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Risk Factors for Ketosis in Dairy Cows and Associations with Some Blood Metabolite Concentrations

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Abstract : Ketosis has become a very common and important metabolic disorder that causes substantial economic loss in modern dairy herds. We determined the risk factors for ketosis and associations with some blood metabolite concentrations in dairy cows. Blood from 475 Holstein cows on four dairy farms was collected weekly until 4 weeks after calving to measure blood β-hydroxybutyrate (BHBA) concentrations using electronic handheld meters. Cows were grouped based on the BHBA concentration into two groups: a ketosis group (≥ 1.2 mmol/L, n = 150) and a non-ketosis group (< 1.2 mmol/L, n = 325). Peripartum health status (dystocia, retained placenta, and metritis), cow parity, and calving season were recorded to identify the risk factors for ketosis. Serum albumin, total cholesterol (TCH), and haptoglobin concentrations were compared between sub-groups of cows selected from the ketosis (n = 92) and non-ketosis (n = 50) groups 1 week postpartum (7.5 ± 0.2 days). The farm influenced the incidence of ketosis ($P < 0.05$). Cows calved during summer tended to have a higher risk (odds ratio [OR]: 1.61, $P < 0.1$) of ketosis than cows calved during spring. Cows with parities of two (OR: 1.95, $P < 0.05$) and three or higher (OR: 2.55, $P < 0.01$) were at higher risk than primiparous cows. Cows with metritis had a higher risk (OR: 7.02, $P < 0.0001$) of ketosis than cows without metritis. Serum albumin and TCH concentrations were lower ($P < 0.01$) in the ketosis group than in the non-ketosis group, whereas haptoglobin concentration was higher ($P < 0.05$) in the ketosis group than in the non-ketosis group. In conclusion, our results indicate that farm, summer calving, parity greater than one, and postpartum disease (metritis) were risk factors for ketosis. In addition, lower serum albumin and TCH concentrations and higher haptoglobin concentration were also associated with the incidence of ketosis in dairy cows.

***Key words* :** blood metabolite, dairy cow, ketosis, risk factor.

Introduction

Ketosis is a common and important postpartum metabolic disorder that occurs predominantly in high-producing dairy herds. Severe negative energy balance (NEB), representing an inability to meet the required energy demand for milk production after calving, leads to excessive lipid mobilization and elevation of circulating non-esterified fatty acids (NEFA) and ketone bodies in tissues and milk (14). Circulating ketone bodies can be used to a certain extent as an energy source by several organs, including the heart, liver, and mammary tissue (10). However, excessive ketogenesis leads to higher concentrations of circulating ketone bodies (hyperketonemia) (26). Cows with hyperketonemia may show clinical signs, including poor feed intake and weight loss (the clinical form), or may not show signs (the subclinical form) within 4 weeks postpartum (24,37). Subclinical ketosis can cause great economic loss, because of increased incidence of postpartum disorders and consequent removal of individuals from the herd, and because of poorer production and reproductive outcomes (7,23,30).

The incidence of ketosis is associated with the nutritional status of the animals, environmental factors that influence their biological responses, and regional geography (5,12,33). In particular, higher parity, prepartum over-conditioning of the animals, calving season, dry period length, previous lactation length, and the amount of colostrum produced were reported to be risk factors for the incidence of ketosis in Dutch dairy herds (33). In addition, a Canadian research group demonstrated that automatic milking systems, cattle breed, longer calving interval, older age at first calving, and milk fat yield during the previous lactation were within-herd risk factors in a large number of dairy herds (31). A large number of risk factors have also been reported in other countries and regions (5,8,34).

The overall incidence rate of ketosis varied from 21% to 97% among the studies undertaken internationally (3,5,21,24, 31,36). Risk factors for ketosis may also vary among regions or countries because of differences in nutritional and health management, differences in the environment, including climate and altitude, and differences in facilities, in addition to others. The determination of region-specific risk factors is likely to be worthwhile as part of a strategy to avoid the severe loss caused to dairy enterprises by ketosis. Thus, the first objective of this study was to determine the risk factors for ketosis

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in dairy cows in Korea.

Several blood metabolites have been used to monitor systemic metabolic changes, especially during the transition period (18,29). It has been well known that decreased glucose and increased NEFA and β-hydroxybutyrate (BHBA) concentrations were associated with severe energy deficit during early lactation (3,35). Moreover, other blood metabolites, such as aspartate aminotransferase and haptoglobin, are useful to monitor liver function following calving (1,6,27). Nevertheless, the relationships between ketosis and some blood metabolites have not been clarified. Thus, determining the relationships between ketosis and blood concentrations of some important metabolites, along with the identification of risk factors for ketosis, may help to establish preventative and therapeutic strategies for ketosis in dairy cows. Therefore, the second objective of this study was to investigate the relationships between ketosis and blood concentrations of some metabolites (albumin, total cholesterol (TCH), and haptoglobin) 1 week postpartum.

Materials and Methods

Animals

The study was performed on four dairy farms, designated A-D, located in Chungcheong Province. Each farm contained 80-200 milking cows, which were maintained in loose housing systems. They were fed total mixed rations and milked twice daily. The mixed rations were based on brewers' grain, alfalfa hay, cotton seed, beet pulp, corn silage, tall fescue, timothy hay, and mineral and vitamin additives. A total of 475 Holstein cows, with 2.6 ± 1.4 lactations (mean ± standard deviation; range: 1-7 lactations), were included in the study. All cows received weekly reproductive health checks by veterinarians on the research team. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University.

Blood sampling and study design

Blood was collected weekly from the tail vein of the cows until 4 weeks after calving to measure blood BHBA concentration using electronic handheld meters and β-ketone test strips (FreeStyle Optimum, Abbott Diabetes Care Ltd., Witney, UK). Cows were grouped based on these BHBA concentrations into two groups: a ketosis group (≥ 1.2 mmol/L, n = 150) and a non-ketosis group (< 1.2 mmol/L, n = 325).

Blood samples from sub-groups of cows from the ketosis (n = 92) and non-ketosis (n = 50) groups were collected 1 week postpartum (7.5 ± 0.2 days) to measure serum albumin, TCH, and haptoglobin concentrations. Ten milliliters of blood from each cow was added to plain plastic centrifuge tubes, and these were immediately placed in an ice bath. The samples were then centrifuged at 2000 × g for 10 min at 4°C, and the serum was harvested and frozen at −80°C until assayed.

Data collection and case definition

Data were collected from 475 Holstein cows, which included peripartum health status, cow parity, and calving date. Table 1 lists the independent variables that describe peripartum health status (including dystocia, retained placenta, and metritis), cow parity, and calving season. The definitions of the health disorders encountered around birth that were used in the present study were similar to those described in previous publications (9,22,28). Calving difficulty was ranked according to the degree of assistance required (1 = no assistance, 2 = minor assistance, 3 = force required, 4 = marked force, and 5 = caesarian section). Cows with a calving score > 2 were considered to have dystocia. Retained placenta was defined as the retention of the fetal membranes for longer than 24 h. Metritis was defined by the presence of fever (≥ 39.5°C) and a watery, fetid uterine discharge. Subclinical ketosis was defined as a BHBA concentration of 1.2-2.9 mmol/L, while clinical ketosis was defined by a BHBA concentration of ≥ 3.0 mmol/L.

Table 1. Descriptive statistics for the 475 dairy cows included in the study

Variable	Level	Number of cows		
		Calved	Ketosis	Percentage
Farm	A	119	37	31.1
	B	108	45	41.7
	C	139	36	25.9
	D	109	32	29.4
Calving season	Spring	109	32	29.4
	Summer	161	68	42.2
	Autumn	137	31	22.6
	Winter	68	19	27.9
Cow parity	1	114	24	21.1
	2	149	45	30.2
	≥ 3	212	81	38.2
Dystocia	No	450	138	30.7
	Yes	25	12	48.0
Retained placenta	No	419	121	28.9
	Yes	56	29	51.8
Metritis	No	442	125	28.3
	Yes	33	25	75.8

Measurement of metabolite concentrations in serum samples

The concentrations of albumin and TCH were measured in serum samples with a 7180 Biochemistry Automatic Analyzer 710 (Hitachi Ltd., Tokyo, Japan) using commercial enzyme assay kits (Wako Pure Chemical Ltd., Osaka, Japan), according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were < 5% for all the assays.

The concentration of haptoglobin was determined using a commercially available bovine haptoglobin ELISA test kit (Life Diagnostics, Inc., West Chester, PA, USA) according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were 3.1% and 6.7%, respectively.

Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM). For statistical analyses, cow parity was categorized as either 1, 2, or ≥ 3, while calving season was categorized as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February). Statistical analyses were performed using the SAS program (version 9.4, SAS Inst., Cary, NC, USA).

To determine the risk factors for ketosis, logistic regression was undertaken using the LOGISTIC procedure. The logistic regression model included the farm (A-D), calving season, cow parity, peripartum health status (recording the presence of dystocia, retained placenta, or metritis), group (non-ketosis and ketosis), and the interactions between these variables. Backward stepwise regression was used in all models, and elimination was performed based on the Wald statistic criterion, when $P > 0.15$. The odds ratio (OR) and 95% confidence interval (CI) were computed by logistic regression. Results are presented as proportions and ORs with their respective 95% CIs.

Statistical analysis of the blood concentrations of albumin, TCH, and haptoglobin was carried out using the general linear model procedure. The model included cow parity, calving season, and group. Haptoglobin concentrations were not normally distributed; therefore, values were transformed to their natural logarithms for data analysis, although non-transformed data, expressed as mean and SEM, are presented herein.

A P-value ≤ 0.05 was considered significant, and $0.05 < P < 0.1$ was considered to indicate a tendency toward significance.

Results

Risk factors for ketosis

The overall incidence of ketosis (150 out of 475 cows) in four dairy farms was 31.6% (herd range: 25.9-41.7%). The percentages of cows with ketosis that developed 1, 2, 3, and 4 weeks postpartum were 70.0%, 20.7%, 6.7%, and 2.7%, respectively (Fig 1). The incidence rates for clinical and subclinical ketosis were 12.6% and 19.0%, respectively. The logistic regression model revealed that the farm, calving season, cow parity, and presence of metritis affected the incidence of ketosis ($P < 0.05$) (Table 2), while dystocia and retained placenta were eliminated from the final regression model ($P > 0.1$). The risk of ketosis on farm B was greater (OR: 1.83, $P < 0.05$) than that on farm A. Cows that calved during summer tended to have a higher risk (OR: 1.61, $P < 0.1$) of ketosis than cows that calved during spring. Cows with parities of two (OR: 1.95, $P < 0.05$) and three or higher (OR: 2.55, $P < 0.01$) had a higher risk than primiparous cows. Cows with metritis had a higher risk (OR: 7.02, $P < 0.0001$) of ketosis than cows without metritis.

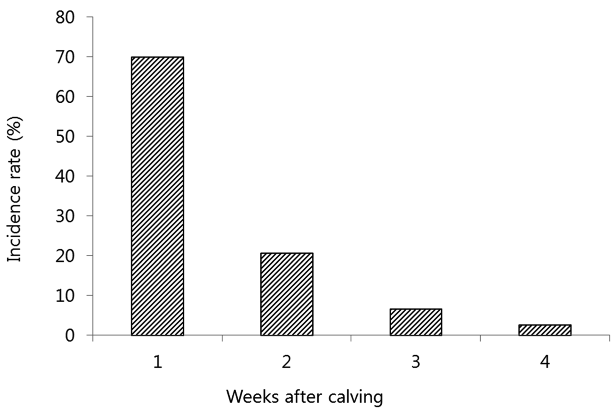

Fig 1. Relative incidence of ketosis 1, 2, 3, and 4 weeks after calving.

Relationships between ketosis and serum metabolites concentrations

Fig 2 shows the concentrations of blood metabolites in the non-ketosis and ketosis groups 1 week postpartum (7.5 ± 0.2 days). Serum albumin ($P < 0.01$, Fig 2A) and TCH ($P < 0.001$, Fig 2B) concentrations were lower in the ketosis group than in the non-ketosis group, whereas haptoglobin ($P < 0.01$, Fig 2C) concentration was significantly higher in the ketosis group than in the non-ketosis group.

Discussion

We determined the risk factors for ketosis in dairy cows, and the relationships between ketosis and serum concentrations of some metabolites, which are indicative of nutrition status and the degree of lipid mobilization postpartum. Our results indicate that herd, calving in the warmer months, higher parity, and the presence of postpartum disease (metritis) were risk factors for ketosis. In addition, lower serum albumin and TCH concentrations, and higher haptoglobin concentration, which likely reflect severe energy deficit and reduced liver function, were also associated with the incidence of ketosis.

The overall incidence of ketosis (31.6%) in the present study was higher than in one previous study (21%) (31), but lower than in other studies (ranges: 43.2-97%) (3,21,22,33). The great variation in the incidence of ketosis among these

Table 2. Odds ratio and confidence interval (CI), and P-value for each of the variables included in the final logistic regression model of the risk of ketosis, using data from 475 dairy cows

Variable	Odds ratio	95% CI	P-value
Farm			< 0.05
A	Reference		
B	1.83	1.013-3.307	< 0.05
C	0.79	0.424-1.482	> 0.1
D	0.92	0.498-1.680	> 0.1
Calving season			< 0.05
Spring	Reference		
Summer	1.61	0.923-2.817	< 0.1
Autumn	0.71	0.385-1.318	> 0.1
Winter	0.84	0.397-1.761	> 0.1
Cow parity			< 0.01
1	Reference		
2	1.95	1.039-3.661	< 0.05
≥ 3	2.55	1.422-4.570	< 0.01
Metritis			
No	Reference		
Yes	7.02	2.944-16.739	< 0.0001

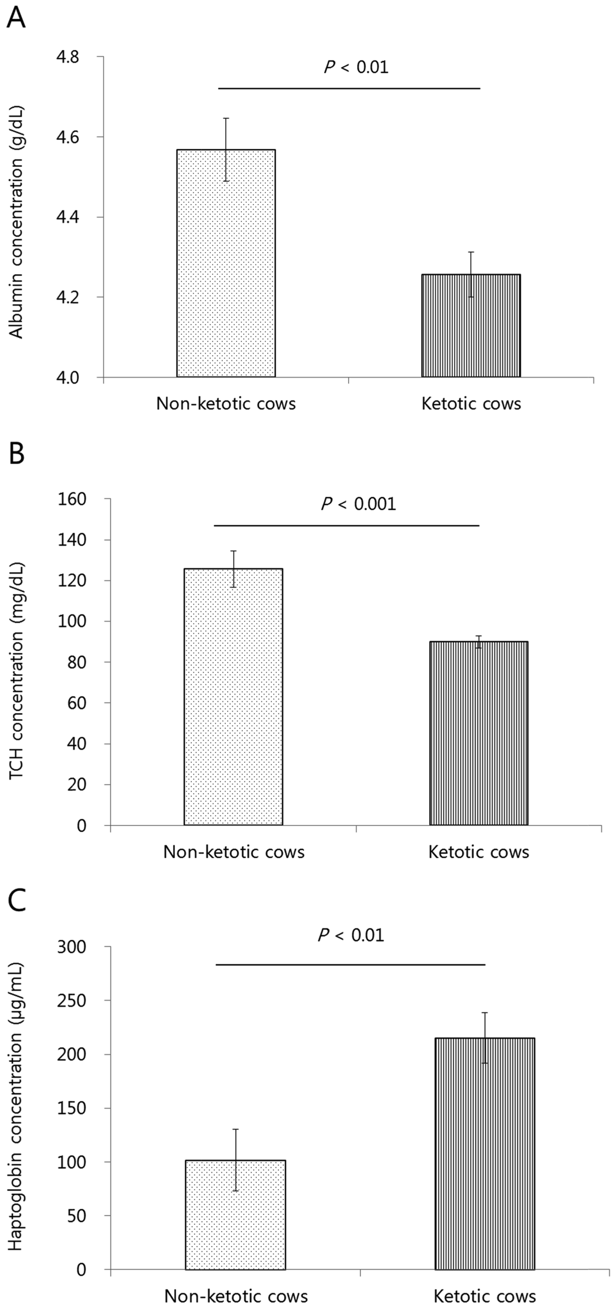

Fig 2. Serum albumin (A), total cholesterol (TCH; B), and haptoglobin (C) concentrations in cows without (n = 50) and with ketosis (n = 92) 1 week postpartum (7.5 ± 0.2 days).

studies might be due to the use of different diagnostic methods and BHBA thresholds, and the time intervals used for evaluation, as well as regional and national differences in dairy cow husbandry (including feeding strategy, milk yield, and disease prevalence) (5,21,31,33). In the present study, more than 90% of ketotic cows were identified within 2 weeks postpartum, which is similar to the period reported in a previous study (24), in which the peak incidence of subclinical ketosis occurred at 5 days postpartum. Our observation that approximately 40% of the 150 ketotic cows demonstrated the clinical form of the disorder was not consistent with other studies, in which ranges of 16-20% for clinical ketosis were reported (33,36). The reasons for the higher rate of clinical ketosis in the present study are not clear; however, this might in part be due to the use of more intensive production systems, resulting in higher milk yield in the present study.

Herd affected the incidence of ketosis in the present study, demonstrated by a variation in incidence of 25.9-41.7% among the farms. A previous study reported a similar variation (26.4-55.7%) in the incidence of ketosis among the four herds studied (23). In addition, figures of 38.2-51.0% were reported by a third study of three farms (21). The reasons for this variation in incidence rate in each study are likely to be related to differences in herd productivity and/or nutritional and health management (5,31).

Several publications report that calving season is associated with the incidence of ketosis in dairy cows (5,13,30,33). The present study showed that cows that calved during the summer tended to have a higher risk of ketosis than cows that calved during other seasons, which might be associated with the higher temperature and humidity during the summer (June to August) in Korea. In a previous European study, the highest incidence of ketosis was observed in cows that calved in the second quarter (April to June) of the year (5), while another study reported higher levels of ketosis in May to June, compared with July to September (30). These reports are not consistent with our data, probably because of differences in climate, weather, and geography.

Our observation that higher parity was an important risk factor for ketosis is generally consistent with previous studies (5,21,33). One study suggested that the higher incidence of ketosis in multiparous, compared with primiparous cows, might be due to concurrent gestation and lactation in the former, and thus more marked depletion of energy reserves (5). In addition, the higher milk yield and more severe metabolic imbalance experienced during the transition period by multiparous cows may also have an impact (19,33).

In the present study, cows with metritis had a significantly higher probability of developing ketosis than cows without this disease, which is consistent with previously published data (11). However, it is difficult to define a cause-effect relationship in the present study, because the diagnosis of metritis was made around the same time as the diagnosis of ketosis. Nevertheless, it has been suggested that ketosis might occur secondary to a decrease in appetite and/or difficulty standing to eat (13). Consistent with this, previous studies observed a peripartum decrease in feed intake in cows with metritis (15,20).

Blood albumin and TCH concentrations may reflect postpartum energy balance in dairy cows (4,25). In the present study, serum albumin and TCH concentrations were lower in the ketosis group than in the non-ketosis group. This finding was not consistent with a previous paper that showed no difference in TCH concentration between cows with and without ketosis (36). However, other previous studies show that low peripartum TCH concentrations are associated with fatty liver (16,32), consistent with our observations. The lower albumin concentration in the present study might be due to an increase in albumin catabolism induced by a severe energy deficit (4). Haptoglobin may be a useful indicator of liver function, because its presence in the blood reflects ongoing acute inflammatory processes after calving (2), and its blood concentration also increases during hepatic lipidosis (17). Our finding of a higher haptoglobin concentration in cows with ketosis is consistent with that of a previous study (1).

In summary, herd, summer calving, higher parity, and the presence of postpartum metritis were risk factors for ketosis. In addition, lower serum albumin and TCH concentrations, and higher haptoglobin concentration, which likely reflect severe energy deficit and reduced liver function, were also associated with the incidence of ketosis. Based on these data, we suggest encouraging higher peripartum feed intake, reducing heat stress during the calving season, and preventing postpartum uterine disease as strategies to help reduce the incidence of ketosis in dairy cows. Furthermore, interventions aimed at reducing ketogenesis in the liver might be needed as part of a ketosis prevention and treatment strategy in dairy cows.

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