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Glutamine andGlutathione peroxidase activity (GPX) and Total Antioxidant Capacity (TAS) inPrepartum in Holstein Dry Cows

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ABSTRACT

The objectives of this study was to investigate whether consuming of protected glutamine (PG) before parturition in close up period would affect dry matter intake ($DMI_{kg/d}$), blood urea nitrogen ($BUN_{mg/dl}$), body condition score (BCS) and biomarkers of oxidative stress, glutathione peroxidase activity ($GPX_{units/ml\ PCV}$) and Total Antioxidant Status ($TAS_{mmol/L}$). Thirty six pregnant Holstein dairy cows were assigned into two treatment groups based on their BCS and expected calving date in t student examination. Treatment groups consisted of 1)glutamine supplementat - ion100 g/d per cow from 21d before calving until parturition (F), 2) glutamine unsupplementation from 21d before calving until parturition (N). There weren't any significant differences among treatments in DMI and BCS on d -21,-14 ,-7 and calving day before parturition. There weren't significant differences in The total antioxidant status(TAS) but plasma glutathione Peroxidase activity (GPX) was significant difference between two group and was greater for (F) group at -7d before calving (57.44 vs 47.94 and $p \leq 0.05$ respectively) and -14 (45.87 vs 41.72 and $p \leq 0.04$ respectively) before parturition. It seems that supplementation diets with glutamine on the close up period can enhance plasma glutathione Peroxides activity (GPX) and the best level of formaldehyde for protection of glutamine is 1%.

Key words: Holstein Cow, Glutamine, Glutathione Peroxides Activity, Total Antioxidant Status.

INTRODUCTION

The nonlactating phase of the dairy cow is regularly referred to as the dry period. Throughout this period, the mammary gland undergoes remarkable changes in histology and physiology, characterized as involution, which is believed to be necessary for maximal milk production in the subsequent lactation [1]. The high-producing dairy cow experiences vast metabolic changes during the transition from the dry period in late conception to the start of milk productions in early lactation [2]. The periparturient period is associated with increasing risk of metabolic-production related diseases; because of inadequate metabolic homeorhetic adaptation [1]. Approximately one half of economic losses in treatments of dairy industry assigned to transition period [3]. An imbalance between increased production of ROS and the availability of antioxidant defenses needed to reduce ROS accumulation during the periparturient period may expose cows to increased oxidative stress. There are now several more recent studies to support the concept that oxidative stress is a significant causal factor to dysfunctional host immune and inflammatory responses that can increase the weakness of dairy cattle to a variety of health disorders, particularly during the transition period [4]. It has been hypothesized that an involvement of oxidative stress during transition period is the etiology of some diseases and disorders in dairy cows [4]. During the transition period, immunosuppression commonly occurs and cows exhibit great susceptibility to a number of diseases [5]. A number of components of the host defense system are changed during this period including neutrophil function, lymphocyte responsiveness to mitogen stimulation, antibody responses, and cytokine production by immune cells [6]. Impaired neutrophil function prior to parturition has been linked to the occurrence of mastitis, metritis, and retained placenta in dairy cows [7]. A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in both humans and dairy cows [8]. Supplementing dairy cows with sufficient levels of both vitamin E and Se was shown to increase the phagocytosis, bacterial killing, and oxidative metabolism of peripheral blood and mammary gland neutrophils when compared to cows that were otherwise deficient in this micronutrient before calving [9]. Some earlier studies presented effective treatment of vitamin A on udder health. Cows with mastitis had lower levels of plasma vitamin A and cows supplemented with b-carotene prior to involution had lower rates of new intramammary infections during the dry period when compared to un-supplemented animals [11]. An increased occurrence of mastitis and the severity of clinical symptoms were associated with decreased concentrations of plasmatic vitamin C [12]. Cytosolic glutathione peroxidase (GPX) is the selenoenzyme usually associated with antioxidant functions in cattle [13]. By increasing use of NADPH in oxidative stress (for reducing glutathione peroxides) the ability of neutrophils to destroy microbes will be diminish and immune system will be suppressed [14].*in vitro* studies have shown that glutamine can provide about 38% metabolizable energy for macrophages [15]. Other evidence suggested that proliferative response of rat, mouse and humans lymphocytes to mitogens are depend upon availability of glutamine [16]. Considering what was mentioned above, we hypothesized that increasing glutamine in prepartum period can decrease oxidative stress and consequently improve immune function performance and positive effects on acute phase responses.

MATERIALS AND METHODS

2.1. 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 to November 1. 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(n=16) based on their BCS, parity, and expected calving date. One group became control group (N) and another received glutamine (100g/d) per cow until calving (F). Two groups received a ration as TMR for based on their requirement to supply their need in close up period based on NRC (2001) recommendations [17]. Experimental are showed 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 until calving. Dry matter of feeds were measured weekly by drying in a oven at 105°C for 48 hour.

Table 1 Ingredient and chemical composition of experimental diet

Ingredient composition (% of DM)	
Alfalfa hay	36.82
Corn silage	10.1
Beet pulp	4.14
Wheat straw	4.23
Barley ground grain	4.89
Corn ground grain	13.8
Cotton seed whole	2.2
Cotton seed meal	1.08
Canola meal	1.36
Soybean meal	6.23
Wheat barn	11
Anionic salt[2]	2.5
Di-calcium phosphate	0.15
Calcium carbonate	0.35
Mineral mix[3]	0.25
Vitamin mix[4]	0.9
Chemical composition (dry basis)	
$NE_{L,}$ Mcal/kg	1.62
CP (g/kg)	142
NFC (g/kg)	388
ADF (g/kg)	240
NDF (g/kg)	380
Ash (g/kg)	78.9
EE (g/kg)	311

[1]Experimental diets were different only in glutamine supplementation (100 g/d). Protected glutamine was added to (F).
[2]Contained 15% calcium carbonate, 24.2% magnesium sulfate, 10.8% chloride ammonium, 18.8% calcium chloride.
[3]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).
[4]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.

2.2. Measurements and sampling

In the trail period 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 [10]. Body condition score (BCS) was scored (five point scale where 1 = emaciated and 5 = obese) by three skilled individuals in 0, +10 and +21. Feed intake was determined daily by measuring supplied feed and refusals for TMR and was averaged per wk. Dry matter intake (DMI) in calving day measured

individually and reported but DMI in 0, - 7, -14 and -21 days before 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. 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 (GPX),total antioxidant status in plasma (TAS) and blood urea nitrogen (BUN). BUN was analysed by manual colorimetric method [31]. GPX activities were determined by a kinetic method with a commercial kit (RANSEL by Randox laboratories ltd).The method was based on Paglia and Valentines [18]. 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 340nm 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 $ABTS^+$[32]. This has a relatively stable blue–green colour which is measured at 600 nm.

2.3. Amino acid protection method and feeding

In order to protect glutamine, we used formaldehyde [19]. To ensure and apply the best level of formaldehyde we designed an experiment by the use of fresh clarified rumen fluid (CRF) and *anaerobic defined medium in serum bottle* (without nitrogen). We prepared anaerobic defined mediums that haven't any sources of nitrogen and the only source of nitrogen for proliferative or growth of bacteria was 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 spring on the 5gr glutamine and after reaction dried them in an oven at 40°C at 24 hour (Davies et al., 1993). After preparation of anaerobic defined mediums in bottles that haven't any source of nitrogen the protected glutamine with different levels of formaldehyde and (CRF) from dairy cattle injected to bottles (n=3). It was hold in $39^{\circ C}$ for 24 h,then we repeated and continued inoculation third sub culture. It was clear that the amount of bacteria growth depend on glutamine availability. Therefore we measured the amount of offence (representation of bacteria population) in bottles by use of a spectrophotometer and then compared them with t-student. Then, we observed that the best level of formaldehyde is 1% and there wasn't significant between levels more than 1%, but protection with 1% was better than 0.5%.

2.4. Statistical analysis

The means of two group comparated by using *t student* between groups. Data measured over time (DMI and other parameters) within the period of interest were subjected to procedure of SAS. 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

3.1. DMI, TAS, GPX, BUN and BCS.

The two ration were isonitrogenous and isoenergetic except adding 100 gram protected glutamine (PG) per cow/d. The dry matter intake (DMI), BUN, BCS changes, TAS, GPX have been shown in Table 2. There was no significant difference in DMI among groups in 0, -10 and -

21 day before parturition, and this finding is in agreement with previous studies that abomasal infusion of glutamine did not affect DMI [20]. To investigate the redox conditions of plasma dynamically and biologically, measuring TAS is an effective method that provides valuable information [21]. There weren't any significant difference in TAS at calving day,-7,-14 and -21d among treatments. Miller et al. [4]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 we suppose that the increasing antioxidant capacity just before calving could have a confusing effect on the responses.

Table 2.TAS,GPX,BUN,DMI and BCS assay

	Teratment		P-Value
	F	N	
TAS(mmol/L)			
Days			
0	0.398	0.4	0.33
-7	0.311	0.327	0.29
-14	0.255	0.276	0.25
-21	0.215	0.227	0.51
GPX(units/ml PCV)			
Days			
0	55.5	53.5	0.39
-7	57.44	47.94	<0.0001
-14	45.87	41.72	0.04
-21	30.87	33.73	0.27
BUN(mg/dl)			
Days			
0	14.62	14.3	0.24
-10	13.64	13.82	0.98
-21	13.57	13.47	0.77
DMI (kg/d)			
Days			
0	8.44	8.8	0.18
-10	13.29	13.4	0.22
-21	13.2	13.36	0.12
BCS			
Days			
0	3.56	3.66	0.75
-21	3.25	3.34	0.89

There were significant differences among treatments at -7 and -14 day before parturition in GPX condition, but there wasn't significant differences between treatments in GPX condition at 0 and -21 day before calving. In high producing dairy cows especially in the transition period with increasing milk production, more than 1kg of milk protein is secreted daily, equal to more than 30% of plasma protein flux [22]. Glutamine is the most abundant amino acids in the plasma and milk and during early lactation a decline of 25 to 75% was reported for the plasma [23] and free pool of GLU in the muscle [24]. Halliwell et al. [25] reported that plasma glutathione peroxidase could be related to plasma lipid peroxidation and content. Yang et al. [26] showed that mitochondria from the fatty livers produce more superoxide anion ($\cdot o_2$) and H_2O_2 compared other cows and possibly supplementation with PG reduced lipid peroxidation due to alleviated

negative energy balance and enhanced GPX plasma activity. 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 [21]. Cysteine required for glutathione synthesis and liver has the unique and predominantly ability to convert the sulphor amino acids methionine to cysteine [27]. Glutathione biosynthesis is strictly dependent on precursors amino acids concentration and competes with albumin synthesis for the available cysteine [28]. It is very important to know that the kinetic characteristics expressed by the km rate for amino acids activating enzymes (the rate limiting enzymes for protein synthesis) is 0.003 mmol/L, while that for gamma glutamyle cysteine synthase (the rate limiting enzymes for glutathione synthesis) is 0.035 mmol/L. 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 [30]. Glutamine has defiantly effects on glutamate availability for glutathione synthesis and in addition by means of save methionine from oxidation on cysteine availability [29]. Providing glutamine with effects on saving methionine and providing glutamate can have increasing effects on glutathione synthesis and GPX activity that can be seen at-7 and -14 daybefore calving.

CONCLUSION

Result of this study showed that protection glutamine with 1% formaldehyde is the best level compare to other levels. Increasing the amount of glutamine in close up period period has effective effects on enhancement antioxidant capacity by increasing glutathione peroxides activity in plasma.

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