Short Paper

Effect of protected-glutamine supplementation on performance, milk composition and some blood metabolites in fresh Holstein cows

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Summary

The present study was conducted to study the effect of protected-glutamine (Gln) supplementation on dry matter intake (DMI), milk yield (MY) and composition, somatic cell counts (SCC) and blood parameters in fresh cows. Forty Holstein cows at zero day of parturition (calving day = day 0) were divided into four groups (n=10), and fed (*ad libitum*) with one of the diets including: basal diet (control), basal diet supplemented with 150 (low Gln, LG), 250 (medium Gln, MG) or 350 (high Gln, HG) g of Gln protected with formaldehyde/cow per day. The DMI and MY were recorded from 0 to 21 days post-calving. Milk fat and protein were assessed on days 7, 14 and 21, and blood was collected on days 0, 7, 14, and 21 after parturition. The DMI and MY at 21 days in milk (DIM) in HG group were compared with control ($P<0.05$). The DMI at 14 and 21 DIM and the MY at 21 DIM were higher in MG group compared with control group ($P>0.05$). Glucose concentration at 7, 14 and 21 DIM increased in both HG and MG groups compared with control group ($P>0.05$). The milk SCC of Gln groups was lower ($P<0.05$) compared with control, at 14 and 21 DIM. Glutamine supplementation increased the blood concentrations of total protein and albumin, but lowered the β-hydoxybutyrate (BHBA), non-esterified fatty acids (NEFA) and aspartate amino transferase (AST) concentrations ($P<0.05$). These results indicate that rumen protected Gln supplementation at 250 g/heat/day to fresh Holstein cows improved the SCC in milk and health status.

Key words: Blood, Fresh cow, Milk, Protected-glutamine, SCC

Introduction

Glutamine (Gln) is a nonessential amino acid synthesized in mammalian tissues. Glutamine is the most abundant and glutamate the third most abundant amino acids (AA) in casein (a major protein in milk) (Meijer *et al*., 1993; Doepel *et al*., 2007). Glutamine, through enhancement in hepatic gluconeogenesis, may increase post-splanchnic glucose (Doepel *et al*., 2007). Initiation of lactation imposes a great metabolic challenge on dairy cows in terms of both protein and energy demands (Jafari *et al*., 2006). During early lactation, a decline of 25 to 33% was reported for the concentration of free Gln in the plasma of dairy cows (Meijer *et al*., 1993). Glutamine has been used in high amounts in immune cells, such as lymphocytes, macrophages and neutrophils (Ardawi and Newsholme, 2001). Since mastitis mostly occurs in early lactation, we hypothesized that Gln, because of modulation in immune system, can reduce the incidence of mastitis and decline milk somatic cell count (SCC) which later affect animal performance, but there are limited studies that have been focused on this regard. Thus, the aim of this study was to investigate the effects of Protected-Gln supplementation on performance, milk quality and SCC and some blood parameters in fresh cows.

Materials and Methods

Forty multiparous Holstein cows were divided into four groups (n=10) which were fed with the basal diet (control), basal diet + 150 (low Gln, LG), 250 (medium Gln, MG) or 350 (high Gln, HG) g of Gln protected with formaldehyde/cow per day. The Gln, for protection, was sprayed with 2% formaldehyde for 72 h, and dried at room temperature and finally formaldehyde residues were evaporated. Glutamine was mixed with calcium carbonate as a carrier. The cows' average body weight (BW) was 796 ± 58 kg and body condition scores were about 3.25 ± 0.35 (Edmonson *et al*., 1989). Fresh cows were investigated for 21 days postpartum.

The basal diet was formulated based on National Research Council (NRC, 2001) recommendations (Table 1). The cows were fed the total mixed rations (TMR) *ad libitum* individually at 07:00 h and 13:00 h. Samples of TMR and refusal of individual cows were collected daily during the data collection period, dried at 60°C in a forced air oven for 48 h, ground to pass through a 1-mm

screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), and were combined per cow for each experimental period. The feeds and refusal samples were analyzed for DM, ash, crude protein (CP) (AOAC, 1995) and neutral detergent fiber (NDF; Van Soest *et al*., 1991). The NDF was determined without α-amylase and sodium sulfite, and expressed exclusive of residual ash. Apparent nutrient digestibility was measured during the last week of the experiment using acid-insoluble ash (AIA) as an internal marker (McGeough *et al*., 2010). The faecal samples (approximately 250 g wet weight) were collected from the rectum of cows twice daily for 5 days beginning on day 15. The faecal samples were combined across sampling times per each cow, dried at 60°C in a forced air oven for 72 h, ground to pass a 1-mm screen, and stored for chemical analyses. Chemical composition of the faecal samples was determined according to the procedures mentioned previously.

Table 1: Ingredient and chemical composition of the basal diet

Ingredient composition	% of DM
Alfalfa hay	21
Corn silage	23
Wheat bran	5
Ground barley	25
Bakery waste	11.2
Soybean meal	6
Corn gluten meal	5
Calcium carbonate	1.1
Salt	0.5
Mineral-vitamin premix	2.2
Chemical analysis	% DM
Dry matter (%)	63.4
Organic matter	90.3
Crude protein	16.3
Natural detergent fiber	32
NE_L (Mcal/kg DM)	1.68

Cows were milked at 05:00 and 16:00 and milk yield (MY) was recorded individually. Individual milk samples were collected, 20 ml per sample, from consecutive morning and afternoon and milk samples were analyzed for protein and fat contents using Milk-O-Scan 133B (Foss Eletric, Hillerod, Denmark). Milk samples were analyzed for SCC by Delaval Cell Counter 4988 (DCC, Sweden). Dry matter intake (DMI) and MY were determined from 0 to 21 days post-calving. Milk fat, protein and SCC were assessed at 14 and 21 days after parturition.

On days 0, 7, 14 and 21 after calving, blood samples (5 ml) were collected, 4 h after morning meal, from jugular vein, and transferred to tubes containing the ethylene diamine tetraacetic acid. The samples were centrifuged at 3000 × g for 15 min to obtain plasma. The plasma concentrations of glucose, total protein, albumin and blood urea nitrogen (BUN) were determined by EXIGO auto-analyzer, vet model. Plasma samples were tested for aspartate amino transferase (using Pars Azmoon kits, Iran), non-esterified fatty acids (NEFA) and β-hydoxybutyrate (BHBA) (using Randox Company's kits, England) by colorimetric methods (Perkin-Elmwr-35).

Statistical analysis

The data were analyzed in a completely randomized design, using the PROC MIXED procedure (SAS, 2003). When differences were significant ($P<0.05$), Tukey's test was used to compare means.

Results

Nutrients digestibility

The apparent digestibility of DM, organic matter (OM), CP, and NDF (Table 2) were similar for cows fed the experimental diets ($P>0.05$).

Dry matter intake, milk yield and composition

Fresh cows of HG group on 7, 14 and 21 and of MG group on 14 and 21 days after calving consumed higher DM ($P<0.05$: Table 3). But, no differences in DMI were observed between LG and control on all the sampling days ($P>0.05$).

The MY was higher in HG group on 7, 14 and 21 DIM and in MG group just on 21 DIM compared with control group ($P<0.05$). There are no significant differences in the MY between LG and control on all the sampling days ($P>0.05$). A higher ($P<0.05$) DMI was observed in groups fed with 250 and 350 g Gln/cow at 21 DIM, which could result in higher MY compared to control group.

Milk protein and fat were not influenced by Gln supplementation ($P>0.05$). Dietary supplementing of Gln in present study, reduced milk SCC on both 14 and 21 DIM ($P<0.05$).

Blood parameters

Cows fed Gln (HG, MG, and LG) had higher plasma concentration of glucose at 14 and 21 DIM compared with control treatment ($P<0.05$; Table 3), but there was no significant difference in blood glucose concentration

Table 2: Total tract digestibility of experimental diets

Digestibility (% of intake)	Treatments [1]				SEM [2]	P-value
	Control	LG	MG	HG		
Dry matter	64.02	65.38	65.19	68.46	1.08	0.38
Organic matter	67.23	67.71	68.22	70.31	1.59	0.41
Crud protein	79.44	78.08	78.75	77.25	2.45	0.53
Natural detergent fiber	49.66	52.64	54.20	54.32	1.73	0.13

[1] Control group: Cows fed control diet; LG: Low Gln, cows fed basal diet supplemented with 150 g; MG: Medium Gln, cows fed basal diet supplemented with 250 g; HG: High Gln, cows fed basal diet supplemented with 350 g Gln protected with formaldehyde/cow per day. [2] SEM: Stand error of means

Table 3: Milk yield, milk composition and plasma concentrations of some blood parameters of experimental treatments

Parameters [1]	Days post calving	Treatments [2]				SEM [3]	P-value
		Control	LG	MG	HG		
DMI (kg/day)	0	7.5	7.7	7.5	7.4	0.95	0.62
	+7	10.0^{b}	13.3^{ab}	14.0^{ab}	15.8^{a}	1.48	0.02
	+14	13.0^{b}	16.4^{ab}	18.5^{a}	19.6^{a}	1.31	0.01
	+21	18.0^{b}	20.0^{ab}	21.7^{a}	21.1^{a}	0.81	0.04
Milk yield (kg/day)	0	14.5	13.9	14.4	14.0	1.17	0.81
	+7	19.5^{b}	22.5^{ab}	23.5^{ab}	24.1^{a}	1.52	0.01
	+14	31.5^{b}	32.7^{ab}	33.3^{ab}	34.5^{a}	0.95	0.02
	+21	35.0^{b}	38.0^{ab}	38.5^{a}	39.2^{a}	1.04	0.01
Milk fat (%)	+7	3.68	3.49	3.61	3.72	0.32	0.58
	+14	3.66	3.43	3.55	3.72	0.33	0.62
	+21	3.54	3.42	3.49	3.62	0.24	0.74
Milk protein (%)	+7	3.30	3.31	3.38	3.36	0.07	0.47
	+14	3.28	3.24	3.38	3.35	0.08	0.36
	+21	3.28	3.29	3.41	3.38	0.06	0.22
SCC (*10^3/ml)	+14	231^{a}	145^{b}	139^{b}	141^{b}	46.8	0.03
	+21	482^{a}	161^{b}	184^{b}	178^{b}	58.9	0.02
Blood metabolites							
Glucose (mg/dl)	0	46.50	46.00	45.60	46.20	1.02	0.63
	+7	47.40^{b}	51.32^{ab}	53.53^{a}	54.30^{a}	1.34	0.02
	+14	48.00^{b}	53.52^{a}	55.86^{a}	56.84^{a}	1.51	0.01
	+21	48.80^{c}	55.00^{b}	59.20^{ab}	60.50^{a}	1.68	<0.01
Total protein (g/dl)	0	5.66	5.77	5.45	5.52	0.29	0.78
	+7	5.20^{b}	5.81^{ab}	5.93^{ab}	6.21^{a}	0.21	0.03
	+14	5.11^{c}	5.84^{b}	6.82^{a}	6.87^{a}	0.19	0.01
	+21	5.02^{c}	5.98^{b}	7.10^{a}	7.20^{a}	0.17	<0.01
Albumin (g/dl)	0	3.56	3.47	3.54	3.44	0.08	0.34
	+7	3.38^{b}	3.46^{b}	3.85^{a}	3.87^{a}	0.05	<0.01
	+14	3.34^{b}	3.40^{b}	3.67^{a}	3.82^{a}	0.07	0.02
	+21	3.54^{b}	3.62^{b}	3.96^{a}	3.98^{a}	0.06	<0.01
Urea-N (mg/dl)	0	10.20	10.70	10.52	10.10	0.42	0.49
	+7	10.30	11.43	11.83	12.24	0.68	0.24
	+14	10.62	12.11	12.74	13.43	1.02	0.18
	+21	10.94	13.23	13.94	14.72	1.31	0.12
AST (U/L)	0	110.0	107.2	114.7	113.1	1.72	0.49
	+7	114.2^{a}	98.3^{b}	97.5^{b}	96.4^{b}	3.57	0.01
	+14	121.7^{a}	89.4^{b}	84.1^{b}	82.8^{b}	4.91	<0.01
	+21	132.5^{a}	82.1^{b}	73.3^{b}	71.3^{b}	6.32	<0.01
BHBA (mmol/L)	+21	1.22^{a}	0.85^{b}	0.78^{b}	0.75^{b}	0.083	<0.01
NEFA (mmol/L)	+21	0.93^{a}	0.62^{b}	0.51^{b}	0.49^{b}	0.048	<0.01

[1] DMI: Dry matter intake, SCC: Somatic cell count, AST: Aspartate amino transferase, BHBA: β-Hydoxybutyrate, NEFA: Non-esterified fatty acids. [2] Control: Cows fed basal diet; LG: Low Gln, cows fed basal diet supplemented with 150 g protected Gln/d; MG: Medium Gln, cows fed basal diet supplemented with 250 g protected Gln/d; HG: High Gln, cows fed basal diet supplemented with 350 g protected Gln/d. [3] SEM: Stand error of means. [a, b, c] Means within a row with different superscripts differ (P<0.05)

between control and LG treatments at 7 DIM. This indicates the conversion of Gln to glucose.

Cows fed HG had higher plasma total protein than cows fed control diet on day 7 after calving (P<0.05). Dietary supplementation of Gln increased total protein concentrations compared with control group at 14 and 21 DIM (P<0.05). The HG, MG, and LG treatments had higher plasma albumin concentrations than control group on 7, 14 and 21 days after calving (P<0.05).

The plasma concentration of BUN was not influenced by dietary supplementation of Gln (P>0.05). The blood plasma concentrations of NEFA and BHBA were lower in all Gln groups compared with control group (P<0.05).

Discussion

The efficiency of microbial growth and cellulose degradation ability differs greatly in animals fed different diets, even within similar diets. There is a strong positive correlation between diet protein (amino acids) content and DMI. It seems that nitrogen resources which have lower rates of ruminal degradation tend to improve the forages digestion and increase the passage rate and DMI (Calsamiglia *et al*., 2010).

Doepel *et al*. (2007) in a study on Holstein cows receiving abomasal infusions of water or 300 g/d of Gln (unprotected-Gln), reported that Gln increased MY but

DMI had tendency to increase.

Texeira *et al*. (2003) showed that there is a major tendency for increase in milk SCC at the beginning of lactation with tendency to decrease until 6 weeks of lactating; thus Gln supplementation can decrease the SCC in critical period, i.e. the start of lactation. Caroprese *et al*. (2013) stated that Gln is required to maintain the proper function of immunological responses by maintaining appropriate levels of lymphocyte activations and enhancing immune responses and this can explain the lower milk SCC levels found in cows that received Gln.

Homeostatic controls maintain circulating glucose concentrations within a narrow range; thus, increases in gluconeogenic activity are reflected in plasma concentrations (Bell, 1995; Roche *et al*., 2013). But Doepel *et al*. (2007) stated that Gln had no effect on glucose metabolism. It can be stated that because Gln increased glucose concentration, it subsequently can help to improve MY in fresh cows (Brown and Allen, 2013).

It is well-known that Gln is an essential precursor for protein synthesis (Plaizier *et al*., 2001).

The lower plasma NEFA concentrations for Gln treatments compared with control group might be due to their higher DMI, and reflect a more favorable energy status and lower mobilization of fatty acids from adipose tissue on 21 DIM (Lohrenze *et al*., 2010; Roche *et al*., 2013). By increasing the NEFA concentration in the liver cells, liver cells are damaged and their functions are disrupted. The liver enzymes concentration such as aspartate amino transferase (AST) increases in cows with fatty liver. The increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (Meyer and Harvey, 1995; Bobe *et al*., 2004). So reducing NEFA concentration can predict a better health status for liver cells, which is characterized by a decrease in liver enzymes. In current study, serum AST concentration reduced significantly with Gln supplementation, that can confirm the better status of liver cells.

From the results of the present study, according to economic aspect, it could be concluded that rumen protected-Gln at 250 g/head/d to fresh Holstein cows improved MY and health status via decreasing AST, NEFA, and BHBA concentrations, and decreased milk SCC.

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