Animal Nutrition 2 (2016) 119–126

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original research article

Reproductive stage associated changes in plasma fatty acid profile and proinflammatory cytokine expression in rat mammary glands

Sen Lin [1], Jia Hou [1], Fang Xiang [1], Xiaoling Zhang, Lianqiang Che, Yan Lin, Shengyu Xu, De Wu, Zhengfeng Fang*

Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education, Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form
29 February 2016
Accepted 8 March 2016
Available online 16 April 2016

Keywords:
Mastitis
Reproductive stage
Proinflammatory cytokine
Fatty acid
Rat

ABSTRACT

Mastitis is a common disease for mammals all around the world. Figuring out why mastitis mainly occurs around parturition may be helpful for dealing with the disease. Lipolytic activity and oxidative stress take place around parturition, which may leads to alteration in fatty acids profile and proinflammatory cytokine expression. Thus, the aim of the present study was to further our understanding about the high incidence of mastitis around parturition by comparison of plasma fatty acid profile and mammary inflammation indicators at different reproductive stages. A total of 47 female rats were included in the present study. After mating, all the pregnant and non-pregnant rats began to receive the same experimental diet. Blood samples were collected at day 1 and 14 of gestation as well as day 3 postpartum. Mammary samples were collected at day 14 of gestation and day 3 postpartum from pregnant and non-pregnant rats. The results showed that rats at d 3 postpartum had greater *($P < 0.05$)* plasma concentrations of non-esterified fatty acids (NEFA), arachidonic acid (ARA) and docosahexaenoic acid (DHA) as well as ARA: eicosapentaenoic acid (EPA) ratio than those at d 14 of gestation. The mRNA abundances of interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNF-α*), *IL-8* and xanthine oxidoreductase (*XOR*) in mammary of the pregnant rats were greater *($P < 0.05$)* than those in age-matched non-pregnant rats. Rats at d 3 postpartum had higher *($P < 0.05$)* protein expression levels of IL-1β and TNF-α as well as meloperoxidase (MPO) activity and polymorphonuclear neutrophils (PMN) prevalence than those at d 1 of gestation. The rats at d 3 postpartum also had greater *($P < 0.05$)* IL-1β and MPO activity than those at d 14 of gestation. The results indicated that elevated mammary expression of proinflammatory cytokines and *XOR* as well as altered fatty acid profile around parturition might facilitate the recruitment of neutrophils into mammary glands.

1. Introduction

Mastitis is a common disease for mammals all over the world that occurs frequently around parturition (Compton et al., 2007). Previous studies showed the average prevalence of mastitis in sows was about 13% (Gerjets and Kemper, 2009) while it was about 25.5% in lactating heifers in Dutch (Santman-Berends et al., 2012). Piglet mortality around one week in the litters of coliform mastitis-affected sows varies from 5.0% to 38.6% (Gerjets and Kemper, 2009). Cows often have mastitis without obvious clinical symptoms named subclinical mastitis (Hansen et al., 2004). Subclinical mastitis results in leakage of plasma constituents into milk and causes gut damage of infants. Antibiotics are often used to deal with mastitis while overuse of antibiotics may lower the quality and safety of animal products and thus threaten the health of humans (Hortet and Seegers, 1998; Seegers et al., 2003).

Up to date, we know little about how to effectively prevent mastitis, and there is little knowledge about the underlying

* Corresponding author.
E-mail address: fangzhengfeng@hotmail.com (Z. Fang).
[1] These authors contributed equally to this work.
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2016.03.008

mechanism. Although *Escherichia coli* has been proposed to play critical roles in triggering mastitis through the toll-like receptor (TLR) pathway (Porcherie et al., 2012) and infusion of lipopolysaccharide (LPS) has been proved to potentially stimulate expression of proinflammatory cytokines (Frank et al., 2003), why mammary glands are more susceptible around parturition (Burvenich et al., 2007) remains to be elucidated. In humans, increased concentration of non-esterified fatty acid (NEFA) can stimulate systemic immune response and is linked to inflammatory-based diseases (Wood et al., 2009). Notably, increased lipolysis takes place around parturition and thus results in large increases in NEFA concentration (Drackley et al., 2001). In addition, enhanced lipolytic activity around parturition also results in break down of fat depots and rapid changes of plasma fatty acid composition (Amusquivar et al., 2010). It was reported that saturated fatty acids (SFA) concentration in plasma of women at parturition was greater than that at week 24 of gestation (Stark et al., 2005). Saturated fatty acids were convinced to be capable of stimulating TLR-mediated proinflammatory signaling pathways (Huang et al., 2012). Moreover, our previous study indicated that consumption of fish oil could attenuate mammary inflammation which might be linked to reduced plasma concentration of SFA (Lin et al., 2013). These results suggested that increased fatty acids metabolism around parturition may play roles in regulation of inflammatory responses in mammary glands. Therefore, detecting the variation in fatty acid composition of mammals across the reproductive cycle may be helpful for understanding why mammary inflammation occurs frequently around parturition.

On the other hand, it is well documented in cows (Castillo et al., 2005) and sows (Berchieri-Ronchi et al., 2011; Xie et al., 2015) that oxidative stress occurs at late gestation and early lactation due to severe catabolic status. And oxidative stress was known to be strongly linked to production of proinflammatory cytokines (Escobar et al., 2009; Yin et al., 2013; 2014; 2015), which has been known to be key factors in inducing mastitis (Oviedo-Boyso et al., 2007). In consequence, enhanced oxidative stress around parturition may affect the expression of proinflammatory cytokines in mammary gland. However, the variation of the proinflammatory cytokines across the reproductive cycle in mammary gland of mammals is poorly understood.

In the present study, the pregnant and age-matched non-pregnant rats were used as models to evaluate changes in plasma fatty acids profile and mammary proinflammatory cytokines expression in different time point of a reproductive cycle, which may further our understanding about the high incidence of mammary inflammation around parturition.

2. Materials and methods

2.1. Animals and facilities

All procedures outlined in this study were approved by the Animal Care and Use Committee of the Animal Nutrition Institute, Sichuan Agricultural University. All experiments involved animals were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The experimental rats (Virgin female Sprague-Dawley rats) were purchased from Sichuan Academy of Medical Sciences-Sichuan Provincial People's Hospital Experimental Animal Research Institute (Sichuan, China), and housed in galvanized-steel cages with bedding and maintained at a controlled temperature (22 ± 2°C) and relative humidity (60 ± 10%) with a 12-h light/dark cycle.

Table 1
Ingredients and composition of the experimental diet (air-dry basis).

Ingredient	Content, %	Composition	Content, %
Corn starch	39.75	Crude protein	16.23
Casein	20	ME, Mcal/kg	3.81
Gelatinization starch	13.2	Lysine	1.53
Sucrose	10	Methionine	0.57
Fat[1]	7	Calcium	0.50
Fiber	5	Available phosphorus	0.16
Mineral premix[2]	3.5		
Vitamin premix[3]	1		
L-cysteine	0.3		
Choline Chloride	0.25		
Total	100		

[1] The 7 kg fat was composed of 5 kg lard and 2 kg soybean oil in the lard diet.
[2] Provided per kg of diet: calcium 5000 mg, phosphorus 1561 mg, potassium 3600 mg, sodium 1019 mg, chlorine 1517 mg, magnesium 510 mg, iron 35 mg, zinc 30 mg, manganese 10 mg, copper 6 mg, selenium 0.15 mg, iodine 0.2 mg.
[3] Provided per kg of diet: vitamin A 4000 IU, vitamin D_3 1000 IU, vitamin K_3 0.75 mg, vitamin B_1 6.0 mg, vitamin B_2 7.0 mg, vitamin B_6 6.0 mg, vitamin B_{12} 0.02 mg, nicotinic acid 30.0 mg, D-calcium pantothenate 15.3 mg, folic acid 2.0 mg, biotin 0.2 mg.

2.2. Diets and treatments

The experimental diet (Table 1) was formulated to meet or exceed the nutrient requirements of gestating and lactating rats as recommended by AIN-93G. To set a proposed level of dietary SFA and n-3 polyunsaturated fatty acid (PUFA), the 7 kg fat included in the experimental diet was composed of 5 kg lard and 2 kg soybean oil. The fatty acid (FA) composition of the diet is showed in Table 2. The diet was stored at −20°C to avoid PUFA oxidation during the experimental period. A total of 47 rats were included in the experiment. When rats grew to sexual maturity, 1 female rat (231 ± 5 g) per cage was housed together with 1 male rat (weighing 300 to 330 g) in the same cage to complete mating. The females

Table 2
Fatty acid composition of the lard (g/100 g) and the diet (g/kg) (as fed basis).

Fatty acid[1]	Lard	Lard diet
C14:0	1.25	0.42
C16:0	26.14	9.91
C18:0	19.89	7.20
C20:0	0.34	0.17
C16:1	1.48	0.52
C18:1	37.62	15.70
C20:1	0.91	0.40
C22:1	ND	0.17
C18:2n6	10.20	10.05
C18:3n3	0.98	0.96
C20:5n3	ND	0.087
C22:6n3	ND	ND
Other	1.19	0.41
∑FA	100	46
∑SFA	47.62	17.70
∑MUFA	40.01	16.80
∑PUFA	11.18	11.09
∑SFA/∑FA	47.62	38.48
∑MUFA/∑FA	40.01	36.52
∑PUFA/∑FA	11.18	24.11
∑n-3	0.98	1.04
∑n-6	10.2	10.05
∑n-6/∑n-3	10.41	9.62

ND = not detected; FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[1] ∑FA means the sum of content of all fatty acids evaluated; ∑SFA means the sum of C14:0, C16:0, C18:0 and C20:0 content; ∑MUFA means the sum of C16:1, C18:1, C20:1 and C22:1 content; ∑PUFA means the sum of C18:2n6, C18:3n3, C20:5n3 and C22:6n3 content; ∑n-3 means the sum of C18:3n3, C20:5n3 and C22:6n3 content; ∑n-6 means the content of C18:2n6.