Jugular infusion of arginine has a positive effect on antioxidant mechanisms in lactating dairy cows challenged intravenously with lipopolysaccharide[1]

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ABSTRACT: The main purpose of this work was to evaluate the effects of jugular L-arginine infusion on antioxidant mechanisms in lactating dairy cows challenged intravenously with lipopolysaccharide (**LPS**). Eight multiparous Holstein cows (609 ± 32 kg) at midlactation were randomly assigned to 5-d jugular infusions of Control (saline), Arginine (Arg, 18 g/d), LPS (0.2 μg/kg BW per day), and LPS + Arginine (0.2 μg/kg BW per day of LPS and 18 g/d of Arg) in a replicated 4 × 4 Latin square design with 4 infusion periods separated by 10-d. Jugular solutions of saline, Arg, LPS, and LPS + Arg were continuously infused using peristaltic pumps for approximately 6 h/d. Jugular vein serum samples were obtained on the last day of each infusion period before infusion (0 h) and at 3- and 6-h postinfusion. Compared with LPS treatment, Arg infusion increased the total antioxidant capacity and activity of glutathione peroxidase, but decreased malondialdehyde concentration ($P < 0.05$). The concentration of nitric oxide in serum and the activity of nitric oxide synthase were greater in LPS treatment compared with saline and Arg ($P < 0.05$). The Arg treatment significantly increased the serum insulin concentration at 3-h postinfusion compared with the saline treatment ($P < 0.05$), and that of LPS and LPS + Arg treatments were in between Arg and LPS treatments. No treatment effect was observed on the activities of superoxide dismutase and catalase ($P > 0.05$). In conclusion, enhancing the supply of Arg during an inflammatory challenge enhances antioxidant mechanisms in lactating dairy cows.

Key words: antioxidant mechanisms, arginine, dairy cow, lipopolysaccharide

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INTRODUCTION

In humans, Arg is classified as a semiessential amino acid and conditionally essential nutrient particularly during injury or stress (Stechmiller et al., 2004). Because of its role in multiple physiologic functions including its use for synthesis of nitric oxide (**NO**), polyamines, proline, and agmatine (Hamasu et al., 2009), Arg is also nutritionally important in livestock (Jobgen et al., 2006; Mateo et al., 2007; Zhang et al., 2018). In addition to these roles, Arg serves as a secretagogue for growth hormone,

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prolactin, and insulin, all of which could impact performance during lactation.

The role of Arg in helping alleviate immune challenges has been well established in non-ruminants (Li et al., 2007, Tan et al., 2009). For instance, Zhu et al. (2013) reported that dietary Arg supplementation protects and enhances intestinal mucosal immune barrier function and maintains intestinal integrity in weaned pigs. As to antioxidant mechanisms, Hu et al. (2016) reported dietary Arg supplementation enhances the antioxidant activity in broiler chickens, but the opposite result came out when supplemented with high concentration of Arg (>20.0 g/kg). Petrovic et al. (2008) reported N^{ω}-nitro-L-arginine methyl affected both early and late acclimation through attenuation and a decrease in the antioxidative defense response in rats (*Rattus norvegicus*). That strongly suggest the L-arginine/NO pathway is involved in the regulation of antioxidant mechanisms. Until now, a large number of current studies have focused on the immune function of Arg; few studies determined the regulatory effects of Arg on the antioxidant ability in animals, especially in ruminants. Therefore, we hypothesized that Arg supplementation may have a beneficial effect on antioxidant mechanisms during inflammatory challenges in lactating dairy cows. The present study was conducted to test this hypothesis and to gain a better understanding of the potential applicability of dietary Arg supplementation in the lactating dairy cow.

MATERIALS AND METHODS

All animal procedures in this study were conducted under the Guide for the Care and Use of Laboratory Animals by the Chinese Academy of Science.

Animals, Diets, and Treatments

Eight multiparous Holstein cows with an average BW of 609 kg and average days in milk of 201 d at the beginning of this study were chosen. Cows were housed in a free stall barn and were fed ad libitum to achieve a minimum of 5% refusals on an as-fed basis and had free access to fresh water. A common total mixed ration (Supplementary Table S1) was mixed at 0800 h and offered daily throughout this experiment. Diets were formulated to meet all nutrient requirements for a 600-kg Holstein cow producing 20 kg of milk containing 4.0% milk fat and 3.0% milk protein as evaluated according to NRC (2001). Cows were provisionally fitted with catheters (173 mm: 1.2 mm i.d:2.0 mm o.d.; Jiangxi Huali Medical Instrument, Ganzhou, China) in the jugular vein for 5 d preceding each infusion period.

Cows were randomly assigned to an infusion sequence of 4 treatments arranged in a 4 × 4 Latin square design. Each infusion periods lasted 5 d. To minimize carryover effects, 4 infusion periods were separated by 10-d intervals. Treatments included saline (Con), Arginine (Arg, 18 g/d), lipopolysaccharide (**LPS**; 0.2 μg/kg BW per day), and LPS + Arginine (0.2 μg/kg BW per day of LPS and 18 g/d of Arg). Cows assigned to the Con treatment received a jugular infusion of 1 L of 0.9% saline, cows assigned to the Arg treatment received 1 L of infusion solution containing 18 g of Arg (purity > 99%; Ajinomoto, Japan), cows assigned to the LPS treatment received a jugular infusion of 1 L of LPS infusion solution with 0.2 μg/kg BW of LPS (*Escherichia colli* O55:B5; Sigma–Aldrich, lot 2880, USA), and cows assigned to the LPS + Arg treatment received a jugular infusion of 1 L of LPS and Arg solution with 0.2 μg/kg BW of LPS and 18 g of Arg. The infusion solutions of LPS and Arg used for the jugular treatments were prepared each morning before infusions using glassware. The pH of the infusion solution (Arg, LPS + Arg) was adjusted to 7.5 with HCl in a daily volume, which was then filtered through 0.22-μm membrane filters (Millipore, Billerica, MA) into sterile bottles. The infusion dose of LPS was 0.2 μg/kg of BW (Waldron et al., 2003; Smith et al., 2015), and the length of time was approximately 6 h/d (Ning et al., 2018). The infusion dose of arginine was 18 g/d, which was calculated to account for 20% of baseline Arg in plasma (i.e., 90 g/d, unpublished data). Every day during the trial, infusions began 30 min after the morning milking, and infusions were delivered at a rate of 2.7 mL/min using peristaltic pumps (Longer BT100-1 L, Baoding, China). Catheters were flushed and filled with saline containing 100 U/mL heparin after the infusion to prevent coagulation.

Body Temperature

Body temperature was measured at 0900 h during each 5-d infusion period. Measurement was conducted with a veterinary clinical thermometer, with the glass part being disinfected with an alcohol swab and inserted into the rectum to a depth of approximately two-thirds of it. Body temperature was presented in Supplementary Fig. S1.

Blood Sampling and Analyses

On day 5 of each infusion period, blood samples (10 mL) were collected into serum tubes

(Becton Dickinson Vacutainer System, Franklin Lake, NJ) from the jugular vein before infusion (0 h) and at 3- and 6-h postinfusion. Samples of serum tubes were centrifuged (1,801 × *g*, at 4 °C for 10 min) 2 h after collection and a portion of serum was stored at −20 °C for the analysis of growth hormone, insulin, and prolactin. Then, a portion of serum aliquot (0, 3, and 6 h) in each period for each cow was pooled prior to storage at −20 °C for the analysis of total antioxidant capacity (**T-AOC**), malondialdehyde (**MDA**), glutathione peroxidase (**GSH-Px**), superoxide dismutase (**SOD**), catalase (**CAT**), NO, and nitric oxide synthase (**NOS)**. Bovine growth hormone, insulin, and prolactin were measured using commercial ELISA kits (MyBioSource Inc., USA, catalog numbers #MBS743413, #MBS2609963, #MBS2609707) following the manufacturer's instructions. Assay sensitivities were 0.1 ng/mL, 0.5 mIU/L, and 0.05 ng/mL, respectively. T-AOC, MDA, GSH-Px, SOD, CAT, NO, and NOS were assayed by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; catalog numbers A015-1, A003-1, A005, A001-3, A007-1-1, A012-1, A014-2), according to the manufacturer's instructions. Assay sensitivities were 0.2 U/mL, 0.5 nmoL/mL, 20 U, 0.5 U/mL, 0.2 U/mL, 1.0 μmol/L, and 5.95 U/mL, respectively. The inter- and intra-assay variation coefficients were no greater than 15% and no greater than 10%.

In addition, serum cytokines [IL-1β, IL-6, tumor necrosis factor (**TNF-α**)] were assayed in our previous (Zhao et al., 2018) to estimate the LPS effects. And blood samples were obtained at 9 and 10 d of the noninfusion period preceding each infusion to determine whether or not carryover effects of treatments occurred. These data are showed in Supplementary Table S2.

Statistical Analysis

The data were analyzed using general linear model procedures in SAS (version 9.2; SAS Institute Inc., Cary, NC), with treatment considered as fixed effect and period and cow as random effect. Period × treatment interactions were initially included and found to be not significant; hence, they were excluded from the final analyses. Duncan's multiple comparison test was used to determine treatment effects, and significant difference was declared at $P \leq 0.05$. All data are reported as the means with pooled standard errors (SEM).

RESULTS AND DISCUSSION

The results of inflammatory cytokines indicated that LPS infusion remarkably increased serum IL-1β, IL-6, and TNF-α compared with Con and Arg treatments during infusion periods. In addition, we also found that the body temperature was higher in LPS treatment than that in the Con and Arg treatments. Therefore, an inflammatory response induced by LPS of lactating dairy cows was established in our trial.

Glutathione peroxidase is one of the most important components of the antioxidant system of the body and can help remove lipid hydroperoxides from the body. A decrease in GSH-Px activity can result in accumulation of hydrogen peroxide, which can lead to tissue damage and activation of nuclear factor-κB-related inflammatory pathways (Yu and Chung, 2006). In the present study, we found that the activity of GSH-Px and the level of T-AOC were lower ($P < 0.05$) in cows challenged with LPS, whereas LPS + Arg treatment enhanced the activity of GSH-Px and the level of T-AOC. The combined action of SOD, CAT, and GSH-Px serves as a potent antioxidant system, but the concentrations of SOD and CAT were not modified by any of the treatments ($P > 0.05$). Malondialdehyde is the most abundant reactive aldehyde resulting from lactoperoxidase activity and has been widely used as an index of oxidative deterioration of lipids in food and biological samples (Garcia-Castro and Gaya, 1984). In the current study, compared with the saline treatment ($P < 0.05$), we also found that LPS treatment led to a significantly greater level of MDA, but LPS + Arg treatment decreased that response.

Together, the responses observed with LPS + Arg treatment indicated an overall positive effect of Arg on whole-body antioxidant mechanisms. As such, the present data agree with those of Petrovic et al. (2008) and Hu et al. (2016) in rats and broiler chickens in which Arg supplementation enhanced antioxidant mechanisms. Petrovic et al. (2008) working with rats also pointed out that the effect of Arg encompassed the L-arginine/NO pathway. Those data seem to agree with our finding that concentration of NO and activity of NOS were greater with LPS treatment compared with saline and Arg ($P < 0.05$). This may have resulted from the Arg infusion decreasing the activity of inducible nitric oxide synthase (Zhao et al., 2018) and indirectly influencing the activity of constitutive nitric oxide synthase although no difference was found for serum NO and NOS

between LPS + Arg and LPS in the current study ($P > 0.05$) (Table 1). Colasanti et al. (1995) suggested that both sodium nitroprusside (SNP, NO donor) and authentic NO solution are able to inhibit LPS-induced inducible nitric oxide synthase mRNA expression. Moreover, Tadié et al. (2013) suggested L-arginine supplementation was associated with an increased ornithine synthesis and reputed a preferential usage of the available arginine by arginase, only a few of arginine is catalyzed by NOS. That may give evidence to support our observation. Arg is a powerful secretagogue, stimulating the release of growth hormone, prolactin, and insulin (Newsholme et al., 2005). Xu et al. (2018) reported that Arg increased the concentration of growth hormone in broiler chickens. Similarly, Cochard et al. (1997) reported a significant increase in growth hormone when Arg was injected into jugular vein of 35-d-old piglets. The reasons for the lack of difference in serum growth hormone concentration among treatments in the present study ($P > 0.05$) are unknown. Because cows used were already past midlactation, it could be possible that sensitivity to Arg was less than typically observed at the onset and through peak lactation (Herbein et al., 1985).

Table 1. Effects of jugular-infused arginine on the oxidative status and the serum NO and NOS of lactating dairy cows challenged intravenously with LPS

	Experimental treatments[1]					
Item	Con	Arg	LPS	LPS + Arg	SEM	*P*-value[2]
Oxidative status[3]						
T-AOC, U/mL	8.90[a]	8.51[a]	4.87[b]	6.02[ab]	1.55	0.037
MDA, nmol/mL	2.19[b]	2.00[b]	3.61[a]	2.83[ab]	0.53	0.022
GSH-Px, μmol/L	247.47[a]	232.42[a]	168.34[b]	238.65[a]	34.66	0.031
SOD, U/mL	149.77	153.28	126.48	172.47	21.61	0.264
CAT, U/mL	8.80	7.97	7.17	6.30	1.45	0.354
NO/NOS						
NO, μmol/L	16.24[b]	18.89[b]	42.37[a]	38.09[a]	2.24	<0.001
NOS, U/mL	38.34[b]	38.62[b]	43.57[a]	41.74[a]	1.22	0.001

[a,b]Values within a row with different letters differ ($P < 0.05$).

[1]Treatments: Saline (Con); LPS (lipopolysaccharide, 0.2 μg/kg BW per day); Arg (Arginine, 18 g/d); LPS + Arginine (0.2 μg/kg BW per day of LPS and 18 g/d of Arg).

[2]Probability of a difference among the 4 treatments.

[3]T-AOC = total antioxidant capacity; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; NO = nitric oxide; NOS = nitric oxide synthase.

Table 2. Effects of jugular-infused arginine on the serum concentrations of growth hormone, insulin, and prolactin in lactating dairy cows challenged intravenously with LPS

	Experimental treatments[1]					
Item	Con	Arg	LPS	LPS + Arg	SEM	*P*-value[2]
Growth hormone, μg/L						
0-h preinfusion	18.60	19.05	19.37	17.37	2.68	0.964
3-h postinfusion	17.26	16.78	19.39	21.6	2.29	0.374
6-h postinfusion	17.01	16.42	18.92	19.69	2.19	0.657
Insulin, mIU/L						
0-h preinfusion	26.88	23.52	23.70	26.43	3.70	0.780
3-h postinfusion	23.30[b]	34.80[a]	26.33[ab]	30.25[ab]	3.51	0.071
6-h postinfusion	26.24	32.14	26.35	30.34	3.27	0.390
Prolactin, ng/L						
0-h preinfusion	286.18[b]	473.20[a]	334.53[b]	373.50[ab]	44.44	0.014
3-h postinfusion	303.32	420.02	306.22	408.48	50.17	0.695
6-h postinfusion	285.74	306.22	415.06	401.63	56.73	0.239

[a,b]Values within a row with different letters differ ($P < 0.05$).

[1]Treatments: Saline (Con); LPS (lipopolysaccharide, 0.2 μg/kg BW per day); Arg (Arginine, 18 g/d); LPS + Arginine (0.2 μg/kg BW per day of LPS and 18 g/d of Arg).

[2]Probability of a difference among the 4 treatments.

Compared with LPS infusion, we also detected greater ($P < 0.05$) concentration of insulin 0 h after infusion of Arg (Table 2). A positive response in concentration of insulin was reported previously by Kim and Wu (2004) in 7- to 21-d-old piglets supplemented with 0.4% Arg in the diet. At this stage of lactation, an increase in concentration of insulin at the same time that an inflammatory response was induced with LPS could help lessen the insulin-insensitive state that characterizes inflammation in cows (Waldron et al., 2003; Graugnard et al., 2013). As such, Arg could help not only the utilization on glucose by peripheral tissues but also synthesis of protein in muscle and mammary gland (Bionaz and Loor, 2011). It can be envisioned that enhancing the supply of Arg during periods where the cow is most susceptible to inflammation and oxidative stress (Loor et al., 2013) could help reduce catabolism of fat and protein.

At least in vitro, the positive effect of Arg on circulating concentrations of prolactin has been associated with the L-arginine/nitric oxide/cyclic guanosine monophosphate pathway specifically in decidual tissue (Kumari and Heffner, 2000). Duvilanski et al. (1995) found that NO exercises an inhibitory control on prolactin release via mediating the inhibitory actions of dopamine and atrial natriuretic factor. Thus, it could be possible that a similar effect was induced by 0-h infusion of Arg in the present study. Study by Sodhi et al. (2008) has shown that treatment of macrophages with prolactin significantly enhanced the production of cytokines, which influences humoral and cellular immunity as well. This result indicating that supplementation with Arg could have direct and indirect effects on immune function.

Overall, the results from the present study demonstrated that serum prolactin, insulin, and the antioxidant ability of lactating dairy cows challenged with LPS were declined. Jugular-infused arginine (18 g/d) was able to counteract those effects. Therefore, Arg could be used to improve the antioxidant capacity of lactating dairy cows challenged with LPS.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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