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*Original article*

# Ultrasonographic image of fatty infiltration of the liver correlates with selected biochemical parameters and back fat thickness of periparturient Holstein-Friesian COWS

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## Abstract

During the transition period, the cow's body activates adaptive mechanisms aimed at adjusting to the changing demand for energy and nutrients, which are necessary for the growing fetus and the subsequent start of milk production. This time is also associated with an increased risk of metabolic diseases and reproductive disorders.

Our study aimed to identify prepartum and postpartum biochemical markers and weight loss patterns that could differentiate cows that would exhibit ultrasonographic signs of liver fatty infiltration during the latter half of the transition period.

The study was performed in a single herd of Holstein-Friesian cows and the animals were divided into two groups: CON (n=13) – cows without ultrasonographic signs of fatty liver, and FL (n=16) – cows with ultrasonographic signs of fatty liver. Backfat thickness and specific biochemical parameters were measured weekly from one week before parturition to 9 weeks postpartum.

Our study highlights the importance of using a combination of monitoring methods to assess the metabolic status of transition dairy cattle. The results showed that ultrasound measurements of backfat thickness, blood NEFA levels, glucose concentration, and AST activity were all different ( $p < 0.05$ ) between the control and FL groups, indicating the usefulness of these parameters in monitoring the health status of transition cows. Additionally, the results suggest that high prepartum glucose levels (4.99 mmol/l) could serve as a potential marker for future FL, while the elevated NEFA levels (0.51 mmol/l) and decreased AST activity (80.56 u/l) in FL animals indicate their potential as indicators of lipid mobilization and liver structural damage, respectively.

**Keywords:** blood biochemistry, dairy cattle, fatty liver disease, ketosis, liver ultrasound, subcutaneous fat, transition period

## Introduction

The transition period in dairy cattle, which occurs between 3 weeks before and 3 weeks after parturition, is marked by various physiological, immunological, and metabolic changes (e.g. transition from gestation to lactation, increased nutrient demands, lipid mobilization, weight loss, immunosuppression, etc.), that can lead to an increased risk of metabolic diseases and reproductive disorders (Bezerra et al. 2014, Redfern et al. 2021). This period is important for the overall health, milk production, and welfare of the dairy cow.

During this period, the cow's body activates adaptive mechanisms aimed at adjusting to the changing demand for energy and nutrients, which are necessary for the growing fetus and the subsequent start of milk production (Ceciliani et al. 2018). Postpartum, dairy cows experience a negative energy balance (NEB) characterized by weight loss, body fat reduction, intensification of gluconeogenesis, mobilization of fat reserves, and decreased peripheral insulin sensitivity (Aschenbach et al. 2010, Contreras and Sordillo, 2011, Abuelo et al. 2016). This phenomenon is common in female mammals, but the duration and extent of these adaptations in high-yielding dairy cows may be considered a biological extreme (Ceciliani et al. 2018), and may lead to metabolic/production diseases such as ketosis or fatty liver syndrome (Bezerra et al. 2014, Esposito et al. 2014, Raboisson et al. 2014).

Fatty liver syndrome is a complex metabolic disease that occurs during the transition period in dairy cattle (Tharwat 2012, Gerspach et al. 2017b). During this time, there is an increase in the concentration of circulating fatty acids and blood flow to the liver, leading to the transport of excessive amounts of fatty acids to the organ (Reynolds et al. 2003). When the liver takes in more lipids in the form of fatty acids than it can oxidize or secrete, triglycerides accumulate in the liver cells (Tharwat 2012). According to Jorritsma et al. (2001), up to 50% of cows may develop hepatic triglyceride accumulation based on the presented mechanism during the first 4 weeks of lactation. Excessive hepatic lipid accumulation during the transition period in dairy cattle can result in anorexia, decreased rumen motility, displaced abomasum, ketosis, increased susceptibility to infections, reduced fertility, and weight loss (Van Dorland et al. 2009, Tharwat et al. 2012). However, some cows with the mild or moderate fatty liver syndrome may not exhibit any visible clinical signs but may still have reduced milk yield, resulting in long-term financial losses for dairy farmers (Ceciliani et al. 2018).

Fatty liver syndrome develops as a result of the negative energy balance and weight loss experienced

by dairy cows postpartum, particularly in those with a higher body condition score near calving (Melendez et al. 2018). Body condition score (BCS) is a simple on-farm tool used to assess body nutrient reserves and fat mobilization (Siachos et al. 2021). Total BCS loss during early lactation has been linked to a higher incidence of metabolic disorders postpartum (Roche et al. 2009). However, ultrasound measurements of back fat thickness can be a more reliable and objective alternative, as it strongly correlates with back fat measurements in carcasses and BCS scores (Brethour 1992, Strieder-Barboza et al. 2015).

Proper diagnosis of fatty liver syndrome and determination of the stage of liver lipid infiltration is often necessary to assess the cow's metabolic status and liver function (Gerspach et al. 2017b). Liver function can be evaluated using various methods such as blood biochemistry (liver profile), liver ultrasound, and examination of liver tissue (e.g., specific gravity or histopathology) obtained through ultrasound-guided biopsy (Bobe et al. 2004, Tharwat et al. 2012, Jawor et al. 2016, Gerspach et al. 2017b). Although liver biopsy remains the gold standard, the constant improvement of ultrasound machines and software makes it worthwhile to continue searching for reliable algorithms to evaluate liver function using ultrasound as a preventive tool on farms to reduce the risk of metabolic diseases in dairy cattle during the transition period.

Given the decreased welfare of dairy cattle, the economic impact, and the high prevalence of fatty liver syndrome worldwide, it is important to search for simple, non-invasive, and reliable on-farm protocols to assess liver health and identify cows that may suffer from excessive NEB and hepatic fatty infiltration postpartum. This is especially crucial now, with the many advances in on-farm diagnostic tools and the increasing metabolic challenges facing high-yielding dairy cows due to genetic selection for increasing milk production, heat stress from climate change, and reduced fertility.

Blood parameters, including NEFA, BHB, and cholesterol, have been shown to reflect the energy balance of dairy cattle and are often included in routine tests for transition cows (Kida 2003, Stengärde et al. 2008). BHB is one of the major ketones, and NEFA concentration reflects the mobilization of lipid reserves (Oetzel 2007, Ospina et al. 2010). High levels of both are associated with a higher incidence of periparturient metabolic diseases such as ketosis or fatty liver (Bobe et al. 2004).

This study aimed to identify prepartum and postpartum biochemical markers and weight loss patterns that could differentiate cows that would exhibit ultrasonographic signs of liver fatty infiltration during the latter half of the transition period.

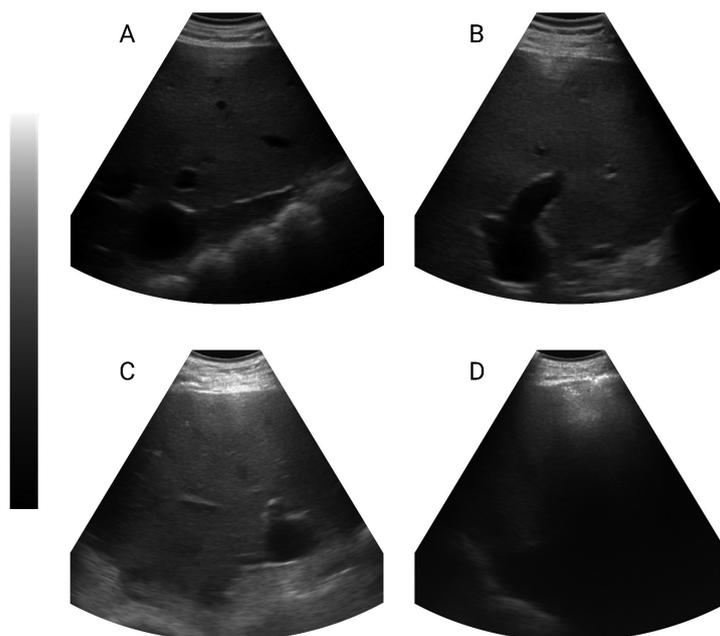


Fig. 1 Ultrasonograms of the cow liver imaged using the 4 Vet Slim ultrasound machine (Dramiński, Olsztyn, Poland) with a convex probe. Panels A and B depict ultrasonograms of a normal liver (CON), demonstrating a homogenous granular echotexture of the parenchyma with sharp vessel margins. Panels C and D depict ultrasonograms of fatty infiltration (FL), with Panel C showing a bright pattern and blurred vessel margins, and Panel D showing a bright pattern, blurred vessels, and deep attenuation.

## Materials and Methods

### Animals and study design

All experimental procedures in this study were approved by The Local Ethics Committee for Animal Experiments in Olsztyn (Resolution No. 28/2020). The study was conducted in a herd of Polish Holstein-Friesian cows and was part of a larger research project on changes in the hepatic transcriptome of periparturient Polish Holstein-Friesian cows. The cows were housed in a free-stall barn, meeting the requirements for animal welfare following EU law, and fed a partially mixed ration. Over six months, all cows in the herd were monitored, and 29 cows entering third or higher lactation with an average milk yield of 9500 kg were eligible to participate in the experiment. Heifers and cows with low milk yield were excluded from the study.

All animals were subjected to weekly body condition score assessments, subcutaneous fat measurements, and blood biochemistry evaluations weekly 10 times from one week before parturition to 9 weeks postpartum. The body condition score was determined based on back fat thickness to investigate the correlation between weight changes and ultrasonographic signs of liver fat infiltration according to Raschka et al. (2016). Blood samples were collected to determine the correlation between the biochemical profile and ultrasonographic signs of liver fat infiltration.

### Sample collection and analysis - liver ultrasound examination and image analysis

The liver ultrasound was conducted twice, once between the 21<sup>st</sup> and 28<sup>th</sup> day postpartum and then again between the 42<sup>nd</sup> and 49<sup>th</sup> day postpartum, using the 4 Vet Slim ultrasound machine with a convex probe (Dramiński, Olsztyn, Poland). The images were captured and analyzed using MicroDicom software (MicroDicom Ltd, Sofia, Bulgaria). The ultrasound images of the liver were evaluated for fatty infiltration, following the method described by Komeilian et al. (2011), which involved examining characteristics such as the brightness pattern, hepatic vessel blurring, presence of deep attenuation, diaphragm visibility, hyperechogenicity of the nearfield, and a dark shadow behind (Komeilian et al. 2011). These image features are used to describe various stages of fatty infiltration, but in the study, we chose not to differentiate between different stages of fatty liver (Fig. 1). The cows were divided into two groups based on the results of the liver image analysis. Cows with no signs of fatty infiltration were designated as the control group (CON, n=13), while those with signs of fatty infiltration were diagnosed with fatty liver syndrome (FL, n=16).

### Sample collection and analysis – body condition

Body condition was assessed once a week by the measurement of a backfat thickness (BFT) in the sacro-

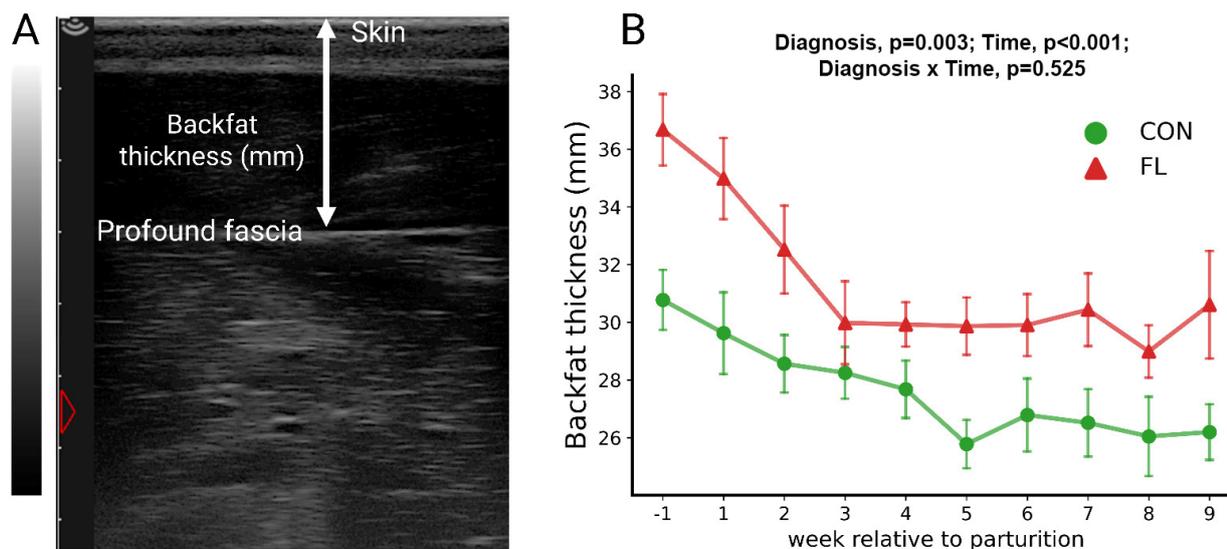


Fig. 2. Exemplary measurement of the backfat thickness (Panel A) and the change in backfat thickness of cow CON and FL groups relative to parturition over the study period (Panel B). The images were obtained using the 4 Vet Slim ultrasound machine (Dramiński, Olsztyn, Poland) with a linear, rectal probe (frequency - 7 MHz, range - 80 mm, focus - 55 mm).

Table 1. Change of the backfat thickness (mm) of cow CON and FL groups.

	1 w. pre p.	1 w. pp.	2 w. pp.	3 w. pp.	4 w. pp.	5 w. pp.	6 w. pp.	7 w. pp.	8 w. pp.	9 w. pp.
FL	36.67* (4.94)	34.98* (5.62)	32.52 (6.09)	29.98* (5.76)	29.93 (3.07)	29.87* (3.98)	29.9 (4.29)	30.43 (5.03)	28.99 (3.63)	30.61 (7.45)
CON	30.77* (3.74)	29.62* (5.1)	28.56 (3.6)	28.25* (3.24)	27.67 (3.59)	25.78* (3.03)	26.78 (4.55)	26.51 (4.22)	26.04 (4.95)	26.19 (3.48)

mean (sd), \* significant difference ( $p < 0.05$ ) between groups, w – week, pp – postpartum

tuberosus region using the 4 Vet Slim ultrasound machine with a linear, rectal probe (Dramiński, Olsztyn, Poland). The measurement was performed as described by Raschka et al. (2016) using the same settings on the ultrasound machine each time (frequency – 7 MHz, range – 80 mm) (Fig. 2). The obtained images were saved and then analyzed using the MicroDicom software (MicroDicom Ltd, Sofia, Bulgaria). All measurements were repeated three times, and the mean values were used during statistical analysis.

### Sample collection and analysis – blood samples

Blood was collected from the coccygeal vein once a week up to 3 hours after morning milking using the vacuumed blood collection system with 20G x 1.5mm needles into three 9ml collection tubes with a clot activator and two 4ml collection tubes with K3EDTA (VACUMED, F.L. Medical, Torreglia, Italy). Samples were transported to the laboratory within an hour after collection in a cooler with an inside temperature of 6°C.

Serum for blood biochemistry was obtained by centrifuging the blood at 4000 rpm for 10 minutes. The analysis was performed using the ACCENT-200

biochemical analyzer with corresponding reagents kits (Cormay Group, ACCENT-200, Łomianki, Poland) and included glucose (GLUC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), non-esterified fatty acids (NEFA), total cholesterol (TC) and  $\beta$ -hydroxybutyrate (BHB). These parameters were measured on the day of sample collection.

### Statistical analysis

The normality and homogeneity of the parameters' distribution were assessed using Shapiro-Wilk and Levene tests, with the R software (version 3.11.2, Python Programming Language, Frederick, Maryland, United States, 1991). Both log-transformed and non-transformed data were analyzed to test the assumptions. Since the data did not follow a normal distribution, log2 transformations were applied for further analysis. A standard cross-sectional cohort study was conducted by comparing the CON group (cows without fatty liver signs) with the FL group (cows with fatty liver) at each time point using t-tests on log2 transformed data, with the Python software (version 4.1.0, R Development Core Team, Vienna, Austria, 2008).

Time-dependent metabolite changes were analyzed over the period from -1 to 9 weeks using a repeated measures analysis of variance. In cases where the assumption of sphericity was not met, the Greenhouse-Geisser correction was applied. Statistical significance was set at  $p < 0.1$ ,  $p < 0.05$ , and  $p < 0.001$ .

## Results

The mean BFT of the CON group was found to be lower than the FL group across all time points. The greatest discrepancy between the two groups was noted prior to parturition, with a mean BFT of 36.67 mm (4.94) for the FL group and 30.77 mm (3.74) for the CON group. The FL group exhibited the largest decrease in BFT from one week before parturition to week 3 postpartum (pp), after which their BFT stabilized around 30 mm which was the starting value observed in the CON group. The BFT in the CON group continued to decrease until stabilization around week 5 pp and remained at a similar level for the duration of the study (Table 1, Fig. 2).

The ALT activity in the FL and CON groups varied over time. The ALT activity in the CON group showed a rapid decrease from the time of parturition to week 4 pp, resulting in a numerically lower concentration compared to the FL group throughout the cows' monitoring ( $p > 0.1$ ), however those changes appeared to be insignificant (Table 2, Fig. 3).

The ALP activity showed a significant decrease over time in both the CON and the FL group ( $p < 0.001$ ). The concentration was highest before the parturition and lowest at the end of the monitoring period. In the CON group, the highest concentration was 68.54 U/l (19.88) before parturition and the lowest was 43.46 U/l (8.45) at week 8 pp. In the FL group, the highest concentration was 58 U/l (16.28) before parturition and the lowest was 45.94 U/l (8.64) at week 6 pp, which remained unchanged until the end of the monitoring period. There was no significant difference in ALP activity between the two groups ( $p = 0.434$ ) (Table 2, Fig. 3).

The AST activity was higher in the CON group compared to the FL group over the time of cow monitoring ( $p = 0.01$ ). There was a significant increase in AST activity over time in both groups ( $p = 0.002$ ). However, the pattern of change in time was similar in CON and FL groups ( $p > 0.1$ ) (Table 2, Fig. 3).

The results indicate an effect of time ( $p = 0.041$ ) on the levels of GLUC. A significant interaction between time and presence of fatty infiltration of the liver ( $p = 0.003$ ) on the levels of GLUC were also found. In the FL group, the GLUC concentration changed over

time, with the highest level of 4.99 mmol/l recorded before parturition and the lowest level of 3.48 mmol/l at week 3 pp. Then, the concentration increased and became similar to the concentration in the CON group. In the CON group, GLUC levels increased from 3.67 mmol/l (0.52) at parturition to 4.34 mmol/l (0.63) at 9 weeks pp (Table 2, Fig. 4).

TG concentration in the CON group was generally lower than that in the FL, except for the week before parturition where the mean concentration of TG was similar in both groups ( $p > 0.05$ ). The mean concentration of TG in both groups decreased, with a significant decrease over time in the control group ( $p < 0.001$ ) (Table 2, Fig. 4).

The TC concentration changed over time in both groups ( $p < 0.001$ ). It increased over the monitoring period, with the lowest concentration at the beginning of monitoring and the highest concentration at the end. In the CON group, the lowest TC concentration was 1.96 U/l (0.74) at week 1 pp and the highest was 5.74 U/l (1.1) at week 9 pp. Similarly FL group had the lowest concentration of 2.19 U/l (0.85) at week 1 pp, and the highest of 5.23 U/l (1.56) at week 9 pp. There was no significant difference in TC concentration between the two groups ( $p > 0.1$ ) (Table 2, Fig. 4).

The NEFA concentration was found to be higher in the FL group compared to the CON group at all time points ( $p = 0.016$ ). Both groups showed a similar trend in NEFA concentration, with an increase until week 2 pp, followed by a decline until the end of the cows' monitoring period (Table 2, Fig. 4).

The LDH, CK activity and BHB concentration did not show any significant difference between the two groups. Neither the passing of time nor the absence of fatty liver signs had an impact on the concentration of those metabolites/enzymes (Table 2, Fig. 3).

## Discussion

Our study aimed to evaluate the weekly changes in selected biochemical parameters, back fat thickness, and ultrasonographic signs of fatty infiltration of the liver in periparturient Polish Holstein-Friesian cows. We hypothesized that cows exhibiting ultrasonographic liver changes characteristic of fatty infiltration postpartum would have different weight loss patterns and biochemical serum profiles during the transition period compared to cows without such changes. This would enable early recognition on-farm of cows that are most likely to experience excessive adaptation mechanisms during the transition period, which would allow for more detailed postpartum examination to ensure their health and future lactation potential.

Table 2. Changes in metabolite serum concentrations in cow CON and FL groups.

	Gluc mmol/l	TG mmol/l	ALT U/l	AST U/l	ALP U/l	CK U/l	LDH U/l	NEFA mmol/l	BHB mmol/l	CHOL mmol/l
1 w. pre partum										
CON	3.67 (0.52)	0.4 (0.78)	46.92 (33.57)	106 (57.08)	68.54 (19.88)	432.38 (1115.78)	3054 (335.26)	0.27* (0.38)	0.71 (0.29)	2.27 (0.77)
FL	4.99 (2.43)	0.41 (0.47)	35.69 (19.6)	80.56 (20.47)	58 (16.28)	204.14 (299.66)	3079.75 (804.54)	0.51 (0.59)	0.73 (0.2)	2.53 (0.89)
1 w. pp.										
CON	3.63 (0.87)	0.11* (0.07)	48.77 (36.8)	116.23 (31.24)	56.77 (14.18)	276.2 (463.58)	3177.23 (464.08)	0.39* (0.37)	0.77 (0.26)	1.96 (0.74)
FL	4.77 (2.31)	0.28 (0.28)	41.06 (24.2)	95.88 (23.85)	56.63 (20.52)	240.93 (214.88)	3277.06 (902.64)	0.71 (0.48)	0.79 (0.23)	2.19 (0.85)
2 w. pp.										
CON	3.88 (0.96)	0.12 (0.02)	38.31 (23.39)	112.69* (19.95)	52.54 (10.51)	161.24 (81.81)	3283.15* (550.05)	0.57 (0.35)	0.7 (0.21)	2.6 (0.56)
FL	3.69 (0.69)	0.23 (0.22)	39.94 (25.03)	91.94 (23.47)	50.88 (12.27)	116.63 (47.31)	2577.79 (1029.14)	0.85 (0.56)	0.74 (0.28)	2.66 (0.86)
3 w. pp.										
CON	3.71 (0.81)	0.13 (0.09)	33.54 (12.73)	99.46 (22.19)	50.62 (16.53)	145.61 (75.25)	3129.77* (319.42)	0.47* (0.32)	0.79 (0.14)	3.51 (0.5)
FL	3.48 (0.71)	0.19 (0.16)	41.31 (24.25)	89.91 (16.9)	49.03 (8.2)	182.22 (249.18)	2812.67 (548.1)	0.84 (0.53)	0.7 (0.25)	3.19 (0.81)
4 w. pp.										
CON	3.58 (0.41)	0.09 (0.04)	31.38 (11.93)	95.62 (22.82)	47.92 (6.2)	185.55 (229.67)	3120.15 (520.3)	0.4 (0.18)	0.74 (0.07)	3.96 (0.68)
FL	3.56 (0.43)	0.15 (0.13)	44 (32.14)	89 (21.77)	46.19 (7.29)	222.67 (265.47)	3022.63 (493.9)	0.65 (0.47)	0.8 (0.16)	3.88 (0.93)
5 w. pp.										
CON	3.99 (0.33)	0.1 (0.02)	31.15 (11.47)	105.08* (28.41)	47.38 (11.39)	108.6 (32.82)	2958.54 (364.48)	0.43 (0.2)	0.76 (0.16)	4.43 (0.65)
FL	3.71 (0.65)	0.16 (0.11)	43.06 (27.49)	84 (23.74)	47.38 (6.9)	104.41 (36.09)	2920.56 (189.21)	0.63 (0.29)	0.78 (0.17)	4.3 (1.13)
6 w. pp.										
CON	4.41 (1.22)	0.12 (0.06)	30.85 (9.84)	105.85 (28.66)	49.92 (6.92)	114.82 (44.11)	3189.62 (409.4)	0.33 (0.18)	0.76 (0.09)	4.77 (1.11)
FL	3.76 (0.59)	0.13 (0.09)	44.81 (26.67)	88.38 (24.92)	45.94 (8.64)	145.71 (108.8)	2994.88 (266.49)	0.47 (0.29)	0.81 (0.16)	4.61 (1.22)
7 w. pp.										
CON	4.04 (0.66)	0.1 (0.03)	31.31 (8.35)	118 (36.93)	51.08 (10.43)	166.64 (114.45)	3032 (235.91)	0.24* (0.16)	0.77 (0.09)	5.03 (1.16)
FL	3.75 (0.62)	0.19 (0.23)	45.13 (28.39)	94.94 (27.14)	46.31 (11.98)	159.69 (127.85)	3042.06 (388.97)	0.47 (0.18)	0.78 (0.13)	4.91 (1.35)
8 w. pp.										
CON	4.2 (0.56)	0.13 (0.07)	30.54 (7.85)	127.15* (38.1)	43.46 (8.45)	225.06 (213.46)	3097.31 (353.87)	0.41 (0.34)	0.76 (0.16)	5.6 (1.13)
FL	3.99 (0.93)	0.13 (0.09)	40.44 (26.28)	94.75 (29.37)	46.44 (12.99)	135.58 (128.26)	2857.84 (857.87)	0.42 (0.25)	0.8 (0.15)	5.1 (1.44)
9 w. pp.										
CON	4.34 (0.63)	0.11 (0.03)	31.23 (9.41)	124.62* (39.11)	45.38 (6.23)	164.7 (96.96)	3075.31 (520.57)	0.26 (0.13)	0.68 (0.22)	5.74 (1.1)
FL	4.16 (0.81)	0.13 (0.1)	38.19 (23.29)	97.19 (30.59)	47.31 (16.45)	168.16 (188.26)	2990.44 (334.96)	0.37 (0.24)	0.78 (0.15)	5.23 (1.56)

mean (sd), \* significant difference ( $p < 0.05$ ) between groups, w – week, pp – postpartum

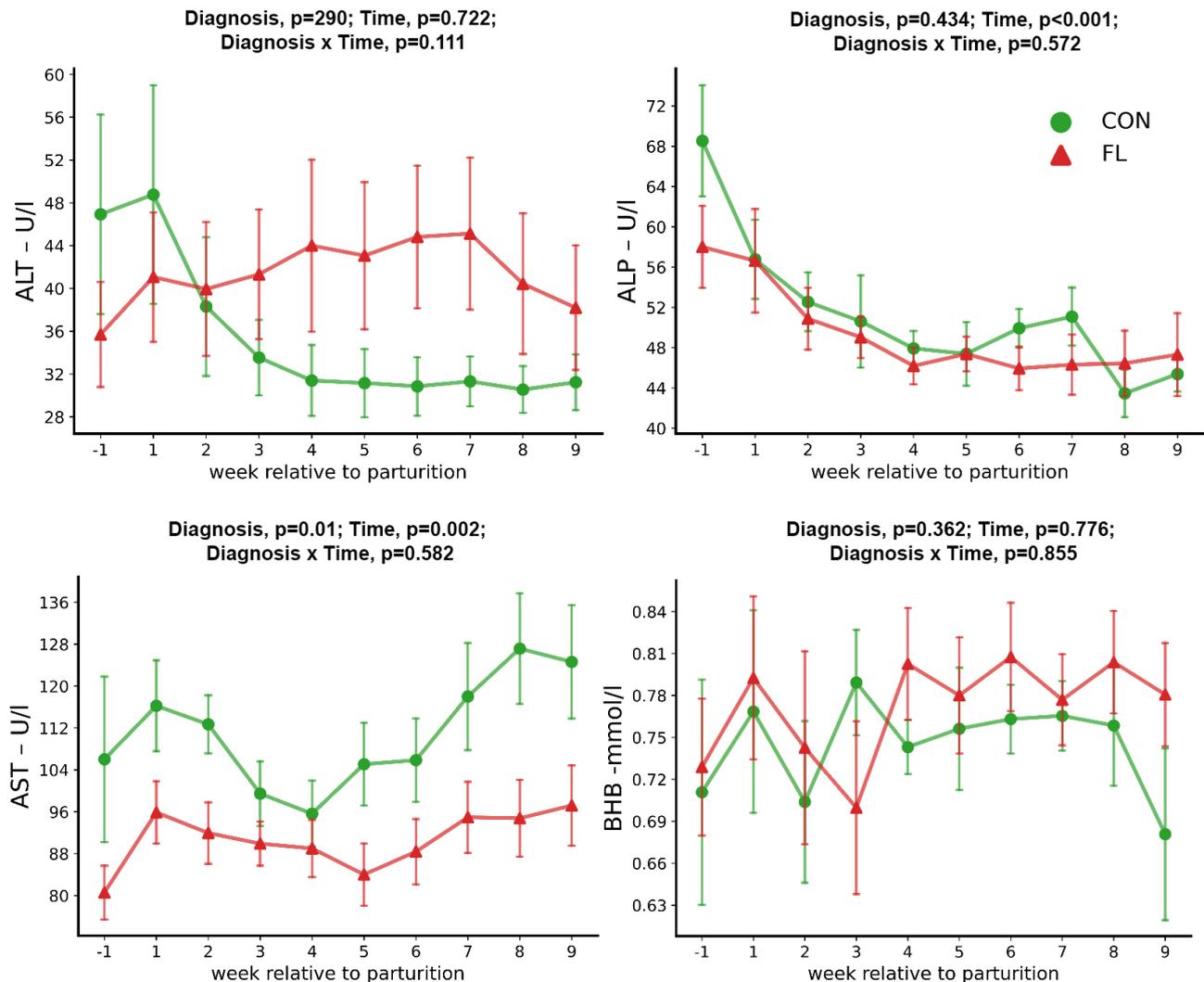


Fig. 3. Change in serum concentration of ALT, ALP, AST, and BHB over time 9 weeks in cow CON and FL groups.

to the liver during the first weeks of lactation (Akbar et al. 2015, Melendez et al. 2018). The bovine species' limited ability to transfer lipids back to the bloodstream is iterated in our findings, which demonstrate that cows with more intense weight loss patterns and lipid mobilization exhibit signs of hepatic fat infiltration (Gerspach et al. 2017a).

Body condition score evaluation, whether assessed by BCS or ultrasound measurement, should be complemented by monitoring biochemical blood parameters and ultrasonographic images of the liver during the transition period. To assess and predict the occurrence of NEB and postpartum metabolic diseases, until now several biochemical markers have been proposed, including BHB and NEFA, which are among the most commonly used markers (Ospina et al. 2010, Strieder-Barboza et al. 2015, Angeli et al. 2019, Luke et al. 2019).

Hussein et al. (2020) studied daily variations of the levels of blood metabolites. Based on their

6 weeks postpartum. This suggests that serum glucose prepartum could be a potential marker for future fatty infiltration of the liver (Schröder and Staufenbiel 2006, Strieder-Barboza et al. 2015, Angeli et al. 2019).

Aspartate aminotransferase activity reflects the function of various organs, including the heart, liver, and skeletal muscles (Puppel and Kuczyńska 2016). According to Sakowski et al. (2012), the highest AST activity occurs during early lactation, which is consistent with our findings. Our results showed that CON animals had the highest AST activity at 8 weeks postpartum. In contrast, FL animals did not reach similar levels of AST activity throughout the experiment and had significantly lower AST activity at 2, 5, 8, and 9 weeks postpartum. Sejersen et al. (2012) reported that cows with high liver TG content in early lactation had higher postpartum plasma concentrations of BHB, NEFA, and AST activity (Sejersen et al. 2012). However, our results did not show significant differences in BHB between groups, and AST activity was lower in

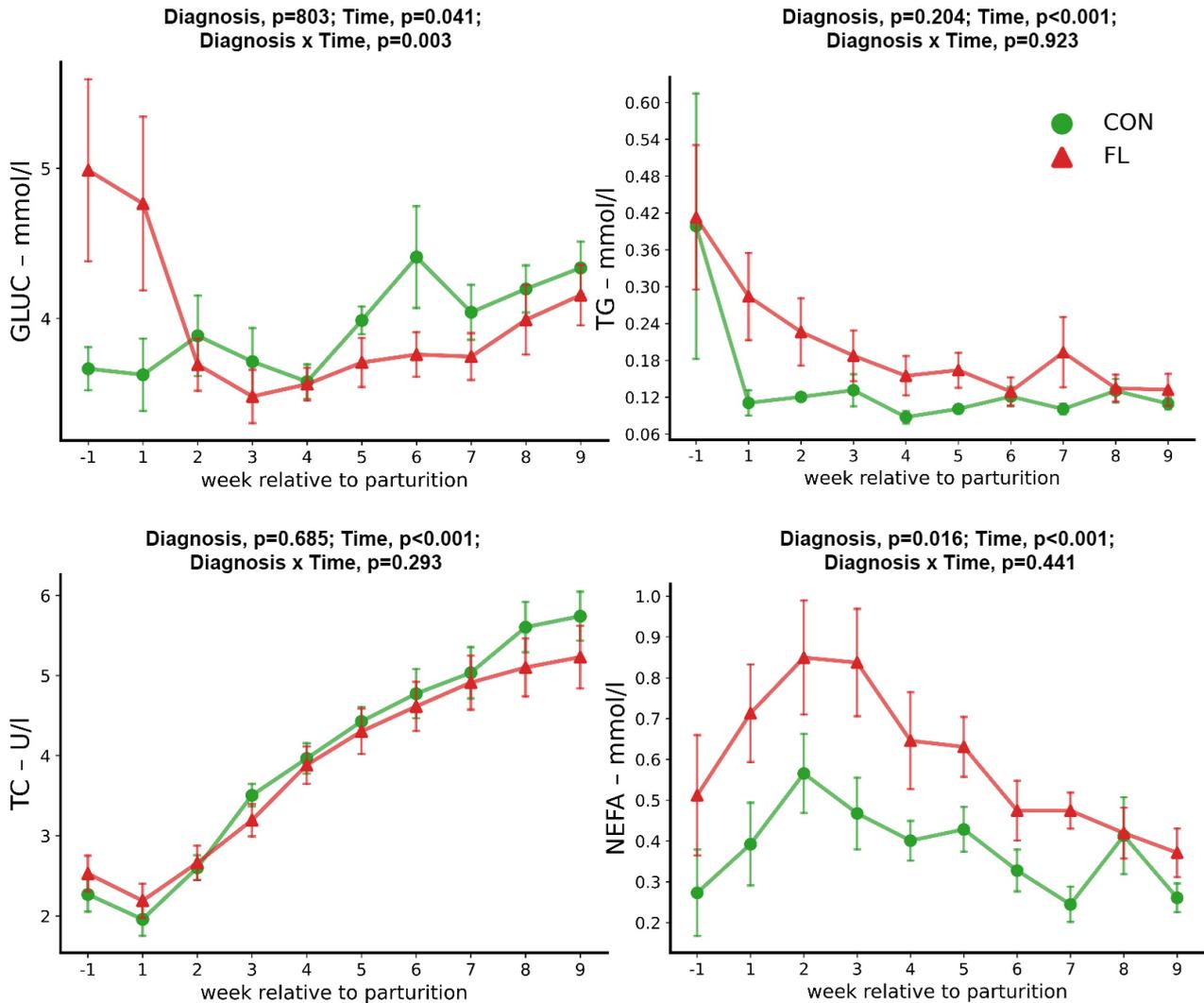


Fig. 4 Change in serum concentration of GLUC, TG, TC, and NEFA over time 9 weeks in cow CON and FL groups.

animals with signs of TG accumulation in the liver. These differences may be due to the absence of severe fatty infiltration in our study. AST activity in the blood can result from increased activity within cells, as well as cell structural damage, which is consistent with our findings.

Stojevic et al. (2005) reported that ALT activity showed significant variations in healthy dry and lactating cows and a statistically significant increase after 46<sup>th</sup> day of lactation up to the dry period, which is consistent with our results. Variations in this parameter demonstrated between groups over time could be useful to differentiate healthy animals from those suffering from transition diseases.

Puppel and Kuczyńska (2016) mentioned that increased ALP activity can be related to the risk of metabolic syndrome, however our results did not show significant differences in ALP between both groups.

## Conclusions

In conclusion, our study highlights the importance of using a combination of monitoring methods to assess the metabolic status of transition dairy cattle. The results showed that ultrasound measurements of backfat thickness, blood NEFA levels, glucose concentration, and AST activity were all significantly different between the control and FL groups, indicating the usefulness of these parameters in monitoring the health status of transition cows. Additionally, the results suggest that high prepartum glucose levels could serve as a potential marker for future FL, while the elevated NEFA levels and decreased AST activity in FL animals indicate their potential as indicators of lipid mobilization and liver structural damage, respectively. It is recommended to complement the traditional body condition score evaluation with monitoring of these parameters to better assess the energy balance and liver health of transition cows. Further research is needed

to validate these findings in a larger population of dairy cattle worldwide.

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## References

- Abuelo A, Hernández J, Benedito JL, Castillo C (2016) Association of oxidative status and insulin sensitivity in periparturient dairy cattle: an observational study. *J Anim Physiol Anim Nutr (Berl)* 100: 279-286.
- Akbar H, Grala TM, Vailati Riboni M, Cardoso FC, Verkerk G, McGowan J, Macdonald K, Webster J, Schutz K, Meier S, Matthews L, Roche JR, Loor JJ (2015) Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. *J Dairy Sci* 98: 1019-1032.
- Angeli E, Rodríguez FM, Rey F, Santiago G, Matiller V, Ortega HH, Hein GJ (2019) Liver fatty acid metabolism associations with reproductive performance of dairy cattle. *Anim Reprod Sci* 208: 106104.
- Aschenbach JR, Kristensen NB, Donkin SS, Hammon HM, Penner GB (2010) Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. *IUBMB Life* 62: 869-877.
- Bezerra LR, de Oliveira Neto CB, de Araujo MJ, Edvan RL, de Oliveira WD, Pereira FB (2014) Major metabolic diseases affecting cows in transition period. *Int J Biol* 6: 85-94.
- Bobe G, Young JW, Beitz DC (2004) Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *J Dairy Sci* 87: 3105-3124.
- Braun U (2009) Ultrasonography of the liver in cattle. *Vet Clin North Am Food Anim Pract* 25: 591-609.
- Brethour JR (1992) The repeatability and accuracy of ultrasound in measuring backfat of cattle. *J Anim Sci* 70: 1039-1044.
- Ceciliani F, Lecchi C, Urh C, Sauerwein H (2018) Proteomics and metabolomics characterizing the pathophysiology of adaptive reactions to the metabolic challenges during the transition from late pregnancy to early lactation in dairy cows. *J Proteomics* 178: 92-106.
- Contreras GA, Sordillo LM (2011) Lipid mobilization and inflammatory responses during the transition period of dairy cows. *Comp Immunol Microbiol Infect Dis* 34: 281-289.
- Esposito G, Irons PC, Webb EC, Chapwanya A (2014) Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Anim Reprod Sci* 144: 60-71.
- Gerspach C, Imhasly S, Gubler M, Naegeli H, Ruetten M, Laczko E (2017a) Altered plasma lipidome profile of dairy cows with fatty liver disease. *Res Vet Sci* 110: 47-59.
- Gerspach C, Imhasly S, Klingler R, Hilbe M, Hartnack S, Ruetten M (2017b). Variation in fat content between liver lobes and comparison with histopathological scores in dairy cows with fatty liver. *BMC Vet Res* 13: 98.
- Hussein HA, Thurmann JP, Staufenbiel R (2020) 24-h variations of blood serum metabolites in high yielding dairy cows and calves. *BMC Vet Res* 16: 327.
- Hussein HA, Westphal A, Staufenbiel R (2013) Relationship between body condition score and ultrasound measurement of backfat thickness in multiparous Holstein dairy cows at different production phases. *Aust Vet J* 91: 185-189.
- Jawor P, Brzozowska A, Słoniewski K, Kowalski ZM, Stefaniak T (2016) Acute phase response in the primiparous dairy cows after repeated percutaneous liver biopsy during the transition period. *Pol J Vet Sci* 19: 393-399.
- Jorritsma R, Jorritsma H, Schukken YH, Bartlett PC, Wensing T, Wentink GH (2001) Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in the Netherlands. *Livest Prod Sci* 68: 53-60.
- Kida K (2003) Relationships of metabolic profiles to milk production and feeding in dairy cows. *J Vet Med Sci* 65: 671-677.
- Komeilian MM, Sakha M, Nadalian MG, Veshkini A (2011) Hepatic ultrasonography of dairy cattle in postpartum period: finding the sonographic features of fatty liver syndrome. *Aust J Basic Appl Sci* 5: 701-706.
- Luke TD, Rochfort S, Wales WJ, Bonfatti V, Maret L, Pryce JE (2019) Metabolic profiling of early-lactation dairy cows using milk mid-infrared spectra. *J Dairy Sci* 102: 1747-1760.
- Melendez P, Whitney M, Williams F, Pinedo P, Manriquez D, Moore SG, Lucy MC, Pithua P, Poock SE (2018) Technical note: Evaluation of fine needle aspiration cytology for the diagnosis of fatty liver in dairy cattle. *J Dairy Sci* 101: 4483-4490.
- Oetzel GR (2007) Herd-level ketosis – diagnosis and risk factors. American Association of Bovine Practitioners 40<sup>th</sup> Annual Conf., Vancouver, BC 67-97.
- Ospina PA, Nydam DV, Stokol T, Overton TR (2010) Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *J Dairy Sci* 93: 1596-1603.
- Pires JA, Delavaud C, Faulconnier Y, Pomiès D, Chilliard Y (2013) Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. *J Dairy Sci* 96: 6423-6439.
- Puppel K, Kuczyńska B (2016) Metabolic profiles of cow's blood; a review. *J Sci Food Agric* 96: 4321-4328.
- Raboisson D, Mounié M, Maigné E (2014) Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review. *J Dairy Sci* 97: 7547-7563.
- Raschka C, Ruda L, Wenning P, von Stemm CI, Pfarrer C, Huber K, Meyer U, Dänicke S, Rehage J (2016) In vivo determination of subcutaneous and abdominal adipose tissue depots in German Holstein dairy cattle. *J Anim Sci* 94: 2821-2834.
- Redfern EA, Sinclair LA, Robinson PA (2021) Dairy cow health and management in the transition period: The need to understand the human dimension. *Res Vet Sci* 137: 94-101.
- Reynolds CK, Aikman PC, Lupoli B, Humphries DJ, Beever DE (2003) Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J Dairy Sci* 86: 1201-1217.
- Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ,

- Berry DP (2009) Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J Dairy Sci* 92: 5769-5801.
- Schröder UJ, Staufenbiel R (2006) Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J Dairy Sci* 89: 1-14.
- Sejersén H, Sørensen MT, Larsen T, Bendixen E, Ingvarsen KL (2012) Liver protein expression in dairy cows with high liver triglycerides in early lactation. *J Dairy Sci* 95: 2409-2421.
- Siachos N, Oikonomou G, Panousis N, Banos G, Arsenos G, Valergakis GE (2021) Association of body condition score with ultrasound measurements of backfat and longissimus dorsi muscle thickness in periparturient Holstein cows. *Animals (Basel)* 11: 818.
- Stengårde L, Tråvén M, Emanuelson U, Holtenius K, Hultgren J, Niskanen R (2008) Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. *Acta Vet Scand* 50: 31
- Strieder-Barboza C, Zondlak A, Kayitsinga J, Pires AF, Contreras GA (2015) Lipid mobilization assessment in transition dairy cattle using ultrasound image biomarkers. *Livest. Sci* 177: 159-164.
- Stojevic Z, Piršljin J, Milinkovic-Tur S, Zdelar-Tuk M, Ljubic BB (2005) Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. *Vet Arhiv* 75: 67-73.
- Tharwat M (2012) Ultrasonography as a diagnostic and prognostic approach in cattle and buffaloes with fatty infiltration of the liver. *Pol J Vet Sci* 15: 83-93.
- Tharwat M, Endoh D, Oikawa S (2012) Hepatocyte apoptosis in dairy cows with fatty infiltration of the liver. *Res Vet Sci* 93: 1281-1286.
- van Dorland HA, Richter S, Morel I, Doherr MG, Castro N, Bruckmaier RM (2009) Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. *J Dairy Sci* 92: 1924-1940.