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Full Length Research Paper

Milk Production Losses Due to Helminth Infections in Sudanese Semi-Intensive Dairy Farms

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The objectives of this study were to investigate whether or not consumption of protected glutamine during transition period would affect biomarkers of oxidative stress and milk production. 36 pregnant Holstein dairy cattle were chosen according to body condition score (BCS) and their parity, after which they were classified into two groups with regards to their expecting calving and parity. Group B received daily (100 g/day) protected glutamine with formaldehyde for 21 days before calving, while Group A received basic ration only. After calving, Group B was divided into two categories; one category continued to receive glutamine (100 g/day) for 21 days (BFAF, n = 9), while the other did not (BFAN, n = 9). Also, group A was classified into two categories; one received glutamine (100 g/day) (BNAF, n = 9), while the other did not (BNAN, n = 9). There were no any significant differences and interaction among treatments in dry matter intake of 0, +7 and +14 after calving, but dry matter intake of d +21 was affected by post partum glutamine feeding and it increased in (BFAF) and (BNAF) as compared to (BFAN) (19.31 and 19.26 versus 18.68 respectively) (p≤0.05). Milk yield and its components did not differ in the treatments. The total antioxidant status (TAS mmol/L) influenced by postpartum glutamine feeding on +7, +14 and +21 after calving was most for (BFAF) and (BNAF) as compared to BFAN and BNAN (p≤0.05). The plasma glutathione peroxidase activity (PGX units/ml PCV) was affected by pre and postpartum treatments and glutamine supplementation had increment effects on (PGX) at 0, +7 and +21 days after parturition. The results suggested that glutamine feeding could enhance total antioxidant status and plasma glutathione peroxidase activity in fresh Holstein dairy cows and the best level of formaldehyde for protection glutamine is 1%.

Key words: Holstein cow, oxidative stress, transition period, glutamine.

INTRODUCTION

Transition period is defined as 21 days before calving to 21 days after it and it is widely accepted (Drackley et al., 1999). Increasing demands of energy and protein to lactate lead cattle to be in catabolic conditions, furthermore increase of catabolism in cattle body may promote production of reactive oxygen metabolites (ROM) (Bernabucci et al., 2005). If the production of ROM is more than the antioxidant mechanisms capability in the body, it leads to oxidative stress (Lorraine et al., 2009). Oxidative stress leads to peroxidation of lipids and other macromolecules especially molecules of membrane and other components of the cells and through this can disturb their essential physiological and metabolic tasks (Miller et al., 1993). Drackley et al. (2001) demonstrated that after calving gluconeogenesis is impaired and this can reduce the ability of immune system. Neutrophils need to produce peroxides through NADPH oxidase in order to diminish microbes and in oxidative stress by using NADPH (to revive glutathione), lack of NADPH may reduce the capability of neuthrophils to diminish microbes

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Abbreviation: BFAF, Before calving feeding after calving Feeding; **BFAN,** before calving feeding after calving not feeding; **BNAF,** before calving not feeding after calving feeding; **BNAN,** before calving not feeding after calving not feeding.

(Hammon et al., 2006). Because high producing dairy cattle lost more weight after calving in comparing to low production cattle, therefore they tend to infect oxidative stress (Bernabucci et al., 2005). On the other hand, some researchers showed that glutamine provides precursors for syntheses purine and pyrimidine (carbamyle phosphatase II) and finally RNA and DNA, especially in cells with rapid proliferative cells (immune cells and mucosal cells in gut after calving) (Calder and Yaqoob, 1999). Increase the density of glutamine in rats rations enhances the glutathione density in plasma (Cao et al., 1998). Some researchers show that glutamine is largely used in intestine tissues (Windmueller et al., 1980).

Generally, following roles are supposed for glutamine in dairy cattle especially in the transition period: (1) glutamine and glutamate comprise 6.5 to 12.5 and 7.2 to 10% of amino acids, respectively which involved in bovine casein (Eigle et al., 1984); (2) Since density of non-essential amino acids in milk is more than blood it seems some of it is made of glutamine (Meijer et al., 1995; Deopel et al., 2006). In early days of calving, density of nonessential amino acids increase in blood, however, the density of glutamine and glutamate decreases nearly 25%, moreover the most released amino acid by muscles is glutamine (Meijer et al., 1995);

(3) Glutamine has a fundamental role in glutathione peroxides structures which is essential component of antioxidant system (Nathalie et al., 2005). Considering what was mentioned in the foregoing, we hypothesized that increasing glutamine in transition period could enhance glutathione peroxidase activity and decrease oxidative stress and consequently, improve production performance.

MATERIALS AND METHODS

Animals and feeding

The study was carried out in a commercial dairy herd (Eshrag) located in Waramin (south -East of Tehran, Iran). The period of trial was between September 12 and November 1. Thirty six pregnant Holstein cows (10 primiparous, 10 at second calving and 16 at third calving with 25±3 days to expected calving) were assigned into two group (n = 16) based on their BCS, parity, and expected calving date (to assay effects of glutamine before calving on animals after calving). One group became control group (A) and another received glutamine (100 g/d) per cow until calving (B). After parturition, cows were assigned to one of 4 dietary treatments: (1) glutamine supplementation (100 g/d) per cow before and after calving (BFAF), 2) glutamine supplementation (100 g/d) per cow before calving and without glutamine after it (BFAN), (3) without glutamine before and glutamine supplementation (100 g/d) per cow after calving (BNAF) and (4) without glutamine pre and postpartum (BNAN). Because glutamine and glutamate comprise 6.5 to 12.5 and 7.2 to 10%, respectively of amino acids in bovine casein (Eigle et al., 1984), our hypotheses was that, to providing requirement glutamine to contribution in casein at production 33 kg milk per day is approximately, 33 kg/day × 0.032 protein × 0.86 casein × (0.065 - 0.125) = 60 to 113 g/day. Protected glutamine with formaldehyde was added and mixed to concentrate before and after calving. Prepartum glutamine supplement or without glutamine and postpartum glutamine feeding or not feeding were used in a 2 × 2 factorial array using randomized complete block.

Two groups before calving and four groups after parturition received a ration as TMR for based on their requirement at close up and then to production 33 kg milk with 3.6% fat and 3.2% protein in 21 days after parturition based on NRC (2001) recommendations. Levels of Glutamine were mixed using ground corn as a carrier and to monitoring the amount of consumed glutamine we adjusted additional glutamine compare to DMI. Experimental diets (before and after parturition) are shown in Table 1. The diets administered throughout the trail consisted of a basal ration given *ad libitum* to achieve 5 to 10% orts as a daily TMR that offered at 0830 before calving and at 0830 and 1630 after calving. Dry matter of feeds were measured weekly by drying in an oven at 105°C for 48 h. Lactating cows were milked 3 times each day at 0800, 1600 and 2400.

Measurements and sampling

Before and after parturition dry matter of diets was determined by forced air oven drying at 105°C to static weight. Samples of feeds were analyzed for CP (AOAC, 2000; ID 984.13), ether extract (AOAC, 2000; ID 920.39) and ash (AOAC, 2000; ID 942.05), ADF and NDF (Van Soest et al., 1991). Body condition score (BCS) was scored (five point scale where 1 = emaciated and 5 = obese) by three skilled individuals in 0, +10 and +21. Cows were milked three times in day with Westfalia Metatron 21 equipment and milk yield were recorded daily for each cow during the study until +21 days. Samples of morning and afternoon milking were collected twice weekly and were sent to local library to analyzing fat and protein. Feed intake was determined daily by measuring supplied feed and refusals for TMR. Dry matter intake (DMI) before and after calving was measured in Groups F and N and BFAF, BFAN, BNAF and BNAN and reported in Tables 2 and 3 and Figure 1. Glutamine was mixed using ground corn as a carrier into the TMR. Concentrate mixtures and forage sources were mixed in a weighing and mixing unit (Iranian Niro, Inc.) and offered once a day before calving at 1100 and after calving twice daily at 0800 and 1600. In any group, orts were collected and recorded daily subtracted from the ration offered and divided by the number of cows fed to determine daily average individual cow intake. Blood samples were obtained by using evacuated tubes from coccygel vein in two distinct tubes in Days 0. +7, +14 and +21 at 4 h after morning feeding. One contains Li-heparin to separate plasma to assay PGX, TAS and BUN. BUN was analyzed by manual colorimetric method (Evans, 1968).

GPX (plasma glutathione peroxides activity) activities were determined by a kinetic method with a commercial kit (RANSEL by Randox laboratories Ltd., Antrim, United Kingdom). The method was based on Paglia and Valentines (1967). Glutathione peroxidasescatalyzes the oxidation of glutathione by cumenehydroperoxde. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was followed at 340 nm for 3 min. Enzyme activity was reported in units per milliliter in plasma. TAS in plasma was measured by using a commercial kit (Randox laboratories Ltd., Antrim, United Kingdom), based on the incubation of ABTS (2, 2´-azino-di-[3-ethylbnzthiazoline sulphonate], Boehringer Mannheim,) with a peroxides (metamyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$ (Ghiselli et al., 2000). This has a relatively stable blue–green color which is measured at 600 nm. The determination of haptoglobin (Hp) was performed by the method of enzyme linked immunosorbent assay using ELISA kits (Tridelta Development, Ltd., Wicklow, Ireland).

Amino acid protection method and feeding

In order to protect glutamine, we used formaldehyde. To ensure and apply the best level of formaldehyde, we designed an experiment by the use of anaerobic defined media (without nitrogen sources) (Davies et al., 1993). We prepared anaerobic defined

Table 1. Ingredient and chemical composition of pre- and postpartum experimental diets.

Composition	Diets	
	Prepartum[1]	Postpartum[1]
Ingredient composition (% of DM)		
Alfalfa hay	36.82	27.50
Corn silage	10.1	15.1
Beet pulp	4.14	5.30
Wheat straw	4.23	0.00
Barley ground grain	4.89	7.50
Corn ground grain	13.8	17.4
Cotton seed whole	2.2	7.8
Cotton seed meal	1.08	1.30
Canola meal	1.36	1.00
Soybean meal	6.23	7.00
Wheat barn	11	0
Fish meal	0.0	2.7
Ca-PFAD[1]	0.0	1.4
Corn gluten meal	0	2
Sodium bicarbonate	0.0	1.4
Salt	0.0	0.3
MgO	0.00	0.05
Anionic salt[2]	2.5	0.0
Di-calcium phosphate	0.15	0.2
Calcium carbonate	0.35	0.7
Mineral mix[3]	0.25	0.30
Vitamin mix[4]	0.9	1.05
Chemical composition (dry basis)		
$NE_{L,}$ Mcal/kg	1.62	1.77
CP (g/kg)	142	170
NFC (g/kg)	388	386
ADF (g/kg)	240	210
NDF (g/kg)	380	340
Ash (g/kg)	78.9	84.0
EE (g/kg)	311	59

[1]Experimental diets were different only in glutamine supplementation (100 g/day). Protected glutamine was added to BFAN, BFAF and BNAF groups. This amount added to ingredients of ration before preparation. [2]Calcium palm fatty acids. [3]Contained 15% calcium carbonate, 24.2% magnesium sulfate, 10.8% chloride ammonium, 18.8% calcium chloride.[4]Contained a minimum of 2% Fe (from ferrous sulfate), 0.6% Cu (from copper sulfate), 4.46% Mg (from magnesium oxide), 2.5% Zn (from zinc oxide),
120 mg/kg Se (from sodium selenite), 24 mg/kg Co (from cobalt sulfat). [5]Contained 2500 KIU/kg of vitamin A, 1250 KIU/kg Vitamin D, 17000 IU/kg Vitamin E, 288 mg/kg biotin, 286 mg/kg niacin.

media that had not any sources of nitrogen and in the other words, the only source of nitrogen for microorganisms were protected glutamine (by formaldehyde) or unprotected glutamine (control). Before initiation of experiment, we add 0.5, 1, 1.5 and 2% (w/w) formaldehyde solution by spraying on the 5 g glutamine and after reaction, we dried those in an oven at 40°C for 24 h (for evaporation of additional formaldehyde that can have destroy effect on microorganisms later). After preparation of anaerobic defined media in serum bottles that have not any sources of nitrogen, the non protected glutamine (control) and protected glutamine with different levels of formaldehyde (1 ml) and fresh clarified rumen fluid (CRF) (0.5 ml) injected to bottles (three bottle for any level 0.5, 1, 1.5 and 2% and control) meanwhile, 0 level as blank (on spectrophotometer). Those were incubated in 39°C for 24 h, and then we repeated and continued inoculation to third sub culture (3×3×3×3 for any level and control). It was clear that the amount of bacteria growth depend on glutamine availability.

Therefore, we measured turbidity (representation of microorganism's population) in bottles by use of a spectrophotometry and then used statistically analyzing. Then, we observed that the best level of formaldehyde is 1% and there were no significant differences between levels more than 1%.

Statistical analysis

The experimental design was a randomized complete block. Statistical computations were performed using SAS (1999) software. Milk production, milk components and other parameters were analyzed by ANOVA using the MIX procedure of SAS with a linear polynomial contrast. In this study, differences among treatments were considered significant if $P<0.05$, whereas when

Table 2. Dry matter intake before calving (Analyzed By T-student).

DMI (kg/day)	Treatment		P-Value
	F	N	
0	8.44	8.8	0.18
-10	13.29	13.4	0.22
-21	13.2	13.36	0.12
-21	3.25	3.34	0.89

[a-b]Means within a row with different superscripts are different (P<0.05). F= fed group. N = not fed group.

Table 3. Effects of PG on, DMI, milk production and composition and BCS change.

Item	Treatment					p-value		
	BFAF	BFAN	BNAF	BNAN	SE	B[1]	A[2]	A×B
DMI, kg/days								
0	8.92	8.88	8.76	9.03	1.04	0.96	0.31	0.19
+7	13.35	14.78	15.24	14.57	1.67	0.27	0.74	0.14
+14	17.53	17.61	17.67	18	0.59	0.19	0.32	0.54
+21	19.31[a]	18.68[b]	19.26[a]	19.14[a]	0.51	0.23	0.03	0.14
0	3.66	3.58	3.66	3.61	0.18	0.27	0.82	0.82
+10	3.41	3.33	3.41	3.36	0.18	0.27	0.82	0.82
+21	3.19	3.08	3.05	3.19	0.18	0.81	0.81	0.54
Milk yield, kg/days								
0	11.65	10.45	11.88	11.38	0.75	0.11	0.80	0.32
+7	22.24	21.66	21.92	21.36	0.84	0.84	0.72	0.99
+14	31.34	28.94	28.58	27.51	1.69	0.31	0.41	0.79
+21	35.54	34.08	34.44	32.11	1.58	0.21	0.14	0.81
Milk fat, g/kg (Days)								
+10	33.3	34.2	33.8	33.8	0.01	0.35	0.33	0.52
+21	34.3	34.7	35.0	34.4	0.01	0.29	0.28	0.11
Milk protein, g/kg (Days)								
+10	31.5	30.7	31.2	31.0	0.01	0.39	0.34	0.29
+21	31.4	30.8	31.0	31.2	0.01	0.36	0.26	0.53

[-b]Means within a row with different superscripts are different (P<0.05). B = Feeding PG before calving. A = Feeding PG after calving. BFAF = before calving feeding after calving feeding, BFAN = before calving feeding after calving not feeding, BNAF= before calving not feeding after calving Feeding, BNAN = before calving not feeding after calving not feeding. B = Feeding PG before calving. A = Feeding PG after calving. BFAF = before calving feeding after calving feeding, BFAN = before calving feeding after calving not feeding, BNAF = before calving not feeding after calving feeding, BNAN = before calving not feeding after calving not feeding.

0.05<P≤0.10, differences were considered to indicate a trend towards significance.

RESULTS

Table 1 shows rations before and after calving. Table 2 shows DMI in A and B groups before calving. There were no significant differences between A and B. Table 3 shows DMI, Changes of BCS, and milk yield and its components in BFAF, BFAN, BNAF and BNAN. DMI on 21 days after calving was significant more in BFAF, BNAF and BNAN compared to BFAN (19.31, 19.26, 19.14 and 18.68 kg/day, respectively). Changes of BCS did not differ among the 4 groups. Milk yield and its components did not differ among the 4 groups. Total antioxidant status (TAS) did not differ among 4 groups on calving day that showed in Table 4. The BFAF and BNAF cows had higher TAS compared to BFAN and BNAN on 7,
14 and 21 days after calving (p<0.05). Glutathione

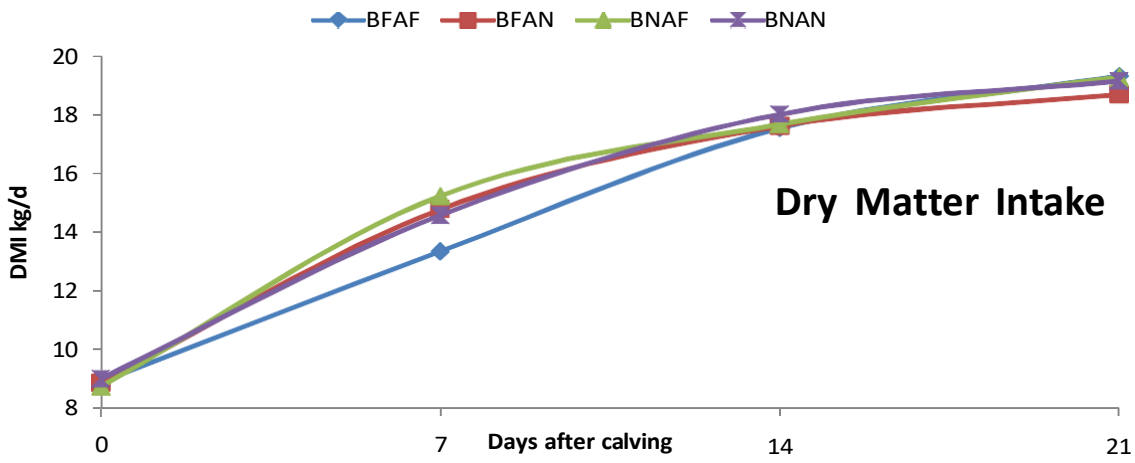

Figure 1. DMI in treatments and control groups after calving. BFAF = before calving feeding after calving feeding, BFAN = before calving feeding after calving not feeding, BNAF= before calving not feeding after calving feeding, BNAN = before calving not feeding after calving not feeding.

Table 4. Blood antioxidants parameters.

Parameter	Treatment					p-value		
	BFAF	BFAN	BNAF	BNAN	SE	A	B	AB
TAS (mmol/L) Days								
0	0.3	0.31	0.36	0.34	0.8	0.91	0.1	0.58
+7	0.34[a]	0.29[b]	0.35[a]	0.3[b]	0.32	<0.0001	0.5	0.97
+14	0.32[a]	0.24[b]	0.31[a]	0.27[b]	0.03	<0.0001	0.44	0.15
+21	0.25[a]	0.21[b]	0.27[a]	0.21[b]	0.03	<0.0001	0.23	0.25
GPX (units/ml PCV)								
Days								
0	57.46[a]	54.77[a]	57.88b[a]	49.25[b]	5.12	0.002	0.14	0.09
+7	52.95[a]	48.17[b]	52.51[ac]	42.37[d]	3.88	<0.0001	0.02	0.04
+14	35.35	37.14	43.86	39.9	15	0.15	0.65	0.34
+21	46.76[c]	40.28[b]	45.48[c]	36.02[a]	2.61	0.003	<0.0001	0.09

[a-b]Means within a row with different superscripts are different (P<0.05). B = Feeding PG before calving.

peroxidase activity (GPX) was higher in BFAF, BFAN, BNAF compared to BNAN on calving day (p<0.05). GPX was higher in BFAF and BNAF groups compared to BFAN and BNAN and GPX was higher in BFAN compared to BNAN on 7 days after calving (p<0.05). GPX did not differ among 4 groups on 14 days after calving. GPX differed among 4 groups on 21 days after calving (p<0.05).

DISCUSSION

DMI, milk yield and composition and BCS

Before and after calving, all rations were the same in terms of energy and nitrogen without regarding to added protected glutamine. Amounts of dry matter intake, milk, varieties of body conditions and milk compositions have been shown in Tables 2, 3 and Figure 1. There were no significant differences between various treatments in terms of DMI in the 7th and 14th day. The findings of this study were supported by those of Plaizier et al. (2001), which showed that abomasal infusion of glutamine did not affect DMI. However, DMI were significant higher in BFAF, BNAF, and BNAN treatments compared to BFAN in 21 days after calving (19.31, 19.26, 19.14 and 18.68 kg/day, respectively) (p≤0.03). There were significant differences on amount of DMI in 21 days after calving by feeding glutamine (p≤0.03). Some researchers have shown that glutamine used extensively by tissues of the digestive system and also demonstrated that weight of intestine increased about 12% after calving(Gibb et al., 1992). Burrin et al. (1991) in their research showed that supplementing piglet rations by glutamine leads to

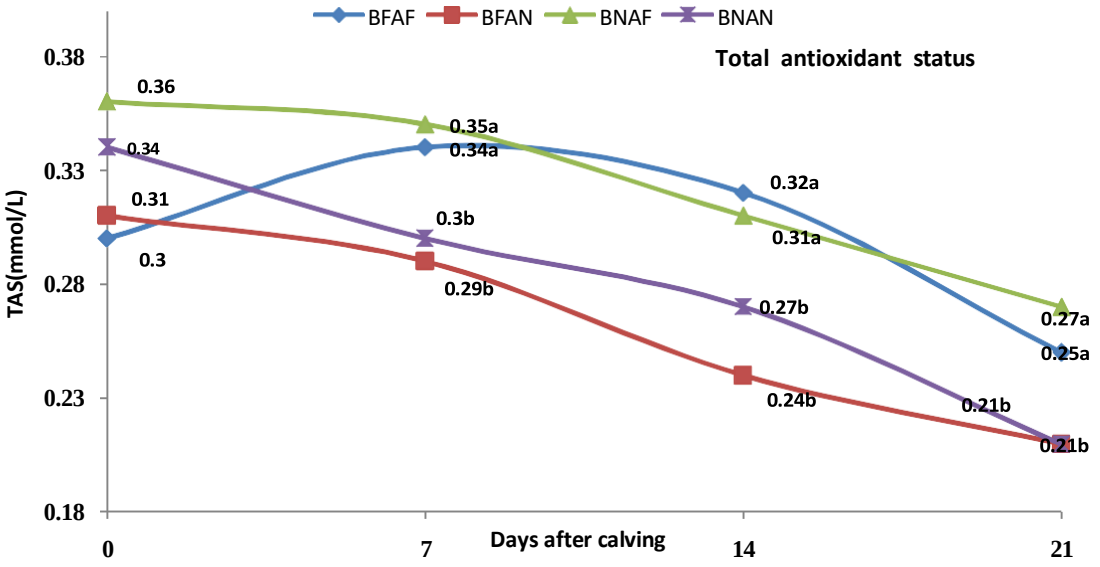

Figure 2. Total antioxidant status in treatments and control groups after calving. BFAF = before calving feeding after calving feeding, BFAN = before calving feeding after calving not feeding, BNAF= before calving not feeding after calving feeding, BNAN = before calving not feeding after calving not feeding.

increase the height of intestinal villi and area in jejunum. On the other hand, Reeds et al. (2000) showed that mucosal cells of the digestive system like other cells which can proliferate and distinct rapidly need the nitrogen results from glutamine to synthesize pyrimidines (Carbamoyl phosphates II) and purines as well as DNA and RNA. Unfortunately, it was not found any findings that show glutamine affect on growth of dairy cattle's digestive system especially in the transition period, but it can be inferred that increasing of DMI in 21 days after calving in BFAF and BNAF possibly due to increase of the digestive system volume and increase of microorganisms due to more accessibility to energy and protein which resulted from added glutamine. Probably lack of significant differences between various treatments may due to not sufficient growth of villi in the digestive system or more time is needed to show the differences by feeding protected glutamine. To examine effects of glutamine on BCS varieties, BCS was considered in 0,10th and 21st day after calving, however, there were no significant differences. Probably lack of glutamine effect on BCS varieties may due to a little amount of energy and protein or due to using glutamine in tissues which have no special relationship with body saving tissues. There were no differences between treatments in milk yield as showed in Table 3. Some researchers showed that infusion or supplementation of glutamine improved milk production (Meijer et al., 1995; Deopel et al., 2006). The lack of significant response possibly is as a result of limited time of experiment and more researches are needed to identify the exact effect of protected glutamine on milk production. There were no significant differences between various treatments in terms of fat and protein of milk in 10 and 21 day after calving and these findings were supported by Plaizer et al. (2001) who demon- strated that there were no significant differences in abomasal infusion of glutamine on fat and protein of milk.

Total antioxidant status (TAS) and glutathione peroxides activity (GPX)

The blood parameters have been shown in Table 4 and Figures 2 and 3. To investigate the redox condition of plasma dynamically and biologically, measuring TAS is an effective method that provides valuable information (Castillo et al., 2006). The blood parameters have been shown in Table 4. To examine redox conditions dynamically and biologically, total antioxidant status (TAS) was measured in 0, 7, 14, and 21 day after the calving (Castillo et al., 2006). There were no significant differences between amount of TAS in the calving day and various treatments. Some researchers (Barja et al., 1992) suggest that the total performance of anti oxidants is controlled by homeostatic. Miller et al. (1993) reported there is probability that decrease of antioxidative stress before the calving relates to increase of antioxidants capacity nearby calving. That is, when oxidative stress increases antioxidant capacity will spontaneously increase too. It is suggested that increase of antioxidant before the calving may lead to not show effects of feeding portions on TAS clearly. In 7, 14 and 21 days after parturition there were significant differences between BFAF and BNAF compared to BFAN and BNAN. Meanwhile, there were not any significant effect between feeding protected glutamine 100 g/day per cow (PG) before calving on TAS condition in 7, 14 and 21 days after parturition. Results showed that feeding protected glutamine can have increasing effects on TAS condition after the calving and the most amount of it relates to BNFA and BFAF treatments. It is demonstrated that SH residuals (SH) results from amino acid cysteine proteins

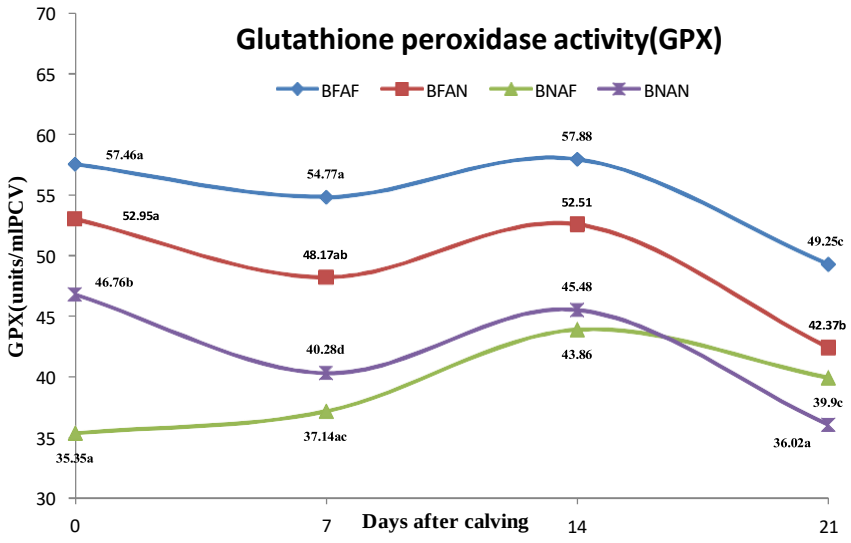

Figure 3. DMI in treatments and control groups after calving. BFAF = before calving feeding after calving feeding, BFAN = before calving feeding after calving not feeding, BNAF = before calving not feeding after calving feeding, BNAN = before calving not feeding after calving not feeding.

which synthesize in the liver especially albumin, L-cysteine and homocysteine have grate roles in body antioxidant defense (Uleand et al., 1996). In other words, in the acute phase response which occurred in early days of calving to encounter with infections, inflammations and stress some amino acids such as phenylalanine may restrict synthesis of the acute phase response protein (Reeds et al., 1994). It seems increase of accessibility to glutamine in intestine by preventing fermentation in the rumen can have protective effects on methionine oxidation as well as phenylalanine with reamination of their oxo-acids. It is obvious that increase of accessibility to methionine can increase physiological levels of cysteine.

According to the assumption in another study demonstrate that glutamine abomasaly leads to increase numbers of the acute phase response proteins such as like haptoglobin, serum amyloid A and lipopolysaccaride binding protein. There were significant differences in glutathione peroxidase activity between BFAF, BNAF BFAN groups compared to BNAN (54.76, 54.77, 57.88 and 49.25 units/ml PCV respectively). There was not significant effect interaction between protected glutamine feeding per and postpartum and levels of protected glutamine before calving (*P*<0.09). Levels of PG feeding after calving have significant effect on GPX (P<0.002) condition at calving day and the results showed (Figure 3) that feeding PG can improve GPX activity at calving day. There were significant differences between treatments at 7 day after parturition on GPX condition. Meanwhile there was significant effect interaction between PG feeding per and postpartum (P<0.04) on the GPX condition at 7 days after parturition. Results show that, PG feeding pre (P<0.02) and post (P<0.0001) calving have significant effect on the GPX condition at 7 days after parturition. We suppose that feeding PG after parturition could increase GPX at 7 day after parturition. There wasn't significant difference between treatments on GPX activity at 14 day after parturition. There were significant differences among treatments at 21 day after parturition on GPX activity, but there was no significant interaction between PG feeding per and postpartum. But levels of PG before calving (P<0.003) and after calving (P<0.0001) has significant effect on GPX activity at 21 day after parturition and the results shows that feeding PG after and before can improve GPX activity at 21 day after parturition. In high milk production dairy cattle, milk production increases more than 1 kg protein through secreted milk especially in early days of calving which is 30% more than total existing protein in plasma (Bequette et al., 1996). Glutamine was the most amino acid in plasma and milk and it has been reported that in earlydays of calving there was 25 to 30% reduction in plasma (Maijer et al., 1993) and 75% reduction in muscles (Palmer et al., 1996).

Halliwell and Chirico (1993) demonstrate that glutathione peroxidase activity has relationship with lipids peroxidation. Young et al. (2000) demonstrate that mitochondria of the cattle liver which have fatty livers produce more superoxide anion ($O_2 \cdot^-$) and H_2O_2 than healthy cattle. There is possibility that feeding protected glutamine to supply more energy and protein can reduce negative balance of energy and protein and through that enhance PGA activity. During negative balance of energy and protein (methionine) in early days of calving cattle experience many inflammatory and infection conditions which stimulate the immune system and acute phase responses (Ametaj et al., 2005). Ametaj et al. (2005) demonstrated necessity of glutamine to synthesize proteins of acute phase responses can evacuate

glutamine repository such as plasma and muscles. It has been shown that glutathione has crucial roles in physiology (Borna et al., 2005). For example it participates in enzyme activities directly and lipids of peroxidants can also protect cells from oxidative stress (Grimble, 2001). Furthermore, when other oxidants are no sufficient glutathione can react with free radical. Regarding to releasing lipids from body reservoir and possible peroxidation can increase use of glutathione. Glutathione is mainly synthesized in a form of de novo in liver by glutamate, cysteine and glycine. Decrease of liver performance typically occurs in early days of calving can have destructive effects on glutathione synthesis (Jeffari et al., 2006). The liver has unique capability to convert nitrogen to cysteine and reduction of its performance can disturb synthesis of glutathione (Kaplowitz et al., 1985). Synthesis of glutathione heavily depends on accessibility to prefabricated amino acids and there is competition on accessible cysteine by synthesizing albumin (Droge et al., 1994). It is very important to know that km enzymes were stimulating amino acids to synthesize proteins is 0/0.03 mmol/L while for gamma glutamyle, cysteine synthesis is 0/0.35 mmol/L. That is biosynthesis of protein happens in density, 166 times lower than gamma glutamile syntheses. Glutamine has definite effects on accessibility to glutamate and by preventing methionine oxidation can have positive effects on increasing cysteine (Blarzino et al., 1994). In a study, where glutamine was given to rats, it was observed that it led to increase of glutathione in intestine tissues (Cao et al., 1998). It is concluded that increase of accessibility to glutamine for animals can have different effects on glutathione synthesis and increase its synthesis (Denno et al., 1996).

Conclusion

The results of this study showed that the better method for glutamine protection is using 1% formaldehyde. Meanwhile, increasing glutamine in transition period has positive effects total antioxidant capacity of plasma (TAS) and glutathione peroxides activity (GPX) in plasma. Glutamine supplementation can lead to increasing dry matter intake and probably production performance.

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