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Original Research Article

Responses of milk production of dairy cows to jugular infusions of a mixture of essential amino acids with or without exclusion leucine or arginine

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ABSTRACT

The purpose of this study was to determine effects of jugular infusion of either balanced or imbalanced amino acid mixture on milk production and composition in dairy cows. Eight mid-lactation Holstein cows were randomly assigned to 5-d continuous jugular infusions of saline (CTL), essential amino acid (EAA) mixture prepared on the profile of casein (CSN, 160 g/d), EAA mixture excluding leucine (Leu) (−Leu, 163 g/d) or EAA mixture excluding arginine (Arg) (−Arg, 158 g/d) in a duplicated 4 × 4 Latin square design with 4 infusion periods separated by a 7-d interval period. The basal diet was formulated with corn grain, soybean meal, cottonseed meal, corn silage, alfalfa hay and Chinese wildrye grass hay according to NRC (2001) and supplied 1.6 Mcal net energy for lactation (NE_L) and 94.4 g metabolizable protein (MP) per kg dry matter (DM) to meet requirements for lactation. The results showed that the dry matter intake (DMI) and normal physiological status were not affected by amino acid mixture infusions. Compared with CTL treatment, the CSN treatment increased milk yield (14.9%, $P < 0.001$), milk lactose yield (14.5%, $P = 0.001$), milk fat yield (16.6%, $P = 0.01$), milk protein yield (18.2%, $P < 0.001$) and the contents of α_{S1}-casein (α_{S1}-CN, 11.8%, $P = 0.007$), β-casein (β-CN, 4.2%, $P = 0.035$) and κ-casein (κ-CN, 8.5%, $P = 0.003$). However, the −Leu and −Arg treatments had lower milk yield (6.3%, $P = 0.058$ and 5.7%, $P = 0.073$, respectively), milk protein yield (8.8%, $P = 0.010$ and 8.2%, $P = 0.011$, respectively) and the contents of α_{S1}-CN (7.3%, $P = 0.057$ and 8.4%, $P = 0.026$, respectively), β-CN (4.2%, $P = 0.033$ and 3.8%, $P = 0.048$, respectively) and κ-CN (5.8%, $P = 0.023$ and 7.6%, $P = 0.003$, respectively) than those of the CSN treatment. Milk lactose yield (5.9%, $P = 0.076$) tended to decrease when Leu was removed from amino acid mixture infusate. In conclusion, the supply of casein profile can increase milk production in dairy cows, but a deficiency of Leu or Arg had negative effects on milk yield and milk protein yield.

1. Introduction

Dairy milk is rich in nutrients and active substances, so it plays a key role in human healthy life. In order to improve the nutrients composition of milk, more and more researches on improving milk quality and safety has been conducted in recent years. Milk protein is an important component of milk and is a sign of core competitiveness in dairy industry. Milk protein response to supplemental metabolizable protein (MP) depends on numerous factors, such as stage of lactation, basal protein and energy status, and the amount and composition of the protein or amino acids (AA) supplied (Aikman et al., 2002). A dairy cow diet consisting of the balanced essential AA (EAA) profile can enhance the efficiency of conversion of MP into milk protein (Rulquin et al., 1993; NRC, 2001). It is reported that casein infusion has increased significantly milk yield and the content of milk lactose in postpartum transition dairy cows (Bach et al., 2000; Larsen et al., 2014).

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Leucine (Leu) and arginine (Arg) are both widely recognized as functional AA, which are absorbed by mammary gland and used for cellular and milk protein synthesis, provision of metabolic intermediates and energy (Mackle et al., 1999; Li et al., 2009) and the activation of signal factors (Bequette et al., 1998; Wang et al., 2014). Branched chain amino acids (BCAA), particularly Leu, can act as signaling molecules to regulate protein synthesis (Kimball and Jefferson, 2004). Leucine has been reported to promote β-lactoglobulin synthesis in mammary epithelial cells (MEC) (Moshel et al., 2006). Moreover, as a metabolic intermediate of Leu, α-ketoisocaproate can suppress the secretion of glucagon, and stimulate the insulin secretion to decrease the protein catabolism (Sener et al., 1982); thereby, the efficiency of protein synthesis in animals was increased.

Arg is absorbed 2 to 3 times the amount which appears in milk protein by the mammary gland beyond the requirement (Mepham, 1982). A large amount of Arg taken up by animals can regulate endocrine secretion and lactation, improve immunity and digestive function, promote self-repairing capability of the tissue, and increase the capacities of antioxidation and anti-stress in the form of itself or metabolite, for example, nitric oxide (NO), polyamine, ornithine or proline (Wu et al., 2009; Kim and Wu, 2009). However, most of researches on functional AA are mainly focused on monogastric animals, the biological roles of functional AA in ruminants is still unclear.

A greater response is generated in the deficiency deleting an AA from the balanced AA profile than in the supplementation of the balanced AA profile (Weekes et al., 2006; Lapierre et al., 2009). Our hypothesis was that deletion of Leu or Arg from the EAA profile of casein would affect milk yield and milk protein yield. The treatments were isonitrogenous to ensure adequate synthesis of the nonessential AA (NEAA) because Leu and Arg provide C and α-amino N for NEAA synthesis (Wohlt et al., 1977). This study was carried out to determine whether the deletion of Leu or Arg affected milk yield or protein synthesis in dairy cows when methionine and lysine supply met the requirement for lactation; the effects of balanced AA mixture infusions on normal physiological status of dairy cows were investigated as well.

2. Materials and methods

2.1. Animals and feedings

The experiment was carried out at the Inner Mongolia Dairy United Technology Co., Ltd., Hohhot, China. Four first-lactation and 4 multiparous Holstein cows averaging 108 ± 11 days (means ± SD) in milk (DIM) and 509 ± 56 kg of body weight were used for this study. The average daily milk yield of the cows was 22.52 ± 3.10 kg/d at the beginning of the experiment, with protein and fat concentrations of 3.17 ± 0.1% and 4.04 ± 0.58% of milk, respectively. Cows were provisionally fitted with catheters (137 mm length, 1.2 mm inner diameter, 2.0 mm outer diameter; Jiangxi Huali Medical Instrument Co., Ltd., Ganzhou, China) in the jugular vein for 5 d preceding each infusion period. Cows were housed in a free stall barn when not receiving infusions and in individual tie stalls when receiving infusions. Cows were fed *ad libitum* to achieve a minimum of 5% refusals and had free access to fresh water. Daily feed intake and refusals of each cow were measured throughout the study. Cows were milked twice daily at 05:30 and 17:30. All experimental procedures and the care of the animals were approved by the Inner Mongolia Agricultural University.

The experimental diet (Table 1) was formulated to meet or exceed all nutrient requirements for a 550 kg Chinese Holstein cow producing 25 kg of milk containing 4.0% milk fat and 3.0% milk protein, and was evaluated according to NRC (2001). Cows were fed the basal diet throughout the experimental period, and the cows were given a 14-d adaptation period before the first infusion.

Table 1
Ingredient and chemical composition of the total mixed ration (TMR).

Item	Quantity
Ingredients, % of dry matter (DM)	
DM, %	55.1
Chinese wildrye grass hay	3.7
Whole plant corn silage	26.7
Alfalfa hay	23.4
Ground corn grain	24.07
Soybean meal	16.5
Cottonseed meal	4.0
Dicalcium phosphate	0.6
NaCl	0.5
Premix[1]	0.53
Nutrient composition, g/kg of DM	
Organic matter	931
Crude protein	164
Ether extract	27.1
Neutral detergent fiber	424
Acid detergent fiber	246
Nutrient supply[2], per kg of DM	
Net energy for lactation, Mcal	1.60
Metabolizable protein, g	94.4

[1] Contained copper sulfate, manganous sulfate, zinc sulfate, cobalt chloride, iodate, selenite, and vitamins A, D and E.
[2] Computed by the NRC (2001) model.

2.2. Experimental design and treatments

The cows were randomly assigned to 1 of 4 treatments in a duplicated 4 × 4 Latin square design. The treatments were 5-d continuous jugular infusions of 1) saline (CTL), 2) EAA mixtures imitating the composition of casein (ideal profile of EAA; CSN), 3) EAA mixtures excluding Leu (−Leu), 4) EAA mixtures excluding Arg (−Arg) (Table 2). To eliminate carryover effects, treatment periods were separated by 7-d noninfusion periods. The profile of EAA in the infusates was the same as the AA composition of casein except that methionine was increased in the total infusion AA, and a 3:1 ratio of lysine to methionine was maintained. The EAA were dissolved in a 0.8% (wt/vol) NaOH solution prepared with ultrapurified water. The pH was adjusted to 7.4 with HCl in a daily volume of 2.5 L/cow (Aikman et al., 2002). The solution was then filter

Table 2
Daily amount of amino acids infused.

Amino acid, g/d	Treatments[1]			
	CTL	CSN[2]	−Leu	−Arg
Thr	0	13.9	13.9	13.9
Val	0	22.3	22.3	22.3
Met	0	9.12	9.12	9.12
Ile	0	19.1	19.1	19.1
Leu	0	30.2	0.0	30.2
Phe	0	16.8	16.8	16.8
Lys	0	27.4	27.4	27.4
His	0	9.36	9.36	9.36
Arg	0	11.9	11.9	0.0
Glu[3]	0	0.0	33.6	10.0

[1] CTL = saline; CSN = essential amino acids (EAA) mixture prepared on EAA composition of casein; −Leu = Leu removed from the EAA mixture; −Arg = Arg removed from the EAA mixture.
[2] The profile of EAA in the infusates was the same as that is found in casein with the exception that methionine was increased in the total infusion AA, a 3:1 ratio of lysine to methionine was maintained.
[3] For a deficiency of Leu and Arg, Glu was used to provide the sources of C and α-amino N for nonessential AA synthesis according to the isonitrogenous principle in the groups of −Leu and −Arg.

sterilized through a 0.22 μm membrane filter into sterile bottles and stored for no more than 3 d at 2 to 4 °C until needed. The solution was infused continuously through a peristaltic pump for 6 h/d.

2.3. Sampling and laboratory analyses

The quantity of the diet offered and orts were weighed daily. Feed ingredients and total mixed ration (TMR) were collected on the last 2 d of each period and stored at −20 °C before subsequent analysis. All feed ingredients and TMR samples were dried at 60 °C to a constant weight to determine DM and then ground. Crude protein (N × 6.25) was analyzed using a HYP-308 Digest Stove and KDN-103F Automatic Nitrogen Determinator (Shanghai Xianjian Instrument Co. Ltd., Shanghai, China), whereas ADF and NDF were determined using an Ankom200I fiber analyzer (Ankom Technology, Macedon, NY) with amylase and sodium sulfite according to the procedures described by Van Soest et al. (1991). Feed ingredients and TMR samples were pooled by period before analysis.

Milk yield was recorded at each milking, and milk samples were taken at the last 2 d of each experimental period. Daily milk samples were mixed with a ration of twice milk production for each cow and measured by Bentley FTS/FCM 400 Combi (Bentley Instrument, Inc., Minnesota, USA) to determine protein, fat, and lactose yields. Milk samples were pooled by each cow within period before the analysis. Milk samples of α_{s1}-casein (α_{s1}-CN), α_{s2}-casein (α_{s2}-CN), β-casein (β-CN) and κ-casein (κ-CN) were assayed using ELISA with MD-SpectraMax M5 plate reader (Molecular Devices Corporation, USA). For the assay of α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN, removal of milk fat by centrifugation preceded ELISA as described by Chen et al. (2013). The enzymatic kits were used for α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN (α_{s1}-CN ELISA kit, α_{s2}-CN ELISA kit, β-CN ELISA kit and κ-CN ELISA kit, respectively; Shanghai Xinyu biotechnology Co. Ltd., Shanghai, China). Blood samples from jugular veins were taken on the last day of each experimental period before the morning feeding for hematological examination using XFA 6030 Animal Blood Cell Analyzer (Nanjing Perlong Medical Devices Co. Ltd., Nanjing, China).

2.4. Statistical analysis

Before statistical analysis, dry matter intake (DMI), milk yield, and milk composition were averaged over the last 2 days of each infusion period. The data were analyzed by ANVOA using general linear models procedures of SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA):

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk,}$$

where Y_{ijk} = response variable value of the kth cow subjected to the ith treatment in the jth period, μ = the grand mean, $e_{ijk,}$ = the random error, T_i = fixed effect of the ith treatment (i = CTL, CSN, −Leu and −Arg), P_j = fixed effect of the jth period (j = 1, 2, 3, and 4), C_k = fixed effect of the kth cow. The maximum number of available observations (n = 8) was used. Treatment differences were determined by LSD multiple comparison procedure and were considered significant if $P \leq 0.05$ and as a trend for $0.05 < P \leq 0.10$. All data are reported as means with pooled standard errors (SEM).

3. Results

3.1. Dry mater intake and milk production

The DMI, milk yield and milk composition of the treatments are reported in Table 3. Compared with the CTL treatment, DMI had no obvious change after cows were jugular-infused the EAA profile of casein. The DMI was slightly higher for the removal Leu from AA mixture infusate ($P = 0.065$), but the −Arg treatment had no effect on DMI than the CSN treatment. Milk yield, the yields of protein, fat and lactose and the contents of α_{s1}-CN, β-CN and κ-CN were significantly increased by 14.9%, 18.2%, 16.6%, 14.5%, 11.8%, 4.2% and 8.5% respectively in CSN treatment than those of CTL. The deletion of Leu (CSN vs. −Leu) and Arg (CSN vs. −Arg) from the infusate decreased the milk yield (6.3%, $P = 0.058$; 5.7%, $P = 0.073$), milk protein yield (8.8%, $P = 0.010$; 8.2%, $P = 0.011$), α_{s1}-CN (7.3%, $P = 0.057$; 8.4%, $P = 0.026$), β-CN (4.2%, $P = 0.033$; 3.8%, $P = 0.048$) and κ-CN (5.8%, $P = 0.023$; 7.6%, $P = 0.003$) compared with CSN treatment. Moreover, there was a trend to decrease the lactose yield (5.9%, $P = 0.076$) by the deletion of Leu infusion.

3.2. Hematological examinations

The immunocytes and other blood parameters of hematological examination are presented in Table 4. Infusion of the AA profile of casein tended to increase the monocyte percentage (0.93%, $P = 0.077$), but decrease the hemoglobin (4.8%, $P = 0.073$) compared with the CTL treatment, and the other parameters were not modified by the CSN treatment. Furthermore, the deletion of Leu or Arg from the infusate had no effect on the immunocytes or other blood parameters ($P > 0.1$).

4. Discussion

4.1. Feed intake

The DMI had no obvious change after infusion of the EAA profile of casein in dairy cows. It is possible that short-term continuous

Table 3
Effects of jugular infusion of AA mixtures on dry matter intake and the yield and composition of milk in lactating dairy cows.

Item	Treatments[1]				SEM	*P*-value		
	CTL	CSN	−Leu	−Arg		CTL vs. CSN	CSN vs. −Leu	CSN vs. −Arg
DM intake, kg/d	14.9	14.8	16.2	15.6	0.47	0.909	0.065	0.267
Milk yield, kg/d	20.5	23.5	22.0	22.2	0.48	<0.001	0.058	0.073
Protein yield, kg/d	0.65	0.77	0.70	0.71	0.02	<0.001	0.010	0.011
Fat yield, kg/d	0.79	0.92	0.87	0.88	0.03	0.010	0.286	0.378
Lactose yield, kg/d	1.04	1.18	1.11	1.14	0.02	0.001	0.076	0.226
α_{S1}-CN, g/L	6.82	7.63	7.07	6.99	0.19	0.007	0.057	0.026
α_{S2}-CN, g/L	2.26	2.42	2.27	2.29	0.08	0.178	0.219	0.278
β-CN, g/L	9.99	10.41	9.97	10.02	0.13	0.035	0.033	0.048
κ-CN, g/L	2.73	2.96	2.79	2.74	0.05	0.003	0.023	0.003

DM = dry matter; α_{S1}-CN = α_{S1}-casein; α_{S2}-CN = α_{S2}-casein; β-CN = β-casein; κ-CN = κ-casein.
[1] CTL = saline; CSN = essential amino acids (EAA) mixture prepared on EAA composition of casein; −Leu = Leu removed from the EAA mixture; −Arg = Arg removed from the EAA mixture.

Table 4
Effects of jugular infusion of AA mixtures on blood immunocytes and other parameters in lactating dairy cows.

Item	Treatments[1]				SEM	*P*-value		
	CTL	CSN	−Leu	−Arg		CTL vs. CSN	CSN vs. −Leu	CSN vs. −Arg
WBC, 10^9/L	9.31	9.24	8.04	9.74	0.65	0.934	0.213	0.591
Lymphocyte count, 10^9/L	3.61	3.18	3.07	3.53	0.25	0.219	0.774	0.333
Monocyte count, 10^9/L	0.79	0.83	0.71	0.84	0.07	0.692	0.267	0.855
Neutrophil count, 10^9/L	4.99	5.24	4.26	5.37	0.48	0.711	0.172	0.848
Lymphocyte percent, %	36.4	39.0	39.6	36.0	2.28	0.433	0.857	0.373
Monocyte percent, %	8.50	9.43	8.96	8.76	0.36	0.077	0.370	0.206
Neutrophil percent, %	54.9	52.7	50.6	52.7	3.13	0.619	0.649	0.997
RBC, 10^{12}/L	6.39	6.10	6.07	6.39	0.14	0.139	0.876	0.166
Hemoglobin, g/L	102	96.9	96.6	99.6	1.85	0.073	0.909	0.320
HCT, %	30.1	28.7	28.5	29.1	0.62	0.109	0.857	0.657
PLT, 10^9/L	387	388	441	387	49.1	0.984	0.460	0.984

WBC = white blood cell; RBC = red blood cell; HCT = hematocrit; PLT = platelet.
[1] CTL = saline; CSN = essential amino acids (EAA) mixture prepared on EAA composition of casein; −Leu = Leu removed from the EAA mixture; −Arg = Arg removed from the EAA mixture.

infusion did not affect DMI. Appuhamy et al. (2011) also observed nonsignificant changes in DMI when dairy cows were infused with Lys and Met (12 and 21 g/d, respectively) or plus BCAA (Leu: 35 g/d, Ile: 15 g/d, and Val: 15 g/d, respectively) in the jugular vein. In this study, DMI was slightly increased when Leu was removed from AA mixture infusate. This result may be caused by the offset effects of changing DMI for the imbalanced AA supply. Conversely, Robinson et al. (2000) reported a significant decrease in DMI when 16 g/d methionine was infused abomasally in cows fed a corn and timothy silage diet. But this effect was not observed in the −Arg treatment. Varvikko et al. (1999) did not observe any negative effects on DMI in response to an abomasal infusion of 40 g/d methionine when cows were fed a grass silage diet.

4.2. Milk production

Lactation performance of dairy cows depends on many factors, such as lactation stage, basal protein and energy status (Aikman et al., 2002), nitrogen content in rumen, digestion and metabolism of carbohydrates and lipids, the integration and redistribution of the metabolites by the liver, and the amount and composition of the protein or AA supplied to the mammary gland. Bauman et al. (2006) reported that the content and composition of precursor of milk directly affect the synthesis of milk fat and milk protein. In our study, the infusion of AA mixture based on casein profile significantly increased the milk yield and protein yield in dairy cows, and enhanced the contents of α_{s1}-CN, β-CN and κ-CN. These results are similar to findings of Doepel and Lapierre (2011). This indicates that the supply of balanced AA profile could improve milk production and transformation efficiency of nitrogen. In this study, fat yield and lactose yield were also increased in the CSN treatment, which may be due to the fact that the balanced profile of AA promoted the growth and proliferation of MEC, and improved the uptake of acetate and butyrate (precursors of milk fat) and propionate (precursor of milk lactose) from blood. Above results are similar to previous reports. Doepel and Lapierre (2011) have demonstrated that the uptake efficiency of glucose by mammary gland trended to increase when 356 g NEAA/d was abomasally infused into cows. And the increases of fat yield were previously reported for proline (Pro) supplementation both in cows (Bruckental et al., 1991) and goats (Alumot et al., 1983). Whereas Rius et al. (2010) found that the yields of milk, protein, fat and lactose had no significant changes with abomasal infusion of casein (0.86 kg/d). The inconsistency may be associated with the different infusion tissues, since the milk composition synthesis under jugular infusion of AA is greater than postruminal infusion of AA (Schei et al., 2007).

Amino acids are not only building blocks for the milk protein but also act as key regulators of the signal transduction pathways during milk protein synthesis (Bolster et al., 2004). Recent studies demonstrated that some bioactive amino acids modulate mRNA translation through downstream targets of the mTOR signaling pathway (Wu, 2009). The deletion of Leu significantly decreased the β-lactoglobulin synthesis of mammary epithelial cells in vitro, and inhibited the activation of 4EBP1 and S6K1 which are downstream targets of the mTOR signaling pathway, suggesting that Leu had effects on regulating the protein synthesis and modulating signaling pathway related to milk protein synthesis (Moshel et al., 2006). Lei et al. (2012) also reported that increasing the circulating amount of Leu was beneficial to lactation. The negative effect on the yield of milk, protein and lactose observed in the −Leu treatment could possibly be a result of an imbalance among BCAA supplied or a direct deficiency of Leu, which suppressed the growth and proliferation of MEC and inhibited the uptake of AA by MEC. These results are in agreement with the observations of Iburg and Lebzien (2000) and Rulquin and Pisulewski (2006) when Leu was infused into dairy cows.

Doepel and Lapierre (2011) proposed that abomasal infusion of the Arg-deleted AA mixture into cows had almost no effect on yield and composition of milk when the supply of other EAA was sufficient. However, in the current study, we observed significant decreases in milk protein yield and the contents of α_{s1}-CN, β-CN and κ-CN, and milk yield had a tendency to decrease when cows were infused the Arg-deleted AA mixture. This is in agreement with Chew et al. (1984), who reported that milk production for the first 22 wk of lactation tended to be higher for cows jugular-infused with 0.1 g Arg/kg body weight, compared to cows infused with saline. In present study, the decreases of milk protein yield and the contents of α_{s1}-CN, β-CN and κ-CN were possibly caused by a low uptake of Arg into MEC when AA mixture infusate is imbalance. Since Arg could stimulate proliferation of MEC and regulate the transcription and translation of casein gene by activating Jak2-Stat5 and mTOR pathways (Xu, 2012).

4.3. Physiological status

Hematological examinations are usually used for assessing the physiological status of animals and mainly measure the blood cells, including red cells, white cells and platelets. Hence, we observed the quantitative change and species distribution to judge whether animals is in the normal physiological status. We observed the monocyte percentage was trended to increase, and conversely, the hemoglobin was trended to decrease with infusion of the AA profile of casein in respect to the CTL treatment, but the numerical values were still in the normal range. No significant difference was presented between the other comparative treatments. These results are in agreement with the report of Ding (2016), which is likely to be due to a small amount of infusion or short infusion time, the normal physiological status of animals has no obvious change.

5. Conclusion

Overall, we observed DMI was not changed by infusing the AA mixtures in dairy cows. Jugular-infusion of the AA mixture (160 g/d) of casein profile could increase the milk yield and protein yield in dairy cows, but these parameters were decreased by deletion of Leu or Arg from the infusate compared with the infusion of casein profile AA mixture. No significant change on physiological status appeared across treatments when dairy cows were given the short-term durative infusion.

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