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Metabolic Characteristics of Lame Cows During Puerperium and the Beginning of the Reproductive Period

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Simple Summary: It has been demonstrated that lameness and metabolic imbalance are connected, and there is a debate as to whether this metabolic imbalance affects, and to what extent, the liver function, body condition loss, and milk production in lame cows. The current study presents findings from two experimental processes that compared liver enzyme activities, glucose levels, milk production, body condition score, and back fat thickness alterations among lame and sound cows in two time periods: early in puerperium and later at the onset of the reproductive period. Lame cows exhibited a poorer physical state in both studies, and milk production was decreased in lame cows of the early experimental study. Liver function, as expressed by certain enzymatic parameters, was found to be mildly altered for lame compared to sound cows, indicating a weak effect if lameness is timely diagnosed.

Abstract: This study presents findings from two discrete experimental processes that examined the impact of lameness events on two consecutive, critical time points in the annual production cycle of dairy cattle (early in puerperium—first study, and later at the onset of the reproductive period—second study) regarding liver function, glucose levels, milk production, body condition score, and back fat thickness. In the first study, 47 cows (lame group n = 22, control group n = 25) were monitored from 10 days ante partum (ap) to 46 days post-partum (pp). In the second study, 79 cows (lame group n = 52, control group n = 27) were monitored from day 28 ± 5 pp to day 65–72 ± 5 pp. Lame cows had greater gamma-glutamyl transferase (GGT) concentrations in the blood serum compared to control cows (25.83 vs. 23.56, p = 0.02, respectively) early in puerperium, whereas the two groups did not differ in the second study. The concentration of glutamate dehydrogenase (GLDH) was lower for lame compared to control cows in both studies (17.24 vs. 24.60, respectively, p = 0.02 in the first study, and 30.50 vs. 51.10, respectively, p = 0.02 in the second study). The concentrations of aspartate transaminase (AST) and glucose did not differ between groups in both studies. Lame cows had a lower body condition score (BCS) and backfat thickness (BFT) scores compared to the control in both studies overall. The lame cows of the first study experienced a significant loss of milk production, especially during the second month of lactation, while in the second study, milk production remained unaffected. Conclusively, lame cows have lower BCS and BFT values, whereas milk yield can be negatively affected only if lameness occurs early in the puerperium, probably beginning at the dry period. However, the current research shows that acutely lame cows, as described in this study, exhibit only mild alterations in liver function compared to non-lame ones.

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Academic Editor: Phillip Lancaster

Received: 27 November 2024
Revised: 20 January 2025
Accepted: 23 January 2025
Published: 5 February 2025

Citation: Praxitelous, A.; Katsoulos, P.D.; Tsaousioti, A.; Schmicke, M.; Basioura, A.; Boscos, C.M.; Tsousis, G. Metabolic Characteristics of Lame Cows During Puerperium and the Beginning of the Reproductive Period. *Ruminants* **2025**, *5*, 8. https://doi.org/10.3390/ruminants5010008

Keywords: dairy cattle; lameness; BCS; BFT; metabolism; liver enzymes

1. Introduction

Lameness challenges the metabolic system of the dairy cow. As a pain inducer, lameness alters behavior and leads to less time spent walking and feeding [1,2]. Such changes have been associated with an increased risk of ketosis. It has been observed that cows with longer standing behavior in the days before calving had lower dry matter intake and were more likely to develop clinical or subclinical ketosis after calving which, in turn, was associated with a significant degree of liver damage in these cows [3]. Furthermore, the inflammatory processes present in the periparturient period have been implicated in impairing liver function through the action of cytokines on the hepatic synthesis of negative acute-phase proteins, which are important for normal liver metabolism, thereby imposing greater oxidative stress [4,5]. Lameness may affect liver function in both of these ways as it is associated with subclinical ketosis when it occurs during the dry period [6] and results in an inflammatory response [7] that may be a causal factor in oxidative stress in the cow. However, whether lameness results from, coexists with, or acts as an additional inflammatory factor that exacerbates oxidative stress [8] and thus further impairs liver function is still under investigation.

Ketone bodies are the by-products of the oxidation of non-esterified fatty acids (NEFAs). High concentrations of NEFAs can accumulate in the liver and impair its metabolic capacity. In the study by Calderon and Cook, higher levels of β-hydroxybutyrate (BHBA) were found in cows with moderate to severe lameness compared to healthier herd mates [9]. In addition, Collard et al. found a deterioration in the energy balance of cows with compromised locomotion [10]. González et al. demonstrated a direct relationship between lipomobilization and liver function during early lactation in high-yielding dairy cows [11]. Previous research by our working group has suggested a possible relationship between energy status and lameness in dairy cows [12,13]. Blood serum NEFA concentrations were higher in lame cows compared to the control group. However, these differences were not reflected in the corresponding BHBA concentrations. To our knowledge, there is very little scientific evidence on liver function parameters in lame dairy cows, especially during the critical puerperal period.

This study presents findings from two discrete experimental processes that examined the impact of lameness on the hepatic function of dairy cows on two consecutive, critical periods, that of puerperium and at the onset of reproduction. Additional data related to nutritional efficiency (glucose concentrations, milk yield, body condition score, and back fat thickness) from the two experimental periods are presented.

2. Materials and Methods

2.1. Ethical Statement

This study was approved by the Assembly of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki (69/30 June 2016). All procedures complied with the EU Directive 2010/63/CE.

2.2. Data Acquisition

The data in the current publication are derived from two different studies regarding the effect of lameness on the reproductive system of dairy cows at two distinct periparturient periods: one during puerperium, close to calving (first study) [12], and the other at the onset of the reproductive period [13]. Concurrently, both studies aimed to investigate the possible

involvement of the energy status of these animals, quantified by their blood NEFA and BHBA concentrations, in their reproductive performance. The results of these experimental protocols can be referred to for detailed information on the experimental procedures.

2.3. Animals and Housing

The present studies were conducted from July 2016 to December 2019 on three dairy farms with Holstein Friesian cows located in the region of Central Macedonia, Greece, around Lake Koroneia. A single dairy farm (farm A, with 200 lactating cows) was utilized for the experiments of the first study [12], whereas two additional farms (farm B, with 300 cows, and farm C, with 400 cows) were incorporated for the requirements of the second study [13]. It was ensured that all farms were similar with regard to housing, feeding, and management. In one of the farms (farm A), during the puerperal period, cows were housed in a straw bedded pack barn. Otherwise, the farms were equipped with free-stall barns, which were characterized by concrete or rubber floors, automatic scraper systems, and cubicles furnished with mattresses. All farms were equipped with mechanical ventilation systems. During the dry period, cows were housed in ventilated stalls on a bedding of composted manure, and an outdoor exercise area was available on two of the farms (farm A and C). The average milk production per cow per year was 10,259 kg. The cows were fed once daily during the dry period and twice during the lactation period, in accordance with the recommendations of the National Research Council at the time of the experiments [14]. Free access to water was provided at all times.

2.4. Experimental Design and Lameness Status

All cows included in both studies were multiparous, to reduce confounding factors associated with aspects of the initial studies [12,13]. General condition and health were routinely assessed by clinical examination; cows with retained fetal membranes, metritis, mastitis, or any other signs of clinical disease other than lameness were excluded from this study [12,13].

The studies included blood sampling for the determination of selected metabolic parameters and glucose concentration. The experimental animals were scored for body condition, and back fat thickness was measured.

Lameness status was assessed when exiting the parlor using Sprecher's 5-point scale [15] to evaluate all cows in the respective interventions according to the experimental protocols (Figure 1). Cows with a lameness score (LSC) of 3 or 4 were considered lame. Severely lame cows (LSC 5) were treated at diagnosis for ethical reasons and excluded from this study. Lesions were identified and recorded in the trimming chute for each cow entering the experimental procedure. Only cows with claw horn disruption lesions (sole ulcer, white line disease, diffuse hemorrhages, and laminitis) or infectious diseases (interdigital dermatitis, digital dermatitis) fulfilled the experimental procedure. The control cows for both studies were selected at the same time, with parity of the lame counterpart considered. They were defined by normal gait (LSC 1 in all measurements) and the absence of any foot lesions during trimming in the chute.

In the first study, cows were monitored from 10 days ante partum (ap) to 46 days post-partum (pp). Lameness events were identified, showing an LSC of 3 or 4 in at least two consecutive measurements with a 10-day interval up to day 15. At this point, the lameness etiology was confirmed at the trimming chute (day 15 $\pm$ 1 pp) and appropriate treatment was initiated. Forty-seven (47) cows were finally enrolled in this study (lame group n = 22; control group n = 25).

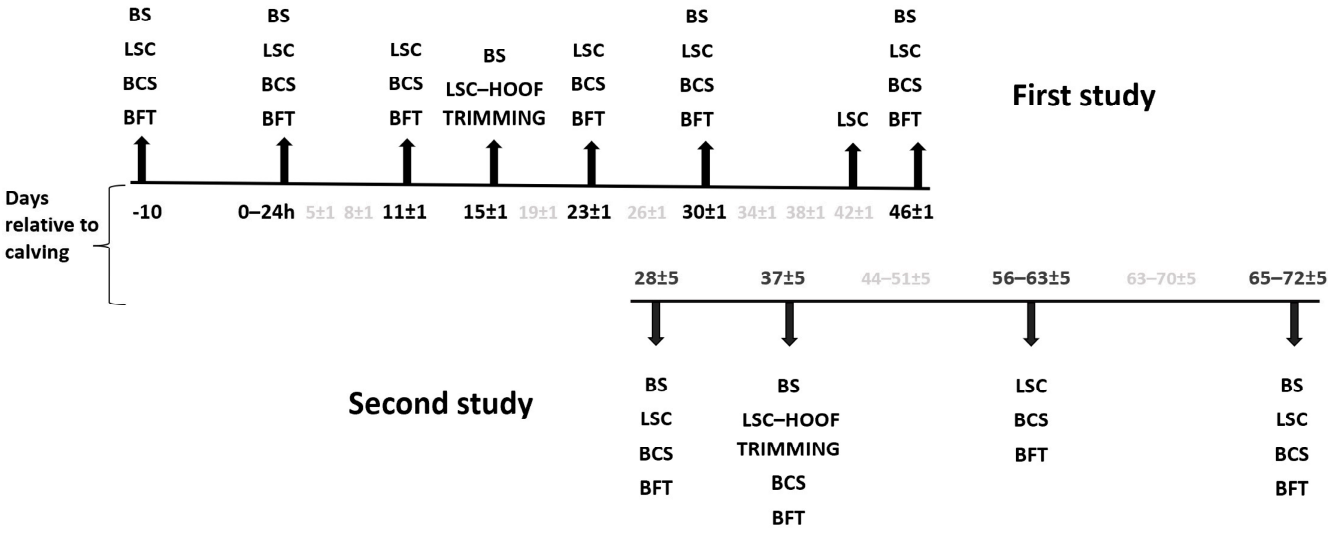

Figure 1. Experimental design–schematic representation. BS = blood sampling, LSC = locomotion scoring, BCS = body condition score, and BFT = back fat thickness.

In the second study, cows were monitored from day 28 ± 5 to 65–72 ± 5 pp. Lameness events were identified with an LSC of 3 or 4 at 28 ± 5 and at 37 ± 5 days pp. At this point, the lameness etiology was confirmed at the trimming chute (day 37 ± 5 pp) and appropriate treatment was initiated. A total of 79 cows were finally enrolled in the second study, with 52 assigned to the lame group and 27 to the control group. This was due to the primary experiment comprising two groups of lame and one group of control cows [13]. Blood samples for the enzymatic analysis were collected from a subgroup of 55 cows, comprising 36 lame and 19 control cows.

Figure 1 summarizes the above-mentioned time points of all the measurements and samplings. Regarding the etiology, in the first study, lameness was caused mainly from claw horn disruption lesions (CHDLs, n = 11, comprising sole or bulb ulcers, white line disease lesions, diffused sole/bulb hemorrhages with or without signs of laminitis) or CHDLs combined with infectious diseases of the claw (ID, n = 6), whereas only 5 cows showed solely ID (comprising interdigital dermatitis with or without the presence of interdigital hyperplasia). In the second study, CHDLs were present in 37 out of 52 lame cows (in 11 cows as the sole problem and in 26 cases as the major cause of lameness) and ID for 15 out of 52 lame cows (in 10 cows as the sole problem and in 5 cows as the major cause). Additionally, Figure S1 in the Supplementary Materials shows the progression of the lameness score in the cows during the experimental period. In the first experiment, no cow was completely free of lameness by the end of the study (day 46); however, 4 cows were scored with LSC 2, and cows with LSC 4 were reduced from 20 to 9 from day 15 to day 46 pp. In the second experiment, 2 cows were lameness-free by the end of it, and LSC 4 cows were reduced from 41 to only 5 from day 37 to day 65–72 pp.

2.5. Blood Sampling and Analytic Assays

Blood was collected via coccygeal venipuncture into 10 mL vacuum polyethylene tubes without an anticoagulant (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) after the morning milking and before feeding. The glucose concentration was measured immediately after sampling using the Precision Xceed® handheld meter (Abbott Diabetes Care Inc., Abbott Park, IL, USA). An accepted reference range for the glucose concentration in bovines is 57–79 mg/dL [16]. The samples were transported to the laboratory on ice and centrifuged (3000 rpm for 20 min) approximately 2–3 h after collection. The serum was then transferred to Eppendorf tubes and stored

at −80 °C for the determination of the enzymatic activities using the ABX Pentra system (Axonlabs, Baden-Dättwil, Switzerland). The concentrations of GGT (DIALAB®, A-2351 Wiener Neudorf, Austria), GLDH (Cobas®, Roche Diagnostics GmbH, D-68305, Mannheim, Germany), and AST (Labor + Technik®, Eberhard Lehman GmbH, D-14167, Berlin, Germany) were determined by spectrophotometry (Pentra C400®, HORIBA ABX SAS, Grabels, France). The laboratory's reference values for GGT, GLDH, and AST were <33 U/L, <14 U/L, and <100 U/L, respectively, (Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany). The intra- and interassay coefficients of variation for the above analyses were <10%.

2.6. Body Condition Score and Back Fat Thickness

Changes in body reserves were also recorded for the experimental cows. The body condition score of all cows was initially evaluated using a 5-point scale with 0.25 increments, as described by Edmonson [17]. Subsequently, the back fat thickness was measured using B-mode sonography (Honda HS 101 V with a 5 MHz linear transducer) following the established methods [18]. The skin was shaved to stabilize the measurement site, which was located 10 cm cranially to the tuber ischia. The transducer was placed perpendicular to the imaginary line between the tuber coxae and tuber ischia. For each measurement, three images were captured, frozen, and stored for subsequent processing using the appropriate software (Inkscape® version 1.0, New York, NY, USA). The mean of the three distances between the skin (including skin thickness) and the fascia trunci profunda was calculated for each measurement. The first author conducted all measurements.

2.7. Milk Production

Daily milk records were not available for analysis. Instead, milk recordings obtained once per month were used to derive milk production data for each cow for the first four months of lactation. All cows were measured on a single day, with the first measurement taken after the first 10 days pp. Average days to milk sampling did not differ at any time point between the lame and non-lame groups (all $p \geq 0.28$). Figure S2 in the Supplementary Materials presents boxplots of the development of milk production by experimentation and group (control vs. lame).

2.8. Statistical Analysis

The analysis of the dataset was performed using the SAS® OnDemand for Academics platform (SAS Institute, Cary, NC, USA). The normality of the data was assessed by histograms and by the evaluation of Q-Q plots of residuals. No important violations from the assumptions were detected. Statistical analysis was conducted with a repeated measures mixed model. Group (lame vs. control), time, and their interactions were included in the models as fixed effects, and farm (where applicable) was included as a random effect. Cow was defined as the subject of the repeated observations. Covariance structure was chosen based on the values of the Akaike information criterion (AIC). Pairwise comparisons were performed with the PDIFF command incorporating Tukey's adjustment. To control if the unbalanced experimental design of the second study could be impacting the results and statistical analysis, issues with heteroscedasticity between residuals within groups have been checked with plots of residuals against fitted values. The inspection of plots revealed no issue of different variances of errors across groups. Additionally, we tested the sensitivity of the model outcomes to potential imbalances by down-sampling the larger group (lame cows) and comparing the results with the unbalanced model. These analyses showed consistent estimates and trends in significances, suggesting that the unbalanced design did not bias our conclusions. Logistic regression models were used to estimate BCS and BFT losses per experimental day in each group separately. The results are presented

as least-squares means (±SE in the figures). A significance level of $p \leq 0.05$ was used for all comparisons.

3. Results

3.1. Concentration of Liver Enzymes in Blood Serum of Lame and Control Cows

In the first study, in which lameness occurred earlier in lactation, lame cows exhibited overall greater GGT concentrations in blood serum compared to the controls (25.83 vs. 23.56, $p = 0.02$, respectively, Figure 2a). However, this difference was rather small and was not observed at any specific time point. In the second study, in which lameness occurred at the onset of the breeding period, GGT was found to be equal between groups across the experimental period (28.79 for lame cows vs. 28.83 for control, $p = 0.97$, Figure 2b).

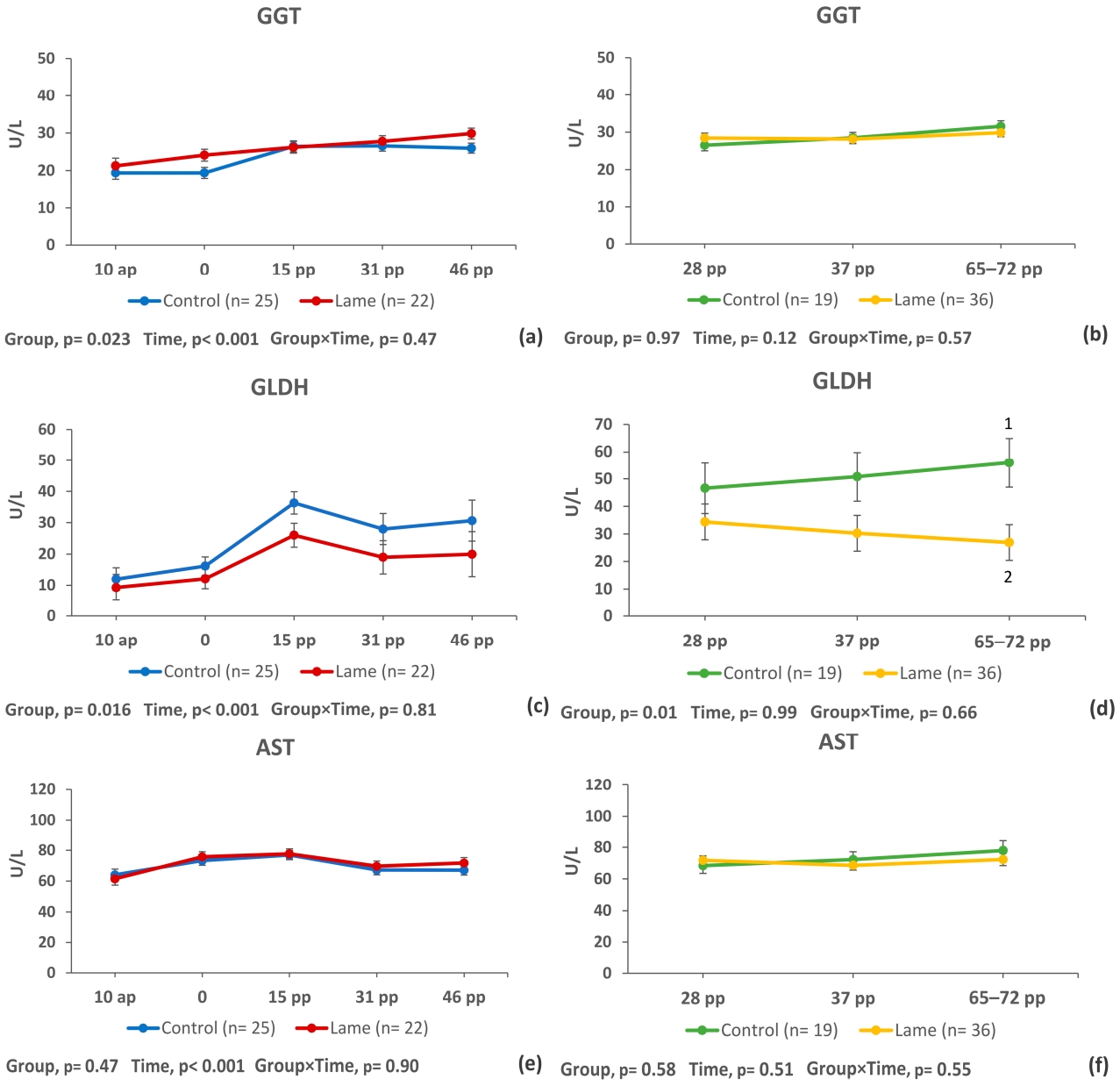

Figure 2. Liver function indices (GGT, GLDH, AST) in the blood serum of lame and control dairy cows in puerperium ((**a**,**c**,**e**), left side) and at the onset of the breeding period ((**b**,**d**,**f**), right side). [1,2] Different numbers denote statistical differences between lame and control cows at specific time points.

The concentrations of GLDH followed a reversed pattern in comparison to GGT. In both studies, lame cows showed lower GLDH values overall compared to the controls (17.24 vs. 24.60, respectively, $p = 0.02$, Figure 2c for the first study and 30.50 vs. 51.10, respectively, $p = 0.01$, Figure 2d for the second study).

Finally, regarding AST concentrations, no significant difference was observed between the lame and control cows in either the first (71.28 vs. 69.71, $p = 0.47$, Figure 2e) or the second (70.84 vs. 72.86, $p = 0.58$, Figure 2f) study.

3.2. Glucose Levels, BCS, BFT, and Milk Production of Lame and Control Cows

There was no difference between lame and control cows regarding glucose levels overall (58.78 vs. 56.26, respectively, $p = 0.15$ for the first study, Figure 3a, and 55.90 vs. 53.08, respectively, $p = 0.18$ for the second study, Figure 3b) or at any specific time point.

The body energy reserves of cows in both experiments demonstrated a decline over time and across groups, as anticipated ($p < 0.01$). Lame cows had an overall lower body condition score compared to the controls in both experiments (2.90 vs. 3.17, respectively, $p < 0.01$, for the first study, and 2.65 vs. 2.84, respectively, $p < 0.01$, for the second study). In the first study, at the beginning of the experimental period, both lame and non-lame cows had similar BCS values (3.34 vs. 3.51 for lame and non-lame cows ap, respectively, $p = 0.94$). Additionally, post hoc analysis revealed that BCS was lower for lame cows compared to the controls on day 30 and 45 pp in the first study ($p < 0.01$ in both days, Figure 3c). Regression analysis revealed that lame cows lost 0.017 BCS for every day of the experimental period, compared to 0.012 of the control group. Similarly, in the second study, post hoc analysis revealed that lame cows were thinner than the control cows at the end of the experimental period (2.55 vs. 2.76 at 65–72 days pp for lame and non-lame cows, respectively, $p = 0.04$, Figure 3d). In this period, BCS loss per day of lactation was 0.005 and 0.004 for lame and control cows, respectively. A similar pattern was observed regarding back fat thickness. Lame cows exhibited lower overall BFT values compared to the controls in both studies (11.42 vs. 14.26, respectively, $p < 0.01$, in the first study, Figure 3e and 8.75 vs. 9.67, respectively, $p = 0.02$, in the second study, Figure 3f). Loss of BFT per day of the experimental period was 0.13 mm for lame and 0.12 mm for control cows in the first study, whereas the two groups lost the same amount of BFT (0.07 mm per day of lactation) in the second study. Additionally, post hoc analysis revealed that BFT was lower for lame cows compared to the controls on day 45 pp in the first study ($p = 0.04$, Figure 3e). Finally, milk production only differed for the cows in the first study, where lame cows experienced a significant loss during the second month of lactation. In this study, milk production for lame cows was lower compared to the control cows (32.17 vs. 40.58, $p = 0.01$, Figure 3g). However, this finding was not repeated in the second study (Figure 3h).

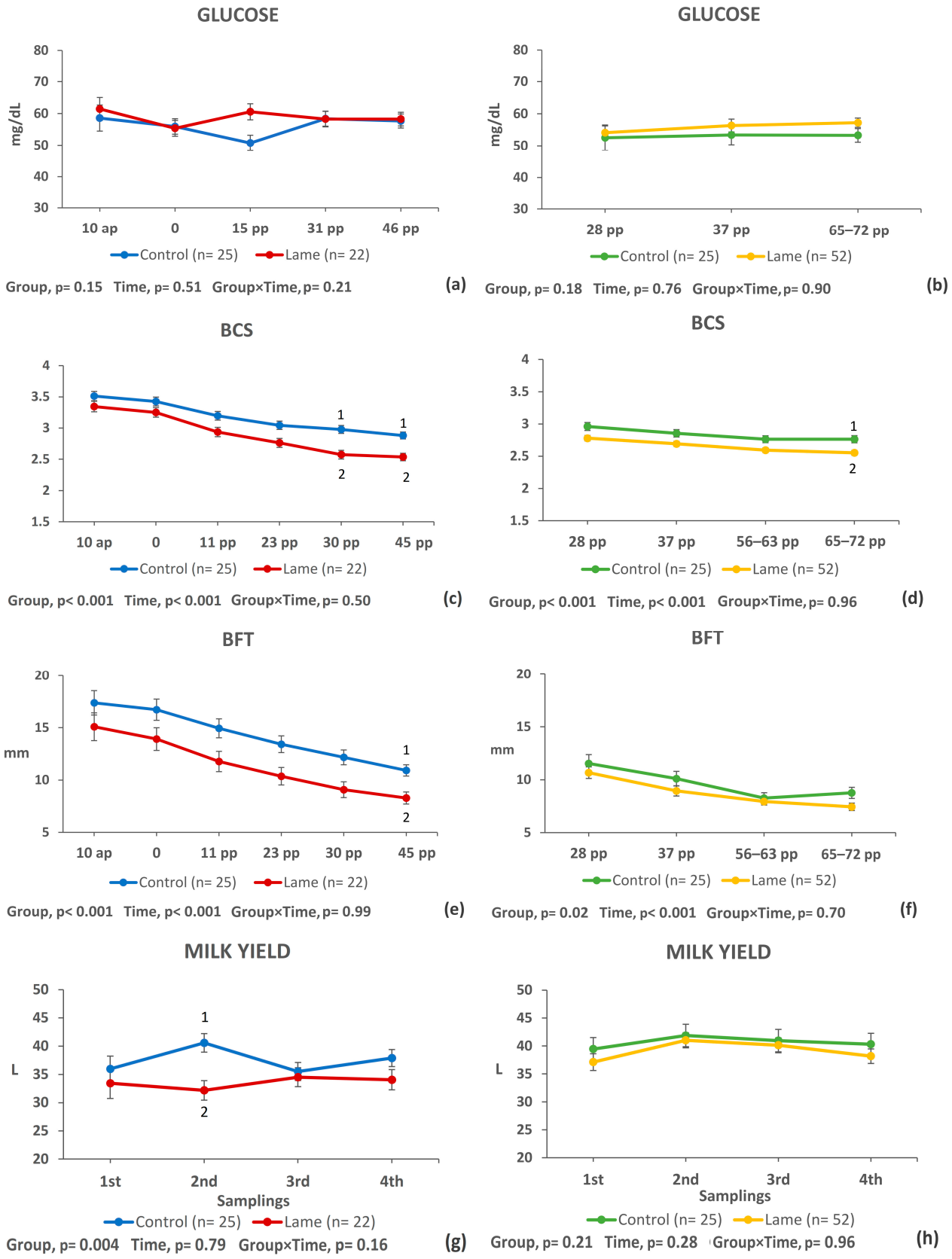

Figure 3. Indices of energy metabolism of lame and control dairy cows in puerperium ((**a**,**c**,**e**,**g**), left side) and at the onset of the breeding period ((**b**,**d**,**f**,**h**), right side). [1,2] Different numbers denote statistical differences between lame and control cows at specific time points.

4. Discussion

Isolating the effects of lameness in high-yielding periparturient cows can be challenging. Research results can often be conflicting due to differences in the identification of lameness, the time frame of the studies, and the parameters examined. In both experimental procedures, lameness was considered as a binary variable, i.e., present or absent. The animals in the first study were assigned to the study 10 days before calving, so the lame cows were treated earlier (day 15 pp) than the lame cows in the second study, which were assigned to the study after 4 weeks of lactation and treated around day 37 pp due to the design of the initial studies [12,13]. Parameters relating to different lesion types or severity were not examined separately, as this would result in numerous subgroups and a reduction in the number of animals per analysis. However, all lame cows included in this study had significant foot and sole lesions diagnosed in the trimming chute, rather than merely being scored for lameness. Furthermore, the severity was rather uniform, with a score of 3 or 4, excluding milder or graver cases. It is important to note that the results presented here were derived from two separate studies. A uniform longitudinal study design that included both the puerperal and early breeding periods would have been optimal for analyzing the fluctuations of the reported indices.

The periparturient period in dairy cows is characterized by an inflammatory response and significant metabolic changes to achieve calving and initiate and maintain milk production [19]. Reduced feed intake and fat mobilization prior to calving are thought to lead to an increase in proinflammatory cytokines in the bloodstream, which in turn adversely affect liver function. Conversely, it is also plausible that systemic inflammation in the periparturient cow may be a primary factor contributing to anorexia and promoting adipose tissue lipolysis through alterations in hepatic synthesis [20,21]. Lameness during this critical period may contribute to the induction of further systemic inflammation or lead to a reduction in dry matter intake, which could subsequently exacerbate the negative energy balance [2,7]. For all the above reasons, metabolic profiling has been recently used in machine learning models in order to test lameness susceptibility in cows [22]. Metabolomes, sets of metabolites that are used to investigate disease pathways, reveal a different metabolic signature in lame compared to sound cows which is largely attributed to the excessive lipolysis and possible muscle catabolism in cows with lameness [23,24]. GLDH, AST, and GGT are commonly used biomarkers of liver function that have been utilized in previous studies to assess liver cell damage [19,25]. In the current analysis, no thresholds were used to determine enzyme activities; instead, raw values were compared between groups.

AST and glucose levels did not differ between groups and were within the normal ranges in both studies. Consistent with our findings, glucose levels were equal between healthy and moderately lame cows (score 3 or 4) and were elevated only in severely lame cows (score 5) in the study by Sun et al. [26]. Furthermore, the study of Dervishi et al. revealed that glucose was downregulated at 6 weeks after the lameness configuration [23], and in the study of Zheng et al., cows with footrot exhibited reduced ATP synthesis and lower glucose concentrations [27]. This was not observed in our study, possibly due to the varying etiology and severity of lameness. In the first study [23], six cows with a locomotion score of 5, indicating reluctance to move and bear weight on the affected limb, were utilized. In the second study, only cows with footrot were used [27], which is a condition that causes significant discomfort to the cow [28]. AST is an enzyme that can be elevated in the blood due to liver damage, muscle injury, or systemic inflammation, all of which could be relevant to the pathophysiology of lameness in cows. Blood serum activities of the AST of lame cows were found to be similar to those observed in this study, and no significant disparities were identified between lame and sound cows [27,29]. The GGT

levels were overall higher in lame cows compared to the controls only in the first study. However, the detected difference should be considered of no clinical significance since it is only 2 U/l and the activities did not exceed the upper reference range. Similarly, in the study of Paiano et al., the GGT levels in lame cows (assigned with a lameness score > 2) did not differ to those of sound cows over a period of 3 weeks ap to 8 weeks pp [30]. Additionally, GLDH exceeded the upper reference range for both lame and control cows in both experiments pp. This could be attributed to the fat mobilization that occurs in the post-partum period. Interestingly, although fat mobilization, as confirmed by the BCS and BFT changes, was greater in lame than in control cows, GLDH activity was consistently lower for lame cows in both experiments compared to the controls. Lower GLDH activity has also been detected in cows with metritis and hypocalcemia [31] and was attributed to a reduced hepatic function rather than to hepatic failure or damage. Since lame cows generally show decreased feeding times and milk yield, and therefore decreased metabolic rate, reduced hepatic function cannot be excluded. Considering that the hoof lesions included in this study are associated with inflammation and acute-phase response [32], the lower GLDH activity could reflect the diversion of liver synthesis towards the production of positive acute-phase proteins [7], but such a hypothesis needs further investigation.

As demonstrated by numerous studies, bovines afflicted with lameness have been observed to exhibit a reduced body condition score across diverse reproductive periods when compared with their healthy counterparts [27,29]. Additionally, research has identified lameness as a crucial factor of body condition loss [30,33]. Investigating the relationship between lameness and body condition loss, Bicalho et al. demonstrated that body condition loss in dairy cattle between the first month and day 120 of lactation was correlated with the thinning and probably altering of the composition of the digital cushion that supports the soft tissue of the claw [34]. The same hypothesis is supported by the fact that cows with a BCS less than 3 between calving and early lactation were 3 to 9 times more likely to be lame compared to cows of greater body condition [35]. Moreover, Hut et al. reported that cows with a BCS loss greater than 0.75 early post-partum were 1.75 times more likely to develop lameness [36]. Nevertheless, the findings of our studies indicate that the reverse route is also a possibility. Specifically, in the first study, control cows exhibited a daily body condition score loss of 0.012 points, while for lame cows, this value was 0.017 points. However, at the beginning of the experimental period (10 days ante partum and at calving), both groups had similar BCS values. Furthermore, in the second study, both lame and non-lame cows exhibited a daily body condition score loss of 0.004–0.005 points. However, lame cows tended to be thinner by the end of the experimental period since lame cows continued to lose and non-lame started to gain BCS. The BFT values corresponded fairly well to those of BCS. In the second study, the return of non-lame cows to positive energy balance by day 70 pp, and the lag of the lame group, was even more evident regarding BFT compared to the BCS values (Figure 3f). Our findings are consistent with a bilateral relationship between BCS loss and lameness. However, longitudinal studies are more appropriate for addressing these questions. In an 8-year study conducted by Randall et al., it was demonstrated that lameness was influenced independently by both BCS loss and the absolute BCS value [37].

To further investigate the relationship between negative energy balance and liver health in early lactating cows, in the study by Bertoni et al. [19], cows in their first month of lactation were divided into four groups based on their liver activity index as determined by plasma negative acute-phase proteins. The low and intermediate–low groups exhibited elevated BHBA and NEFA levels during the first week of lactation, followed by a decline. It is noteworthy that cows in the intermediate–low group showed a slower reduction in BHBA and NEFA levels compared to the low group and a more unfavorable health

situation and reproductive performance. This group also had a high incidence of lameness in the first month of lactation (21%). Conversely, liver enzymes (alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl transferase, glutamic oxaloacetic transaminase) and glucose levels were not significantly different between the four groups in this study. These results support the concept that lameness may be a contributing factor to negative energy balance in dairy cows. However, its direct effect on liver function remains uncertain. Further targeted studies, probably taking into account the severity, the chronicity, and the type of lesion, are required to investigate this interpretation in more detail.

Furthermore, high milk production has been linked to an increased risk of lameness. Barkema et al. demonstrated that for every 100 liters of additional production in the initial 100 days of the milking period, there was a 6% increased likelihood of lameness in cows [38]. In a similar study by Ristevski et al., cows with a daily milk yield above 30.9 kg were twice as likely to suffer from lameness compared to cows with lower production, and the odds increased to five times higher with a milk yield of 39.1 kg/day [39]. Green et al. observed that cows that developed lameness exhibited a 1.2 L per-day increase in milk production compared to healthy herd mates [40]. The majority of researchers concur that the most probable pathway linking high milk production with lameness is due to negative energy balance, resulting in body condition loss and the thinning of the protective digital cushion, as previously discussed [34]. However, other studies have demonstrated that once lameness occurs, this relationship can be reversed. Lameness has been shown to negatively affect milk production, probably by reducing feed consumption, resulting in insufficient energy intake to maintain production [2]. In a study by Hernandez et al., cows that developed lameness within the first month after calving exhibited a total decrease in milk production, with a range of 81 to 620 liters in the lactation period [41]. Furthermore, milk loss in lame cows has been found to increase in proportion to the degree of lameness, the severity of the underlying lesion, and the type of lesion. In the current studies, only monthly records of milk production were available. Our data suggest that when lameness was initiated earlier in lactation or even during the dry period (first study), cows showed a detrimental loss of milk yield in the second month of lactation, where the peak of production was absent. This was not the case for the cows in the second study, where lameness occurred later, after the transition period, and which were treated probably more promptly compared to the cows of the first study. In accordance with this finding, Archer et al. demonstrated that cows at the end of their lactation period that had developed lameness 4, 6, and 8 months earlier had a lower production of 0.51, 0.66, and 1.55 liters per day compared to their healthy counterparts [42]. This effect was more pronounced the earlier in lactation the lameness occurred. From our findings, it is obvious that the interactions between body condition score development, milk production, and lameness onset, along with its characteristics (type and severity of lesions), can be complex and form vicious cycles in the production life of a dairy cow.

5. Conclusions

The findings of this study indicate that lameness, as described in the present manuscript, which typically presents with an acute onset and is promptly treated, has a relatively mild impact on liver function. However, lame cows experience both lower values and a greater decline in BCS and BFT during puerperium and up to the onset of the breeding period. Milk yield can be affected, especially in cows that are already lame at the onset of lactation. These findings underscore the necessity for prompt diagnosis and treatment of lameness prior to the onset of lactation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ruminants5010008/s1, Figure S1: Progression of lameness score (LSC) in cows during puerperium (Experiment 1, Figure a) and the onset of the breeding period (Experiment 2, Figure b). Cows with LSC 5 were treated promptly and excluded from analysis due to ethical reasons. a.p.: ante partum, p.p.: post partum. Figure S2: Box-plots of the progression of milk yield in control (blue boxes) and lame (red boxes) cows of the two experiments during the first four months of lactation from milk recordings obtained once a month with the first measurement taken after the first 10 days p.p.

Author Contributions: Conceptualization, A.P., P.D.K. and G.T.; methodology, A.P., P.D.K., C.M.B. and G.T.; investigation, A.P., M.S., A.B. and A.T.; formal analysis, P.D.K. and G.T.; writing—original draft, A.P. and P.D.K.; writing—review and editing, all authors; visualization, A.P., P.D.K. and G.T.; supervision, C.M.B. and G.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was approved by the Assembly of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki (69/30 June 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data are available upon request to the authors.

Acknowledgments: The authors thank the farms of Darginakis, Koukoutzelis, and Rokkos for letting us conduct the experimentations. We would also like to thank NUEVO SA for covering the costs of the blood samples analysis and Martin Höltershinken and the technicians in the laboratory department at the University of Veterinary Medicine Hannover. We are grateful to Chrysovalantis Malesios, Agricultural University of Athens, for his contribution to the statistical analysis of this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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