



Biochemical indicators of adaptation and stress across seasons in Brown Swiss dairy cows

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Abstract

Investigation of the seasonal dynamics of blood biochemical indicators in dairy cows is a key prerequisite for effective herd management, prevention of metabolic disorders and improvement of animals' adaptive resilience under various housing conditions. This article presents the results of a comprehensive study on the seasonal changes of blood biochemical parameters in Brown Swiss cows at 130–140 days of the second lactation under year-round tie-stall housing in the central region of Ukraine. It was found that the seasonal factor has a significant impact on several metabolic and adaptive biomarkers. In summer compared to spring, aspartate aminotransferase (AST) activity increased by 19.1 % (up to 103.9 ± 22.3 U/L; $p = 0.0457$), while in autumn the increase was 24.3 % (up to 108.5 ± 22.5 U/L; $p = 0.0106$). Alanine aminotransferase (ALT) in summer increased by 27.1 % (up to 47.4 ± 10.8 U/L; $p = 0.0325$). In autumn, the concentration of total calcium decreased by 16.2% compared to spring (2.07 ± 0.07 mmol/L versus 2.47 ± 0.08 mmol/L; $p = 0.00001$), and the Ca/P ratio fell by 25 % (1.11 ± 0.11 versus 1.48 ± 0.18 ; $p = 0.00005$). Autumn glucose levels increased by 52.9 % (up to 3.44 ± 0.14 mmol/L; $p = 0.00003$), while β -hydroxybutyrate decreased by 50.5 % in summer (to 0.32 ± 0.10 mmol/L; $p = 0.0001$). Total lipoproteins in autumn decreased by 19.3 % compared to spring (to 1054.8 ± 189.4 mg%; $p = 0.0002$). In summer, β -globulins were 37.8 % lower than in spring (15.8 ± 4.9 % versus 25.4 ± 8.9 %; $p = 0.0051$). High cortisol concentrations in summer and autumn (up to 37.2 ± 27.1 nmol/L; $p = 0.0001$ – 0.0008) confirm the activation of the stress axis under adverse conditions. The results of this study may be used for the implementation of metabolic status monitoring systems, the development of adaptive feeding and veterinary strategies, and for the improvement of heat stress prevention programmes, as well as increased productivity of cows during different seasons.

Keywords: cow; Brown Swiss breed; seasonality; blood biochemical indicators; adaptation; stress; metabolic status.

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1. Introduction

Under modern livestock farming conditions, seasonal fluctuations in the microclimate remain one of the key challenges for maintaining the health and productivity of dairy cows. Physiological adaptation to changes in temperature, humidity, feeding, and photoperiod is accompanied by significant shifts in metabolism, energy balance, immune responses, and the overall physiological status of animals (Giri et al., 2017; Tejaswi et al., 2022). Numerous studies have demonstrated that even in the absence of clinical signs of disease, the biochemical profile of blood sensitively reflects the strain of adaptive processes and the risks of developing subclinical pathologies throughout the year (Blond et al., 2024; Mylostyvyi, 2025a).

Seasonal stresses, primarily heat and cold stress, often result in disturbances of protein, lipid, mineral, and energy

metabolism, as well as in disorders of hormonal homeostasis and immune reactivity (Hadžimusić & Hadžijunuzović-Alagić, 2024; Baccouri et al., 2025). Accordingly, blood biochemical markers are increasingly regarded as a reliable tool for early diagnosis of physiological disturbances, optimisation of nutrition, and herd management across different seasons (Stoop et al., 2016; Bahrami-Yekdangi et al., 2022).

Particular attention has been paid by researchers to the fact that the response of metabolic and enzymatic indicators shows considerable variability depending on breed, productivity, stage of lactation, and housing conditions (Hu et al., 2023; Alrhoun et al., 2025). In this context, the establishment of reference ranges and seasonal dynamics for specific cow populations enables improvements in health control strategies and enhances the effectiveness of metabolic and productive disorder prevention (Mylostyvyi et al., 2021; Lora et al., 2024).

Systematic monitoring of enzymes, metabolites, macroelements, and protein fractions allows not only the assessment of the degree of strain on adaptive processes but also the timely identification of risks such as energy deficiency, metabolic stress, hypocalcaemia, ketosis, or immunodeficiency. This is particularly relevant against the background of increasing frequency of climatic extremes (Blond et al., 2024; Santos et al., 2024).

Despite substantial progress in this field, the peculiarities of the seasonal dynamics of biochemical markers in Brown Swiss cows under Ukrainian conditions remain insufficiently studied, thus necessitating targeted research that takes into account local feeding and management practices (Aggarwal et al., 2019; Tejaswi et al., 2022).

The aim of this study was to identify the most informative biochemical markers of seasonal adaptation in Brown Swiss cows maintained in a year-round loose housing system.

2. Materials and methods

The study was conducted at a typical dairy farm in the central region of Ukraine during 2023. Clinically healthy Brown Swiss cows in their second lactation at 130–140 days in milk were selected for the experiment. The cows were maintained under loose housing conditions with a standard ventilation system, reliable water supply, and total mixed ration (TMR) feeding in accordance with NRC (2001) recommendations. Groups were formed taking into account average productivity and the absence of any signs of acute or chronic diseases.

Blood samples were collected during four characteristic seasons: winter (January), spring (April), summer (July), and autumn (October). Sampling was carried out in the morning hours (6:00–7:00), before feeding, from the jugular vein into vacuum tubes without anticoagulant. The blood

was immediately transported in refrigerated containers (+4 °C) to the laboratory. Serum was separated by centrifugation at 3000 rpm for 10 minutes.

Biochemical parameters (total protein, urea, blood urea nitrogen, creatinine, AST, ALT, alkaline phosphatase, glucose, calcium, inorganic phosphorus, carotene, total lipoproteins, beta-hydroxybutyrate) were measured using a BioSystem A25 automatic analyser (Spain) with certified diagnostic kits. Protein fractions (albumin, α -, β -, γ -globulins) were determined by agarose gel electrophoresis, and cortisol concentration was measured by immunoenzymatic assay (ELISA, DRG Instruments, Germany) according to the manufacturer's instructions.

The monitoring of animal status and housing conditions (temperature, humidity, microclimate parameters, ration composition) was carried out according to the methodology previously described by Mylostyvyi et al. (2024). For each season, 10–15 animals were examined ($n = 10$ –15).

Statistical analysis was performed using Statistica 12 (StatSoft Inc., USA). Data are presented as mean \pm standard deviation ($M \pm SD$). The significance of differences between seasons was assessed using the non-parametric Mann–Whitney U test. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1 Results

The analysis of the results (Table 1) revealed both trends and statistically significant differences between seasons for a number of parameters reflecting the status of protein, carbohydrate, lipid and mineral metabolism, as well as hormonal balance. The obtained data demonstrate alterations in protein, carbohydrate, lipid and mineral metabolism, and hormonal status, which accompany the adaptation of animals to seasonal environmental factors.

Table 1
Seasonal changes in blood biochemical parameters of Brown Swiss cows

Parameter	Season of the year			
	Winter, n = 10	Spring, n = 10	Summer, n = 15	Autumn, n = 15
Total protein, g/L	75.2 \pm 5.84	74.6 \pm 4.37	71.3 \pm 1.71	72.2 \pm 3.23
Urea, mmol/L	4.76 \pm 0.60	5.12 \pm 0.88	5.64 \pm 1.05	5.01 \pm 1.76
Urea nitrogen, mg%	9.08 \pm 1.13	9.79 \pm 1.72	10.77 \pm 1.99	9.56 \pm 3.38
Creatinine, μ mol/L	57.9 \pm 5.97	62.7 \pm 5.64	62.2 \pm 5.37	54.6 \pm 4.05*
AST, U/L	85.7 \pm 17.08	87.3 \pm 12.76	103.9 \pm 22.25*	108.5 \pm 22.46*
ALT, U/L	33.2 \pm 7.19	37.3 \pm 14.12	47.4 \pm 10.84*	44.3 \pm 7.16
De Ritis index (AST/ALT)	2.68 \pm 0.78	2.80 \pm 1.51	2.38 \pm 1.16	2.48 \pm 0.50
Alkaline phosphatase, U/L	78.5 \pm 20.66*	101.9 \pm 24.79	81.2 \pm 21.34	93.2 \pm 44.47
Glucose, mmol/L	2.59 \pm 0.23*	2.25 \pm 0.27	2.53 \pm 0.32*	3.44 \pm 0.14*
Total calcium, mmol/L	2.38 \pm 0.12	2.47 \pm 0.08	2.33 \pm 0.06*	2.07 \pm 0.07*
Inorganic phosphorus, mmol/L	2.03 \pm 0.09*	1.67 \pm 0.17	1.66 \pm 0.16	1.89 \pm 0.15*
Ca/P ratio, units	1.18 \pm 0.04*	1.48 \pm 0.18	1.39 \pm 0.15	1.11 \pm 0.11*
Carotene, μ g%	970 \pm 132.59	801.7 \pm 242.81	743.9 \pm 151.76	667.2 \pm 159.98
Total lipoproteins, mg%	1482.4 \pm 169.62*	1307.3 \pm 59.73	1303.5 \pm 130.71	1054.8 \pm 189.41*
Thymol test, S-H units	5.96 \pm 1.52	4.56 \pm 1.46	4.66 \pm 2.83	5.79 \pm 1.95
β -hydroxybutyrate, mmol/L	0.445 \pm 0.090*	0.652 \pm 0.166	0.323 \pm 0.102*	0.473 \pm 0.113*
Albumins, %	36.28 \pm 10.47	39.31 \pm 7.64	40.16 \pm 7.36	33.04 \pm 6.84
α -globulins, %	16.94 \pm 3.52	19.18 \pm 10.20	23.04 \pm 10.30	24.58 \pm 8.68
β -globulins, %	22.38 \pm 4.67*	16.14 \pm 4.62	21.02 \pm 6.07*	21.06 \pm 10.03
γ -globulins, %	24.37 \pm 6.48	25.36 \pm 8.92	15.76 \pm 4.90*	21.31 \pm 5.86
Cortisol, nmol/L	4.47 \pm 1.13*	34.57 \pm 24.65	37.23 \pm 27.09	33.87 \pm 21.92

Note: * significant differences relative to the spring period ($P < 0.05$)

Monitoring protein and nitrogen metabolism is of major importance for assessing the adaptive capacity and metabolic state of dairy cows across different seasons, since changes in these parameters may indicate disturbances in protein turnover, deficiencies of nutrients or excessive protein load. In summer compared to spring, total protein decreased by 4.4 % (a tendency towards reduction), while in autumn it decreased by 3.2 % (a tendency). However, a statistically significant decrease was recorded when comparing winter and summer ($p = 0.0020$). For urea and urea nitrogen, an increase of 10.2 % and 10.0 %, respectively, was observed in summer (statistically significant compared to winter: $p = 0.0242$ and $p = 0.0212$), whereas in relation to spring these changes did not reach statistical significance, although a tendency to increase was noted. Creatinine in summer showed almost no change, whereas in autumn compared to spring a significant decrease of 12.9% was observed ($p = 0.0014$).

Monitoring enzyme activity and hepatic function is essential for evaluating the adaptive response of dairy cows to seasonal stressors. In the summer period, AST activity increased by 19.1 % relative to spring ($p = 0.0457$), and in autumn by 24.3 % ($p = 0.0106$). ALT levels in summer increased by 27.1 % ($p = 0.0325$) compared to spring, with a more pronounced increase compared to winter ($p = 0.0007$). The De Ritis index decreased in the same seasons, although no significant differences were found and a tendency towards reduction was evident. Alkaline phosphatase was significantly higher in spring than in winter ($p = 0.0449$), with changes in other seasons being of a tendency character.

Assessment of the dynamics of carbohydrate and energy metabolism is key for understanding the metabolic adaptation of dairy cows to seasonal changes in feeding and housing conditions. Glucose concentration in summer was 12.4 % higher compared to spring ($p = 0.0312$), and in autumn the increase reached 52.9 % ($p = 0.00003$). The content of beta-hydroxybutyrate in summer was reduced by 50.5 % ($p = 0.0001$), and in autumn by 27.5 % ($p = 0.0090$) compared to spring.

Studying seasonal changes in mineral metabolism indices is important for ensuring optimal bone tissue function, energy metabolism and the prevention of metabolic disorders in dairy cows. Total calcium content in summer was significantly reduced by 5.7 % ($p = 0.0004$), and in autumn by 16.2 % ($p = 0.00001$) compared to spring. Inorganic phosphorus in winter increased by 21.6 % compared to spring ($p = 0.0002$), and in autumn by 13.2 % ($p = 0.0066$). The Ca/P ratio in autumn decreased by 25 % ($p = 0.00005$), and in summer a tendency towards reduction was observed.

Assessment of seasonal changes in lipid and antioxidant status parameters makes it possible to monitor the efficiency of fat metabolism and vitamin A provision, which are important factors for maintaining productivity and resilience to oxidative stress throughout the year. The content of carotene in winter was 21 % higher than in spring (a tendency to increase), while in summer and autumn a tendency to decrease was observed. Total lipoproteins were significantly reduced in autumn by 19.3 % compared to spring ($p = 0.0002$), and in summer a significant reduction was recorded compared to winter ($p = 0.0158$).

Investigation of the seasonal dynamics of serum protein fractions is important for a comprehensive assessment of the state of protein metabolism, immune response and adaptive

reactions of the organism of dairy cows during the year. The content of albumins in autumn compared to summer was significantly decreased ($p = 0.0191$), while in other seasons only a tendency to changes was observed. The content of alpha-globulins in autumn compared to winter was significantly increased ($p = 0.0213$). In summer, gamma-globulins were significantly reduced compared to spring ($p = 0.0051$).

Monitoring changes in hormonal status, particularly cortisol levels, is essential for assessing the degree of activation of the stress axis and the adaptive response of dairy cows to seasonal environmental changes. Cortisol in all comparisons of the summer and autumn periods with winter and spring was significantly higher ($p = 0.0001$ – 0.0008), indicating statistically confirmed activation of the stress axis in these seasons.

Thus, the study of the seasonal dynamics of blood biochemical parameters in Brown Swiss cows allowed the identification of the group of the most sensitive and stable biomarkers of physiological status that reliably respond to seasonal changes. These include hepatic enzymes (AST, ALT), glucose, indices of mineral metabolism (total calcium, inorganic phosphorus, Ca/P ratio), total lipoproteins, β -hydroxybutyrate and β -globulins. These indicators should be considered as reliable biochemical markers of cow adaptation to the impact of seasonal factors, as they clearly reflect the metabolic and adaptive response of the organism under changing temperature, feeding and stress levels throughout the year.

3.2 Discussion

The results obtained confirm that among the wide range of blood biochemical indicators, it is the activity of hepatic enzymes (AST, ALT), concentrations of glucose, calcium, inorganic phosphorus, the Ca/P ratio, total lipoproteins, β -hydroxybutyrate, and β -globulins that demonstrate the clearest and most stable response to seasonal changes in housing and feeding conditions in Brown Swiss cows. The activity of AST and ALT are sensitive markers of hepatic load and the functional state of hepatocytes. Increases in these enzymes during the summer and autumn are attributable to the activation of hepatic transaminases in response to heat stress, the intensification of protein and energy metabolism, and the need to detoxify metabolic products (Aggarwal et al., 2019; Mylostyvyi, 2025a). Similar seasonal fluctuations in enzymatic activity have been reported in other dairy cattle breeds, which highlights the universal nature of hepatic adaptation mechanisms (Mekroud et al., 2021).

The concentration of glucose in blood reflects the balance between energy requirements, feed intake and quality, as well as environmental stressors. Seasonal increases in this parameter, particularly in autumn, are associated with enhanced gluconeogenesis, the effect of cortisol, adaptation to a reduction in dietary energy content, and the transition to winter management. Mechanistically, under the influence of cortisol and adrenaline, glycogenolysis is activated and the blood glucose level rises, which enables the organism to cope more effectively with the consequences of heat or cold stress (Hu et al., 2023; Mylostyvyi et al., 2023; Pawliński et al., 2023).

The levels of total calcium, inorganic phosphorus, and their ratio are important markers of metabolic status, especially in the context of the prevention of hypocalcaemia, osteomalacia, and the maintenance of milk production. Seasonal variations in these indicators are determined not only by the

mineral composition of the diet, but also by hormonal regulation (an increase in parathyroid hormone, changes in calcitriol levels), as well as by the redistribution of calcium and phosphorus to support lactation and bone metabolism. A decrease in calcium and the Ca/P ratio in autumn may indicate increased utilisation of minerals to maintain osteomineralisation and adaptation to changes in the ration (Bahrami-Yekdangi et al., 2022; Hadžimusić & Hadžijunuzović-Alagić, 2024). The significant decrease in calcium and the Ca/P ratio in autumn indicates a potential micronutrient deficiency which requires correction of mineral feeding during this period (Baccouri et al., 2025; Mylostyvyi, 2025b).

The reduction of total lipoproteins in autumn and summer is consistent with the findings of other studies which point to the effect of seasonality, ration, and energy balance on the blood lipid profile. This dynamic is due to changes in feed composition (lower energy, fats, vitamin E, and carotene) and the activation of lipolysis under stress, which is accompanied by increased utilisation of fats as an energy source. Heat stress also stimulates fatty acid oxidation in the liver, leading to a decrease in lipid reserves in the blood (Aggarwal et al., 2019; Mylostyvyi et al., 2021; Blond et al., 2024).

The level of β -hydroxybutyrate is a marker of mobilisation of fat reserves, energy deficiency, and the risk of ketosis. Its reduction in the summer and autumn reflects adaptive metabolic remodelling under heat stress, when the intake of carbohydrates increases and mobilisation of fats decreases due to changes in appetite, energy balance, and hormonal status (in particular, increased insulin and cortisol), resulting in reduced ketone body formation in the liver (Melendez et al., 2020; Zachut et al., 2020; Thammacharoen et al., 2021).

Seasonal variability in β -globulins may be associated with both the overall status of protein metabolism and the immune response of the organism to environmental influences. The reduction in β -globulin levels in summer points to possible immunosuppression caused by prolonged heat stress, which suppresses immunoglobulin synthesis, increases the risk of infectious diseases, and weakens the specific immune response (Stoop et al., 2016; Važić et al., 2020; Gutjy et al., 2024).

Thus, the results confirm the key role of these biochemical indicators as reliable markers of the physiological state and adaptive response of dairy cows to seasonal changes, in line with current literature data, and they can be used to develop adaptive management strategies for herds in different periods of the year.

4. Conclusion

The study established that among the wide range of blood biochemical parameters in Brown Swiss cows, the most informative for assessing seasonal adaptation are the activities of hepatic enzymes (AST, ALT), concentrations of glucose, total calcium, inorganic phosphorus, the Ca/P ratio, total lipoproteins, β -hydroxybutyrate, and β -globulins.

The dynamics of these parameters clearly reflect the tension of adaptive mechanisms under the influence of seasonal fluctuations in microclimate, feeding, and photoperiod. In particular, the summer and autumn periods are characterised by the activation of hepatic transaminases, alterations in carbohydrate and lipid metabolism, a tendency towards a decrease in calcium, lipoproteins and β -hydroxybutyrate, as well as variability in protein fractions. An increase in AST

and ALT levels is a sensitive indicator of hepatic load under heat stress, while the dynamics of glucose, lipids, and minerals reflect the remodelling of metabolic pathways to maintain homeostasis.

The data obtained expand current understanding of the physiological adaptation of high-yielding cows to environmental changes and can be applied to optimise preventive and nutritional measures in farms practising year-round loose housing.

Conflict of interest

The authors of this study declare no conflict of interest.

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