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Feeding a high-concentrate corn straw diet increased the release of endotoxin in the rumen and pro-inflammatory cytokines in the mammary gland of dairy cows

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Abstract

Background: The objective of this study was to investigate the effects of feeding a high-concentrate corn straw diet on the release of endotoxin in the rumen and the changes of pro-inflammatory cytokines in the mammary gland of dairy cows in comparison with a low-concentrate corn straw diet and a low-concentrate mixed forage diet. Thirty second-parity Chinese Holstein cows in mid-lactation with a body condition score of 2.86 ± 0.29, weighing 543 ± 57 kg and producing 24.32 ± 3.86 kg milk per day were randomly assigned to 1 of the 3 diets ($n = 10$ per treatment): 1) low-concentrate mixed forage diet (LCF) with a concentrate to roughage ratio of 46 : 54; 2) high-concentrate corn straw diet (HCS) with a concentrate to roughage ratio of 65 : 35; 3) low-concentrate corn straw diet (LCS) with the same concentrate to roughage ratio (46 : 54) as LCF. The experiment lasted 6 weeks, and samples were collected in the last week. Milk samples were analyzed for conventional components, rumen fluid samples were analyzed for pH and endotoxin, and mammary arterial and venous plasma samples were analyzed for concentrations of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α).

Results: Concentrations of endotoxin in rumen fluid and feces of cows fed HCS were significantly higher than those of cows fed LCS and LCF. Feeding HCS increased the release of IL-1β, IL-6 and IL-8 in the mammary gland compared with feeding LCS. Concentrations of cytokines (IL-1β and IL-8) in mammary venous plasma had a negative correlation with milk production efficiencies.

Conclusions: Results indicated that the high-concentrate corn straw diet increased the concentrations of endotoxin in rumen fluid and feces. Furthermore, feeding the high-concentrate corn straw diet stimulated the mammary gland to release more pro-inflammatory cytokines. The results suggest that feeding a high-concentrate corn straw diet induce a higher pro-inflammatory response in the mammary gland and thus may partly decrease the milk production efficiencies in dairy cows.

Keywords: Cytokine, Dairy cow, Endotoxin, Mammary gland

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Background

Feeding dairy cows diets containing high proportions of concentrate to support high milk production is associated with the high incidence of subacute ruminal acidosis (SARA) [1-3]. SARA, a well recognized digestive disorder, poses a health threat to lactating dairy cows. The high rumen digestibility of most grains in concentrate mixtures increases the rumen's production of volatile fatty acids and causes a corresponding drop in rumen pH [1,4,5], which can result in alterations in the rumen environment, leading to changes in the composition of rumen microbiota [6,7] and accumulation of endotoxin (or lipopolysaccharide, LPS), a potentially harmful cell-wall component of all gram-negative bacteria [2,8,9]. LPS can translocate into the bloodstream across the epithelium barrier of the gastrointestinal tract wall [4,10,11] and trigger inflammatory responses in cows [12-14]. The systemic inflammation caused by feeding cows diets rich in concentrate is associated with a variety of metabolic and immunologic alterations [2,15]. Furthermore, the systemic inflammation may reduce supply of milk component precursors for milk synthesis by repartitioning more nutrients for synthesis of immune molecules [3]. Nevertheless, the association between the rise of free endotoxin in rumen fluid during the feeding of diets high in concentrate and the local inflammation in the mammary gland in dairy cows has not yet been documented. In addition, in many countries, corn straw is frequently used in the diets of cows due to a lack of quality roughages. Consequently, the proportion of concentrate has to be raised to meet the nutrient requirements for lactation. Therefore, we hypothesized that increasing the concentrate proportion in a corn straw-based diet will result in increased release of endotoxin in the rumen, which may ultimately elicit a local inflammation in the mammary gland. Therefore, the objective of this study was to evaluate the effects of feeding a high-concentrate corn straw diet on the release of endotoxin in the rumen and pro-inflammatory cytokines in the mammary gland of dairy cows in comparison with a low-concentrate corn straw diet and a low-concentrate mixed quality forage diet.

Methods

Animals, diets and experimental procedures

Thirty second-parity Chinese Holstein cows in mid-lactation with a body condition score of 2.86 ± 0.29, averaging 543 ± 57 kg of BW and producing 24.32 ± 3.86 kg milk per day at the onset of the experiment, were randomly assigned to 1 of the 3 diets ($n = 10$ per treatment): 1) low-concentrate mixed quality forage diet (LCF) with a concentrate to roughage ratio of 46 : 54, containing Chinese wildrye, alfalfa hay, and corn silage. This diet is commonly regarded as an ideal diet type for lactating cows and is served as control in the study; 2) high-concentrate corn straw diet (HCS) with a concentrate to roughage ratio of 65 : 35, containing corn straw as the only roughage; 3) low-concentrate corn straw diet (LCS) with the same concentrate to roughage ratio (46 : 54) as LCF. The LCS diet also contained corn straw as the only roughage and is served as another control in the study. The concentrate of all the 3 diets consists of corn, soybean meal, whole cottonseed, $CaHPO_4$, NaCl and premix. The ingredients and nutrient compositions of the experimental diets are presented in Table 1. Diets were mixed and offered as total mixed ration (TMR) twice daily (08:30 and 17:30 h). Orts were recorded and discarded before the next feeding each day and the amount of feed was adjusted to ensure a 5% feed residual. Cows were milked twice daily at 08:00 and 19:00 h, and milk yield was recorded electronically throughout the experiment period. The experiment lasted 6 weeks, and the last week was samples collection period. Cows were housed with playground and with free access to water. Cows in the experiment showed no clinical signs of

Table 1 Diet composition of the experimental diets

Item	Diet		
	LCF	HCS	LCS
Ingredient (% of dry matter)			
Chinese wildrye	3.7	-	-
Corn silage	26.7	-	-
Alfalfa hay	23.4	-	-
Corn straw	-	35.0	53.8
Corn	24.6	35.26	24.6
Soybean meal	14.8	20.82	14.8
Whole cottonseed	5.1	7.18	5.1
Dicalcium phosphate	0.6	0.84	0.6
Salt	0.5	0.5	0.5
Premix[1]	0.6	0.6	0.6
Total	100	100	100
Concentrate to roughage ratio	46:54	65: 35	46:54
Nutrient composition (% of dry matter)			
Net energy (Mcal/kg)	1.50	1.54	1.40
Crude protein	16.8	16.9	13.9
Neutral detergent fiber	37.6	34.4	46.0
Acid detergent fiber	23.9	19.9	26.7
Non-fiber carbohydrate[2]	37.0	41.3	32.6
Ether extract	3.4	3.2	2.5
Calcium	0.50	0.45	0.42
Phosphorus	0.38	0.41	0.35

[1]Contained Cu (as sulfate), 2,142 mg/kg; Mn (as sulfate), 15,428 mg/kg; Zn (as sulfate), 15,428 mg/kg; Co (as chloride), 28 mg/kg; I (as iodate), 231 mg/kg; Se (as selenite), 57 mg/kg; vitamin A, 2,285,000 IU/kg; vitamin D, 457,000 IU/kg; and vitamin E, 11,400 mg/kg.

[2]Non-fiber carbohydrate = 100 − (% Neutral detergent fiber +% Crude protein +% Ether extract +% Ash).

infectious diseases. This experiment was conducted at the Inner Mongolia Dairy United Technology Co., Ltd., Hohhot, China. All experimental procedures and the care of the animals were approved by the Inner Mongolia Agricultural University, and the ethical approval for conducting this study was also granted by the University. The methodology employed in this study was in accordance with the REFLECT guidelines (http://www.reflect-statement.org/statement/docs/reflectstatementchecklist.pdf).

Milk sampling and analysis and milk production efficiency calculation

Milk samples ($n = 10$ per treatment) were obtained twice daily at 08:00 and 19:00 h in the last week of the experiment, and the daily samples were mixed with a ratio of 6 : 4 and analyzed for milk fat and protein contents by mid-infrared spectroscopy (MilkoScan FT120, Foss). Milk fat and protein yields were calculated from milk yield and milk fat and protein contents, and milk fat and protein synthesis efficiencies were calculated by dividing milk fat and protein yields with measured dry matter intake (DMI), respectively. Milk synthesis efficiency and 4% fat corrected milk (FCM) synthesis efficiency were calculated by dividing milk yield and 4% FCM with DMI, respectively. DMI measures were obtained by multiplying the TMR intake by the dry matter content of the TMR.

Rumen fluid and feces sampling and analysis

Rumen fluid samples ($n = 10$ per treatment) were collected into two plastic containers 3 hours after the morning feeding on day 4 of the last week by an oral stomach tube equipped with a strainer and a syringe, as described by Shen et al. [16]. The initial 50 mL rumen fluid samples were discarded in order to avoid saliva contamination. The pH of rumen fluid was determined immediately by a mobile pH meter (Rex PHS-3E, INESA Scientific Instrument Co., Ltd). Fecal grab samples ($n = 10$ per treatment) were collected from the rectum on day 3 of the last week. The rumen fluid and feces samples were stored at −20°C until being analyzed for LPS contents.

Concentrations of free LPS in the rumen fluid and feces were determined by the chromogenic end-point assay using the limulus amebocyte lysate (LAL) test reagent kit purchased from Xiamen Limulus Experiment Factory. The initial processing before LPS determination was as described by Khafipour et al. [14] with some modifications. In brief, rumen fluid samples were thawed quickly and centrifuged (Sigma 3-18 K, Sigma-Aldrich) at 6,000 × *g* for 15 min, and 1.5 mL of the supernatant was centrifuged again at 10,000 × *g* for 30 min. The supernatant was then passed through a disposable 0.22 μm sterile, pyrogen-free filter (Millex, Millipore Corporation). The filtrate was collected into a sterile, depyrogenated glass tube (previously heated at 250°C for 2 h) and heated at 100°C for 30 min. Samples were cooled at room temperature and diluted using pyrogen-free water and pyrogen-free test tubes until their LPS concentrations were in the range of 0.1 to 1 endotoxin units (EU)/mL relative to the reference endotoxin (*Escherichia coli* O111:B4) provided by the manufacturer. In this analysis, rumen fluid samples were diluted 40,000-fold. Samples were analyzed according to the manual for the optical density at 545 nm on a microplate reader (Synergy H4, BioTek). Thawed feces samples were vortex mixed with an equal amount of physiological saline (9 g/L NaCl) for 15 min. The mixture was then immediately processed for LPS assay using the same procedure as described above for rumen fluid samples.

Blood sampling and analysis

Blood samples ($n = 10$ per treatment) were obtained shortly before the morning feeding from the mammary (external pudendal) artery and the mammary vein, respectively, on day 6 and 7 of the last week of the trial. Cows were in a standing posture and restrained from movement during the whole process of blood sampling. In order to keep cows sedated, 20 mL procaine hydrochloride was injected into the muscles near the artery blood collection point, which was the triangle centre between the femoral joint and the hip joint. The operator then put one hand into cow's rectum against the external pudendal artery, and used the other hand to hold the sterile needle (18#, 20 cm long) which was vertically penetrated into the fossa formed through the converging of the internal abdominal oblique muscle, the vastus lateralis muscle and the gluteus medius muscle, after shearing and disinfection. The mammary vein blood was collected at the site where the superficial abdominal vein can be easily located, after shearing and disinfection. Blood samples were collected into 10-mL vacuum tubes (Shu Guang Jian Shi) containing heparin sodium anticoagulant and were stored in ice and centrifuged (Feige TDL-40B, Shanghai Anting Scientific Instrument Factory) within 10 min at 3,000 × *g* and 4°C to harvest plasma. The plasma was divided into 1 mL aliquots, labeled immediately, and stored at −20°C until being analyzed for cytokine concentrations.

The concentration of interleukin (IL)-1β in plasma was determined by a commercially available human IL-1β ELISA kit (Invitrogen Corporation) according to the manufacturer's instructions, as described by Shuster et al. [17]. The specificity of the IL-1β activity was verified by blocking with recombinant human IL-1 receptor antagonist [17]. All samples including the standards were tested in duplicate, and the optical density values were read on an automatic microplate reader (ELx808; BioTek, Winooski,

VT) at 450 nm. The minimum detection limit of the assay was 3.9 pg/mL. The concentration of IL-6 in plasma was quantified in duplicate by means of a commercially available human IL-6 ELISA kit (Invitrogen Corporation). The human IL-6 kit has been used as the assay standard for bovine IL-6 determination [17]. According to the manufacturer, the minimum detection limit of the assay was 7.8 pg/mL as defined by the linear range of standard curves. The samples were tested in duplicate, and the optical density at 450 nm was measured on a microplate reader (BioTek). Concentrations of IL-8 in plasma were determined with a commercially available human IL-8 ELISA kit (Invitrogen Corporation), which has been reported to cross-react with bovine IL-8 [17]. The measurable concentrations ranged from 15.6 to 1000 pg/mL. Plasma samples were tested in duplicate, and the optical density at 450 nm was measured on a microplate reader (BioTek). Concentrations of tumor necrosis factor alpha (TNF-α) in plasma were measured by a commercially available bovine TNF-α ELISA kit (Bethyl Laboratories, Inc.) according to the manufacturer's instructions. All samples including the standards were tested in duplicate, and the optical density values were read on a microplate reader (BioTek) at 450 nm. The measurable concentrations ranged from 0.078 to 5 ng/mL.

Statistical analyses

The general linear model (GLM) of SPSS (v.18) was used in the data analysis. The GLM included the random cow effect and the fixed effect of diets, and mean differences for all variables were separated and compared using Duncan multiple comparison procedure. Data are presented as means ± standard deviation. Significance was declared at $P < 0.05$ and a tendency was considered if $0.05 < P < 0.10$. Statistical correlation was performed using GraphPad PRISM 5.0, and standard error, *P*-value, and R^2 were computed and used to evaluate the goodness of fit.

Results

DMI, milk yield, milk composition and milk production efficiencies

Data for DMI, milk yield, milk composition, and milk production efficiencies are shown in Table 2. Cows fed LCS had less DMI than cows fed LCF or HCS ($P < 0.05$), however, DMI was not different between LCF and HCS ($P > 0.05$). Milk yield and 4% FCM were significantly different among the diets ($P < 0.01$). Cows fed LCF had higher milk yield and 4% FCM than cows fed HCS, and cows fed LCS had the lowest milk yield and 4% FCM. The contents of milk fat and protein in cows fed HCS and LCS were comparable ($P > 0.05$), which were lower than cows fed LCF ($P < 0.05$). Cows fed LCS had the lowest daily milk fat and protein yield ($P < 0.05$), whereas cows fed LCF had the highest daily milk fat and protein yield ($P < 0.05$). Cows fed LCF had higher milk production efficiencies than cows fed HCS or LCS, whereas the milk production efficiencies in cows fed HCS were not different from those in cows fed LCS.

Ruminal pH, and LPS in rumen fluid and feces

There were no significant differences in ruminal pH between the LCF group and HCS group ($P > 0.05$), whereas the ruminal pH values of cows fed LCF and HCS were lower ($P < 0.01$) than those of cows fed LCS (Figure 1). The highest LPS content of rumen fluid was observed in cows fed HCS ($P < 0.01$), and cows fed LCS and LCF had similar concentrations of ruminal LPS ($P > 0.05$) (Figure 2).

Table 2 Effect of different diets on milk production performance in dairy cows

Items	Diets[1]			*P*-value
	LCF	HCS	LCS	
Dry matter intake (kg/d)	20.74 ± 1.87^{a}	20.33 ± 2.23^{a}	18.52 ± 0.97^{b}	0.022
Milk yield (kg/d)	25.64 ± 4.71^{A}	21.75 ± 3.89^{B}	16.84 ± 4.60^{C}	<0.001
4% fat corrected milk (kg/d)	26.27 ± 3.99^{A}	20.38 ± 3.72^{B}	16.74 ± 5.00^{C}	<0.001
Milk fat content (%)	4.23 ± 0.69^{a}	3.58 ± 0.42^{b}	3.95 ± 0.52^{b}	<0.001
Milk protein content (%)	3.18 ± 0.19^{a}	3.07 ± 0.22^{b}	3.04 ± 0.13^{b}	0.012
Milk fat yield (kg/d)	1.07 ± 0.18^{a}	0.78 ± 0.16^{b}	0.67 ± 0.22^{c}	<0.001
Milk protein yield (kg/d)	0.81 ± 0.13^{A}	0.66 ± 0.11^{B}	0.51 ± 0.14^{C}	<0.001
Milk synthesis efficiency (g/g)	1.23 ± 0.18^{a}	1.08 ± 0.16^{ab}	0.98 ± 0.22^{b}	0.019
4% fat corrected milk synthesis efficiency (g/g)	1.23 ± 0.18^{a}	1.01 ± 0.14^{b}	0.95 ± 0.25^{b}	0.011
Milk fat synthesis efficiency (%)	4.91 ± 0.97^{a}	3.83 ± 0.58^{b}	3.74 ± 1.16^{b}	0.016
Milk protein synthesis efficiency (%)	3.86 ± 0.42^{a}	3.30 ± 0.38^{ab}	2.94 ± 0.67^{b}	0.001

[1]LCF is low-concentrate mixed forage diet with a concentrate to roughage ratio of 46 : 54; HCS is high-concentrate corn straw diet with a concentrate to roughage ratio of 65 : 35; LCS is low-concentrate corn straw diet with the same concentrate to roughage ratio (46 : 54) as LCF.
$^{A-C}$Means within a row differ ($P < 0.01$).
$^{a-c}$Means within a row differ ($P < 0.05$).

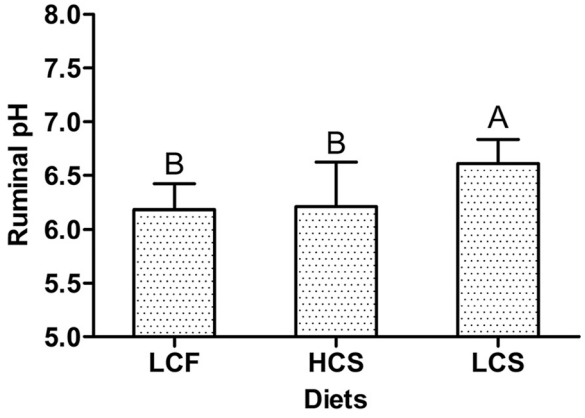

Figure 1 Effect of different diets on ruminal pH of dairy cows. LCF is low-concentrate mixed forage diet with a concentrate to roughage ratio of 46 : 54; HCS is high-concentrate corn straw diet with a concentrate to roughage ratio of 65 : 35; LCS is low-concentrate corn straw diet with the same concentrate to roughage ratio (46 : 54) as LCF. $^{A-B}$Means between treatments differ ($P < 0.01$).

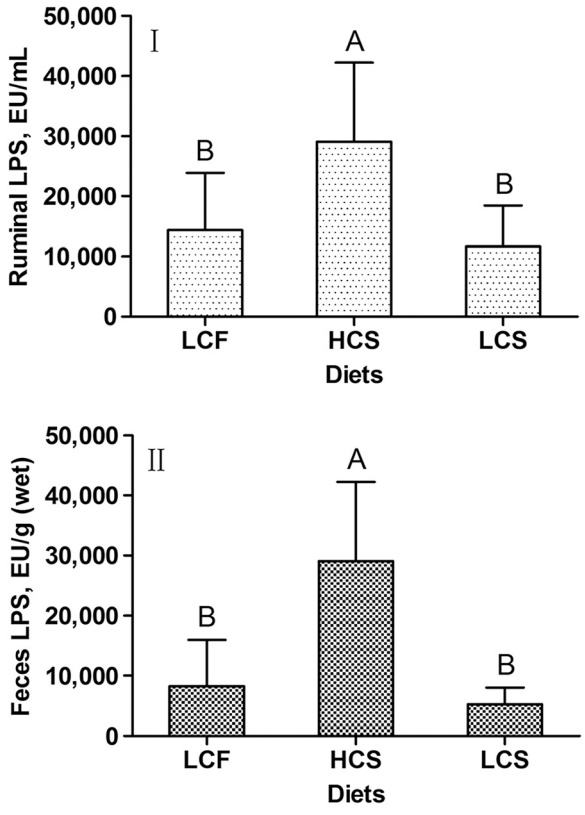

Figure 2 Effect of different diets on the concentrations of endotoxin (LPS) in rumen fluid (I) and feces (II) of dairy cows. LCF is low-concentrate mixed forage diet with a concentrate to roughage ratio of 46 : 54; HCS is high-concentrate corn straw diet with a concentrate to roughage ratio of 65 : 35; LCS is low-concentrate corn straw diet with the same concentrate to roughage ratio (46 : 54) as LCF. $^{A-B}$Means between treatments differ ($P < 0.01$).

Both groups of cows fed LCF and LCS had lower concentrations of LPS in the feces compared with the group fed HCS ($P < 0.01$). There were no differences in fecal LPS contents between the groups of cows fed LCF and LCS ($P > 0.05$).

Cytokines in mammary artery and vein blood

No differences in mammary arterial plasma contents of IL-1β, IL-6, IL-8 and TNF-α among the experiment diets were observed ($P > 0.05$; Table 3). However, the concentrations of mammary venous plasma IL-1β and IL-8 in the HCS group were higher than those of other groups ($P < 0.05$). The concentrations of mammary venous plasma IL-6 in the HCS group were higher than those of the LCS group ($P < 0.05$), but not different from those of the LCF group ($P > 0.05$). The mammary venous plasma TNF-α contents in the HCS group were numerically higher than those of the other two groups, but the data were highly variable and the difference did not attain statistical significance.

Relationships between mammary venous plasma cytokines and milk production efficiencies

The relationships between concentrations of mammary venous plasma IL-1β, IL-6, IL-8 and milk production efficiencies (milk synthesis efficiency, 4% FCM synthesis efficiency, milk fat synthesis efficiency, and milk protein synthesis efficiency) in dairy cows are presented in Figures 3, 4, 5, respectively. The increase in concentration of mammary venous plasma IL-1β was associated with a decline in milk production efficiencies (except milk protein synthesis efficiency; Figure 3). However, there were no significant relationships between mammary venous plasma IL-6 concentration and milk production efficiencies (Figure 4). The increase in concentration of mammary venous plasma IL-8 was also associated with a decline in milk production efficiencies (except milk synthesis efficiency; Figure 5).

Discussion

Khafipour et al. [14] reported that DMI, milk yield, and milk fat percentage were reduced when they used pellet containing 50% wheat and 50% barley to replace 21% of the dry matter (DM) of the control diet with a concentrate to roughage ratio of 50:50. Results of our study showed that milk yield, 4% FCM, milk fat percentage, milk protein percentage and milk production efficiencies were lower in cows fed HCS than those in cows fed LCF, although the nutrient levels were similar between these two diets. The reduction in milk production performance in cows fed HCS may be due to the adverse effects resulting from the increased concentrate proportion [3,10,11] as well as the low quality of corn straw. As demonstrated in this study, feeding a high-concentrate diet induced more

Table 3 Effect of different diets on cytokine contents in mammary arterial and venous plasma of dairy cows

Items[1]		Diets[2]			*P*-value
		LCF	HCS	LCS	
Artery	IL-1β (pg/mL)	217.12 ± 32.93	226.08 ± 32.20	221.05 ± 27.03	0.407
	IL-6 (pg/mL)	63.43 ± 20.96	67.09 ± 20.03	64.35 ± 19.39	0.683
	IL-8 (pg/mL)	77.82 ± 17.39	91.12 ± 17.96	81.97 ± 17.18	0.273
	TNF-α (ng/mL)	0.55 ± 0.85	0.72 ± 0.99	0.32 ± 0.38	0.546
Vein	IL-1β (pg/mL)	211.42 ± 25.45^{b}	246.99 ± 36.62^{a}	210.98 ± 20.27^{b}	0.030
	IL-6 (pg/mL)	86.10 ± 7.12^{b}	82.73 ± 9.08^{b}	74.77 ± 9.22^{a}	0.050
	IL-8 (pg/mL)	76.26 ± 11.45^{B}	98.58 ± 11.71^{A}	77.34 ± 9.52^{B}	0.001
	TNF-α (ng/mL)	0.55 ± 0.88	0.82 ± 1.10	0.38 ± 0.44	0.323

[1]IL = interleukin, TNF = tumor necrosis factor.
[2]LCF is low-concentrate mixed forage diet with a concentrate to roughage ratio of 46 : 54; HCS is high-concentrate corn straw diet with a concentrate to roughage ratio of 65 : 35; LCS is low-concentrate corn straw diet with the same concentrate to roughage ratio (46 : 54) as LCF.
$^{A-B}$Means within a row differ ($P < 0.01$).
$^{a-b}$Means within a row differ ($P < 0.05$).

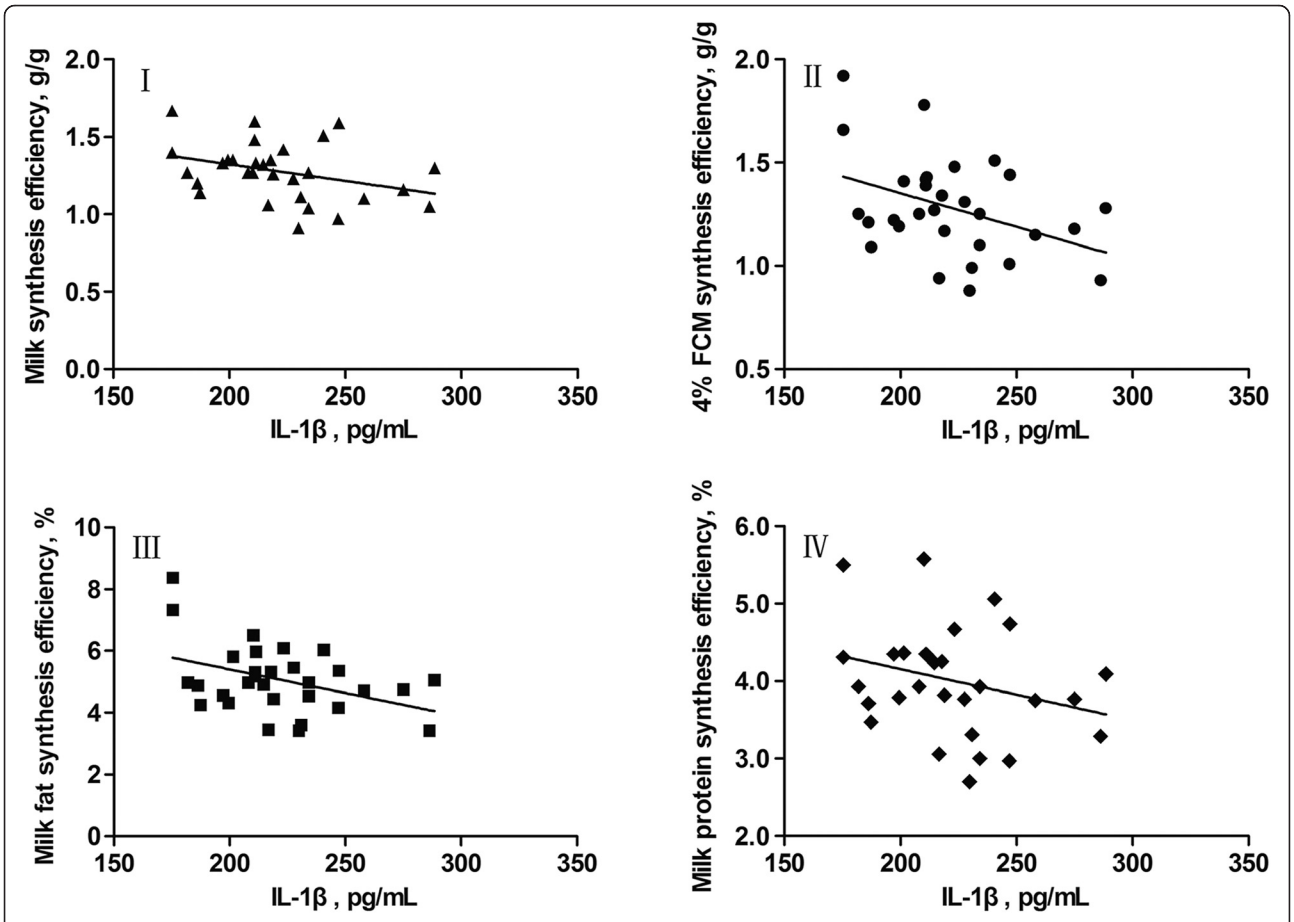

Figure 3 Linear models fitted to data of milk synthesis efficiency (I), 4% FCM synthesis efficiency (II), milk fat synthesis efficiency (III), and milk protein synthesis efficiency (IV), which are expressed as *y*, in response to concentrations of IL-1β (expressed as *x*) in mammary gland venous plasma of dairy cows. I, $y = -0.0022x + 1.753$ ($R^2 = 0.1183$, $r = -0.3439$, SE = 0.1771, $P = 0.0627$, $n = 30$); **II**, $y = -0.0033x + 2.003$ ($R^2 = 0.1606$, $r = -0.4007$, SE = 0.2244, $P = 0.0282$, $n = 30$); **III**, $y = -0.0153x + 8.457$ ($R^2 = 0.1678$, $r = -0.4096$, SE = 1.026, $P = 0.0246$, $n = 30$); **IV**, $y = -0.0066x + 5.474$ ($R^2 = 0.0795$, $r = -0.2820$, SE = 0.6769, $P = 0.1311$, $n = 30$).

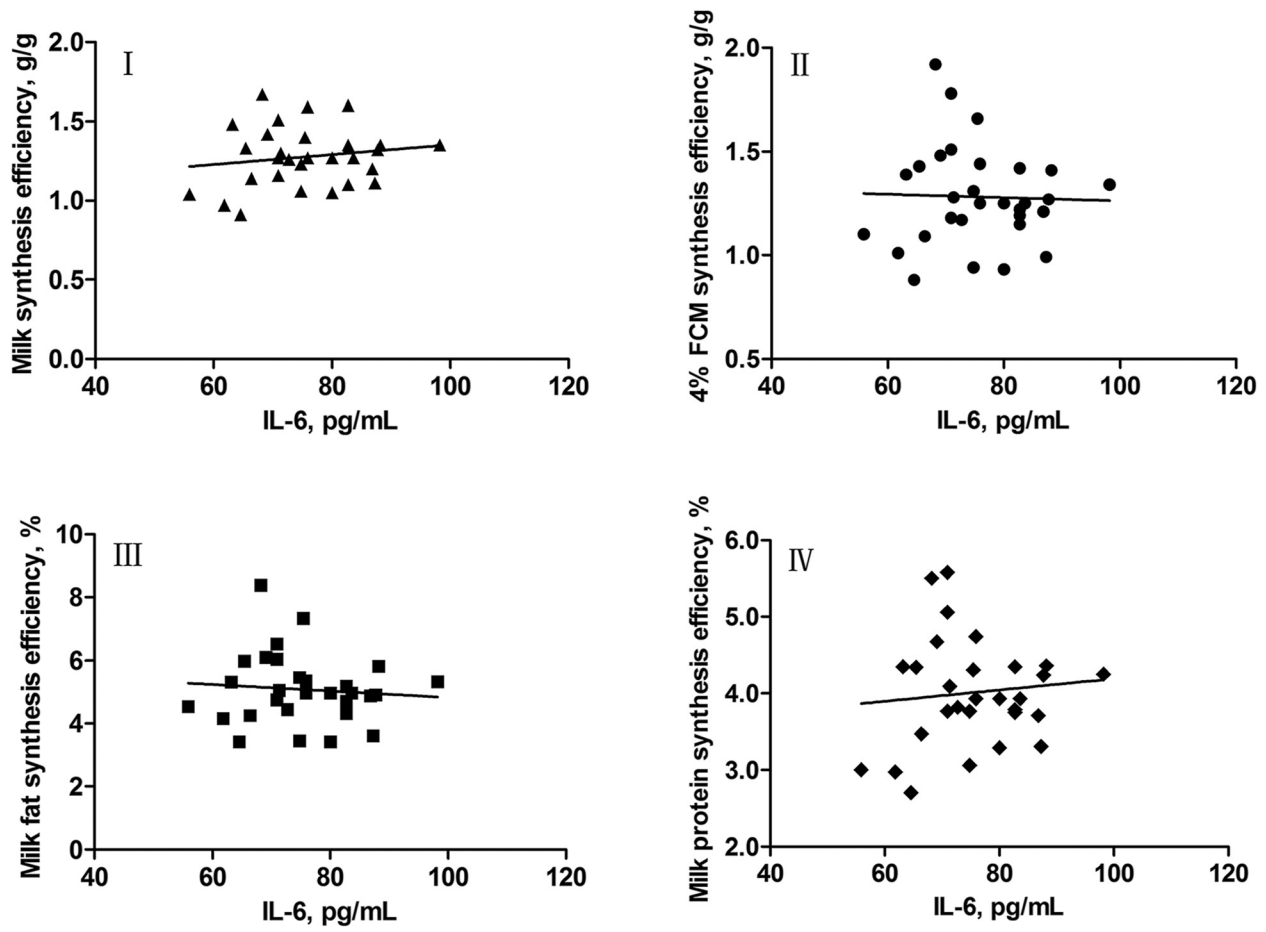

Figure 4 Linear models fitted to data of milk synthesis efficiency (I), 4% FCM synthesis efficiency (II), milk fat synthesis efficiency (III), and milk protein synthesis efficiency (IV), which are expressed as *y*, in response to concentrations of IL-6 (expressed as *x*) in mammary gland venous plasma of dairy cows. I, $y = 0.0031x + 1.041$ ($R^2 = 0.0257$, $r = 0.1603$, SE = 0.1862, $P = 0.3979$, $n = 30$); **II**, $y = -0.0008x + 1.343$ ($R^2 = 0.0010$, $r = -0.0316$, SE = 0.2448, $P = 0.8671$, $n = 30$); **III**, $y = -0.0103x + 5.856$ ($R^2 = 0.0079$, $r = -0.0889$, SE = 1.120, $P = 0.6408$, $n = 30$); **IV**, $y = 0.0074x + 3.455$ ($R^2 = 0.0102$, $r = 0.1001$, SE = 0.7019, $P = 0.5956$, $n = 30$).

LPS in the rumen, which can elicit systemic inflammatory response and local inflammatory response in the udder after translocation of LPS into blood [3]. Under the circumstances, nutrients will be directed to support proinflammatory events. The redirection or repartition of nutrient use in addition to a low nutrient supply due to the low quality of corn straw will decrease nutrient availability for milk synthesis. Furthermore, the LPS entering the mammary tissue may directly exert harmful effects on the mammary epithelial cells, and those effects include reducing proliferation of the epithelial cells, increasing cell apoptosis, and suppressing the activity of key enzymes in milk component synthesis such as fatty acid synthetase, acetyl-CoA carboxylase and lipoprotein lipase [3].

Results of this study also showed that DMI, milk yield, 4% FCM, milk fat percentage, milk protein percentage and milk production efficiencies were lower in cows fed LCS than those in cows fed LCF. The poor milk production performance in cows fed LCS could be attributed to the low DMI and low dietary energy and protein levels in comparison with cows fed LCF. In this study, cows fed HCS had higher DMI, milk yield, and 4% FCM than cows fed LCS, which could be attributed to the higher dietary energy and protein levels in the HCS diet due to the increased concentrate proportion and the reduced straw proportion. However, the milk production efficiencies between the two groups of cows were not different and the reason may be that the HCS diet induced more ruminal LPS, the adverse effects of which compromised the milk production efficiencies.

A large number of studies indicate feeding cows diets high in concentrate is associated with low rumen pH [14,18]. Accordingly, in our study the ruminal pH of cows fed the high-concentrate diet (HCS) was significantly lower than that of cows fed the low-concentrate diet (LCS). Interestingly, the ruminal pH in cows fed the low-

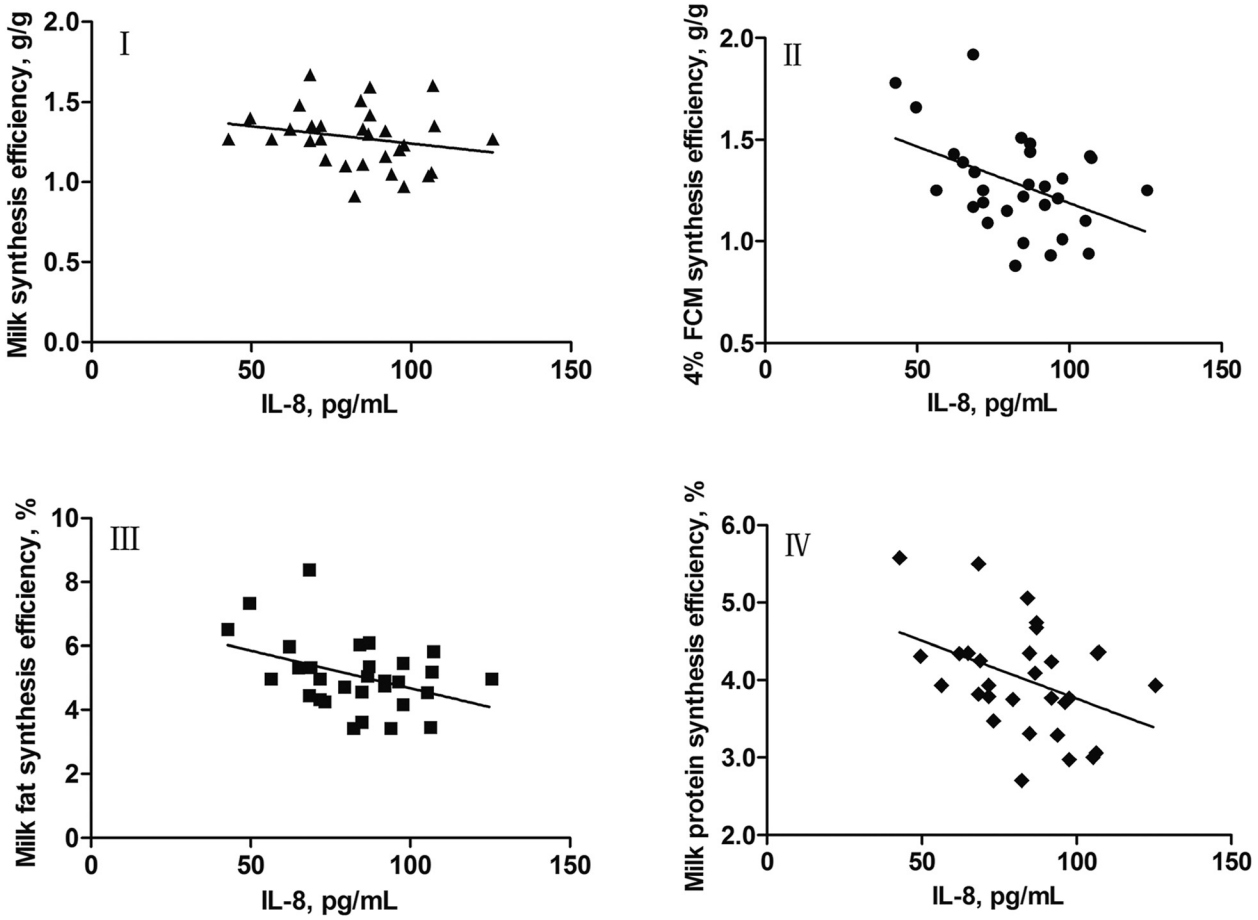

Figure 5 Linear models fitted to data of milk synthesis efficiency (I), 4% FCM synthesis efficiency (II), milk fat synthesis efficiency (III), and milk protein synthesis efficiency (IV), which are expressed as *y*, in response to concentrations of IL-8 (expressed as *x*) in mammary gland venous plasma of dairy cows. I, $y = -0.0022x + 1.457$ ($R^2 = 0.0477$, $r = -0.2184$, SE = 0.1840, $P = 0.2462$, $n = 30$); **II**, $y = -0.0056x + 1.746$ ($R^2 = 0.1879$, $r = -0.4335$, SE = 0.2207, $P = 0.0167$, $n = 30$); **III**, $y = -0.0236x + 7.037$ ($R^2 = 0.1595$, $r = -0.3394$, SE = 1.031, $P = 0.0288$, $n = 30$); **IV**, $y = -0.0149x + 5.255$ ($R^2 = 0.1622$, $r = -0.4027$, SE = 0.6458, $P = 0.0273$, $n = 30$).

concentrate diet (LCF) was not different from that in cows fed the high-concentrate diet (HCS). In this study, the LCF diet contained 26.7% (DM basis) of corn silage that was rich in lactic acid, which may serve as an explanation for the low ruminal pH observed for this low-concentrate diet.

Our results showed that feeding dairy cows the high-concentrate diet (HCS) was associated with increased concentrations of LPS in the rumen fluid and feces. Cows receiving 65% concentrate (HCS) had higher amounts of free LPS in rumen fluid and feces compared with cows receiving 46% concentrate (LCS or LCF). As expected, no differences in the amount of free LPS in rumen fluid and feces between the groups fed LCF and LCS were obtained. Our findings are consistent with previous studies demonstrating enhanced content of LPS in rumen fluid from feedlot steers or dairy cows fed diets containing high proportions of grain [19,20]. Emmanuel et al. [12] reported a 10-fold increase in the concentration of LPS in rumen fluid of cows when dietary barley grain increased from 15% to 45%. Another study reported the concentration of LPS in rumen fluid increased about 3-fold when steers were fed a 61% concentrate diet compared with those fed an all-forage diet [21]. Results of this study also showed the concentration of LPS in feces increased with increasing the diet concentrate. This is in agreement with the study by Li et al. [22] who reported the grain based SARA challenge increased LPS in feces from 12,832 to 118,522 EU/g.

LPS produced in the gastrointestinal tract can be translocated into the bloodstream, thus the concentration of peripheral blood LPS increases [14,23]. Khafipour et al. [14] reported that the concentrations of both ruminal and blood LPS increased, when they replaced 21% of the DM of the control diet with a concentrate to roughage ratio of 50:50 with pellet containing 50% ground wheat and 50% ground barley. It was also observed in other studies that SARA led to a rise of blood LPS concentrations [24,25].

Our study is the first to report the concentrations of pro-inflammatory cytokines in mammary arterial and venous plasma. Results of this study demonstrated that the release of cytokines (IL-1β, IL-6, IL-8 and perhaps TNF-α) increased in the mammary gland when cows fed the high-concentrate diet (HCS) compared with cows fed the low-concentrate diet (LCS). The increases in the release of cytokines in the mammary gland provide evidence of LPS translocation into this tissue and activated local inflammation in the tissue. A study by Lee et al. [26] also showed that the concentrations of IL-8 and TNF-α in milk increased immediately after administering healthy cows with 100 μg LPS. IL-8 is one of the most important chemokines for recruitment of neutrophils to inflammatory sites [27]. TNF-α is stimulated and secreted after activation of nuclear factor-κB pathway via toll-like receptors [28], and causes pathophysiological events similar to that of endotoxaemia or septic shock [29]. IL-1β is produced as a pro-cytokine upon initial stimulation of immune cells via toll-like receptors [30]. IL-6 is a complex cytokine in the roles of enhancing or limiting the immune response [30]. In our study, the high content of IL-6 detected in cows fed LCF may be attributed to that IL-6 is a duplicitous cytokine playing both pro- and anti-inflammatory roles.

The negative relationships between the concentrations of mammary venous plasma IL-1β and IL-8 and milk production efficiencies in dairy cows were demonstrated in our study. The adverse effects of LPS on lactation were detailed in a review by Dong et al. [3]. The reduced milk production efficiencies in dairy cows may be partly attributed to the local inflammation in the mammary tissue induced by LPS translocated into the body from the digestive tract. As a result of the local inflammation, more nutrients or precursors of milk components will be directed to support immune processes, resulting in less precursors being used for synthesizing milk components. LPS may also exert harmful effects on the mammary epithelial cells [31,32] and suppress the activity of enzymes involved in milk component synthesis [33-35].

Conclusions

Feeding dairy cows a corn straw-based diet with a high proportion of concentrate was associated with higher concentrations of LPS in ruminal fluid and feces. Furthermore, feeding the high-concentrate corn straw diet increased pro-inflammatory cytokines in the mammary venous blood, and the concentrations of cytokines (IL-1β and IL-8) in mammary venous plasma had a negative correlation with milk production efficiencies. The results suggest that feeding a high-concentrate corn straw diet induce a higher pro-inflammatory response in the mammary gland and thus may partly decrease the milk production efficiencies in dairy cows.

Competing interests
None of the authors has any financial or personal competing interests that could inappropriately influence or bias the content of the paper.

Authors' contributions
JZ and GD conceived the overall idea of this study and wrote the manuscript. CA and KE contributed to animal experiment design. SZ, MQ, XW, YW, LJ, CL and ZZ participated in animal experiment and samples analysis. All authors read and approved the manuscript.

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