A comparative analysis of rumen pH, milk production characteristics, and blood metabolites of Holstein cattle fed different forage levels for the establishment of objective indicators of the animal welfare certification standard

Dong Jin Baek¹, Hyoun Chul Kwon², Ah Lyum Mun¹, Joo Ri Lim¹, Sung Won Park¹, and Jin Soo Han³,*

Objective: This study was conducted to obtain an objective index that can be quantified and used for establishing an animal welfare certification standard in Korea. For this purpose, rumen pH, ruminating time, milk yield, milk quality, and blood components of cows reared in farms feeding high forage level (90%) and farms feeding low forage level (40%) were compared.

Methods: Data on rumen pH, ruminating time, milk yield, milk fat ratio, milk protein ratio, and blood metabolism were collected from 12 heads from a welfare farm (forage rate 88.5%) and 13 heads from a conventional farm (forage rate 34.5%) for three days in October 2019.

Results: The rumination time was longer in cattle on the welfare farm than on the conventional farm (p<0.01), but ruminal pH fluctuation was greater in the cattle on conventional farm than the welfare farm (p<0.01). Conventional farms with a high ratio of concentrated feed were higher in average daily milk yield than welfare farms, but milk fat and milk production efficiency (milk fat and milk protein corrected milk/total digestible nutrients) was higher in cattle on welfare farms. Blood test results showed a normal range for both farm types, but concentrations of total cholesterol and non-esterified fatty acid were significantly higher in cows from conventional farms with a high milk yield (p<0.01).

Conclusion: The results of this study confirmed that cows on the animal welfare farm with a high percentage of grass feed had higher milk production efficiency with healthier rumen pH and blood metabolism parameters compared to those on the conventional farm.

Keywords: Animal Welfare; Blood Metabolites; Holstein; Ruminal pH; Ruminating Time

INTRODUCTION

Korea has established certification standards so that farm animals can live normally and maintain their natural behavior. Farms that comply with these standards are certified as animal welfare farms [1]. In ruminants, the production of saliva is activated by ruminating and is related to microorganism activity in the rumen, which is directly related to the occurrence of metabolic diseases in ruminants [2].

A high-yield breeding method for Holstein cows has been adopted to increase economic efficiency by greatly increasing the production of milk [3]. However, in high-yield and efficiency cow-oriented feeding management livestock systems, several problems have been reported. These include animal welfare issues and a shortened economic life of the cows, and it was shown that the management of feeding to sustain animal welfare was insufficient [4]. This intensive livestock system in Korea has resulted in, frequently occurring metabolic diseases in dairy cows, such as sub-acute ruminal acidosis [5].
To ensure the health and welfare of ruminant dairy cows, it is important to properly maintain the ruminal environment so that it can function optimally [6]. Ruminants are dependent on rumen microorganisms, which supply most of the nutrients necessary for life and production [7]. For rumen microorganisms to survive, ruminal pH must be properly managed, and this factor is widely used as an indicator of normal rumen functioning [4]. In this regard, the World Animal Health Organization’s cow welfare standard stipulates that normal rumen function should be maintained by providing unlimited amount of grass feed for cows. This will prevent ruminal digestive disorders that are caused by supplying a high percentage of concentrated feed [6] and will comply with animal welfare approved (AWA) dairy cattle standards that require 60% roughage for lactating dairy cows [8]. Korea’s dairy cow animal welfare certification standards also make it mandatory to provide more than 60% of the dry matter as grass feed [1], and this is the main difference between animal welfare farms and conventional farms.

This study aimed to obtain a quantifiable and objective index for establishing welfare standard for Holstein cows in Korea by comparing rumen pH, ruminating time, milk yield, milk quality, and blood components among cows from farms feeding high (welfare farms) and low (conventional farms) forage level.

MATERIALS AND METHODS

Animal care

The experimental procedure was approved by the Institutional Animal Care and Use Committee of the Animal and Plant Quarantine Agency (No. 2019-494), Korea.

Animals and housing

The study was conducted at two dairy cow farms in Ansung City, Gyeonggi Province, Republic of Korea, and 25 dairy cows were selected. The first was an animal welfare certified farm, and the other was a conventional dairy farm in a similar region with, comparable herd size, breeding, and management.

Twelve healthy cows were selected randomly from the welfare certified farm: two primiparous and ten multiparous heads. The average parity of the two farms was 2.6±1.4 for welfare farm and 2.6±1.7 for the conventional farm. The average number of milking days of the two farms were 176.1±73.6 d for the welfare farm and 180.2±88.7 d for the conventional farm. The average milk yield was 24.8±6.2 kg and 33.9±6.2 kg for the welfare and conventional farms, respectively. The housing facility was an open-type barn structure, and in accordance with the animal welfare certification standards, had a milking cow’s bedding area of 8 m²/head and a total area of 16.5 m²/head [1].

The shape of the barn was the bedding barn, and the barn was covered with sawdust. To provide comfort to the experimental animals in accordance with the animal welfare standards, a sufficient amount of sawdust was provided to keep the rest area clean and dry.

Diet

Total mixed ration was used as the experimental feed and cows were fed ad libitum. Dry matter intakes were measured by subtracting the remaining amount of feed from the weight of the total amount supplied to each cow. All cow had access to water ad libitum.

The ingredients and chemical composition of the diets of both farms are shown in Tables 1 and 2.

Table 1. Chemical composition of the diet on the conventional and welfare farms

| Items                     | TMR  | Concentrate |
|---------------------------|------|-------------|
| Ingredients (kg/head)     |      |             |
| Beet pulp                 | 2.50 | -           |
| Cotton seed whole         | 2.50 | -           |
| Alfalfa hay               | 2.17 | 4.00        |
| Timothy hay               | 2.17 | -           |
| Oats hay                  | 3.67 | 4.00        |
| Tall fascue strew         | 2.17 | -           |
| Meadow hay                | -    | 4.00        |
| Algoie grass hay          | -    | 12.00       |
| Concentrate feed          | -    | 14.17       |
| Vit and Min premix        | 0.12 | -           |
| Chemical composition (%)  |      |             |
| CP                        | 13.47| 13.41       |
| NDF                       | 57.10| 61.49       |
| eNDF                      | 47.81| 55.66       |
| NSC<sup>2</sup>           | 19.33| 18.51       |
| Energy content            |      |             |
| TDN (% DM)                | 68.20| 59.06       |
| NE (Mcal/kg)              | 2.36 | 2.04        |
| NEm (Mcal/kg)             | 1.48 | 1.19        |
| NEg (Mcal/kg)             | 1.54 | 1.32        |
| NEI (Mcal/kg)             | 0.89 | 0.62        |

TMR, total mixed ration; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; eNDF, effective neutral detergent fiber; NSC, nonstructural carbohydrate; TDN, total digestible nutrients; NE, net energy; NEm, net energy for maintenance; NEg, net energy for grain; NEI, net energy for lactation.

<sup>1</sup> C, conventional farm; W, welfare farm.

<sup>2</sup> NSC = 100−(CP+NDF+Fat+Ash+pectin).
Table 2. Feed intake and nutrient composition of experimental animals

| Item                  | C\(^1\)  | W\(^1\)  |
|-----------------------|-----------|-----------|
| DMI (kg/d)            | 27.74     | 21.19     |
| Forage ratio (%)      | 34.54     | 88.46     |
| NDF (kg/d)            | 9.96      | 8.39      |
| NSC (kg/d)            | 6.76      | 4.93      |
| TDN (kg/d)            | 19.80     | 13.32     |
| NEI (Mcal/d)          | 451       | 301       |
| Forage DMI (kg/d)     | 9.57      | 18.74     |
| Concentrate DMI (kg/d)| 18.17     | 2.45      |

DMI, dry matter intake; NDF, neutral detergent fiber; NSC, nonstructural carbohydrate; TDN, total digestible nutrients; NEI, net energy for lactation.

\(^1\) C, conventional farm; W, welfare farm.

The particle size of the feed was measured using a Penn State Particle Size Separator (Table 3).

Ruminal pH and temperature, and rumination time

To continuously monitor the ruminal pH value, a monitoring sensor (smaXtec PREMIUM sensor, Animal Care Sale GmbH, Graz, Austria) was orally inserted into the rumen using a dedicated insertion gun. Prior to insertion, calibration was performed using pH 4.0 and 7.0 buffer solutions according to the manufacturer’s protocol, and real-time information of pH and temperature was collected through the inserted sensor (length: 12 cm, 3.5 cm in diameter, and 210 g in weight), every 10 min [9]. The rumination time was measured using a collar-attached type sensor, HR-Tag (SCR, Allflex, Netanya, Israel) which was placed on the left side of the neck and rumination data were automatically collected. This logger has a built-in microphone that allows for the recording of the sound of rumination. Time spent ruminating was recorded and stored in 2-h intervals [10].

Milk yield and composition

Both farms used an automatic robotic milking machine (Lely Astronaut system, Lely Industries N.V., Maassluis, The Netherlands) to collect information on milk yield, and milk fat and protein content. Cows only accessed the automatic robotic milking system associated with their pen. The cows had free access to the automatic robotic milking system 24 h/d. Milking occurred on a voluntary basis, but if an individual cow had not visited the automatic robotic milking system for over 12 h, she was fetched by farm staff to be milked [11].

Blood sampling and analysis

Blood samples were collected from each experimental cow 4 to 5 h after the 7 o’clock morning feed. Blood was drawn from the coccygeal vein of 12 cows from the certified welfare farm and 13 cows from the conventional farm.

The samples were placed in a blood collection tube (VA-CUETTE serum clot, 9 mL) and serum was separated by centrifugation (1,500×g at 4°C for 5 min). Serum samples were tested for alanine aminotransferase, aspartate aminotransferase, total bilirubin, and total cholesterol levels using a serum biochemical analyzer (Beckman Coulter AU480; Beckman Coulter, Inc., Brea, CA, USA). Total protein, albumin, albumin/globulin ratio, glucose, blood urea nitrogen (BUN), creatine, Ca, P, Mg, and non-esterified fatty acid (NEFA) levels were also analyzed.

Statistical analysis

Data were analyzed using the MIXED procedure in the SAS package program (SAS Inst. Inc., Cary, NC, USA) as a completely randomized design. The experimental model was as follows:

\[ Y_{i(t)} = \mu + Ti + E_{i(t)} \]

where \( \mu \) is the average value, \( Ti \) is the treatment value and \( E_{i(t)} \) is the error value. The fixed-effect of the welfare farm and random effects were not considered. Least squares mean was assessed using pairwise comparisons and the orthogonal contrast method. Statistical differences and tendencies were accepted at \( p \)-values less than 0.05 and 0.10, respectively. All means are presented as the least square means.

RESULTS

Ruminal pH and temperature, and rumination time

The average ruminal temperature of all the cows from both farms was 39°C±1°C, and the daily average ruminal pH was 6.2±0.2 for the welfare farm and 6.3±0.2 for the conventional farm. The lowest daily pH values from the welfare and conventional farms were 5.9±0.2 and 5.8±0.2 (\( p<0.05 \)), respectively. All means are presented as the least square means.

Table 3. The measurement of the particle size of total mixed ration

| Item                  | C\(^1\) (g) | W\(^1\) (g) | C\(^1\) (%) | W\(^1\) (%) |
|-----------------------|-------------|-------------|-------------|-------------|
| 1 sieve               | 218         | 162         | 43.6        | 32.4        |
| 2 sieve               | 63          | 76          | 12.6        | 15.2        |
| 3 sieve               | 63          | 97          | 12.6        | 19.4        |
| 4 sieve               | 156         | 165         | 31.2        | 33          |
| Total                 | 500         | 500         | 100         | 100         |

C, conventional farm; W, welfare farm.
Comparison of ruminal pH value between conventional farm and welfare farm for 3 days

| Item               | C  | W  | p-value |
|--------------------|----|----|---------|
| Mean pH            | 6.25±0.16 | 6.24±0.21 | 0.81 |
| Min pH             | 5.79±0.22 | 5.90±0.23 | <0.05 |
| Max pH             | 6.67±0.18 | 6.53±0.23 | <0.01 |
| Max-min pH         | 0.87±0.21 | 0.62±0.16 | <0.01 |
| Drinking frequency | 5.9±1.7   | 6.7±1.9   | -   |

\(^{1)}\) C, conventional farm; W, welfare farm.

The highest pH values were 6.5±0.2 and 6.7±0.2 (p<0.01), respectively. The pH fluctuation of the two farms was 0.6±0.2 and 0.9±0.2 (p<0.01), respectively (Table 2). The average rumination time was 508.8±73.8 min for the welfare farm and 435.4±50.5 min for the conventional farm (p<0.01) (Figure 1). The average daily visit to drinking boals during three days was 5.9±1.7 and 6.7±1.9 times, respectively (Table 4).

**Milk yield and composition**

During the three days experiment, the average milk production was 24.0±6.3 kg/d in the welfare certified farm and 33.9±6.1 kg/d in the conventional farm (p<0.01). Milk fat content was 3.8±0.5% in the welfare certified farm (p<0.01) and 3.37±0.17% in the welfare farm, and the milk protein contents were 3.2%±0.2% (p<0.01) and 3.4%±0.2% for the welfare and conventional farms respectively. Fat-and milk protein-corrected milk yield (FPCM) was 24.2 kg/d in on the welfare farm, and 33.4 kg/d on the conventional farms (p<0.01). Milk production efficiency (FPCM/TDN) was 1.8±0.5 and 1.7±0.3 for the two farms, respectively (Table 5).

**Blood parameters**

Blood parameters of cows from both farms were within the normal range as obtained from other reported studies except total cholesterol, albumin, glucose, BUN, creatinine levels, and the A/G ratio, which were significantly higher in cows on conventional farms than in those on welfare farms (p<0.05), and total protein values of cows on the welfare farm were significantly higher than those on the conventional farm (p<0.01) (Table 6).

**DISCUSSION**

Recently, the importance of performing dairy cattle welfare assessment using animal-based measurement was emphasized by the World Animal Health Organization [12,13] and major criteria for welfare assessment were measured in this study.

In this study, the average rumination time of cows on the welfare farm was 508.8±73.8 min, which was significantly longer than that of cows on the conventional farm which was 435.4±50.5 min. This is similar to results of previous studies on welfare farms that provide with a high percentage of grass feed to their cattle [14-16].

![Figure 1](https://www.animbiosci.org)

**Figure 1.** Comparison of ruminating time between conventional farm and welfare farm over 3 days. C, conventional farm; W, welfare farm. * Indicates significant differences between conventional and welfare farms (p<0.01).

**Table 4.** Comparison of ruminal pH value between conventional farm and welfare farm for 3 days

| Item               | C  | W  | p-value |
|--------------------|----|----|---------|
| Mean pH            | 6.25±0.16 | 6.24±0.21 | 0.81 |
| Min pH             | 5.79±0.22 | 5.90±0.23 | <0.05 |
| Max pH             | 6.67±0.18 | 6.53±0.23 | <0.01 |
| Max-min pH         | 0.87±0.21 | 0.62±0.16 | <0.01 |
| Drinking frequency | 5.9±1.7   | 6.7±1.9   | -   |

\(^{1)}\) C, conventional farm; W, welfare farm.

**Table 5.** Comparison of lactation performance between conventional farm and welfare farm for 3 days

| Items              | C  | W  | p-value |
|--------------------|----|----|---------|
| Milk yield (kg/d)  | 33.94±6.06 | 24.03±6.25 | <0.01 |
| Milk fat (%)       | 3.84±0.45 | 4.15±0.48 | <0.01 |
| Milk protein (%)   | 3.37±0.17 | 3.19±0.16 | <0.01 |
| FPCM               | 33.42±6.23 | 24.21±6.06 | <0.01 |
| FPCM/TDN           | 1.68±0.32 | 1.82±0.45 | 0.17 |

FPCM, fat protein correction milk; FPCM/TDN, fat protein correction milk/total digestible nutrients.

**Table 6.** Comparison of metabolic profiles between conventional farm and welfare farm for 3 days

| Item               | C  | W  | p-value |
|--------------------|----|----|---------|
| ALT (U/L)          | 35.64±5.79 | 33.72±12.98 | 0.65 |
| AST (U/L)          | 86.18±16.75 | 94.28±10.98 | 0.19 |
| T-BIL (mg/dL)      | 0.23±0.05 | 0.22±0.07 | 0.73 |
| T-Cholesterol (mg/dL) | 276.38±66.10 | 198.75±35.96 | <0.01 |
| T-Protein (mg/dL)  | 7.29±0.34 | 8.01±0.16 | <0.01 |
| Albumin (g/dL)     | 3.42±0.15 | 3.54±0.04 | 0.07 |
| A/G ratio          | 0.89±0.08 | 0.80±0.08 | <0.05 |
| Glucose (mg/dL)    | 61.48±4.17 | 43.67±4.54 | <0.01 |
| BUN (mg/dL)        | 15.77±1.54 | 12.11±1.53 | <0.01 |
| Creatine (mg/dL)   | 0.93±0.08 | 0.84±0.06 | <0.01 |
| Ca (mg/dL)         | 9.80±0.45 | 9.67±0.52 | 0.55 |
| P (mg/dL)          | 6.11±0.87 | 6.47±0.18 | 0.33 |
| Mg (mg/dL)         | 2.65±0.21 | 2.45±0.17 | <0.05 |
| NEFA (uEq/L)       | 151.7±25.69 | 109.2±96.78 | <0.01 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; T-BIL, total bilirubin; T-Cholesterol, total cholesterol; T-Protein, total protein; A/G ratio, albumin/globulin ratio; BUN, blood urea nitrogen; Ca, calcium; P, phosphorus; Mg, magnesium; NEFA, non-esterified fatty acid.

\(^{1)}\) C, conventional farm; W, welfare farm.
Originally, we expected that the welfare farm that fed a high percentage of grass to its cows, would maintain a relatively high pH, compared to that of the conventional farm [15,16]. However, a significant difference in rumen pH was not found between the two farms. This is unexpected results because it is generally accepted that higher roughage feeding results in higher ruminal pH with more ruminations [17]. Current results of present study might be related to differences in particle size of feeds offered to animals of two farms. The forage used in the conventional farm was longer than that used in the welfare farm, which affected ruminal pH. It was concluded that the particle size of the feed grain on the welfare farm had an effect on the decreased rumen pH [18].

However, unlike the comparison of the overall average pH on the two farms, the average daily pH change, calculated from the difference between the maximum and minimum pH values, was significantly different between the two farms; 0.6±0.2 for the welfare farm and 0.9±0.2 for the conventional farms, which indicates that higher forage promotes much more stable ruminal pH environment and hence provides more favorable condition for rumen microbial growth. These results are similar to those of previous studies in which the lower the proportion of grass feed, the wider the range of changes in pH was observed [19]. This suggests that pH is relatively stable in the rumen of cows on welfare farms [20].

The frequency of drinking water was found to be higher on the conventional farm than on the welfare farm. This might be due to the increase in osmolarity of the rumen content of higher concentrate and is attributed to the regulation of pH homeostasis in the rumen of cows on conventional farms where the range of pH changes in the rumen was large, and previous goat research supports this finding [21].

Therefore, it can be concluded that the welfare farm, which had a relatively high percentage of grass feed, showed positive results as compared to the conventional farm with respect to the rumen state.

The average daily milk yield of cows from the welfare farm was 9 kg higher than those from the conventional farm. However, the milk production efficiency (a value obtained by dividing the corrected milk fat milk protein flow rate by the plasticized total nutrients) was 1.8±0.5 for the welfare farm and 1.7±0.3 for the conventional farm, showing a higher production efficiency of the welfare farm. Current result is expected one and high energy diets would promote higher milk yield, but the same diet is not always superior in milk production efficiency [22].

The results of the blood analysis confirmed that there were no major differences in most of parameters among cows from both farms and those values were within the normal range, as shown in previous studies that measured the same blood component [23]. However, blood components related to energy balance, such as total cholesterol, total protein, BUN, and NEFA, were significantly different between the two farms. This implies that feeding different levels of forages influenced metabolic rates of cows and resulted in high milk yields in conventional farms, which provided high-energy feed to cows [24-26] This may in turn, have a negative impact on the economic lifespan of cows by exerting a high metabolic burden [27,28].

In summary the level of forage affected rumen pH, milk production characteristics, and blood metabolism components and these results may provide some useful index in establishing the guideline for welfare standard in Korea. Further research by using more animals with different types and levels of forage sources under various environmental conditions are warranted to provide more valid criteria for the animal welfare certification policy system in Korea.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

FUNDING

This work was supported by the Animal and Plant Quarantine Agency (Republic of Korea; No.F-1543071-2019-20-01).

REFERENCES

1. Animal welfare farm certification standards for dairy cattle [Internet]. Animal Protection Management System [cited 2021 Feb 4]. Available from: https://www.animal.go.kr/front/community/show.do?boardId=contents&seq=79&menuNo=300000019
2. Beauchemin KA. Invited review: Current perspectives on eating and rumination activity in dairy cows. J Dairy Sci 2018;101:4762-84. https://doi.org/10.3168/jds.2017-13706
3. RDA (Rural Development Administration). Korean feeding standard for Dairy Cattle. 3rd ed. Jeonju, Korea: National Institute of Animal Science; 2017.
4. Aschenbach JR, Penner GB, Stumpff F, Gäbel G. Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J Anim Sci 2011;89:1092-107. https://doi.org/10.2527/jas.2010-3301
5. Son HI, Baek SG, Moon JY, Ahn EY, Lee HJ, Son YS. Effect of subacute ruminal acidosis on plasma concentrations of lipopolysaccharide in dairy cattle. J Korean Soc Grassl Forage Sci 2013;33:313-8. https://doi.org/10.5333/KGFS.2013.33.4.313
6. OIE [Internet]. Terrestrial Animal Health Code Chapter 7.11. Animal welfare and dairy cattle production systems [cited 2021 Feb 4]. Available from: https://www.oie.int/index.
7. Castillo-González AR, Burrola-Barraza ME, Domínguez-Viveros J, Chavez-Martínez A. Rumen microorganisms and fermentation. Arch Med Vet 2014;46:349-61. doi:10.1007/S00301-732X201400030003
8. AWA (Animal Welfare Approved by AGW standard) [Internet]. Dairy Cattle Standards [cited 2021 Feb 4]. Available from: https://agreenerworld.org/certifications/animal-welfare-approved/standards/dairy-cattle-and-calf-standards/
9. Antanaitis R, Juozaitienė V, Rutkauskas A, Televičius M, Stasiulevičitė I. Reticulorumen temperature and pH as indicators of the likelihood of reproductive success. J Dairy Res 2018;85:23-6. doi:10.1017/S0022029918000018
10. Stangaferro ML, Wijma R, Caixeta LS, Al-Abri MA, Giordano JO. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. J Dairy Sci 2016;99:7422-33. doi:10.3168/jds.2016-11352
11. Elischer MF, Arceo ME, Karcher EL, Siegford JM. Validating the accuracy of activity and rumination monitor data from dairy cows housed in a pasture-based automatic milking system. J Dairy Sci 2013;96:6412-22. doi:10.3168/jds.2013-6790
12. OIE. Animal Welfare and Dairy Cattle Production System. In Terrestrial Animal Health Code; Chapter 7.11. Paris, France: World Organization for Animal Health/OIE; 2015.
13. Spigarelli C, Zuliani A, Battini M, Mattiello S, Bovolenta S. Welfare assessment on pasture: a review on animal-based measures for ruminants. Animals 2020;10:609. doi:10.3390/ani10040609
14. Lee WS, Lee BS, Oh YK, et al. Effects of concentrate to roughage ratios on duration and frequencies of rumination and chewing in Hanwoo steers. J Anim Sci Technol 2004;46:55-60. doi:10.5187/JAST.2004.46.1.055
15. Schirmann K, von Keyserlingk MA, Weary DM, Veria DM, Heuwieser W. Validation of a system for monitoring rumination in dairy cows. J Dairy Sci 2009;92:6052-5. doi:10.3168/jds.2009-2361
16. Soriani N, Trevisi E, Calamari L. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. J Anim Sci 2012;90:4544-54. doi:10.2527/jas.2011-5064
17. Jiang FG, Lin XY, Yan ZG, et al. Effect of dietary roughage level on chewing activity, ruminal pH, and saliva secretion in lactating Holstein cows. J Dairy Sci 2017;100:2660-71. doi:10.3168/jds.2016-11559
18. Mao SY, Huo WJ, Zhu WY. Microbiome-metabolome analysis reveals unhealthy alterations in the composition and metabolism of ruminal microbiota with increasing dietary grain in a goat model. Environ Microbiol 2016;18:525-41. doi:10.1111/1462-2920.12724
19. Krause KM, Combs DK, Beauchemin KA. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J Dairy Sci 2002;85:1947-57. doi:10.3168/jds.S0022-0302(02)74271-9
20. Pitt R, Pell A. Modeling ruminal pH fluctuations: interactions between meal frequency and digestion rate. J Dairy Sci 1997;80:2429-41. doi:10.3168/jds.S0022-0302(97)76195-2
21. Desnoyers M, Duvaux-Ponter C, Rigalma K, Rousel S, Martin O, Giger-Reverdin S. Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. Animal 2008;2:1802-8. doi:10.1080/1751731108003157
22. Keady TWJ, Mayne CS, Fitzpatrick DA, McCoy MA. Effect of concentrate feed level in late gestation on subsequent milk yield, milk composition, and fertility of dairy cows. J Dairy Sci 2001;84:1468-79. doi:10.3168/jds.S0022-0302(01)70180-4
23. Jung SH, Jung YH, Choe C, et al. Reference intervals for blood metabolite profiles of Holstein cows in Korea. Korean J Vet Serv 2019;42:121-6. doi:10.7853/kjvs.2019.42.2.121
24. Adewuyi AA, Gruys E, Van Eerdenburg FJCM. Non-esterified fatty acids (NEFA) in dairy cattle. A review. Vet Q 2005;27:117-26. doi:10.1080/01652176.2005.9695192
25. Jóźwik A, Strzalkowska N, Bagnicka E, et al. Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows. Czech J Anim Sci 2012;57:353-60. doi:10.17221/6270-CJAS
26. Mohebbi-Fani M, Nazifi S, Rowghani E, Bahrami S, Jamshidi O. Thyroid hormones and their correlations with serum glucose, beta hydroxybutyrate, nonesterified fatty acids, cholesterol, and lipoproteins of high-yielding dairy cows at different stages of lactation cycle. Comp Clin Pathol 2009;18:211-6. doi:10.1007/s00580-008-0782-7
27. Lee BH, Nejad JG, Kim HS, Sung KI. Effect of forage feeding level on the milk production characteristics of Holstein lactating cows. J Korean Soc Grassl Forage Sci 2013;33:45-51. doi:10.5333/KGFS.2013.33.1.45
28. De Vries A, Marcondes M. Overview of factors affecting productive lifespan of dairy cows. Animal 2020;14(S1):155-64. doi:10.1017/S1751731119003264