153	118	191
10	con	90	6	142	81
Ave		132.00	-24.60	116.40	113.00
s.d.		95.81	48.39	53.94	52.18

Gilt 21-1

week 2

Piglet	trt	day -1	0	1	2	3	4
1	con	148	140	88	88	33	2
2	con	106	119	84	127	69	71
3	con	242	241	188	228	186	204
4	con	259	266	227	258	237	184
5	LPS	33	21	1	147	63	60
6	con	158	181	169	213	167	69
7	LPS	59	9	48	-12	44	66
8	con	136	175	133	170	140	107
9	con	238	191	180	194	190	73
10	con	55	23	44	136	4	-45
Ave		143.40	136.60	116.20	154.90	113.30	79.10
s.d.		82.22	92.45	74.19	77.91	80.11	74.14

week 3

Piglet	trt	day -1	0	1	2
1	con	-75	-29	-11	-46
2	con	115	77	113	109
3	con	249	157	195	259
4	LPS	239	-23	88	75
5	con	144	85	120	94
6	con	171	100	147	182
7	con	132	52	126	97
8	con	181	121	149	127
9	LPS	206	98	94	130
10	con	-46	-16	-30	-55
Ave		131.60	62.20	99.10	97.20
s.d.		110.30	64.71	70.14	94.07

Table 39. 24 h weight gains, in grams, of piglets nursing gilts 26-2 & 21-1 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS-piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

Table 40 represents the raw data for 24 h weight gains in grams, of piglets nursing gilt 19-4 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation, and used for subsequent statistical analysis.
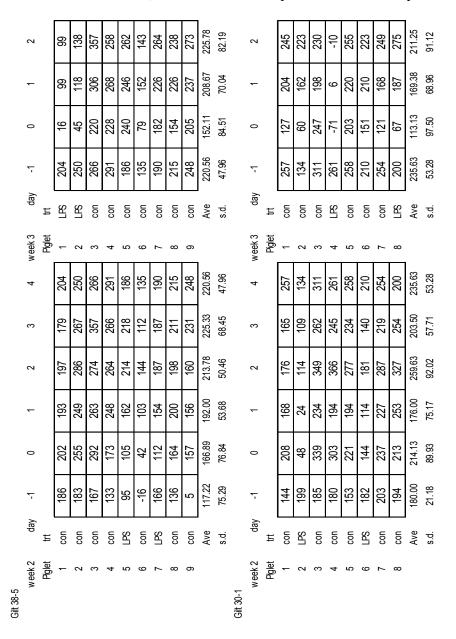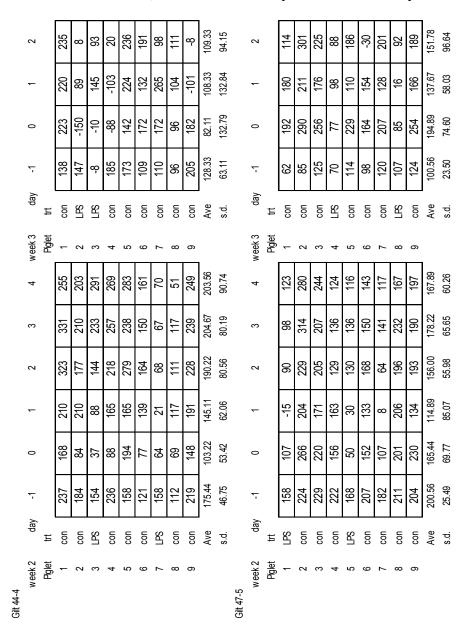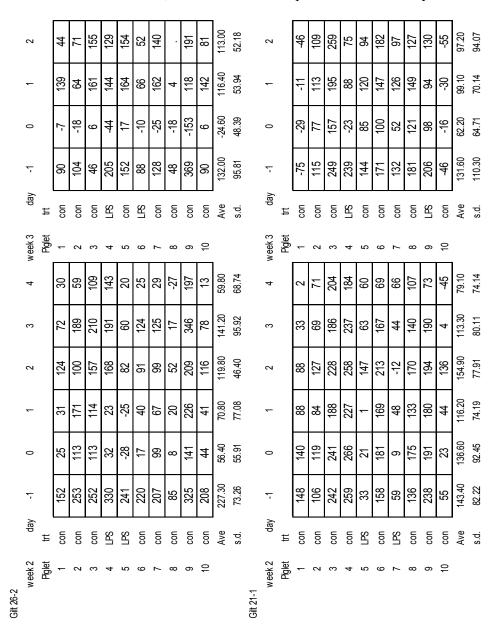
week 3

Piglet	trt	day -1	0	1	2
1	con	245	244	258	242
2	LPS	192	64	41	95
3	con	164	191	167	205
4	con	197	164	184	236
5	con	245	231	161	216
6	con	270	296	256	245
7	LPS	168	60	46	102
8	con	240	330	286	280
9	con	62	97	116	120
10	con	244	288	262	301
Ave		202.70	196.50	177.70	204.20
s.d.		61.33	98.16	89.25	73.65

Gilt 19-4

week 2

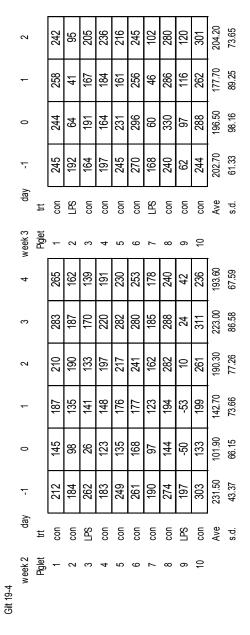| Piglet	trt	day -1	0	1	2	3	4
1	con	212	145	187	210	283	265
2	con	184	98	135	190	187	162
3	LPS	262	26	141	133	170	139
4	con	183	123	148	197	220	191
5	con	249	135	176	217	282	230
6	con	261	168	177	241	280	253
7	con	190	97	123	162	185	178
8	con	274	144	194	282	288	240
9	LPS	197	-50	-53	10	24	42
10	con	303	133	199	261	311	236
Ave		231.50	101.90	142.70	190.30	223.00	193.60
s.d.		43.37	66.15	73.66	77.26	86.58	67.59

Table 40. 24 h weight gains, in grams, of piglets nursing gilt 19-4 at specified days relative to LPS infusion of mammary glands during weeks two and three of lactation. LPS- piglet nursed an endotoxin-infused mammary gland. Con- piglet nursed a non-infused mammary gland. Day- day relative to endotoxin infusion.

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        Kauf, A. C. W., S. M. Hough, and R. D. Bowditch. 2001. Recognition of
            fibronectin by the platelet integrin alpha IIb beta 3 involves an extended
            interface with multiple electrostatic interactions. Biochem. 40:9159-9166.
        Kauf, A. C. W., and R. S. Kensinger. 2002. Purification of porcine beta-casein, N-
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            80:1863-1870.

Abstracts
        Numerous published abstracts in animal science and biochemistry.