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Effect of Glutamine Enhancement on Oxidative Stress and Reproduction in Holstein Dairy Cows During Transition Period

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Abstract: The objective of this study was to investigate whether consuming of protected glutamine before and after parturition would affect biomarkers of oxidative stress, dry matter intake and production performance. Thirty six pregnant Holstein dairy cows were assigned into four treatment groups based on their BCS, parity and expected calving date in a completely randomized design. Treatment groups consisted of glutamine supplementation Before and After Calving (BFAF), glutamine supplementation before calving and without glutamine after it (BFAN) without glutamine before and glutamine supplementation after calving and (BNAF) and without glutamine pre and postpartum (BNAN). There were not any significant differences and interaction among treatments in DMI but DMI on days 21 was affected by post partum glutamine feeding. Milk yield was not affected by treatments. The Total Antioxidant Status (TAS) influenced by postpartum glutamine feeding and was most for BFAF and BNAF. The Plasma Glutathione Activity (PGA) was affected by pre and postpartum treatments and the effect of post calving feeding was more clear and there was only an interaction effect on 7 days.

Key words: Holstein cow, oxidative stress, transition period, glutamine, haptoglobin, Iran

INTRODUCTION

Transition period is defined as 21 days before calving to 21 days after it. This definition has been widely accepted (Drackley, 1999). This period is the most sensitive time of dairy cattle life that production diseases and metabolic disorders such as ketosis, metritis, abomasum displacement, milk fever, ovarian cystic, immune suppression, retained placenta, mastitis and lameness are happens in this time. After calving the intake of NEl and metabolizable protein by healthy dairy cattle are less than requirement (Bell, 1995). Increasing demand of energy and protein for lactation cause animal to be in catabolic situation and catabolic pathways increase the production of Reactive Oxygen Metabolites (ROM) and consequently oxidative stress (Bernabucci *et al*., 2005). Drackley *et al*. (2001) demonstrated that after calving gluconeogenesis is impaired and this can reduce the ability of immune system because neutrophils use peroxide to killing germs by NADPH oxidas pathway. By increasing use of NADPH in oxidative stress (for reducing glutathione peroxidas) the ability of neutrophils to destroy microbes will be diminish and immune system will be suppressed and would effect on reproduction system (Hammon *et al*., 2000). On the other hand, it shows that glutamine provides precursors for syntheses purine and pyrimidine (carbamile phosphatase 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). Because uptakes of non essential amino acids from blood are less than their concentration in milk it seems that some of them are synthesized from glutamine and during early lactation the plasma concentrations of most nonessential amino acids increased but in the same time both glutamine and glutamate decreased by 25% and the most deleted free amino acids in muscle is glutamine (Meijer *et al*., 1995). Another important role of glutamine is incorporation in structure of glutathione peroxidase, one of the elements of antioxidant system (Spears and Weiss, 2008). Proteins of the acute phase are a kind of plasma that will increase or decrease when animals expose an external stressful factor such as transformation, separation, bacteria inflammation or surgery (Murata *et al*., 2004). Proteins of the acute phase of positive responses in dairy cattle which will secrete in response to glucocorticoids and/or cytokines involve Serum Amyloids A (SAA), Haptoglobin (HP), Fibrinogens Reactive Protein (CRP) and proteins of

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the acute phase of negative responses include albumins, transferrin and paraxonas (Murata *et al*., 2004). Most studies have been shown that there is a close relationship between the concentration of HP in plasma and mastitis after calving (Huzzey *et al*., 2009). For example Regasa and Novakoz (1991) established ewes which have mastitis in comparing to healthy ewes have higher concentration of HP. Williams *et al*. (2007) indicated bacterial infections of the uterine in dairy cattle are related to increase HP concenteration. Generally because there is significant difference between HP in serum of healthy cattle and suffered ones, scientists interested in measuring it in order to determine inflammations (Eckersall, 2000). Huzzey *et al*. (2009)'s study showed that there is 6-7 more possibility of suffering severe or mild metrits, the cattle which have more than 1 g L^{-1} HP concentration in plasma after 3 days of calving. On the other hand, Hiss *et al*. (2009) study variation of the acute phase proteins especially HP and lactoferin by varying of animals energystatus and indicate that the cattle in which concentration of BHBA in their serum is >8/6 mm L^{-1} in last week of pregnancy, the concentration of HP in their serum, milk and lactoferin would be more. HP is the most important joints to hemoglobin in most vertebrata and mammals (Jayle *et al*., 1952). A major physiological task of HP is to combine with free hemoglobins and neutralize and block them for available microbes and prevention of oxidative stress (Wang *et al*., 2001). Getting inactive enzymes which involved in producing steroeids can lead to decrease reproduction performance. Normal reproduction performance depends on enough concentration of progesterone and estrogens. Sensitivity of steroid genesisenzyms in cytochrome p-450 to lipid peroxidation in high producing dairy cattle can lead to decrease reproduction performance during oxidative stress and negative energy balance (Staats *et al*., 1988). In many researches have been shown that oxidative stress in first steps of embryos and fetus formation may cause physical deformity and slowing down the growth (Fleming *et al*., 2004). On the other hand, it has been indicated that glucose 6-phosphate hydrogenise cycle is one of the most important protective mechanisms against internal and external stressful factors (oxidative stress) and if there is not right function due to the lack of glucose it can be caused to death of the premature embryo increase sensitivity to viral illnesses be physically deformity and degenerate embryo (Ho *et al*., 2007). It has been indicated that there is relationship between a vitamin E reserves of the pregnant cattle at the beginning and end of the pregnancy and fetus growth (Scholl *et al*., 2006). There are a lot of documents that indicate making supplementary of the feeds of buffalo that are repeat breeder and anestrus with vitamin E as well as selenium leads to decrease oxidative stress by decreasing of lipids per oxidations and increase superoxidedismutase and glucose phosphate 6-dehydrogenase activity and beta carotene concentration in plasma (Nayyar *et al*., 2003; Anita *et al*., 2003). In another study has been demonstrated that oxidative stress leads to produce limitation of progesterone in repeat breeder cattle (Rizzo *et al*., 2007). The researchers hypothesized that increasing glutamine in transition period can decrease oxidative stress and consequently improve reproduction performance and haptoglobin.

MATERIALS AND METHODS

Animals and feeding: The experiment was carried out in a commercial dairy herd. The average milk yield per lactation (305-DIM) of the herd was >9100 kg. The period of trial was between September 12 to 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 based on their BCS, parity and expected calving date. About 25 days before expected parturition, cows were assigned to one of 4 dietary treatments arrangement: Glutamine supplementation Before and After calving (BFAF), glutamine supplementation before calving and without glutamine after it (BFAN) without glutamine before and glutamine supplementation after calving and (BNAF) and without glutamine pre and postpartum (BNAN). Prepartum glutamine supplement or without glutamine and postpartum glutamine feeding or not feeding were used in a 2×2 factorial array using complete randomized design. Four groups received a ration as TMR for based on their requirement to production 33 kg milk with 3.6% fat and 3.2% protein in 21 days after parturition based on NRC (2001) recommendations.

Experimental diet has been shown in Table 1. The diets administered throughout the trail consisted of a basal ration given *ad libitum* to achieve 5-10% 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 a 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 55°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

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

Diets	Prepartum[1]	Postpartum[1]
Ingredient composition (% of DM)		
Alfalfa hay	36.82	27.50
Corn silage	10.10	15.10
Beet pulp	4.14	5.30
Wheat straw	4.23	0.00
Barley grain	4.89	7.50
Corn grain	13.80	17.40
Cotton seed whole	2.20	7.80
Cotton seed meal	1.08	1.30
Canola meal	1.36	1.00
Soybean meal	6.23	7.00
Wheat barn	11.00	0.00
Fish meal	0.00	2.70
Ca-PFAD[1]	0.00	1.40
Corn gluten meal	0.00	2.00
Sodium bicarbonate	0.00	1.40
Salt	0.00	0.30
MgO	0.00	0.05
Anionic salt[2]	2.50	0.00
Di-calcium phosphate	0.15	0.20
Calcium carbonate	0.35	0.70
Mineral mix[3]	0.25	0.30
Vitamin mix[4]	0.90	1.05
Chemical composition (dry basis)		
NE_L (Mcal kg^{-1})	1.62	1.77
CP (g kg^{-1})	142.00	170.00
NFC (g kg^{-1})	388.00	386.00
ADF (g kg^{-1})	240.00	210.00
NDF (g kg^{-1})	380.00	340.00
Ash (g kg^{-1})	78.90	84.00
EE (g kg^{-1})	311.00	59.00

[1]Experimental diets were different only in glutamine supplementation (100 g $days^{-1}$). 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^{-1} Se (from sodium selenite), 24 mg kg^{-1} Co (from cobalt sulfat). [5]Contained 2500 KIU kg^{-1} of vitamin A, 1250 KIU kg^{-1} vitamin D, 17000 IU kg^{-1} vitamin E, 288 mg kg^{-1} biotin, 286 mg kg^{-1} niacin

942.05), ADF and NDF. 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 with WestfaliaMetatron 21 equipment and milk yield recorded daily by this system during experiment and also after experiment period. Milk samples were collected weekly from consecutive am and pm milkings, composited and analyzed by Milk-o-Scan minor (78110; Foss, Denmark). Feed intake was determined daily by measuring supplied feed and refusals for TMR and was averaged per week. Dry Matter Intake (DMI) in calving day measured individually and reported but DMI in 7, 14 and 21 days after parturition was the average of DMI in days (0-7), (7-14) and (14-21). The refusals were monitored to avoid selection in rations by cows. NRC (2001) requirements were used for diet formulation. Throughout the experiment cows monitored to disorders such as dystocia, milk fever, metritis, retained placenta, abomasum replacement, acidosis and mastitis but until 3 weeks postpartum anything of them didn't occur.

Blood samples spontaneously were obtained by using evacuated tubes from coccygel vein in two distinct tubes, one containing Li-heparin to separate plasma to assay Plasma Glutathione Activity (PGA) and Total Antioxidant Status in plasma (TAS). PGA activities were determined by a kinetic method with a commercial kit (RANSEL by Randox laboratories Ltd). The method was based on Paglia and Valentine (1967). Glutathione peroxidase catalyzes 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 the kit supplied by Randox laboratories Ltd, 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 colour which is measured at 600 nm. For accuracy and reproducibility control a commercial kit (Randox TAS kit, Randox laboratories Ltd.).

Amino acid protection method and feeding: Glutamine amino acid was provided amount 80 kg in powder form. In order to protect, the researchers used formaldehyde (Davies *et al*., 1993). To ensure and apply the best level of formaldehyde researchers designed an experiment by the use of fresh rumen liquid. The researchers prepared cultures that the only sources of nitrogen was protected glutamine by formaldehyde. Before initiation of experiment the researchers add 0.5, 1, 1.5 and 2% (w/w) formaldehyde solution by spring on the 5 g glutamine and after reaction dried them in an oven at 40°C at 24 h (Davies *et al*., 1993). After preparation of cultures in bottles that haven't any source of nitrogen the protected glutamine with different levels of formaldehyde and rumen liquid from dairy cattle injected to bottles (any level protection in 3 bottles). After 24 h holding in 39°C researchers repeated and continued inoculation third sub culture. It was clear that the amount of bacteria growth depend on glutamine availability. Therefore, the researchers measured the amount of offence (representation of bacteria population) in bottles by use of a spectrophotometer and then compared them with t-student. Then, the researchers observed that the best level of formaldehyde is 1% and there was not significant between levels >1% but protection with 1% was better than 0.5%.

Statistical analysis: The complete randomized design model included fixed effects of treatment random effects of cows. Data measured over time (DMI, milk yield and components) within the period of interest were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS. For all analyses, least squares means were calculated. Means were evaluated by Duncan's multiple range test. In this study, differences among treatments were considered significant if $p<0.05$ whereas when $0.05<p<0.15$, differences were considered to indicate a trend towards significant.

RESULTS AND DISCUSSION

DMI, milk yield and composition and BCS: All rations pre and postcalving were isonitrogenous and isoenergetic except adding Protected Glutamine (PG). The Dry Matter Intake (DMI), milk yield, milk composition and BCS changes have been shown in Table 2. There was no significant difference in DMI among groups in calving day, 7 and 14 days after parturition and this finding is in agreement with previous studies (Plaizier *et al*., 2001) that abomasal infusion of glutamine did not affect DMI. But DMI in 21 days after parturition were different ($p<0.03$) among groups with the highest for BFAF and lowest for BFAN. The results showed that there was not any interaction between feeding glutamine before and after calving on DMI (Table 2). Some researchers showed that glutamine is used largely in gut tissues (Windmueller and Spaeth, 1980) and meanwhile the others suggested that after parturition the wet weight of gut tissue increases approximately 12% in dairy cattle. On the other hand, Reeds *et al*. (1994) showed that mucosal cells of digestive tract like other cells that have rapid proliferative (immune system), require glutamine as a provider N for synthesis purines via cytosolic carbamoyl phosphatase II and incorporation on pyrimidines synthesis. The researchers did not find any study which investigated the effects of glutamine on the growth of rumen and gut in transition period in dairy cattle. However, increasing the DMI at 21 day after parturition in BFAF and BNAF groups can be justified based on the increment of capacity in gut and rumen due to more availability of energy and protein for cells in this area and subsequently more growth and proliferative. The lack of a PG effect on DMI immediately after calving can be a result of insufficient growth of gut villi or absorptive area to produce a significant response.

There were no difference and interaction between treatments in milk yield (Table 2). However, a tendency was observed for more milk in after calving glutamine supplemented groups ($p<0.14$). Some researchers showed that infusion or supplementation of glutamine improved milk production (Meijer *et al*., 1995). The lack of significant response possibly is as a result of limited time of experiment and more researches is needed to identify the exact effect of PG on milk production because results showed that 21 days after parturition DMI starts to increasing and this phenomena could lead to increasing milk production.

There were no difference between treatments in milk fat and protein percentage at 10 and 21 days after parturition. This is in agreement with previous studies (Plaizier *et al*., 2001) that showed abomasal infusion of glutamine did not affect milk fat and protein. There was no difference among treatments in BCS at calving day, 10 and 21 days after parturition.

Table 2: Effects of PG on, DMI, milk production and composition and BCS change

	Treatments					p-value		
Item (days)	BFAF	BFAN	BNAF	BNAN	SE	B[1]	A[2]	A×B
DMI (kg days^{-1})								
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	18.00	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
BCS change								
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 day^{-1})								
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^{-1})								
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^{-1})								
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

Table 3: Blood antioxidants parameters

Blood antioxidants parameters	Treatments				p-value			
	BFAF	BFAN	BNAF	BNAN	SE	A	B	AB
TAS (mmol L^{-1}) (Days)								
0	0.30	0.310	0.360	0.340	0.80	0.9100	0.1000	0.58
7	0.34^{a}	0.290^{b}	0.350^{a}	0.300^{b}	0.32	<0.0001	0.5000	0.97
14	0.32^{a}	0.240^{b}	0.310^{a}	0.270^{b}	0.03	<0.0001	0.4400	0.15
21	0.25^{a}	0.210^{b}	0.270^{a}	0.210^{b}	0.03	<0.0001	0.2300	0.25
PGA (units mL^{-1} PCV) (Days)								
0	57.46^{a}	54.770^{a}	57.880^{ba}	49.250^{b}	5.12	0.0020	0.1400	0.09
7	52.95^{a}	48.170^{b}	52.510^{ac}	42.370^{d}	3.88	<0.0001	0.0200	0.04
14	35.35	37.140	43.860	39.900	15.00	0.1500	0.6500	0.34
21	46.76^{c}	40.280^{b}	45.480^{c}	36.020^{a}	2.61	0.0030	<0.0001	0.09

TAS and PGA: The blood parameters have been shown in Table 3. To investigate the redox conditions of plasma dynamically and biologically, measuring TAS is an effective method that provides valuable information (Castillo *et al*., 2006). There were not any significant difference in TAS at calving day among treatments. Miller *et al*. (1993) reported that the decrease of oxidative stress before parturition might be ascribed to the increase of antioxidant protection that occurs in that particular physiological stage.

This means that when the risk of oxidative damage increases, endogenous antioxidant protection increases too. Therefore, the researchers suppose that the increasing antioxidant capacity just before calving could have a confusing effect on the responses. In 7, 14 and 21 days after parturition there were not any significant interaction between B (consume protected glutamine before calving) and A (consume protected glutamine after calving) on the amount of TAS. Meanwhile, there was not any significant effect between levels of feeding PG before calving on TAS condition in 7, 14 and 21 days after parturition. But levels of feeding PG after parturition have significant effect on TAS condition after parturition. With using LSD (Least Significant Differeance) it seems that feeding PG can enhance the TAS condition in post calving. It is declared that proteins that are synthesized in the liver, especially albumin, L-cysteine and homocysteine by means of their SH residuals (originated from cysteine) have significant effect on the antioxidant defense in oxidative stress (Uleand *et al*., 1996). On the other words in the acute phase response proteins in the liver some amino acids like phenylalanine can limit acute phase response proteins by liver (Reeds *et al*., 1994). The researchers suppose that increasing the availability of PG in the gut by protection that from fermentation in the rumen and consequently increasing its uptake by intestine can save methionine and phenylalanine by reamination its oxo-acids (especially methionine and then phenylalanine) and reduce their oxidation in liver (Blarzino *et al*., 1994). Definitely protection of methionine from oxidation can enhance physiological level of cysteine.

There were significant differences among treatments at calving day in PGA condition but there was not significant effect interaction between PG feeding per and postpartum and levels of PG before calving ($p<0.09$). Levels of PG feeding after calving have significant effect on PGA ($p<0.002$) condition at calving day and the results shows that feeding PG can improve PGA activity at calving day. There were significant differences between treatments at 7 day after parturition on PGA condition. Meanwhile, there was significant effect interaction between PG feeding per and postpartum ($p<0.04$) on the GPA 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 GPA condition at 7 days after parturition.

The researchers suppose that feeding PG after parturition could increase PGA at 7 day after parturition. There was not significant difference between treatments on GSH activity at 14 day after parturition and the researchers do not have explained for this. There were significant differences among treatments at 21 days after parturition on PGA activity but there wasn't significant interaction between PG feeding per and postpartum. But levels of PG before calving (0.003) and after calving ($p<0.0001$) has significant effect on PGA activity at 21 days after parturition and the results shows that feeding PG after and before can improve PGA activity at 21 days after parturition. In high producing dairy cows especially in the transition period with increasing milk production, >1 kg of milk protein is secreted daily, $\geq$30% of plasma protein flux (Bequette *et al*., 1996). Glutamine is the most abundant amino acids in the plasma and milk and during early lactation a decline of 25-30 and 75% was reported for the plasma (Meijer *et al*., 1995) and free pool of GLU in the muscle. Halliwell (2007) reported that plasma glutathione peroxidase could be related to plasma lipid peroxidation and content. Yang *et al*. (2000) showed that mitochondria from the fatty livers produce more superoxide anion ($\bullet O_2$) and H_2O_2 compared other cows and possibly supplementation with PG reduced lipid peroxidation due to alleviated negative energy balance and enhanced PGA plasma activity. Through the negative

energy and protein (methionine) balance during transition period in dairy cattle that cows experience many inflammatory conditions and activation of general immune responses like innate (phagocytosis) and acquired (immunoglobulin secretion) and acute phase response (Ametaj *et al*., 2005). Amino acids requirement for protein synthesis especially glutamine and phenylalanine that compromise in acute phase response could to shrink their pools of amino acids (plasma and muscle) (Meijer *et al*., 1995). It is demonstrated that glutathione is extremely important because of its numerous functions (Grimble, 2001).

It acts as a substrate or cosubstrate in the enzymatic reactions; it reacts directly with free radicals and lipid peroxides and it can protect cells (Grimble, 2001). In addition, when other antioxidant is insufficient, glutathione is also can conjugated by peroxyl radical. This might lead to net consumption of glutathione and these phenomena can be take place in early lactation (Castillo *et al*., 2006). Glutathione is mainly synthesized *de novo* from glutamate, cysteine and glycine within the liver and reduction of liver function that is usually observed in the early lactation might have deleterious effect on this pathway (Castillo *et al*., 2006). Cysteine required for glutathione synthesis and liver has the unique and predominant ability to convert the sulphor amino acids methionine to cysteine (Kaplowitz *et al*., 1985). Glutathione biosynthesis is strictly dependent on precursors amino acids concentration and competes with albumin synthesis for the available cysteine (Droge *et al*., 1994). It is very important to know that the kinetic characteristics expressed by the kilometer for amino acids activating enzymes (the rate limiting enzymes for protein synthesis) is 0.003 mmol L^{-1} while that for gamma glutamyle cysteine synthase (the rate limiting enzymes for glutathione synthesis) is 0.035 mmol L^{-1}. This means that the biosynthesis pathways for protein works maximally at concentration approximately 166 fold lower than for glutathione synthesis whose production is subsequently impaired in greatly amounts than that for protein at low cysteine availability (Grimble, 2001). Glutamine has defiantly effects on glutamate availability for glutathione synthesis and in addition by means of save methionine from oxidation on cysteine availability (Blarzino *et al*., 1994).

Providing glutamine with effects on saving methionine and providing glutamate can have increasing effects on glutathione synthesis and PGA activity that can be seen in 0, 7, 21 days after calving. There were not any significant difference among treatments in milk fat and protein percentage at 10 and 21 days after parturition. And this finding is consisted with previous studies (Plaizier *et al*., 2001) that abomasal infusion of glutamine hasn't any effect on milk fat, protein and composition.

CONCLUTION

The results of this study shows that increasing the amount of glutamine in transition period has effective effects on increasing antioxidant capacity and by means of development in gut tissue can lead to increasing dry matter intake. To assay effects on milk production more time is needed. More researches is needed to investigation of effects of improvement of antioxidant capacity on health and production in dairy cattle.

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