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Relationship Between Blood Metabolic Profile and Milking Performance in Holstein Cows

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홀스타인 젖소의 혈중 대사 물질과 산유성적과의 관계

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Abstract This study was conducted to evaluate the relationship between blood metabolic profile and milking performance in Holstein cows. 858 cows were each allocated to four or five groups according to lactation stage, milk yield, and parity. Milk performance and serum metabolites were collected and analyzed with respect to experimental conditions. Mean serum glucose concentration was lower in the early- and mid-lactation groups ($p < 0.05$), but non-esterified fatty acid (NEFA) concentration was higher in early-lactation and dry-period groups ($p < 0.05$). Serum blood urea nitrogen and Mg concentrations were higher in the early- and mid-lactation groups than in the late-lactation and dry-period groups. NEFA, albumin, and cholesterol concentrations were highest in the > 40 kg/d group ($p < 0.05$). Glucose, cholesterol, and Mg concentrations were higher in the parity 1 group than in the other parity groups ($p < 0.05$), and total protein and globulin concentrations were higher in the parity 4 and 5 groups than in the lower parity groups. Thus, we recommend that blood metabolic profiles be considered with lactation stage, milk yield, and parity to more accurately determine the nutritional and health statuses of Holstein cows.

요 약 본 연구는 홀스타인 착유우에서 혈중 대사물질 농도와 산유 성적의 연관성을 검증하기 위해 수행하였다. 총 885두의 암소는 비유 단계, 산유량 및 산차에 따라 각각 4(비유 초기, 비유 중기, 비유 후기 및 건유기), 4(< 30, 30 – 35, 36 - 40 및 > 40 kg/일) 혹은 5(1, 2, 3, 4 및 5 산차)개의 그룹으로 배치되었다. 산유성적과 혈청 대사물질은 시험조건에 따라 수집 및 분석되었다. glucose 농도는 비유 초기 및 중기 그룹에서 낮았지만($p < 0.05$), 혈청 non-esterified fatty acid (NEFA) 농도는 비유 초기 및 건유기 그룹에서 높았다($p < 0.05$). 혈청 blood urea nitrogen과 Mg의 농도는 비유 후기 및 건유기 그룹에 비해 비유 초기 및 중기 그룹에서 높았다($p < 0.05$). 혈청 NEFA, albumin 및 cholesterol 농도는 다른 그룹에 비해 > 40 kg/일 그룹에서 높았다($p < 0.05$). 혈청 glucose, cholesterol 및 Mg 농도는 다른 산차 그룹에 비해 1산차 그룹에서 높았다($p < 0.05$). 혈청 total protein 및 globulin 농도는 저산차 그룹(1, 2 및 3 산차)에 비해 고산차 그룹(4 및 5 산차)에서 높았다($p < 0.05$). 따라서 본 연구의 결과에서 홀스타인 착유우의 영양 및 건강 상태를 보다 정밀하게 진단하기 위해서는 비유 단계와 산유량 및 산차를 함께 고려할 필요가 있는 것으로 판단된다.

Keywords : Blood Metabolic Profile, Holstein Cows, Lactation Stage, Milk Yield, Parity

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1. Introduction

The productivity of dairy cows can be assessed by metabolic status and is closely related to the cow's health. The metabolic status in dairy cows can be determined using the metabolic profile test (MPT). Rowlands [1] defined the MPT as a set or combination of blood constituents analyzed together in one test. Recently, interest in the use of MPT for obtaining information on the nutritional and health status of livestock has increased considerably [2].

Lactation stages significantly affect the blood metabolic profile [3]. For example, cows during the transition period must adapt their metabolism to the high demands of lactation and to a different diet in order to meet their requirement. However, negative energy balance (NEB) can be resulted by the inadequate dry matter intake. The NEB can induce dramatic changes in blood metabolites such as the non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (BHB) concentrations [4]. Therefore, the farmer can avoid the NEB condition by giving the adequate nutrition to the cows.

Milk yields also affect the changes in the blood metabolic profile of dairy cows [5]. Elevation of milk production and dry matter intake requires the additional intake of crude protein and energy in the diet [6] and this can be one of the reasons for changes in the blood metabolic profile. Milk production can be affected by some factors such as lactation stage, parity, and season of production, which can also affect the blood metabolic profile in normal lactating cows [7].

However, until recently, studies on MPT according to lactation stages have been mainly conducted, a few study about MPT and lactation performance according to milk yield and parity.

Thus, this study was conducted to investigate the relationship between blood metabolic profile and the performance of Holstein cows according to the different lactation stages, milk yields, and parities.

2. Material and Methods

2.1 Experimental animals and design

In the present study, a total of 858 Holstein cows from 39 farms in South Korea were selected. No special dietary treatment used in this experiment in order to evaluate the blood metabolic status. The cows were separated in early-lactation (7 to 109 days from calving), mid-lactation (110 to 219 days from calving), late-lactation (220 to 305 days from calving) and dry period (-60 to -1 days from calving). The cows were divided into four groups based on milk yields: 〈 30, 30 – 35, 36 – 40, and 〉 40 kg/d. In addition, the cows were separated according to parity number (parity 1, parity 2, parity 3, parity 4, and parity 5).

2.2 Feeding management

The cows at all farms were fed with a total mixed ration (TMR) within two times a day. The supply of each nutrient to the requirement of cows was calculated for each lactation stage based on the NRC [8]. The TMR was provided at 34 - 37 kg (22 - 24 kg of dry matter [DM]), and the average percentage of forage was 35 - 40% based on DM of TMR. In the early-lactation or high-yielding cows, an additional 2 kg of concentrate were supplemented for every 5 kg of increased in milk yield to meet the nutrient requirement compared to the average milk yield. The ingredient composition (formula) of the TMR differ depending on the forage and raw 89 materials at each farm. Table 1 shows the ingredients and chemical composition of the concentrates used for TMR or individual feeding in this study. The chemical compositions of the concentrates were analyzed according to AOAC [9]. The water was set to be freely available at all times. Other management was conducted in accordance with the practice of the farm.

2.3 Milk collection and measurements

Cows were milked twice daily, and individual milk yields were recorded. Milk yield (kg) was determined on a daily basis both at the morning and evening milking.

Table 1. Ingredients and chemical composition of the experimental diets

Items	Concentrates[1]			
	Con I	Con II	Con III	Con IV
Ingredients composition (%)				
Corn grain	25.99	28.33	26.17	18.22
Wheat grain	10.00	10.00	10.00	11.00
Cane molasses	4.50	4.00	4.00	4.50
Tapioca residue	3.00	2.00	-	10.00
Wheat flour	-	2.00	4.00	-
Wheat bran	1.00	1.10	1.00	9.22
Corn gluten feed	5.78	17.00	16.00	15.65
Soybean meal	19.14	18.58	16.77	2.98
Rapeseed meal	4.00	4.00	4.00	5.00
Distillers grain	-	-	-	2.00
Coconut meal	6.50	-	-	6.00
Corn gluten meal	-	0.78	3.00	-
Palm meal	12.00	7.00	10.00	13.00
Cottonseed whole	3.00	-	-	-
Salt & etc.	5.09	5.21	5.06	2.43
Chemical composition (%, as-fed basis)				
Dry matter	88.32	88.32	88.56	88.29
Crude protein	18.35	18.50	19.50	14.20
Ether extract	3.91	3.50	3.64	3.43
Crude fiber	6.65	5.29	5.28	8.20
Calcium	1.10	1.10	1.10	0.65
Phosphorus	0.53	0.41	0.43	0.43
Crude ash	7.44	7.26	7.11	6.14
TDN[2]	72.02	73.05	73.89	71.10

[1]Con I and II for cows with 25 - 30 kg milk yields, while Con III for cows with 30 - 40 kg milk yields and Con IV for dry cows; [2]TDN: total digestible nutrients (calculated values)

2.4 Blood collection and analytical methods

Blood samples were collected from 8 am to 9 am in all farms. Approximately 10 ml of blood was taken from the jugular vein using an 18-gauge needle and a vacuum blood vessel (Vacutainer; Becton-Dickinson, NJ, USA). Blood was stored in a refrigerator at 4°C for 24 hours after centrifuged at 1,250 × g for 10 min to separate the blood serum. The separated blood serum was used for metabolic profile analysis using an automated blood analyzer (Hitachi 7020, Hitachi Ltd., Tokyo, Japan). Glucose (GLU), non-esterified fatty acid (NEFA), total protein (TP), albumin (ALB), globulin (GLB), blood urea nitrogen (BUN), cholesterol (CHOL), triglyceride (TG), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), calcium (Ca), magnesium (Mg), and inorganic phosphorus (IP) were analyzed.

2.5 Statistical analysis

All the experimental results were analyzed by one-way ANOVA (Analysis of variance) for variance using the GLM (General Linear Model) procedure of the SAS [10] 9.1 Software Package. Duncan's multiple range test was used to compare differences in parameter mean values. Significant differences were accepted if $p < 0.05$.

3. Results and Discussion

The blood metabolic profiles related to lactation stages are shown in Table 2.

Table 2. Changes in blood metabolic profile according to the lactation stage in Holstein cows

Items	Lactation stage				SEM[1]	P-value
	Early	Mid	Late	Dry		
GLU[2] (mg/dL)	45.80^{bc}	44.23^{c}	48.38^{ab}	50.57^{a}	0.46	0.00
NEFA[3] (μeq/L)	248.68^{a}	168.36^{b}	161.66^{b}	263.75^{a}	5.01	0.00
TP[4] (g/dL)	7.26	7.24	7.25	7.25	0.02	0.99
ALB[5] (g/dL)	3.05	3.10	3.09	3.05	0.00	0.08
GLB[6] (g/dL)	4.19	4.31	4.15	4.20	0.02	0.10
BUN[7] (mg/dL)	18.95^{ab}	19.91^{a}	18.55^{b}	16.12^{c}	0.19	0.00
CHOL[8] (mg/dL)	204.37^{c}	282.21^{a}	257.93^{b}	190.82^{d}	2.38	0.00
TG[9] (mg/dL)	26.12	28.31	26.64	22.58	1.07	0.42
AST[10] (U/L)	95.10^{a}	97.65^{a}	94.21^{a}	88.70^{b}	0.89	0.01
GGT[11] (U/L)	25.21^{bc}	28.94^{a}	27.44^{ab}	22.91^{c}	0.45	0.00
Ca[12] (mg/dL)	9.16	9.23	9.24	9.34	0.07	0.91
Mg[13] (mg/dL)	2.42^{a}	2.44^{a}	2.36^{b}	2.29^{c}	0.01	0.00
IP[14] (mg/dL)	6.06	6.20	6.06	6.07	0.03	0.31

[a,b,c]Means within the same row with different letters are significantly different ($p < 0.05$).
[1]SEM: standard errors of the mean; [2]GLU: glucose; [3]NEFA: non-estrified fatty acid; [4]TP: total protein; [5]ALB: albumin; [6]GLB: globulin; [7]BUN: blood urea nitrogen; [8]CHOL: cholesterol; [9]TG: triglyceride; [10]AST: aspartate aminotransferase; [11]GGT: γ-glutamyl transferase; [12]Ca: calcium; [13]Mg: magnesium; [14]IP: inorganic phosphorus.

GLU concentration was lower in early- and mid-lactation than that in late-lactation and dry period ($p < 0.05$). NEFA concentration was higher in early-lactation and dry period than that in

mid- and late-lactation ($p < 0.05$). There was no difference in TP concentration between the lactation stages. Concentrations of ALB, GLB, TG, and IP were slightly but not significantly higher in mid-lactation than those in the other lactation stages. Concentrations of BUN and Mg were higher in early- and mid-lactation than those in late-lactation and dry period ($p < 0.05$). CHOL concentration was higher in mid-lactation than that in other lactation stages ($p < 0.05$). Concentrations of AST and GGT were higher in early-, mid-, and late-lactation than those in dry period ($p < 0.05$), and the highest concentrations were found in mid-lactation. Ca concentration tended to increase with the progressing lactation stage; however, there was no statistically significant difference.

In the present study, GLU concentrations were increased through the lactation stage. The present study was in agreement with a previous study [11] which showed increasing GLU concentrations through lactation stages. GLU is a precursor that is used as an energy source for animals or synthesized as a fat source [12]. In addition, increase in GLU concentration may reflect the increase of feed intake and energy status of the cow [3]. The NEFA concentration in early lactation was higher, which indicated that the dairy cow was in negative energy balance condition, which is characterized by increased mobilization of NEFA. This was also in agreement with a previous study [13] showing that cows had high lipomobilization in early lactation, whereas cows in mid-lactation showed low lipomobilization.

Piccione *et al.* [14] found that decreased TP concentration can also be indicated as the effect of a decreased GLB fraction whereas changes in the plasma ALB were marginal. These decreases may indicate the maternal requirements of proteins for milking and providing immunoglobulins [15]. Piccione *et al.* [14] also reported that the decrease in γ-globulins should be considered as one of the factors associated with the higher predisposition of postpartum dairy cows to infection. During the early-lactation stage, the lower concentration of ALB was considered to be due to negative energy balance. The present study is also in agreement with a previous study [16] which found that the ALB concentration was lower at early lactation as a consequence of negative energy balance.

In the present study, BUN concentration in the mid-lactation stage was increased compared to that in the early or late-lactation stages. The lowest concentration of BUN was in the dry period. This is because there was an elevated feed intake in order to provide adequate energy for pregnancy and the next lactation stage, which is in line with a previous study [17]. Peterson and Waldern [18] also reported that BUN concentration was the lowest in dry cows, which consistent with the results of the present study. The CHOL concentration increased in mid-lactation stage may be due to a greater energy demand than that supplied by the offered diet [19]. However, a decrease in the dry period is likely because of the increased requirement of fetal tissues as well as the maternal glands for steroid hormone synthesis [20]. The highest TG concentration in the present study was observed at mid-lactation stage, which was in contrast to the previous study [11,19] reporting the highest TG concentration at the dry period due to the cessation of milk fat formation during pregnancy. TG concentration was higher at the mid-lactation stage possibly because of the decrease in catabolism or due to overproduction [21].

The maximum AST concentrations in the present study were lower than those reported by González *et al.* [13] at 184.5 U/L. In addition, AST concentrations higher than 100 U/L may indicate the hepatic lesions [13]. It thus seemed that the AST concentration in the present study revealed early signs of liver damage in dairy cows. The GGT concentration in the present study was in consistent with that in a previous

study [22] which reported that the GGT concentration in late pregnancy much lower than that in the first week after calving, and that the activity increased 6 weeks after delivery. Semacan and Sevinç [23] found that the GGT concentration in healthy cows was 22.33 U/L whereas the GGT concentration in diseased cows was 42.4 U/L.

In the present study, the low concentration of Ca in early-lactation stage might result from Ca transport by blood into the mammary gland for milk synthesis during lactation. Seifi *et al.* [19] also reported decreasing of Ca during early-lactation due to input of blood minerals into milk with initiation of lactation. The Mg concentrations were decreased in the dry period in the present study. A previous study [24] found that the dry period had the lowest Mg concentration compared to the other lactation stages. This decrease was related with the decreasing dietary intake and absorption in the digestive tract. Payne and Payne [25] reported that low Mg concentrations are also observed with a low DMI (Dry matter intake). The IP concentration during all lactation stages was in the normal range. Kida [26] reported that the IP concentration range was 2.6 - 8.6 mg/dL. Kalantzopoulos [27] found that this situation to be related with reduction in the mammary gland uptake of phosphorous from blood depending on the deterioration of udder health.

The blood metabolic profiles according to milk yields are shown in Table 3. Concentrations of GLU and IP were higher in 〈 30 kg/d and 30 - 35 kg/d groups than those in the 36 - 40 kg/d and 〉 40 kg/d groups (p 〈 0.05). TG concentration was lower in the 〈 30 kg/d and 30 - 35 kg/d groups than that in the 36 - 40 kg/d and 〉 40 kg/d groups (p 〈 0.05). The highest concentrations of NEFA, ALB, and CHOL were found in the 〉 40 kg/d group (p 〈 0.05). Concentrations of TP and GGT were slightly but not significantly different along with the increasing milk yield. Concentrations of GLB, BUN, and Ca were similar between milk yield groups.

Table 3. Changes in blood metabolic profile according to the milk yield in Holstein cows

Items	Milk yield (kg/d)				SEM[1]	P-value
	〈 30	30 - 35	36 - 40	〉 40		
GLU[2] (mg/dL)	47.00[a]	46.93[a]	42.89[b]	42.27[b]	0.51	0.00
NEFA[3] (μeq/L)	177.59[b]	178.38[b]	204.64[b]	249.92[a]	4.72	0.00
TP[4] (g/dL)	7.21	7.25	7.31	7.33	0.02	0.22
ALB[5] (g/dL)	3.05[b]	3.11[a]	3.13[a]	3.17[a]	0.01	0.00
GLB[6] (g/dL)	4.14	4.12	4.18	4.16	0.02	0.85
BUN[7] (mg/dL)	18.38	19.75	18.95	18.97	0.23	0.12
CHOL[8] (mg/dL)	242.91[c]	271.24[b]	270.01[b]	293.84[a]	2.46	0.00
TG[9] (mg/dL)	23.82[b]	24.42[b]	35.19[a]	33.65[a]	1.32	0.01
AST[10] (U/L)	93.07[b]	100.68[a]	94.66[ab]	97.51[ab]	0.92	0.00
GGT[11] (U/L)	27.24	27.57	27.09	27.98	0.54	0.96
Ca[12] (mg/dL)	9.29	9.30	9.18	9.18	0.09	0.97
Mg[13] (mg/dL)	2.35[b]	2.44[a]	2.39[ab]	2.43[a]	0.01	0.00
IP[14] (mg/dL)	6.20[a]	6.16[a]	5.87[b]	5.84[b]	0.03	0.00

[a,b,c]Means within the same row with different letters are significantly different (p 〈 0.05).
[1]SEM: standard errors of the mean; [2]GLU: glucose; [3]NEFA: non-estrified fatty acid; [4]TP: total protein; [5]ALB: albumin; [6]GLB: globulin; [7]BUN: blood urea nitrogen; [8]CHOL: cholesterol; [9]TG: triglyceride; [10]AST: aspartate aminotransferase; [11]GGT: γ-glutamyl transferase; [12]Ca: calcium; [13]Mg: magnesium; [14]IP: inorganic phosphorus.

The result of the present study was in consistent with that of a previous study [28] that also found low GLU concentration in the high producing group. The low concentration of GLU in high producing dairy cows was associated with the high demand for GLU in lactose synthesis. This was also in agreement with a previous study [29] reporting that about 60 - 85% of the total GLU used in the body of lactating cows is used in the mammary gland tissue. The highest NEFA concentration was found in the higher milk yield group. Blum *et al.* [30] also found the highest NEFA concentration in high producing cows. In addition, they also added that this condition can occur due to fat mobilization. The TP concentrations were increased in higher milk yield groups. The result of the present study is in agreement with that of the previous study [30] showing that TP had a high correlation with milk

yield. Bobbo *et al*. [16] reported that increasing the dietary crude protein significantly increased the ALB and TP concentrations in Holstein cows.

The highest BUN concentration was found in the 30 - 35 kg/d group. A similar result was also reported by Jones *et al*. [31] who found the highest BUN concentration in the medium-yield group. In addition, the BUN concentrations in dairy cows was increased as the stage progressed from the dry period to early lactation [32].

The concentration of serum ALB similar to our results has been reported by Jones *et al*. [31] and Bobbo *et al*. [16]. They found that the high producing group had the highest ALB concentration. Bobbo *et al*. [16] found that the diets given to modern high producing herds could impact the concentration of blood ALB. The GLB concentrations did not differ significantly, but there was a slight increase along with increasing of milk yields. This result was similar to that of a previous study [31].

The CHOL concentrations were increased along with changes in milk yield in the present study. This finding was similar to the result of Adedibu *et al*. [33] who found a positive relationship between the milk yield and serum CHOL. In general, CHOL is known to increase as feed intake increases [34], and it is considered that this is because dairy cows with high milk yield have relatively high feed intake than dairy cows with low milk yield. The highest TG concentration was found in the 36 - 40 kg/d group. This result was similar with the result of Nozad *et al*. [35] who found the highest TG concentration at high producing cows with 27.5 mg/dL.

The highest Ca concentration was found in the 30 - 35 kg/d group which was inconsistent with a previous study [31] which found high producing cows had higher blood Ca concentration. In the present study, the highest Mg concentration was found in the 30 - 35 kg/d group. Similarly, a previous study [31] showed that the highest concentration of Mg was found in the medium-yield group. Moreover, the highest concentration of IP was found in the < 30 kg/d group in the present study, whereas in a previous study [31], the highest IP concentration was found in the medium-yield group.

The blood metabolic profiles according to the parity number are shown in Table 4.

Table 4. Changes in blood metabolic profile according to the parity in Holstein cows

Items	Parity					SEM[1]	P-value
	1	2	3	4	5		
GLU[2] (mg/dL)	48.07^{a}	45.21^{ab}	45.06^{ab}	43.99^{ab}	41.03^{b}	0.53	0.00
NEFA[3] (μeq/L)	191.64	212.23	221.36	190.12	245.02	5.55	0.09
TP[4] (g/dL)	7.15^{c}	7.15^{c}	7.29^{bc}	7.36^{ab}	7.48^{a}	0.02	0.00
ALB[5] (g/dL)	3.10	3.11	3.12	3.03	3.04	0.01	0.08
GLB[6] (g/dL)	4.05^{b}	4.04^{b}	4.17^{b}	4.33^{a}	4.44^{a}	0.02	0.00
BUN[7] (mg/dL)	19.14	17.92	18.49	18.53	17.94	0.24	0.36
CHOL[8] (mg/dL)	263.43^{a}	248.98^{ab}	245.20^{ab}	236.19^{b}	231.32^{b}	2.75	0.00
TG[9] (mg/dL)	26.88	27.41	25.93	35.33	24.35	1.33	0.34
AST[10] (U/L)	97.33	95.31	96.69	94.07	87.22	0.94	0.12
GGT[11] (U/L)	26.20	27.87	27.37	28.57	30.05	0.56	0.44
Ca[12] (mg/dL)	9.32	9.54	9.08	8.99	8.91	0.10	0.37
Mg[13] (mg/dL)	2.42^{a}	2.40^{ab}	2.39^{ab}	2.31^{bc}	2.25^{c}	0.01	0.00
IP[14] (mg/dL)	6.39^{a}	6.17^{ab}	5.97^{bc}	5.90^{cd}	5.71^{d}	0.03	0.00

^{a,b,c}Means within the same row with different letters are significantly different ($p < 0.05$).
[1]SEM: standard errors of the mean; [2]GLU: glucose; [3]NEFA: non-estrified fatty acid; [4]TP: total protein; [5]ALB: albumin; [6]GLB: globulin; [7]BUN: blood urea nitrogen; [8]CHOL: cholesterol; [9]TG: triglyceride; [10]AST: aspartate aminotransferase; [11]GGT: γ-glutamyl transferase; [12]Ca: calcium; [13]Mg: magnesium; [14]IP: inorganic phosphorus.

The GLU, CHOL, Mg and IP concentrations of the parity 1 group were the highest compared to those in the other parity groups ($p < 0.05$). The TP and GLB concentrations were higher in the higher parity groups (parity 4 and 5) than those in the lower parity groups (parity 1, 2, and 3)($p < 0.05$). The concentrations of AST and Ca were slightly but not significantly decreased along with increasing parity number; however, the GGT concentration was slightly but no significantly increased along with the increasing parity number.

The present result was similar to the previous result of Janovick *et al*. [36]. They found that the GLU concentrations were decreased in the higher

parity group. This was because in parity 1, the ages of the cows are still young and are related with the growth process, which requires moderate quantities of energy [37]. Although GLU is the primary metabolic fuel and is definitely essential for vital organ function, fetal growth, and milk production, serum GLU had no strong correlations with other energy-metabolites [38]. The parity 5 group showed the highest NEFA concentration in the present study. This is in agreement with the findings of Janovick *et al.* [36] who found that the NEFA concentration also increased along with increasing the parity number. Janovick *et al.* [36] also reported that the increased NEFA concentration in multiparous cows was related with increased mobilization of adipose TG to support greater milk yields and energy requirements compared with that in primiparous cows.

The TP concentrations were increased in the parity 4 and 5 groups compared to those in the parity 1 and 2 groups. The present finding is similar a previous result [37] showing that the TP concentration was higher in multiparous cows. Bobbo *et al.* [16] reported an increasing TP concentration mainly due to an in increase in GLB concentration which increased with parity number. Doornenbal *et al.* [39] found that the ALB levels and the age of cattle had no clear relation; however, the GLB concentration showed increasing numbers along with increasing parity of the cows. Their findings are similar to the findings in the present study. Brscic *et al.* [37] found that the GLB levels were higher in multiparous cows than in primiparous cows due to the better immunological memory in older cows. Their finding was in agreement with the present result which shows the highest GLB concentration in the higher parity groups. There were no variations in BUN concentration among cows at different parity in the present study. This finding was similar to the result of Dias *et al.* [40] who found that the lowest parity number had the highest BUN concentration.

In the present study, the CHOL concentrations were decreased along with increased parity number. High concentration of CHOL may occur due to a greater energy demand than that supplied by the diet [41]. Kim *et al.* [42] reported that CHOL concentrations are essential in the indication and classification of hyperlipoproteinemia. Increasing levels of CHOL, TG, and phospholipids are indicative of copper deficiency [43]. The GGT concentrations were increased along with increasing parity number in the present study. This is in agreement with the findings of Brscic *et al.* [37]. Increasing GGT concentration in multiparous cows most likely occurs due to a possible long-term effect of hepatic stress triggered by previous lactation because GGT is considered as a specific hepatic marker in cattle [37]. In the present study, AST concentrations were decreased with increasing of parity number. Cavestany *et al.* [41] found a positive correlation between CHOL and AST concentration. In the present study, it is considered that AST and CHOL concentrations had a similar pattern as the previous study and were influenced by parity number.

The Ca concentration was decreased along with increased parity number in the present study. Romo *et al.* [44] also found that the Ca concentrations decreased with an increase in parity. The decreased of Ca concentration might be due to the osteoclastic activity in older cows, which was lower than that in young cows [44]. In the present study, the Mg concentration was also decreased along with increased parity. The present result had similar result with that of Cavestany *et al.* [41]. Decreasing of Mg concentrations might be related to the decrease in Ca concentration [41]. It is considered that Ca and Mg concentrations had a positive correlation as the previous study and were influenced by parity number in the present study.

However, the IP concentration also decreased

along with increased parity number in the present study. The present study had the same finding with the previous study [45] who showed that cows with higher parity had lower IP concentration. Doornenbal *et al.* [39] reported that the decreased IP concentration in older animal occurred due to the decreased need of IP for skeletal growth.

4. Conclusion

In conclusion, we found that lactation stage significantly affected the blood metabolic profile of cows in the present study. The cows experiencing negative energy balance were indicated by increasing serum NEFA concentrations in the early-lactation stage. The serum NEFA, ALB, CHOL, TG, AST, and Mg concentrations were increased in higher milk yield groups. Some serum metabolites concentrations were decreased with higher parity number due to increasing age; However, there was no consistent change in blood metabolite concentrations according to the increase in parity. Thus, the present results indicate that blood metabolic profiles are recommended to diagnose the nutritional and health status according the lactation stages of Holstein cows, and that feeding management according to the blood metabolic profile is necessary; however, milk yield and parity need to be considered along with the lactation stage for more accurate diagnosis.

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