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Inhibition of arginase via jugular infusion of N^{ω}-hydroxy-nor-L-arginine inhibits casein synthesis in lactating dairy cows

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ABSTRACT

A previous in vitro study revealed that Arg elicits positive effects on casein synthesis through alterations of the Arg-ornithine pathway in bovine mammary epithelial cells. The main purpose of this work was to determine the effects of arginase inhibition using N^{ω}-hydroxy-nor-L-arginine (nor-NOHA) on milk protein synthesis in vivo. Six healthy Chinese Holstein cows with similar body weight (550.0 ± 20 kg; means ± standard deviation), parity (4), body condition score (3.0), milk yield (21.0 ± 1.0 kg), and days in milk (80 ± 2) were selected and randomly assigned to 3 treatments in a replicated 3 × 3 Latin square design with 22 d for each period (7 d for infusion and 15 d for washout). The treatments were (1) control: saline infusion; (2) nor-NOHA: infusion of 125 mg/L of nor-NOHA; (3) nor-NOHA + Arg: infusion of 125 mg/L of nor-NOHA with 9.42 g/L of Arg. The activity of enzymes related to Arg metabolism, milk protein synthesis, and expression of AA transporters was determined. The infusion of nor-NOHA decreased the activity of arginase but had no effect on the activity of ornithine decarboxylase and nitric oxide synthase in serum, and these responses were the same at the gene expression level in mammary gland. In addition, the infusion of nor-NOHA also reduced protein and fat synthesis in milk but had no effect on milk yield. When Arg was infused with nor-NOHA, the activity of total arginase, ornithine decarboxylase, and nitric oxide synthase, and the concentration of casein, protein, and fat in milk did not change compared with the nor-NOHA group, but the milk protein yield, the expression of some Arg transporters (*SLC7A5* and *SLC7A8*), and milk yield increased. Overall, results verified previous in vitro findings indicating that synthesis of casein protein is closely regulated by the Arg-ornithine pathway in bovine mammary gland.

Key words: arginine, N^{ω}-hydroxy-nor-L-arginine, milk casein synthesis, jugular vein infusion

INTRODUCTION

The synthesis and secretion of milk protein in mammary gland is affected by several factors including nutrition, genetics, environment, and management. As the raw materials of milk protein synthesis, some EAA (lysine, methionine, and histidine) elicit regulation on the synthesis of milk protein (Prizant and Barash, 2008; Kim and Wu, 2009). Recently, some studies revealed that Arg, a conditionally essential AA, also has some positive effects on the synthesis of milk protein (Mabjeesh et al., 2002; Doepel and Lapierre, 2011). Some recent studies (Chen et al., 2013; Wu et al., 2016) have demonstrated that the addition of Arg in culture medium increased casein protein synthesis in bovine mammary epithelial cells (**BMEC**). Moreover, Wang et al. infused Arg through jugular vein in dairy cows to measure the effects of Arg infusion on milk protein synthesis in vivo. They found the infusion of Arg (37.66 g/d) increased milk protein yield significantly [M. Z. Wang, Q. Y. Xu, G. Zhou, J. Zhang, and H. R. Wang (College of Animal Science and Technology, Yangzhou University, Yangzhou, China), and J. J. Loor (Department of Animal Science and Division of Nutritional Science, University of Illinois, Urbana), unpublished data].

Arginine can be degraded through 3 different ways (Wu et al., 2009) in mammals: (1) Arg-Orn, which is regulated by arginase; (2) Arg-nitric oxide, which is regulated by nitric oxide synthase (**NOS**); and (3) Arg-agmatine, which involves arginine decarboxylase. The conversion of Arg to Orn is reported to be the main pathway in mammary tissue (O'Quinn et al., 2002). The final products of the Arg-Orn pathway are polyamines (putrescine, spermidine, and spermine). Chen et al. (2015) reported that putrescine had some positive effects on the proliferation of cells, and the addition of

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spermidine and spermine elicited a positive effect on the synthesis of milk protein (Kong et al., 2014).

As the first rate-limiting enzyme in the Arg-Orn-Polyamine pathway (Wu et al., 2000), we hypothesized that arginase is the main regulator of milk casein synthesis. Decreasing activity of the Arg-Orn pathway with an arginase-specific inhibitor led to a decrease in the synthesis of casein and mammary cell proliferation. However, addition of Orn to the BMEC cultured with arginase inhibitor resulted in a recovery of casein synthesis and rate of cellular proliferation (Wang et al., 2017). These in vitro results provided strong indication that the Arg-Orn pathway is a key regulator of the synthesis of casein. To verify the in vitro results, the present study explored the effects of arginase inhibition on milk production, casein concentration in milk, and mammary gene expression of AA transporters through infusing arginase inhibitor in the jugular vein of lactating dairy cows.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures were approved by the Yangzhou University Animal Care and Use Committee of Jiangsu Province, China. The experiment was carried out at the Experimental Farm of Yangzhou University, Yangzhou city, Jiangsu province, China. Six Chinese Holstein lactating cows with similar BW (550.0 ± 20 kg), parity (4), BCS (3.0), milk yield (21.0 ± 1.0 kg), and DIM (80 ± 2 d) were selected for this experiment. The indwelling catheters (L13712, Jiangxi Huali Medical Instrument Company, Ganzhou, China) were placed in a jugular vein and flushed with heparin and physiological saline (750 IU/mL) twice daily during the week before infusion. The animals were fed a common diet (provided by the Experimental Farm of Yangzhou University) as a TMR according to NRC (2001). The composition and nutrient levels of basal diets are reported in Table 1. The experimental cows were fed twice daily at 0600 and 2000 h, milked thrice daily at 0700, 1500, and 2300 h. Cows were housed in a freestall barn, and had ad libitum access to the TMR and fresh water.

Cows were randomly assigned to 3 treatments in a replicated 3 × 3 Latin square design with 22 d for each period (d 1 to 7 for infusion and d 8 to 22 for washout). The treatments were as follows: (1) saline (control); (2) saline + 125 mg/L of N^{ω}-hydroxy-nor-L-arginine (**nor-NOHA**); (3) saline + 125 mg/L of nor-NOHA + 9.42 g/L of L-Arg (**nor-NOHA + Arg**). The dosage of infused nor-NOHA in the nor-NOHA group and nor-NOHA + Arg group per day was designed according to some previous studies in different animals (Reid et al., 2007; Kövamees et al., 2014). The dosage of Arg infused to cows per day was designed on the basis of the study of Wang et al. (2014). Wang et al. (2014) found that the mRNA expression of *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3* in BMEC cultured with different concentrations of Arg were different. Moreover, the cubic regression analysis shown that the expression of casein genes was highest when the concentration of Arg in medium was 434.44 mg/L, which was almost 1.56 times that (278 mg/L) in the control group. In a later experiment in lactating dairy cows [M. Z. Wang, Q. Y. Xu, G. Zhou, J. Zhang, and H. R. Wang (College of Animal Science and Technology, Yangzhou University, Yangzhou, China), and J. J. Loor (Department of Animal Science and Division of Nutritional Science, University of Illinois, Urbana)], the authors infused Arg through the jugular vein to evaluate the results in vitro. In the pre-experiment, they infused 37.66 g/d of Arg to make 104.91 g/d of supplementation of Arg to the mammary gland, which was 1.56 times that in the control group, and they found the infusion of 37.66 g/L of Arg increased the concentration of protein in milk. We infused this dosage of Arg in this study.

Table 1. Composition and nutrient levels of basal diets (DM basis)

Item	Concentration
Ingredient (g/kg)	
Alfalfa	209.00
Chinese wildrye	38.00
Corn silage	246.00
Cracked corn	277.00
Cottonseed meal	37.00
Soybean meal	135.00
Corn DDGS[1]	38.00
$CaHPO_4$	3.00
NaCl	5.00
Premix[2]	12.00
Total	1,000.00
Nutrient[3]	
NE_L (Mcal/kg)	1.51
CP (g/kg)	166.80
NFC (g/kg)	445.40
NDF (g/kg)	283.90
ADF (g/kg)	177.90
Ether extract (g/kg)	32.60
Ca (g/kg)	11.60
Total P (g/kg)	6.00

[1]Corn DDGS = corn distillers dried grains with solubles that contain 2.31 Mcal/kg of NE_L, 280 g/kg of CP, 390 g/kg of NDF, 150 g/kg of ADF, 110 g/kg of ether extract, 2 g/kg of Ca, and 8 g/kg of total P.

[2]The premix provided the following per kilogram of diet: 25 mg of $CuSO_4$, 75 mg of $FeSO_4 \cdot H_2O$, 105 mg of $ZnSO_4 \cdot H_2O$, 0.0024 mg of Co, 0.016 mg of Na_2SeO_3, 12,000 IU of vitamin A, 10,000 IU of vitamin D_3, 25 mg of vitamin E, 36 mg of nicotinic acid, and 1,000 mg of choline.

[3]The NE_L in the diet was calculated according to the NE_L of ingredients and their percentages; concentrations of the other nutrients were measured values.