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Research Article

Effect of Barn or Grazing on Biochemical Indices in Prepartum, and Milk Composition in Postpartum of Dairy Cows

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ABSTRACT

The present study was designed to determine the effect of barn or cycle of grazing on changes of biochemical metabolites in prepartum and changes of milk composition in postpartum of dairy cows. For this purpose, a total of sixteen 25 months old Holstein primiparous dairy cows were allocated in two groups (n=8) with an average body weight of 571.61 ± 35.30 kg (Barn) and 578.10 ± 39.20 kg (Grazing). The study was conducted from June 2018 to October 2018. Results revealed that barn raised dairy cows had a higher increase in their serum albumin and calcium level on day 14 prepartum. However, the level of palmitic acid, saturated fatty acid increased significantly, and the level of fat, oleic acid, γ-linoleic acid, arachidonic acid and unsaturated fatty acids decreased significantly in barn raised dairy cow's milk on day 14 postpartum. There were no significant differences observed with respect to all other biochemical metabolites, fatty acids and minerals between barn raised and cycle grazing dairy cows during prepartum and postpartum. Our study results could serve to a better understanding of barn raised cow with respect to changes of biochemical metabolites in prepartum and changes of milk composition, fatty acids and minerals content in grazing dairy cows in postpartum for estimating their physiological status.

(**Key words**: Blood, Concentrate, Feed efficiency, Health status, Prepartum, Postpartum)

Ⅰ. INTRODUCTION

Pasture provides a natural habitat for ruminants, and it approach to pasture enhances the animal health. However, numerous of lactating dairy cows have no approach to pasture. Lack of this approach to pasture resulted in higher rates of lameness between other diseases (Hernandez-Mendo et al., 2007). Increasing ratio of grazing helps to reduce the feeding cost, especially as pasture or combined mixed with total mixed rations (TMR), and it has been planned as an essential approach for the efficient use of rural resources (Capper et al., 2009). However, grazing alone cannot meet energy requirements of high yielding dairy cows, mainly in postpartum period (Kennedy et al. 2011). Dairy cows grazing to pasture permanently proved more weight loss and less milk production than that of dairy cows in free-stall barn (Hernandez-Mendo et al., 2007), owing to less dry matter intake (DMI). Besides, the inadequate nutrient content of grass than compound feeds provided indoors found the lowered milk production compared to pasture-kept dairy cows (Soriano et al., 2001). Therefore, supplementation is essential to meet energy requirements to promote the animal's genetic potential. The circumstance of using TMR and high-quality pastures deliver during the grazing season have been investigated (Bargo et al., 2002). It can help to produce high amount of milk yield with enhanced protein and fat contents (Morales-Almaráz et al., 2010), and saved feeding costs (Vibart et al., 2008). Grazing cows produced milk with the desirable fatty acid (FA) content such as conjugated linoleic acid (CLA) and 18:3n-3, which are more supportive for human welfare and health (Belury, 2002; Stockdale et al., 2003; Bargo et al., 2006; Dewhurst et al., 2006; Morales-Almaráz et al., 2011). Previous study has shown that cows feeding fresh cut forage (Ferlay et al., 2006; Auldist et al., 2013) or fish oil with sunflower oils (AbuGhazaleh et al., 2007) produced higher amount of CLA and 18:3n-3 in milk.

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Previous studies have reported that the chemical composition varies in herbage throughout the day, with remarkable changes in fatty acid profile, protein, water soluble carbohydrates, starch and digestible organic matter (Avondo et al., 2008; Gregorini et al., 2009; Vasta et al., 2012). The 18:3n-3 is one of the major components of herbage fatty acids (Elgersma et al. 2003) which play a major role in photosynthesis (Browse et al. 1981), and therefore this would validate its greater content in leaf in the afternoon than in the morning. An earlier study (Gregorini et al. 2008) reported that there was no difference in 18:3n-3 level in herbages sampled between the morning and afternoon. However, Avondo et al. (2008) have found the grazing in day-time can affect the fatty acid profile in goat milk. Over 80% of the total dry matter intake was consumed continuously by dairy cows between 07·00 to 20·00 h (Huzzey et al., 2007), with a maximum intake rate found twice daily after both milkings (Cuadrado et al., 2011). However, there has been no study report on the effect of barn or grazing on changes of biochemical metabolites in prepartum and milk composition in postpartum of dairy cows. Therefore, in this study we aimed to examine the effect of barn or grazing on biochemical metabolites in prepartum and milk composition in postpartum of dairy cows.

Ⅱ. MATERIALS AND METHODS

1. Dairy cow's management and experimental design

A total of sixteen 25 months old Holstein primiparous dairy cows were allocated in two groups (n=8) with an average body weight of 571.61 ± 35.30 kg (Barn) and 578.10 ± 39.20 kg (Grazing). The experiment was performed from June 2018 to October 2018 at Meadow ranch located in Gurye-gun, Jeonnanm, Korea (ROK). All dairy cows were maintained as stated in standard guideline, and the experimental protocol involved in this study was approved by the Institutional Animal Care and Use Committee (IACUC) at National Institute of Animal Science, Korea. Two feeding systems were compared over five months. The 1st group Holstein dairy cows were housed barn and mainly fed a concentrate diet (Alfalfa and Timothy forage), while the 2nd group Holstein dairy cows was maintained with cycle practice at grazing with 5-7 h/d interval for 10-15 days (depends on the growth of grass) at pasture with a various kind grass in range A (Tall fescue - 20%, Orchard grass 25%, Kentucky bluegrass 15%, and wild grass 40%) in pasture, followed by cows were maintained at grazing for 5-7 h/d for next 10-15 days on a various kind grass in range B (Tall fescue - 38%, Orchard grass 12%, Kentucky bluegrass 13%, White clover 7% and wild grass 30%) in pasture, and then cows were maintained at grazing for 5-7 h/d for next 10-15 days on a various kind grass in range C (Tall fescue - 25%, Orchard grass 15%, Kentucky bluegrass 14%, and wild grass 46%) in pasture respectively until end of study, and their chemical compositions were presented in Table 1. The experimental animals' supplemented diet, on a dry matter basis as shown in Table 2.

2. Biochemical analysis

Blood sample was collected from each cow on day 14 prepartum by using a 20 ml syringe into a plain glass tube. Then the blood was allowed 20 min to coagulate. Followed by the coagulation, the sample was centrifuged at 3000 rpm for 30 min at 4 ℃ to collect serum, and the collected serum was stored at -20 ℃ until further use. The level of total protein, albumin, calcium (Ca), creatine kinase (CK), creatinine, gamma-glutamyltranferase (G-GTP), glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), magnesium (Mg), phosphorus (P), total bilirubin, total cholesterol, triglycerides (TG), urea and non-essential fatty acids (NEFA) were determined by using an auto-analyzer (Automatic Humanplus 900s, Human GmbH, Wiesbaden, Germany).

Table 1. Chemical composition of grazing area

Grazing area	A (%)			B (%)			C (%)		
	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF	CP
Site 1	36.92	59.50	7.35	35.00	59.24	8.46	36.75	60.33	6.05
Site 2	36.72	56.69	8.04	35.79	61.48	8.13	36.35	60.98	6.57
Site 3	34.36	57.45	8.60	36.05	61.03	6.14	38.23	62.66	5.31
Average	36.00	57.88	8.00	35.61	60.58	7.58	37.11	61.32	5.98

Table 2. Chemical composition of basal diet for Holstein dairy cows (% DM)

Items	Non-GMO (Milking cows)	Dairy association (dry cows)	Forage for milking cows		Forage for dry cows
			Alfalfa	Timothy	
Moisture content (%)	8.77	10.98	10.72	9.79	9.10
Crude protein (%)	21.66	15.58	10.78	5.19	2.64
Crude fat (%)	5.63	3.72	2.46	2.55	1.65
Crude fiber (%)	5.71	6.86	40.45	35.56	34.30
Crude ash (%)	7.01	5.78	5.14	5.08	4.97
Calcium (mg/kg)	10020.74	12712.05	9569.68	1080.07	1194.71
Phosphorous (mg/kg)	4890.87	4783.87	1165.00	1666.34	893.9
NDF (%)	-	-	52.81	61.70	60.58
ADF (%)	-	-	43.06	36.64	36.11

Basal diet recommended for Non-GMO (milking cows) by dairy association
Basal diet recommended for dry cows by dairy association

3. Milk composition, fatty acids and mineral content analysis

Milk sample was collected from each cow in a 100 ml of aseptic containers on day 14 postpartum. The collected milk sample was kept at 4 ℃ in an ice box to control sample contamination at the time of transportation. The determinations of chemical composition, fatty acids and mineral contents in milk were done by a LactoScope (MK2; Delta Instruments, the Netherlands).

4. Statistical analysis

Data was statistically analyzed in using triplicates by using the SAS Program for Windows (release 9.2; SAS Institute, Cary, NC, USA). The results were stated as means and standard error of the mean on the basis by t-test (SAS Institute, 2007). The significant differences between the mean were declared at $p<0.05$ level.

Ⅲ. RESULTS AND DISCUSSION

A biochemical index plays a key role in determination of physiological condition, organ functions and metabolic process (Scamell, 2006) within body. For example, total protein, albumin, urea, glucose, triglycerides and NEFA are considered an indicator of protein traits and energy metabolic markers, also these levels may vary depending on the nutrient intake and physiological differences of the livestock. Liver function is revealed by the activities of SGOT, SGPT, G-GTP, LDH and CK while creatinine and total bilirubin is considered as important markers for kidney function (Stojevic et al., 2005). Minerals are considering an indicator for the maintenance and growth performance of animals. So, the evaluation of physiological status is essential to analyze the animal’s welfare. Therefore, in this study we have analysed the changes of biochemical metabolites levels in blood. Results revealed that barn raised dairy cows had a higher increase in their albumin (4.03g/dl) and calcium (10.31mg/dl) level of serum than that of grazing dairy cows (albumin 3.25g/dl and calcium 9.48mg/dl) on day 14 prepartum. However, there were no significant changes noted on protein, urea, creatinine and NEFA of serum between the barn (10.12g/dl, 12.81mg/dl, 0.90mg/dl and 69.25mEq/L) and grazing (10.32g/dl, 12.03mg/dl, 0.85mg/dl and 67.50mEq/L) cows respectively on day 14 prepartum. Similarly, there was no significant differences observed on serum total cholesterol, triglycerides, SGOT, SGPT, and enzymes of G-GTP, CK, total bilirubin, LDH, Mg and P in serum between the barn and grazing dairy cows respectively on day 14 prepartum ($p<0.05$; Table 3).

The lowered level of serum albumin in grazing dairy cows might because of four possible mechanisms: lowered synthesis of albumin by the liver, increased the rate of albumin catabolism in the liver, as a consequence of increased blood volume or decreased level of albumin into the gut (Little, 1974). The decreased level of serum albumin might be associated with serum calcium in dairy cows raised from grazing on day 14 postpartum (Goff, 2000; Seifi et al., 2005; Bulent et al., 2006; Liesegang, 2008). In maternal period, cows needed of proteins for milk synthesis and immunoglobulins production and the higher level of protein

Table 3. Effects of TMR compositions and grazing on blood biochemical metabolites during prepartum in Holstein dairy cows

Blood analysis	Barn (n=8)		Grazing (n=8)		P-value
	Mean	SEM	Mean	SEM	
Protein traits and Energy metabolism markers					
Total protein (g/dl)	10.12	1.76	10.32	1.56 ns	0.81
Albumin (g/dl)	4.03	0.33	3.25	0.40*	0.04
Urea (mg/dl)	12.81	4.32	12.03	3.83 ns	0.71
Creatinine (mg/dl)	0.90	0.26	0.85	0.22 ns	0.68
Glucose (mg/dl)	30.00	5.09	28.62	3.70 ns	0.54
Triglycerides (mg/dl)	19.75	6.77	20.25	7.72 ns	0.89
NEFA (mEq/L)	69.25	27.13	67.50	33.08 ns	0.90
Enzymes and hepatic markers					
SGOT (IU/L)	86.50	18.42	90.87	17.64 ns	0.63
SGPT (IU/L)	27.87	10.65	23.87	7.12 ns	0.39
G-GTP (IU/L)	21.12	10.11	21.50	7.67ns	0.93
LDH (IU/L)	949.50	321.91	982.00	289.14 ns	0.83
CK (IU/L)	174.75	133.09	189.37	86.17 ns	0.79
Total cholesterol (mg/dl)	123.75	59.79	95.75	37.43 ns	0.28
Total bilirubin (mg/dl)	0.035	0.02	0.03	0.01 ns	0.82
Minerals					
Ca (mg/dl)	10.31	0.63	9.48	0.63*	0.02
Mg (mg/dl)	2.31	0.27	2.22	0.23 ns	0.50
P (mg/dl)	8.53	1.60	8.61	1.23 ns	0.91

* Means within a row differ; $p < 0.05$.
ns Means within a row did not differ; $p > 0.05$.

content is essential for the development of fetus body tissue in the pregnancy period. The changes of urea and creatinine level are most widely used for the evaluation of the renal function. Among the study results, the non-significance level of urea was noted between the barn and grazing dairy cows, it may because of diet supplemented to the barn and grazing cows had the same amount of protein content and energy requirements that regulated the nitrogen content in diet as well as in blood (Pelletier et al., 1985). Roubies et al. (2006) reported that creatinine plays a major role in the development of foetal musculature. So, the constant level of serum creatinine was observed from the study results, it may be due to the direct physiological activity of cows in prepartum period, for the circulatory system of fetus, believes the fill of toxic waste to the neonates (Ferrel, 1991). The non-significant level of NEFA indicated that the cows raised from barn and grazing are useful to maximize the milk production with normal glucose level in serum during the prepartum period, stimulate a marked mobilization from adipose tissue as confirmed by serum NEFA level (Wheelock et al., 2010). The insufficient availability of glucose precursor for the energy production or from the decreased level of gluconeogenic potential could affected by liver leads to ketosis, and its characterized by its higher level in serum, milk and urine (Rollin et al., 2010).

Total cholesterol and triglycerides are important key factor in milk production. Therefore, the researchers are continuing to assess the changes in lipid metabolism during the prepartum of the dairy cows (Roche et al., 2009). Our study results also presented no effects on total cholesterol and triglycerides between the barn and grazing dairy cows. From the findings, we concluded that the lipid regulatory mechanisms were not affected in cows raised from barn and grazing. In gestational period, endocrine system, lipolysis and lipogenesis are regulated to enhance lipid reserves, and these lipid reserves are used to start of lactation (Roche et al., 2009; Nazifi et al., 2002). Minerals are potential for cow fertility, and these minerals released from the blood to milk during the initiation of lactation. In this study, we observed the lowered level of Ca, and this may be due to the above reason. In early lactation, the physiological status was low, in mid-lactation was rising and in later and lactating pregnant stage was higher in dairy cows (Peterson and Waldern, 1981). Our study results also concluded that cows raised from barn and grazing were maintained the physiological status; thus, Mg and P level was not changed.

Among our study results, we confined that the changes of blood metabolites levels were within physiological reference ranges. In accordance with Pechova et al. (1997), the hepatic markers activity will increase when dairy cows are suffering from liver steatosis or energy metabolism. Our study results also confirmed that the cows raised from barn or grazing did not suffer from liver steatosis during prepartum period. Our study result agreed with Sretenović et al. (2008) reported that the consistent level of hepatic markers in blood of dairy cows during prepartum. There was no significant change observed in G-GTP level between the barn and grazing dairy cows. The results of current study were on the contrary with Abdel-Raheem et al. (2010) who previously reported that the level of G-GTP was increased in lactation as compared to prepartum dairy cows.

The effects of barn or grazing on composition, fatty acid and mineral content in milk of dairy cows during postpartum are shown in Table 4. A significantly higher percentage of milk fat content (3.67%) was showed in grazing dairy cows as compared that of barn raised cow's milk (3.12%) on day 14 postpartum. However, no significant difference were noted on other milk compositions like protein, lactose, citric acid, cells, solids and NPN between the barn and grazing dairy cows on day 14 postpartum ($p<0.05$). Among the study results, the saturated fatty acid (67.29%), with palmitic acid (44.82%) was the most abundant milk fatty acid of cows raised from barn, which was higher than those of milk fatty acid (64.92% and 42.51%) of cows raised from grazing. Monounsaturated fatty acids was the second most represented, with highest percentage of oleic acid (28.85%) in milk's of dairy cows raised from grazing as compared to dairy cows raised from barn (26.84%). The polyunsaturated least represented fatty acids, with γ-linoleic acid in milk's of dairy cows raised from barn (0.09%) had lower than that of dairy cows raised from grazing (0.11%). However, no significant differences were shown in the levels of myristic acid, palmitoleic acid, stearic acid, linoleic acid, and eicosenoic acid between the barn and grazing dairy cows on day 14 postpartum ($p<0.05$). Similar results were shown on minerals content in milk's of dairy cows reared from barn and grazing(Table 4).

From the study results, we concluded that a variety of dietary factors which regulates the milk fat percentage and composition including: the level of unsaturated fatty acids in the diet; the availability of readily degradable starch; the level of intake fibre used to protect the rumen, additionally, the protection may increase ruminal outflow and increasing the acetate to propionate ratio and thus enhancing milk fat percentage (Cruywagen et al., 2015); the management of feeding system; and the use of rumen protecting agents, yeast or the intake of moulds. However, saturated fatty acid with palmitic acid was the most plenty fatty acid in milk's of barn rearing dairy cows on day 14 postpartum (Table 4). Monounsaturated fatty acids were the second most represented, with highest percentage of oleic acid. The polyunsaturated least represented fatty acids, with linoleic acid. The fatty acids compositions of milk exhibited no significant differences in the levels of myristic acid, palmitoleic acid, stearic acid, linoleic acid, and eicosenoic acid among day 14 postpartum ($p<0.05$). Our study results on fatty acids composition of milk are agreed with Hanus et al. (2016) who published previously that Holstein dairy cows raised from silage-based feeding system. In this study, the most prevalent group of fatty acids in the milk was saturated fatty acid followed by monounsaturated fatty acid and polyunsaturated fatty acids which is agreed with the findings of Stádník et al. (2015). Also, our results agreed with one more study findings on milk fatty acid composition involving TMR fed versus grazing (Schroeder et al., 2003; Khanal et al., 2008). Negative energy balance were in high-producing dairy cows at early lactation that resulting in increased level of adipocyte fatty acid, and these increased level of fatty acid included with milk (Palmquist et al., 1993). Generally, fatty acids are stored in adipose tissue in the form of triglycerides in ruminant (Chilliard et al., 2000). This study finding recommends the prospect of the use of the fatty acid composition in milk to monitor the performance of dairy cows.

Minerals play a key role in the regulation of structure and stability of casein micelles (Holt and Jenness, 1984; Gaucheron, 2005). Particularly, Ca and P concentrations of milk rich in proteins have influenced the milk characteristics including rennet coagulation, heat tolerant and ethanol stability (Tsioulpas et al.,

Table 4. Effect of TMR composition and grazing on milk compositions, fatty acids, and mineral content during postpartum in Holstein dairy cows

Milk analysis	Barn (n=8)		Grazing (n=8)		p-value
	Mean	Std	Mean	Std	
Milk composition					
Fat (%)	3.12	0.49	3.67	1.28*	0.02
Protein (%)	3.55	0.53	3.30	0.39 ns	0.30
Lactose (%)	4.46	0.19	4.48	0.62 ns	0.93
Citric acid (mg/kg)	1636.62	200.67	1574.25	207.83ns	0.55
Cells (k cells/ml)	298.75	267.12	297.62	235.11ns	0.99
Solids (%)	11.37	1.38	11.29	1.09ns	0.89
NPN/CU (mg/100g)	17.68	2.05	17.01	1.47ns	0.46
Fatty acids (% of total fatty acid)					
Myristic acid (C14:0)	12.33	0.54	12.11	0.92 ns	0.58
Palmitic acid (C16:0)	44.82	1.49	42.51	1.67*	0.01
Palmitoleic acid (C16:1n-7)	2.82	0.40	2.92	0.64ns	0.71
Stearic acid (C18:0)	10.13	1.39	10.31	1.63ns	0.81
Oleic acid (C18:1n-9)	26.84	1.63	28.85	1.72*	0.03
Linoleic acid (C18:2n-6)	1.77	0.01	1.87	0.01*	0.02
γ- Linolenic acid (C18:3n-6)	0.09	0.00	0.11	0.01*	0.00
Linolenic acid (C18:3n-3)	0.38	0.06	0.42	0.04 *	0.01
Eicosenoic acid (C20:1n-9)	0.63	0.10	0.65	0.08 ns	0.77
Arachidonic acid (C20:4n-6)	0.14	0.22	0.19	0.03*	0.00
Saturated fatty acid	67.29	1.99	64.94	2.24*	0.04
Unsaturated fatty acid	32.70	1.99	35.05	2.24*	0.04
Minerals					
Ca (mg/kg)	1217.36	163.63	1113.05	115.50 ns	0.18
Fe (mg/kg)	0.82	0.31	0.74	0.32 ns	0.64
K (mg/kg)	963.43	86.96	939.86	133.48 ns	0.68
Mg (mg/kg)	109.76	14.07	107.09	10.76 ns	0.68
Na (mg/kg)	395.95	51.25	437.19	99.47 ns	0.32
P (mg/kg)	853.80	70.39	833.62	94.68 ns	0.64

* Means within a row differ; $p < 0.05$.
ns Means within a row did not differ; $p > 0.05$.

2007; Sandra et al., 2012; Horne 2016). Also, these two factors can change the nutritional status of milk and milk products (Cashman 2011; Pirilä et al., 2011). From the study results, it could be concluded that minerals except calcium levels were not changed in barn and grazing dairy cows on day 14 postpartum.

Ⅳ. CONCLUSIONS

This study indicated that when compared with grazing cows, serum albumin and calcium level was higher in barn raised cows during prepartum. Milk fat produced from barn raised cows had a FA profile that can be considered more favorable for the consumers from a health view point. In fact, milk fat from barn presented consistently higher concentrations of oleic acid, γ-linoleic acid, arachidonic acid and lower concentration of unsaturated fatty acids during postpartum. In conclusion of the results indicated that the transition of cows raised barn takes less time for the main milk fat FA concentrations to stabilize than the reverse transition, which suggests a smaller period of time for rumen microbes to adapt.

Ⅴ. ACKNOWLEDGEMENTS

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