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RESEARCH ARTICLE

Investigation of Negative Energy Balance and Biochemical Parameters in Dairy Cows with Hypomagnesemia Developing in the Fresh Period

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Abstract

Hypomagnesemia is a disease in ruminants characterized by biochemically low serum magnesium. Metabolic profile refers to the analysis of blood biochemical parameters used to assess and prevent metabolic and nutritional disorders in dairy farms. This study aims to evaluate the effect of magnesium on energy balance and metabolic profile parameters in fresh postpartum dairy cows. The study included 30 cows diagnosed with postpartum hypomagnesemia and 10 control group cows. Measurements of the parameters urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), total protein, cholesterol, albumin, calcium, phosphorus, beta-hydroxybutyric acid (BHBA), and non-esterified fatty acids (NEFA) were performed on biochemical analyzer. In the hypomagnesemia group, NEFA, total protein, AST and cholesterol levels were higher compared to the control group, while glucose levels were lower. Correlation analysis revealed a negative moderate correlation between magnesium and NEFA, BHBA and AST, positive moderate correlation with glucose and urea. Significant changes were detected in NEFA and glucose parameters, which reflect energy status, and in AST and cholesterol parameters, which provide information about liver function. It was concluded that metabolic adaptation in dairy cows affected by hypomagnesemia may be weakened, and energy balance and liver function may be affected.

Introduction

Magnesium acts as a cofactor in enzymatic processes, intracellular signaling, bone development, and neurotransmission, as well as in maintaining muscle function (Martín-Tereso and Martens, 2014). Therefore, magnesium deficiency can lead to serious biochemical and physiological disturbances (Doncel *et al*., 2019). Magnesium is distributed to different parts of the body in cattle. While 60-70% of magnesium is stored in bones, approximately 30% is found inside cells, and only 1% is present in blood and extracellular fluids (Goff, 1999; Doncel *et al*., 2019). Blood magnesium levels depend on the balance between absorption and excretion. In times of need, magnesium reserves in bones cannot be easily mobilized. Only 30% of these reserves are actively usable (Alfrey and Miller, 1973). Hypomagnesemia also affects animal species such as sheep and goats, but it is stated that cattle, especially dairy cows, are more susceptible (Grunes *et al*., 1970). In dairy cows, magnesium is excreted via milk (4.1-4.9 mmol/L) (Cerbulis and Farrell, 1976), urinary tract (0.017-0.17 mmol/day) (Kemp *et al*., 1961) and digestive secretions (Doncel *et al*., 2019).

Hypomagnesemia is a disease in ruminants characterized by biochemically low serum magnesium (Martín-Tereso and Martens, 2014). The disease may present with clinical or subclinical signs such as anorexia, low milk yield, anemia, mild restlessness, difficult birth, and edema in the mammary gland, or it can lead to sudden deaths. Subclinical or latent hypomagnesemia in dairy cows can persist for a long time and remain present for months without clinical signs (Doncel *et al*., 2019; Hernández-Gotelli *et al*., 2024). Although the severity of clinical disease is greater than that of subclinical cases, a large proportion of subclinical hypomagnesemia goes unnoticed. Subclinical hypomagnesemia is associated with other metabolic imbalances, the incidence of clinical disease, and reproductive performance. Therefore, subclinical hypomagnesemia is an important disease that should be taken into consideration even if it does not show clinical symptoms (Hernández-Gotelli *et al*., 2024). Clinical hypomagnesemia can manifest with mild clinical symptoms such as anxiety and restlessness when serum magnesium levels drop to critical levels. Additionally, in animals with subclinical hypomagnesemia, factors such as stress, weather, housing space, and feed changes can cause latent hypomagnesemia cases to manifest as clinical tetany (Pehrson, 1985; Ghanem, 2013). Serum levels should also be added for the diagnosis of clinical and subclinical hypomagnesaemia.

Metabolic profile refers to the analysis of blood biochemical parameters used to assess and prevent metabolic and nutritional disorders in dairy farms. In addition, it is used as a reliable test for optimizing herd management at a relatively low cost, enabling attempts to achieve maximum future yield, and for the early detection of nutritional deficiencies or metabolic diseases (Radostits, 2007). Additionally, metabolically superior cows can be identified by evaluating their metabolic profiles in circulation. Therefore, information about the circulating metabolic profile is considered a useful diagnostic aid for identifying herd problems (Găvan *et al*., 2010). The fresh period is known to be a time when cows can be extremely susceptible to mineral imbalances due to the rapidly increasing energy requirements, decreased feed intake, and metabolic adaptations following parturition. Based on this, this study aims to determine the effects of magnesium deficiency on critical metabolic processes, particularly lipid mobilization, energy balance, ketone body formation, and liver function indicators.

Materials and Methods

Ethics

This study was initiated after obtaining approval from the Bingöl University Local Ethics Committee for Animal Experiments (B.Ü HADYEK Meeting Number: 2025/02, Decision No: 02/09).

Animal Selection and Grouping

A total of 40 dairy cows were used in the study, including 30 hypomagnesemic cows in the fresh period (30 days postpartum) and 10 control cows. The animals were housed in semi-open barn paddocks. Cows were fed anionic diets in the prepartum period and cationic diets in the postpartum period and a total mixed ration containing hay, soybean meal, corn flakes, corn silage, bypass oil, alfalfa hay, vitamins and minerals. The cows had free access to water. The cows included in the study were Holstein, Simmental, and crossbred breeds. Their body condition was 3.46±0.22, and their milk yield was 31.72±82. Dry matter intake was not measured accurately. To minimize inter-animal differences in biochemical measurements, farms using similar rations were preferred.

Serum magnesium concentrations were measured in animals showing weight loss, decreased milk yield, and restlessness, and those below 1.5 mg/dl (Fielder, 2022) were included in the hypomagnesemia group. Blood samples were taken once from cows in the postpartum fresh period, 4 hours after the morning feeding. The control group was composed of cows determined to be healthy based on systematic clinical examination and biochemical analysis during their fresh period.

Cows that developed metritis, mastitis, hypocalcemia, retained placenta, ketosis, or laminitis during the fresh period, as well as animals that showed no signs of disease during clinical examination and biochemical analysis, were receiving medication, or were outside the fresh period, were excluded from the study.

Sample Collection and Laboratory Analyzes

Blood samples (5 mL) were collected from animals in the hypomagnesemia and control groups included in the study, using gel serum tubes (BD Vacutainer®, BD Vacutainer Systems, Plymouth, UK) according to the appropriate technique. Blood samples taken into gel serum tubes were centrifuged (Hermle Z 36 HK®, Germany) at 7000 rpm for 5 minutes, the sera were transferred to Eppendorf tubes and stored at -20 °C until analysis. Metabolic profile parameters from the obtained sera, including magnesium, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), total protein, cholesterol, albumin, calcium, and phosphorus (BS-2000m, Mindray, China), as well as beta-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA), were measured using an automated biochemical analyzer (Randox Monaco, UK).

Statistical Analysis

The SPSS 26 computer program was used to evaluate the data. The Shapiro-Wilk test was applied to check for the normal distribution of the data. The independent samples t-test was applied to those who met the normal distribution. The Mann-Whitney U test was applied to those who did not meet the normal distribution. In the data analysis, descriptive statistics were presented with mean and standard error (Mean±SE) values. The relationship between the variables was determined using Spearman correlation analysis. Correlation between variables was determined by Spearman rank correlation analysis as described by Little and Hills (1978). High correlation was accepted at r > 0.60, moderate correlation at r < 0.60, and low correlation at r < 0.30. Differences between groups were considered significant when P < 0.05 for all variables.

Results

In the hypomagnesemia group, NEFA (P < 0.001), total protein (P < 0.001), AST (P < 0.001), and cholesterol (P < 0.001) levels were higher compared to the control group, while glucose (P < 0.001) levels were lower. No statistically significant difference was found between the BHBA, urea, GGT, ALT, calcium, creatinine, albumin, and phosphorus levels in the hypomagnesemia and control groups (P > 0.05). The results of the biochemical analysis and statistical significance levels between the hypomagnesemia and control groups are shown in Table 1.

Correlation analysis revealed a negative moderate correlation between magnesium and NEFA (r = -0.588, *P* < 0.001), BHBA (r = -0.394, *P* < 0.012) and AST (r = -0.537, *P* < 0.001), positive moderate correlation with glucose (r = 0.334, *P* < 0.035) and urea (r = 0.344, *P* < 0.030). Detailed results of the correlation analysis are shown in Table 2.

Discussion

Magnesium concentrations are known to decrease, particularly in cows at the beginning of lactation, and cows that develop hypomagnesemia are less likely to experience a healthy fresh period (Ganesan *et al*., 2025). Hypomagnesemia affects dairy cows both subclinically and clinically (Doncel *et al*., 2019). The prevalence of subclinical hypomagnesemia in dairy cattle is reported to be under-measured despite being a significant risk factor for diseases often associated with the development of peripartum metabolic disorders (Silva *et al*., 2020). This study evaluated energy balance and metabolic profile parameters in dairy cattle that developed hypomagnesemia during the fresh period.

Increased energy demands during begining of the post partum period reduced dry matter intake immediately before calving, and milk production lead to a negative energy balance in cows (Ospina *et al*., 2010). The mobilization of lipid and protein stores and the increase in NEFA levels are inevitable during the transition from pregnancy to lactation to support milk production (Adewuyi *et al*., 2005). However, excessive NEFA production has negative effects on the health and production of dairy cows due to energy deficiency and immune suppression (Ospina *et al*., 2010). While NEFA concentrations have been reported to increase near parturition (Adewuyi *et al*., 2005), this increase is even more pronounced in dairy cows that develop transition cow disease (Li *et al*., 2016). Ospina *et al*. (2010) reported that NEFA levels >0.3 mEq/L 1-2 weeks before calving were associated with an increased risk of retained placenta, abomasal displacement, and metritis during the fresh period. In addition, Li *et al*. (2016) stated that glucose levels decreased in cows with ketosis, and Cai *et al*. (2018) reported that glucose levels decreased in cows with hypocalcemia, and NEFA levels increased due to negative energy balance. In this study, as reported by Li *et al*. (2016) and Cai *et al*.

Table 1. Biochemical analysis results and statistical significance levels between the hypomagnesemia and control groups

Parameters	Hypomagnesemia Group	Control Group	*P* value
Magnesium (mg/dL)	1.24±0.02	2.37±0.24	0.001
NEFA (mmol/L)	0.23±0.03	0.09±0.01	0.001
BHBA (mmol/L)	0.62±0.03	0.53±0.04	0.139
Total protein (g/dL)	9.30±0.12	7.80±0.20	0.001
Urea (mg/dL)	37.67±0.86	42.24±3.63	0.249
GGT (U/L)	23.66±1.31	21.40±1.57	0.380
AST (U/L)	162.40±13.97	97.49±4.90	0.001
ALT (U/L)	37.73±2.46	31.51±2.76	0.089
Calcium (mg/dL)	9.03±0.09	9.51±0.28	0.803
Cholesterol (mg/dL)	205.43±14.30	127.35±10.49	0.001
Creatinine (mg/dL)	0.81±0.02	0.78±0.04	0.584
Albumin (g/dL)	3.39±0.04	3.46±0.07	0.987
Glucose (mg/dL)	44.58±1.22	56.31±3.05	0.001
Phosphorus (mg/dL)	7.87±0.26	9.06±2.00	0.365

NEFA: Non-esterified fatty acid, BHBA: Beta-hydroxybutyric acid, GGT: Gamma-glutamyl transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 2. Correlation analysis between magnesium and negative energy balance and metabolic profile parameters

Parametreler	**BHBA (mmol/L)**	**Total protein (g/dL)**	**Urea (mg/dL)**	**GGT (U/L)**	**AST (U/L)**	**ALT (U/L)**	**Calcium (mg/dL)**	**Cholesterol (mg/dL)**	**Creatinine (mg/dL)**	**Albumin (g/dL)**	**Glucose (mg/dL)**	**Phosphorus (mg/dL)**	**Magnesium (mg/dL)**
NEFA (mmol/L)	0.068	0.205	-0.352*	- 0.495**	0.223	-0.173	0.148	-0.024	0.217	0.13	-0.197	-0.111	-0.588**
BHBA (mmol/L)	1	0.094	-0.098	0.17	0.333*	-0.033	0.078	-0.049	0.151	0.038	-0.325*	0.211	-0.394*
Total protein (g/dL)		1	-0.01	0.400*	0.575**	0.299	0.196	0.691**	-0.182	0.268	- 0.412**	0.222	-0.183
Urea (mg/dL)			1	0.265	-0.136	-0.089	-0.148	0.164	-0.138	-0.344*	0.008	0.323*	0.344*
GGT (U/L)				1	0.453**	0.417**	-0.002	0.436**	-0.469**	-0.099	-0.124	0.268	0.054
AST (U/L)					1	0.497**	-0.01	0.311	-0.117	-0.02	-0.344*	0.214	-0.537**
ALT (U/L)						1	0.062	0.171	-0.179	-0.036	-0.019	0.025	-0.129
Calcium (mg/dL)							1	0.015	0.359*	0.044	0.118	-0.216	-0.198
Cholesterol (mg/dL)								1	-0.249	0.132	-0.343*	0.223	0.069
Creatinine (mg/dL)									1	-0.129	-0.048	-0.197	-0.291
Albumin (g/dL)										1	-0.086	-0.112	0.136
Glucose (mg/dL)											1	-0.540**	0.334*
Phosphorus (mg/dL)												1	0.071

BHBA: Beta-hydroxybutyric acid, GGT: Gamma-glutamyl transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, * Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

(2018) in other transition period diseases, NEFA levels were found to be high in the hypomagnesemia group compared to the control group, while glucose levels were low. Additionally, magnesium was found to have a moderate negative correlation with NEFA and a moderate positive correlation with glucose. The results obtained support the idea that NEFA levels increase due to the negative energy balance that occurs in hypomagnesemia and that this magnesium deficiency may indirectly increase the incidence of transition period disease in dairy cows and weaken their metabolic adaptation.

It is known that AST and ALT levels increase due to liver and muscle function damage (Andjelić *et al*., 2022; Uztimür and Ünal, 2024). Krsmanović *et al*. (2016) stated that AST levels were higher during the fresh period compared to late lactation and late pregnancy due to damage to the body's cellular structure (especially in the liver). Similarly, Đoković *et al*. (2017) and Andjelić *et al*. (2022) state that mild fat infiltration in liver cells formed during the fresh period and the release of AST into circulation result in higher levels during early lactation compared to mid and late lactation. In addition, it is stated that magnesium deficiency can lead to liver damage due to dietary intake and excretion mechanisms in various diseases, and that it can also increase the severity of liver damage as a result of impaired mitochondrial function, inflammatory response, oxidative stress, and metabolic disorders (Liu *et al*., 2019). Li *et al*. (2018) reported that high magnesium intake reduces the risk of fatty liver disease. In this study, while no difference was observed in ALT and GGT values compared to the control group in the group that developed hypomagnesemia, the AST level was found to be higher compared to the control group. Additionally, a negative correlation was observed between AST levels and magnesium. The results are consistent with those reported by Li *et al*. (2018) and Liu *et al*. (2019) and support the hypothesis that magnesium deficiency can trigger liver damage.

Magnesium is known to be an important coenzyme for the functioning of lipid metabolism. It can be activated by magnesium requiring enzymes in β-hydroxy β-methylglutaryl-CoA reductase, which is the rate limiting step in cholesterol synthesis (Nartea *et al*., 2023). It has been suggested that low magnesium may disrupt the inactivation of β-hydroxy β-methylglutaryl-CoA reductase via phosphorylation (Rosanoff and Seelig, 2004). Guerrero-Romero and Rodriguez-Moran (2002) reported low magnesium levels in people with metabolic syndrome who had high total cholesterol levels, due to complex pathophysiological pathways. However, it is stated that cholesterol levels in cows during the fresh period are lower compared to late lactation, due to negative energy balance and reduced feed intake (Kessler *et al*., 2014; Sepúlveda-Varas *et al*., 2015). In this study, lower cholesterol concentrations were found in the hypomagnesemia group compared to the control group. The results obtained support the hypothesis that the step that slows down cholesterol synthesis, as stated by Guerrero-Romero and Rodriguez-Moran (2002) and Nartea *et al*. (2023), could not be achieved due to magnesium deficiency.

Conclusion

In this study, evaluated negative energy balance and metabolic profile parameters in dairy cows that developed hypomagnesemia in the postpartum period. Significant changes were detected in NEFA and glucose parameters, which reflect energy status, and in AST and cholesterol parameters, which provide information about liver function. It was concluded that metabolic adaptation in dairy cows affected by hypomagnesemia may be weakened, and energy balance and liver function may be affected. In this context, the rations of postpartum dairy cows should be adjusted for magnesium, and metabolic adaptations should be monitored for energy balance and liver function by taking periodic blood samples.

Ethical Approval

This study was initiated after obtaining permission from the Local Ethics Committee for Animal Experiments of Bingöl University (B.Ü HADYEK, Date: 2025/02, Decision No: 02/09).

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Author Contribution

Cennet Nur ÜNAL contributed to conceptualization, methodology, investigation, formal analysis, and resource acquisition, and was responsible for supervision as well as writing the original draft and reviewing and editing the manuscript. Mert Alperen İNCE contributed to investigation and resource acquisition and participated in reviewing and editing the manuscript. Hakan KEÇECİ contributed to methodology and investigation. Murat UZTİMÜR contributed to writing the original draft and reviewing and editing the manuscript.

Conflict of Interest

The authors of this study declare that there is no commercial, financial or personal conflict of interest in its initiation, development, writing and publication.

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