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Effect of Dietary Supplementation of Glutathione on Blood Biochemical Changes and Growth Performances of Holstein Calves

Jong Hyeong Kim, Lovelia L. Mamuad, Hyun June Lee[1], Kwang Seok Ki[1], Wang Shik Lee[2], Jong K. Ha[3] and Sang Suk Lee*

Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon, Jeonnam 540-742, Korea

ABSTRACT : The objective of this experiment was to evaluate the effect of dietary supplementation of glutathione (GSH) on health, solid feed consumption, nutrient intake, body weight gain (BWG), feed efficiency, blood metabolites and the occurrence of diarrhea in Holstein neonatal calves. The calves were fed plain milk as a control (CON) or milk with GSH supplementation. Sixteen calves were separated from their mothers immediately after birth, moved into individual cages and fed colostrum for the first three days. For GSH supplementation, three grams of GSH powder were mixed in 1.8 L of heat-treated milk and placed in a plastic bottle with a rubber nipple. The calves were fed GSH-supplemented milk only once out of four daily feedings. For the first 25 d, calves were fed 1.8 L of milk four times per day. Milk feeding frequency was reduced to three times per day from days 26 to 30, followed by twice a day from days 31 to 44, and once a day from days 45 to 49, after which they were weaned at day 50. Body weight gain (BWG), feed consumption, and growth performance were monitored until day 70. The dietary supplementation of GSH had no effect on daily feed intake and growth performance in growing calves. Hematological results revealed red blood cell distribution width (RDW) was lower, and mean corpuscular volume (MCV) was significantly higher in calves fed GSH. Serum lactate dehydrogenase (LDH) concentrations were lower in calves fed GSH. Rectal temperature at day 70 was higher in calves that did not receive GSH, while mean frequency of diarrhea and enteritis was less in calves fed GSH. It is concluded from the present study that BW gain, total dry matter intake (DMI), feed efficiency, and breathing rate did not differ between groups. However, there were some positive blood parameters and the mean frequency of diarrhea and enteritis was less in calves fed GSH compared to CON which did not receive GSH. With the results obtained, supplementation of GSH is highly recommended. (**Key Words :** Glutathione, Hematology, Neonatal Calves)

INTRODUCTION

Glutathione (GSH) is a beneficial protein in every cell of the body which functions as an antioxidant, immune system optimizer, detoxifier, energy restorer, and anti-aging protein. It is an important substance particularly with regards to elimination of waste matter from the body and one of the most important connections to the widely distributed tripeptide thiol GSH. Indeed there is a strong evolutionary link between GSH and eukaryotic aerobic metabolism that is reflected in the function of GSH in protection against oxygen toxicity (Fahey and Sundquist, 1991). GSH is not required in the diet of animals but is synthesized in virtually all animal cells by the sequential actions of two enzymes: y-glutamylcysteine synthetase and GSH synthetase. GSH deficiency can be reduced by supplementary administration to animals. Jain et al. (1991) and Meister (1995) found that when such GSH deficiency occurred in newborn rats or guinea pigs, the animals developed multi-organ failure and died within a few days. This result is directly related to the loss of an essential antioxidant system. The animals exhibited focal necrosis in the liver, proximal tubular damage in the kidneys, and disruption of lamellar bodies in the lung. Newborns with GSH deficiency developed brain damage (Martensson and

* Corresponding Author : Sang Suk Lee. Tel: +82-61-750-3237, Fax: +82-61-750-3237, E-mail: rumen@scnu.kr
[1] Dairy Science Division, National Institute of Animal Science, Korea.
[2] College of Applied Life Sciences, Jeju National University, Korea.
[3] Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea.
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Meister, 1991) and also cataracts (Calvin et al., 1986). Calves are exposed to viruses, bacteria, and parasites in the environment which are capable of causing respiratory problems and diseases. These are stress factors that can significantly reduce the GSH reservoir. Reduced GSH levels result in lower energy, higher inflammation, cellular damage, and a weakened resistance to various diseases. Thus, the objective of this experiment was to evaluate the effect of supplemental GSH on health, solid feed consumption, nutrient intake, body weight gain (BWG), feed efficiency, blood metabolites and the occurrence of diarrhea in Holstein neonatal calves.

MATERIALS AND METHODS

Experimental design

Supplementation of GSH in experimental procedures was approved by the National Institute of Animal Science (Gwonsun-Gu, Suwon, South Korea). A total of 16 Holstein calves born between June and August 2007 were immediately separated from their dam within 24 h of birth, weighed, and placed in naturally ventilated individual pens (1.5×2.5 m) with sawdust bedding. The pens had solid iron rod sides with openings in the front and rear to allow calves free access to calf starter (CS) (contained 88.2% dry matter (DM) and 74.7% crude protein (CP)) and chopped mixed hay (contained 90.3% DM and 10.4% CP) from feeding buckets. The chemical composition of the CS and hay provided is shown in Table 1. Drinking bowls in each calf pen allowed free access to water. The experimental design was as follows: i) CON (n = 8) were calves fed only whole milk; and ii) GSH (n = 8) were calves fed milk and GSH. The milk and GSH was mixed at a ratio of 3 g of GSH powder to 1.8 L of warm milk which was only fed at 1100 h. Before feeding, the milk in the steel buckets was heated to 37°C using a water bath. During the first three days, calves were fed colostrums at a volume based upon 10% of their BW. The milk and colostrums compositions shown in Table 2 were analyzed with a MK2 Lactoscope (Delta Instruments, Drachten, The Netherlands). Plastic feeding bottles (2 L capacity) with rubber nipples were used to feed the calves milk sequentially at different frequencies. At days 4 to 25, 7.2 L of milk were fed to the calves at 0500, 1100, 1700, and 2300 h followed by 5.4 L at 0500, 1100, and 2300 h on days 26 to 30, and 3.6 L at 1100 and 2300 h on days 31 to 45. They were weaned gradually from day 46 to 50 with 1.8 L of milk at 1100 h and observed until day 77 with regards to growth, feed intake, and hematological parameters.

Table 1. Mean chemical composition of calf starter and hay[1] on a DM basis

Composition (%)	Calf starter	Hay
DM	88.2±0.27	90.4±0.20
OM	84.6±0.05	84.2±0.29
ADF	4.90±0.11	36.1±1.62
NDF	12.5±0.18	60.1±1.13
Crude fat	2.30±0.53	1.40±0.16
Crude fiber	3.40±0.09	30.4±0.29
Crude protein	25.3±0.13	10.4±0.53

[1] Mixed grass hay contained 40% orchard grass, 40% tall fescue, and 20% white clover on a DM basis.

Values presented are mean±standard error.

ADF = Acid detergent fiber. NDF = Neutral detergent fiber.

Sampling and analysis

BWG, body barrel (BB), head girth (HG), body length (BL), wither height (WH), and hip height (HH) were measured at days 1, 14, 28, 42, 56, 70 and 77. CS and water intake for all calves were measured twice weekly until day 77. CS and feed refusals were collected and analyzed for DM by the Association of Official Agricultural Chemists (AOAC) method (AOAC, 1990). Average BWG, total dry matter intake (DMI) (milk, CS, and hay), feed efficiency, and rectal temperature were recorded preweaning (d 1 to 50), post weaning (d 50 to 77), and in total (d 1 to 77).

Blood samples were collected from the jugular vein of the calves before the second feeding (1100 h) on days 7, 14, 21, 28, 35, 49 and 63 using 10-ml evacuated tubes (BD; Franklin Lakes, NJ, USA) without anticoagulant and with 4 ml ethylenediaminetetraacetic acid (EDTA). The blood samples that were collected without anticoagulant were centrifuged at 1,000×g for 20 min at 4°C and the serum were partitioned into aliquots and stored at -20°C until analyzed for albumin, glucose, blood urea nitrogen (BUN), calcium, creatinine, glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), and globulin with a serum analyzer (Hitachi Chemistry Analyzer 7180, Japan). The blood samples collected with EDTA were used to determine concentrations and percentages of leukocytes (white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils, and basophils), erythrocytes (red blood cells (RBCs)), hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin

Table 2. Mean chemical composition of colostrum and milk

Composition (%)	Colostrum[1]	Milk[2]
Fat	6.21±0.41	3.52±0.20
Protein	13.2±1.22	3.01±0.06
Lactose	2.86±0.23	4.79±0.05
Total solids	23.2±1.23	12.1±0.27
FFA (mEq/dl)	0.99±0.34	0.80±0.12
Citrate (mEq/dl)	0.11±0.02	0.16±0.01

[1] Colostrum samples were collected for analysis twice daily.

[2] Milk was collected in a cooling tank.

Values presented are mean±standard error. FFA = Free fatty acid.

concentration (MCHC), red blood cell distribution width (RDW), thrombocytes (platelet count (PLT)) and mean platelet volume (MPV) using a Hemavet 950 hematology analyzer (Drew Scientific Inc., USA).

Statistical analysis

Data on CS, hay, water, and BWG are expressed as means±standard error of the mean (SEM). Totality differences in feed consumption, BW, BWG, skeletal growth, feed consumption, liquid feed intake, and gain efficiency were evaluated using a Wilcoxon two-sample test (SAS, 2002). Blood metabolite data were analyzed using proc GLM.

RESULTS AND DISCUSSION

Feed intake and growth performance

The average daily milk, CS, hay and water consumption of Holstein calves fed with or without GSH at days 1 to 50, 51 to 70, and 1 to 70 is presented in Table 3. The mean daily consumption of milk was similar in both groups. Moreover, average daily consumption of CS at days 1 to 50 and 51 to 70, mixed grass hay, and water intake were also not significantly different.

The calves were fed using a step-down method. Similar consumption of CS, hay, and water by Holstein calves fed with or without GSH may be due to the similar feed efficiency and the same milking method. With this type of method, adaptation of calves to supplementation of GSH in milk may be one of the factors affecting the similarity of milk consumption. Moreover, using this method in milk feeding helps prevent the problem of depressed solid feed intake associated with *ad libitum* milk feeding and poor BWG encountered with conventional milk feeding in dairy calves (Khan et al., 2007). Significant differences in solid feed consumption were previously reported when calves were offered restricted or *ad libitum* amounts of liquid feed (Hammon et al., 2002; Khan et al., 2007). The results show that addition of GSH did not affect milk palatability for the calves and continuous or further use of GSH supplementation in milk is possible. Moreover, the feed additive yeast extract powder, a product of China, is currently used commercially which supports the palatability of GSH as a supplement. GSH is one of the components of this feed additive and the product claims to improve feed intake significantly, as well as feed conversion rate and animal performance in pigs.

Mean growth measurements of Holstein calves (BW, BL, HG, BB, HH, and WH) are shown in Table 4. Average BW, HG, and BB were similar in both groups at birth, day 50, and day 70. On the other hand, BL (p<0.05) and WH (p<0.01) were significantly different in the two groups at birth but not significantly different at day 50 and day 70. Moreover, HH was significantly different at birth (p<0.01), day 50 (p<0.01), and day 70 (p<0.05) among treated and non-treated animals.

Supplementation of GSH slightly lowered the growth

Table 3. Average daily milk, calf starter, hay, and water consumption of Holstein calves fed with or without GSH supplementation

Parameters	CON	GSH	SE	p-value
Milk intake (kg)				
d 1 to 50	5.2	5.3	0.05	0.39
Calf starter (g)				
d 1 to 50	455.9	476.3	92.7	0.88
d 51 to 70	2,472.0	2,010.0	259.1	0.34
d 1 to 70	1,000.2	921.3	124.1	0.66
Hay (g)				
d 1 to 50	63.7	78.1	10.4	0.38
d 51 to 70	205.0	178.0	32.1	0.56
d 1 to 70	97.7	104.6	11.2	0.68
Water (kg)				
d 1 to 50	2.5	2.6	0.25	0.84
d 51 to 70	10.4	8.6	0.48	0.09
d 1 to 70	5.0	4.6	0.28	0.43

Table 4. Average growth measurements of Holstein calves fed with or without GSH

Parameters	CON	GSH	SE	p-value
Body weight (kg)				
At birth	43.5	41.0	1.36	0.21
d 50	71.4	64.9	3.19	0.17
d 70	88.6	82.3	3.11	0.18
Body length (cm)				
At birth	79.8	74.8	1.36	0.03*
d 50	87.0	84.1	1.51	0.21
d 70	92.1	89.7	1.68	0.33
Head girth (cm)				
At birth	83.7	83.7	1.32	0.99
d 50	97.9	96.0	1.30	0.33
d 70	104.4	103.6	1.28	0.70
Body barrel (cm)				
At birth	88.8	85.0	2.25	0.26
d 50	108.6	108.4	2.79	0.96
d 70	119.2	119.4	2.23	0.97
Hip height (cm)				
At birth	86.2	82.3	0.91	0.01**
d 50	91.9	87.4	0.95	0.01**
d 70	95.1	91.9	1.04	0.04*
Wither height (cm)				
At birth	82.7	78.0	0.68	0.01**
d 50	88.0	85.0	1.60	0.20
d 70	91.1	87.7	1.19	0.06

* Means are significantly different (p<0.05).
** Means are significantly different (p<0.001).

measurements of Holstein calves compared to those fed plain milk but otherwise the calves were generally not affected by the addition of GSH. This result is in contrast to the claims associated with the feed additive yeast extract powder which assert that GSH supplementation increases beef cattle feed intake, promotes growth, and improves daily weight gain. This may be due to the fact that the amount supplemented was only 3 g per 1.8 L which is comparatively less than that recommended and claimed by Kenyhercz and Jorgensen (1997). They claimed that there is an effective way to increase BW, and prevent and treat weight loss in mammals in need of such management by providing GSH in amounts of approximately 200-5,000 mg, administered in two to four substantially equal dosages per day, with each dose being administered between three to eight hours apart.

Table 5 shows the effect of GSH supplementation on BWG, total DMI, feed efficiency, rectal temperature, and breathing rate. Treatment effect on BWG, total DMI, feed efficiency, and breathing rate were not significantly different in all groups monitored. On the other hand, rectal temperature at day 70 was significantly higher ($p<0.05$) in calves fed only milk compared to those fed milk and GSH, but at birth and day 50 results showed no significant difference between the treatments.

Table 5. Average BW gain, total DMI[1], feed efficiency[2], rectal temperature, and breathing rate of Holstein calves fed only milk or milk with GSH added

Parameters	CON	GSH	SE	p-value
BW gain (kg)				
d 1 to 50	28.1	23.9	3.57	0.41
d 51 to 70	16.7	16.4	2.33	0.94
d 1 to 70	45.3	40.9	3.33	0.37
Total DMI (kg)				
d 1 to 50	48.8	48.8	3.22	0.99
d 51 to 70	57.6	42.3	5.71	0.17
d 1 to 70	104.7	90.1	7.62	0.28
Feed efficiency				
d 1 to 50	0.51	0.43	0.08	0.53
d 51 to 70	0.43	0.45	0.07	0.80
d 1 to 70	0.45	0.45	0.05	1.00
Rectal temperature				
At birth	38.6	39.2	0.21	0.06
d 50	38.9	38.7	0.28	0.68
d 70	39.3	38.7	0.16	0.03*
Breathing rate				
At birth	46	40	2.93	0.24
d 50	40	38	3.62	0.76
d 70	43	41	1.91	0.41

[1] Total DMI = Iincludes milk, calf starter, and hay.
[2] Feed efficiency = kg of BW gain/kg of total DMI.
* Means are significantly different ($p<0.05$).

Generally, the effect of GSH on BWG, total DMI, feed efficiency, rectal temperature and breathing rate was similar but slightly lower than those without GSH supplementation. In accordance with the results obtained, Kenyhercz and Jorgensen (1997) mentioned that U.S. Pat. No. 4,466,978 describes the use of GSH to reduce BW, but provides no examples. In addition, the administration of a nutritional composition containing multiple ingredients including GSH was required to affect weight gain in AIDS patients and the administration of GSH alone has been reported to promote the opposite effect, namely weight loss, in overweight persons (U.S. Pat. No. 4,466,978). Moreover, high doses of GSH have previously been reported to promote cachexia in guinea pigs (Martensson et al., 1993).

The results are in contrast to assertions regarding the feed additive yeast extract powder that claim to improve feed intake, daily weight gain, and increase feed conversion in poultry. In this case, the results may be different due to the fact that different species were used, which may experience different effects from GSH supplementation. In addition, Kenyhercz and Jorgensen (1997) cited in their patent that they unexpectedly found that treatment with GSH produced the opposite effect, namely causing an increase in BW. They also claimed that quite unexpectedly, administration of GSH alone can increase BW.

The mean occurrence of diarrhea and enteritis in Holstein calves fed with or without GSH is shown in Table 6. Overall, the mean occurrence of diarrhea and enteritis was lower in calves fed milk and GSH than in calves fed only milk. A high incidence of diarrhea is usually related to sanitation, management, and housing conditions of calves rather than their daily milk intake (Hammon et al., 2002).

These results agree with those of the feed additive yeast extract powder which claims to improve calf disease resistance as well as immunity. It also claims to improve milk yield of cows and goats, milk quality, prolong the milking peak period, and decrease the rate of cow mastitis. Moreover, Iurkiv et al. (1985) reported that diarrhea syndrome was found to decrease markedly after GSH administration at a dose of 1 g/kg BW. In addition, the polyfunctional cellular activity of GSH suggests that the anti-diarrhea effect should be considered as an element of pathogenetic therapy. Greenwell (2001) cited Perlmutter wherein he claimed that intravenous GSH is immediately effective against irritable bowel syndrome and diarrhea.

Table 6. Mean occurrence of diarrhea and enteritis in Holstein calves fed with or without GSH

Parameters	CON	GSH
Diarrhea		
d 1 to 50	2.8±1.0	1.5±0.5
Enteritis		
d 1 to 50	5.0±1.9	3.6±0.7

Furthermore, Roberts (2008) said that having a reduced GSH antioxidant level may severely compromise the defense system, rendering it more at risk to oxidative tissue damage, hindering recovery from inflammatory bowel disease as well as any inflammatory diseases. Therefore, by replenishing essential GSH the bodies' natural defense against inflammation is increased. Thus, supplementation with GSH may promote absorption of iron and decrease the frequency of diarrhea in calves.

Hematology and serum chemistry

Hematological changes in Holstein calves fed with or without GSH are shown in Table 7. In general, WBCs and lymphocytes were similar in all periods except at day 14 wherein lymphocytes were significantly higher ($p<0.05$) in calves fed with milk and GSH compared to CON. On the other hand, RBC and HCT were higher ($p<0.05$) at days 7 and 21 in calves fed milk mixed with GSH versus CON. Moreover, MCV was higher ($p<0.05$) at days 35, 49, and 63 in calves fed milk mixed with GSH versus CON. Also, RDW was only found to be lower ($p<0.05$) at day 28 in calves fed milk mixed with GSH versus CON. In addition, platelet counts were higher ($p<0.05$) on days 28 and 49 in calves fed milk mixed with GSH versus CON.

Normally, the range of RBC is $5.0\text{-}10.0\times10^6/\mu l$ (Feldman et al., 2006) which was found in calves supplemented with GSH. It is noticeable that without GSH supplementation increasing amounts of RBCs were found. Also, at day 7 the RBC count was lower than the normal range and at day 63 it was higher than the normal range. According to this result, GSH supplementation helps stabilize the RBC count in cattle.

Quantitative descriptors of erythrocyte size include both the RDW and the conventional erythrocyte index or MCV (Turgeon, 2005). MCV is the size of the RBCs while RDW is also size-related indicating how much variation of size there is between RBCs. If anemia is observed, RDW test results are often used together with MCV. Turgeon (2005) also mentioned that RDW is independent of high, low, or normal MCV and is an earlier sign of nutritional deficiency than MCV. The relationship of RDW and MCV can characterize various erythrocytic abnormalities. Iron deficiency anemia initially presents with a varied size distribution of RBCs and as such shows an increased RDW. Thus, RDW and MCV are thought to be highly sensitive blood cell parameters in the differentiation of iron deficiency anemia.

In a previous study, Bednarek et al. (1996) found that higher blood leukocyte counts and globulin levels in parenterally selenium-supplemented calves than in control animals, but the differences in erythrocyte count, Hgb concentration, and HCT were not significant. In another study, WBC counts were significantly higher in selenium injected groups of rats than in controls, but the RBC count and the Hgb, PCV, MCV, MCH, and MCH concentration values were apparently not influenced by the injection of selenium (Cay and Naziroglu, 1999). In cows with anemia, it is the MCV measurement that allows classification as either microcytic anemia (below normal range) or macrocytic anemia (above normal range). Furthermore, another study demonstrated for the first time a direct *in vivo* and *in vitro* action of GSH in enhancing intracellular magnesium content and showed significant and independent positive relationships *in vivo* between intracellular

Table 7. Hematological changes in Holstein calves fed with or without GSH

Item	Treatment	Calf age in days							SE
		7	14	21	28	35	49	63	
WBC (k/μl)	CON	8.6	8.9	9.3	9.1	10.1	9.1	11.7	1.43
	GSH	13.3	14.6	11.5	10.5	10.3	10.3	10.6	
Lymphocyte (k/μl)	CON	4.36	4.78	5.06	5.14	5.56	5.47	6.55	0.83
	GSH	5.23	8.19*	5.81	5.49	5.79	5.73	5.76	
RBC (M/μl)	CON	4.97	6.97	7.25	9.93	9.30	9.97	11.2	0.77
	GSH	8.45*	7.91	8.68*	8.50	9.45	9.79	9.09	
HCT (%)	CON	14.1	18.3	17.1	22.1	19.1	20.2	24.4	2.26
	GSH	25.8*	22.0	22.3*	20.5	22.1	23.6	24.2	
MCV (fL)	CON	28.6	26.4	23.5	21.7	20.4	20.1	21.8	0.81
	GSH	30.4	27.5	25.6	23.9	23.3*	24.1*	26.4*	
RDW (%)	CON	28.0	33.9	38.1	40.8	38.6	37.5	38.7	1.25
	GSH	29.3	33.9	35.7	36.2*	35.8	35.5	36.1	
Platelet (k/μl)	CON	568.5	752.0	533.4	681.3	649.5	605.4	492.3	60.4
	GSH	550.0	573.8	512.3	511.5*	517.8	416.4*	483.8	

WBC = White blood cell; RBC = Red blood cell; HCT = Hematocrit; MCV = Mean cell volume; RDW = Red blood cell distribution width.

* Means are significantly different between treatments ($p<0.05$).

Table 8. Serum chemistry changes in Holstein calves fed with or without GSH

Item[1]	Treatment	Calf age in days								SE
		7	14	21	28	35	49	63	77	
LDH (units/L)	CON	558.7	573.0	621.2	748.0	734.4	768.0	781.0	816.3	56.7
	GSH	438.0	388.8*	448.4*	552.4*	534.4*	468.0*	550.5*	628.0	
BUN (mg/dl)	CON	11.90	10.98	11.08	13.78	15.24	14.54	17.20	19.83	1.86
	GSH	8.70	9.03	7.78*	9.14*	15.38	14.44	16.18	16.26	
Triglycerides (mg/dl)	CON	19.7	35.4	64.0	36.6	20.6	21.0	21.0	21.8	5.83
	GSH	31.7	40.0	40.2	25.6	19.6	28.2	24.2	29.8*	
Albumin (g/dl)	CON	2.87	2.94	3.20	3.30	3.28	3.18	3.34	3.20	0.11
	GSH	2.97	2.90	3.20	3.18	3.30	3.40*	3.34	3.36	
GOT (units/L)	CON	34.0	27.6	31.0	39.6	46.4	55.0	71.6	71.3	5.20
	GSH	25.3	22.0	20.2	33.2	32.6	32.8*	58.6	60.4	
GPT (units/L)	CON	7.7	4.4	4.6	4.0	6.2	7.8	12.8	16.0	1.47
	GSH	7.3	3.8	2.8	3.8	5.8	8.6	14.0	21.2	

[1] LDH = Lactate dehydrogenase; BUN = Blood urea nitrogen; GOT = Glutamic-oxaloacetic transaminase; GPT = Glutamic-pyruvic transaminase.
* Means are significantly different between treatments (p<0.05).

magnesium content, GSH/GSSG ratios, and insulin-mediated glucose disposal. The data are consistent with the role of magnesium in mediating the effects of GSH on peripheral insulin action (Barbagallo et al., 1999).

Serum chemistry changes in calves fed with or without GSH are shown in Table 8. Lactate dehydrogenase (LDH) was lower (p<0.05) in calves fed milk with GSH than CON at days 14, 21, 28, 35, 49, and 63. On the other hand, BUN was lower (p<0.05) in calves fed milk with GSH than CON at days 21 and 28. Moreover, calves fed milk with GSH were found to have higher (p<0.05) triglycerides at day 77 and albumin at day 49 but lower GOT at day 49. There was no significant difference between treatments for GPT values.

A higher BUN at days 63 and 77 in CON animals than in those fed milk with GSH may be due to the higher rate of protein degradation as a result of higher protein intake and possibly a more functional rumen (Hadorn et al., 1997). On the other hand, higher LDH was found in CON calves than those that received GSH. Higher LDH in calves is usually related to anomalies of liver metabolism and diarrhea. LDH is often used as a marker of tissue breakdown as it is abundant in RBCs and can function as a marker for hemolysis. Higher LDH enzyme levels are related to the generation of frequent anemia and diarrhea. In the present study, LDH values were lower in calves fed GSH.

CONCLUSION

This study has revealed the effects of GSH on Holstein neonatal calves. BW gain, total dry matter intake (DMI), feed efficiency, and breathing rate did not differ between groups. Mean diarrhea and enteritis frequency were less in calves fed milk with GSH supplementation. Moreover, higher MCV and lower RDW were found in calves fed milk with GSH supplementation. RDW and MCV are highly sensitive blood cell parameters in the differentiation of iron deficiency anemia. Higher LDH enzyme is related to the generation of frequent anemia and diarrhea. In the present study, LDH values were lower in calves fed GSH. With the results obtained, supplementation of GSH is highly recommended.

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