

Article

Relation Between Inflammatory Parameters and Insulin Resistance Indices in Cows During Early Lactation

Marko Cincović ^{1,*} , Dragica Stojanović ¹ , Radojica Djoković ², Mira Majkić ¹, Jože Starič ³ , Miloš Petrović ²  and Zorana Kovačević ¹ 

¹ Faculty of Agriculture, University of Novi Sad, Square Dositeja Obradovića 7, 21000 Novi Sad, Serbia; dragicas@polj.edu.rs (D.S.); miramajkic@gmail.com (M.M.); zorana.kovacevic@polj.edu.rs (Z.K.)

² Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia; radojicadjokovic@gmail.com (R.D.); petrovic.milos87@yahoo.com (M.P.)

³ Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; joze.staric@vf.uni-lj.si

* Correspondence: mcincovic@gmail.com or marko.cincovic@polj.uns.ac.rs; Tel.: +381-21-4853516

Abstract

Background/Objectives: Early lactation in high-producing dairy cows is a critical period characterized by pronounced negative energy balance, enhanced lipomobilization, and the development of insulin resistance (IR), often accompanied by low-grade systemic inflammation. This study aimed to investigate the dynamics of inflammatory markers and IR indices in early-lactation cows, assess their interrelationships, and evaluate the effects of NSAID administration. **Methods:** Thirty Holstein–Friesian cows were included and allocated into a control group ($n = 15$) and a treatment group ($n = 15$), which received ketoprofen (3 mg/kg BW intramuscularly) during the first postpartal week. Blood samples were collected at weeks 0, 1, and 2 postpartum to measure TNF- α , IL-1 β , haptoglobin, fibrinogen, NEFA, glucose, and insulin concentrations. Surrogate indices of IR, including RQUICKI, HOMA-IR, QUICKI, and Adipo-IR, were calculated. **Results:** In the control group, TNF- α , IL-1 β , haptoglobin, fibrinogen, and NEFA progressively increased over the first two weeks, accompanied by elevated adipose tissue IR, evidenced by decreased RQUICKI and increased Adipo-IR. Positive correlations were observed between inflammatory markers and NEFA, as well as between TNF- α and IL-1 β with Adipo-IR. Conversely, negative correlations were found between inflammatory markers and glucose and insulin, and between TNF- α and RQUICKI, as well as IL-1 β and haptoglobin with glucose. **Conclusions:** Ketoprofen administration significantly reduced inflammatory markers and NEFA while improving RQUICKI and Adipo-IR, without altering the overall relationships among the parameters. These findings indicate that inflammatory cytokines and adipose tissue IR indices serve as reliable parameters for monitoring the interaction between inflammation and IR, and for assessing the metabolic effects of NSAID treatment in early-lactation cows.

Keywords: lipolysis; insulin sensitivity; inflammation; nonsteroidal anti-inflammatory drug



Academic Editor: Saulo L. Silva

Received: 22 October 2025

Revised: 12 November 2025

Accepted: 15 November 2025

Published: 20 November 2025

Citation: Cincović, M.; Stojanović, D.; Djoković, R.; Majkić, M.; Starič, J.; Petrović, M.; Kovačević, Z. Relation Between Inflammatory Parameters and Insulin Resistance Indices in Cows During Early Lactation. *Metabolites* **2025**, *15*, 751. <https://doi.org/10.3390/metabo15110751>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Early lactation represents the most critical period in the production cycle of high-yielding dairy cows, characterized by substantial metabolic challenges and exceptionally high energy demands [1]. The onset of lactation inevitably leads to a state of Negative Energy Balance (NEB), in which energy intake is insufficient to meet the requirements for milk production and basal metabolism. The intensity and duration of NEB directly influence the metabolic adaptation, productivity, and overall health of the cows [2]. In

response to NEB, cows mobilize body fat reserves, resulting in elevated concentrations of non-esterified fatty acids (NEFA) in the blood. The increased NEFA concentrations, together with the redirection of glucose toward the mammary gland, are key factors contributing to the development of physiological, and often pathological, insulin resistance (IR) [3].

IR, defined as a reduced ability of target tissues (muscle, liver, adipose tissue) to effectively respond to circulating insulin, represents an essential adaptation during early lactation, allowing for glucose sparing and its redirection toward the mammary gland [3]. However, when this resistance becomes excessive, it is closely associated with metabolic disorders such as subclinical and clinical ketosis, fatty liver, and altered immune function [4,5]. Insulin resistance in adipose tissue during early lactation is physiological, facilitating the mobilization of fat to meet the energetic demands of lactation; however, its regulation is crucial, as excessive adipose tissue insulin resistance can contribute to systemic insulin resistance affecting muscle and liver, potentially leading to metabolic disorders such as ketosis and fatty liver. In ruminant studies, validated surrogate indices based on basal glucose and insulin concentrations are commonly used, providing reliable insights into metabolic status [6,7]. Indices such as the Quantitative Insulin Sensitivity Check Index (QUICKI) and the Homeostasis Model Assessment of IR (HOMA-IR) are widely accepted for evaluating systemic insulin sensitivity [8]. Additionally, the Adipose tissue Insulin Resistance (Adipo-IR) index, which incorporates the relationship between insulin and NEFA, as well as the Revised QUICKI (RQUICKI), which extends the QUICKI index by including NEFA values, offer specific assessments of adipose tissue IR, which is particularly relevant under conditions of intensive lipomobilization during early lactation [9].

An increasing body of evidence suggests that IR is not merely a disorder of carbohydrate and lipid metabolism, but is deeply intertwined with a state of chronic, low-grade systemic inflammation, often referred to as “meta-inflammation” [10]. Metabolic stress, induced by elevated NEFA concentrations and other factors, activates the innate immune system and leads to increased production of pro-inflammatory cytokines [11,12]. These cytokines directly interfere with insulin signaling pathways, thereby exacerbating IR and creating a vicious cycle [13].

In this context, key inflammatory mediators of interest include the pro-inflammatory cytokines Tumor Necrosis Factor-alpha (TNF- α), Interleukin 1 beta (IL-1 β), and others. These cytokines are known to reduce GLUT4 transporter translocation and inhibit insulin receptor phosphorylation, leading to alterations in insulin sensitivity [14]. Furthermore, cytokines may be associated with adipose tissue inflammation and contribute to increased lipolysis [15]. In addition to cytokines, reliable markers of systemic stress and inflammation are acute-phase proteins (APPs). Examples are haptoglobin (Hp) and fibrinogen, whose concentrations increase significantly in response to subclinical or clinical inflammatory events, serving as robust indicators of general health status and metabolic stress response during early lactation [16,17].

Given the strong link between inflammation and IR, there is considerable interest in therapeutic strategies aimed at alleviating inflammatory burden. Ketoprofen, a Non-Steroidal Anti-Inflammatory Drug (NSAID), exerts its effects through non-selective inhibition of cyclooxygenases (COX-1 and COX-2), thereby effectively reducing the synthesis of pro-inflammatory prostaglandins [18]. Although ketoprofen use has traditionally been directed toward the treatment of clinical conditions (e.g., metritis, mastitis, digital dermatitis), an increasing number of studies suggest the potential benefit of NSAIDs in modulating metabolic status by reducing systemic inflammation in high-yielding dairy cows [19,20]. Non-selective inhibition of cyclooxygenases, lipoxygenase inhibition, rapid onset of action, and short milk withdrawal make ketoprofen suitable for this longitudinal study.

It is hypothesized that reducing inflammation may improve insulin signaling and thereby mitigate the pathological aspects of IR. Although the effects of ketoprofen administration on individual metabolic or inflammatory markers have been partially investigated [21], there is a lack of comprehensive studies that simultaneously integrate a broader panel of IR indices and inflammatory markers in the context of ketoprofen use in lactating cows during early lactation. The aim of this study was to examine the effects of ketoprofen administration on inflammatory parameters and indicators of IR, as well as to thoroughly evaluate the relationship between surrogate indices of IR (HOMA-IR, QUICKI, RQUICKI, Adipo-IR) and a panel of key inflammatory markers, including cytokines (TNF- α , IL-1 β) and acute-phase proteins (haptoglobin, fibrinogen). We hypothesize that ketoprofen administration will significantly alter the concentrations of inflammatory markers, carbohydrate and lipid metabolism indicators, and IR indices, and that the correlations will reflect the interplay between inflammation and IR.

2. Materials and Methods

2.1. Animals and Management

A total of 30 clinically healthy cows of Holstein–Friesian breed on a commercial dairy farm were included in the experiment. The cows were in their second or third lactation, exhibited a body condition score typical for the postpartum period (BCS 2.75–3.25, score system 1–5), and had produced 9000–10,000 L of milk in the previous lactation. The cows were divided into two groups: 15 cows treated with ketoprofen and 15 cows in the negative control group. Experimental cows were treated during first week of lactation with ketoprofen with $3 \text{ mg} \times \text{kg}^{-1} \text{ BW}$ of ketoprofen IM injection (Mediprofen[®], Vetmedic, Vršac, Serbia).

Cows were kept in standard free stalls system of cow housing. Water was available *ad libitum*. Cows were fed twice daily using the TMR mixture according to standards [22]. Components of meal (in kg of dry matter) included: corn silage-multiple grains 8.2; Haylage of alfalfa 1.65; Hay of alfalfa 1.94; ensiled beet pulp 1.8; fresh beer trub 0.96; corn grain 5.38; barley grain 0.61; rapeseed meal 0.92; soybean meal 44% 1.1; sunflower meal 33% 1.44; extruded flaxseed 0.55; livestock chalk 0.13; livestock salt 0.06; baking soda 0.06; MgO 0.05; premix 0.18; Phosphozel 0.12; Zenural (urea) 0.18; Bentonite (Mycotoxin adsorbent) 0.02; dairyfat c16 0.39.

2.2. Blood Sampling and Metabolic Parameters Analysis

Blood samples were collected in the 0, 1 and 2 weeks (0, 7 and 14 days after calving, respectively) around calving by venipuncture of *v.coccigea* using 10 mL serum separation tubes. In order to separate the serum better, it was additionally centrifuged for 5 min at 3000 g. The serum samples were then collected and placed in vials and transported by laboratory refrigerators to the Laboratory of Pathophysiology, Department of Veterinary Medicine, University of Novi Sad.

The concentrations of the TNF- α , IL-1 β , Haptoglobin and Fibrinogen inflammatory parameters were measured by standard ELISA kit manufactured by Cloud-Clone Corp (China, Intra-Assay: CV < 10%; Inter-Assay: CV < 12%). The following readers were used for measurements: Fluoroscan Ascent FL reader (Thermo Scientific, USA) and Rayto RT 2100C reader (Rayto Life and Analytical Sciences, China).

We performed analysis for the following endocrine–biochemical parameters: insulin, non-esterified fatty acids (NEFA), glucose (GLU). Standard kits from Randox (UK) for NEFA and BioSystem (Spain) for glucose were used on Rayto Chemray 120 biochemical analyzer (Rayto Life and Analytical Sciences, China). An automated immunoassay analyzer TOSOH AIA-360 (Tosoh Bioscience, Tokyo, Japan) was used for insulin analyses. For the

estimation of IR we used RQUICKI, Adipo-IR, HOMA-IR and QUICKI index according to standard formula [6,23]:

$RQUICKI = 1/[\log(\text{Glucose}) + \log(\text{Insulin}) + \log(\text{NEFA})]$, where *Glucose*—fasting glucose in mg/dL, *Insulin*—fasting insulin ($\mu\text{U/mL}$), *NEFA*—non-esterified fatty acids (mmol/L);

$AdipoIR = \text{Insulin} \times \text{NEFA}$, where *Insulin*—fasting insulin ($\mu\text{U/mL}$), *NEFA*—non-esterified fatty acids (mmol/L);

$HOMA-IR = (\text{Glucose} \times \text{Insulin})/22.5$, where *Glucose*—fasting glucose (mmol/L), *Insulin*—fasting insulin ($\mu\text{U/mL}$);

$QUICKI = 1/[\log(\text{Glucose}) + \log(\text{Insulin})]$, where *Glucose*—fasting blood glucose in mg/dL, *Insulin*—fasting plasma insulin ($\mu\text{U/mL}$).

2.3. Statistical Analysis

Statistical analysis included the use of repeated measures ANOVA to examine the effects of treatment, week, and the treatment $\times$ week interaction on the values of blood parameters. Results are presented in the table. In the second step, linear correlation and regression analyses were performed between the independent variables (TNF- α , IL-1 β , Haptoglobin, and Fibrinogen) and the dependent variables (NEFA, glucose, insulin, RQUICKI, Adipose-IR, HOMA-IR, and QUICKI). The model $B_i = \beta_0 + \beta_1 A_i + \varepsilon_i$ was used, where B_i —represents the value of the dependent variable for observation iii , A_i —denotes the value of the independent variable for the same observation, β_0 —is the intercept, corresponding to the initial value of B when A equals zero, β_1 is the slope, representing the effect of changes in A on B, and ε_i is the residual. For clearer insight into trends during time and correlation between parameters, results were presented both graphically and in formula form. SPSS statistics software version 25 (IBM, Armonk, New York, US) was used. All statistical tests were considered significant if $p < 0.05$.

3. Results

The values of inflammatory parameters and IR indices, as well as the effects of the experimental treatments, are presented in Table 1. Significant changes were observed during the study period in response to ketoprofen administration on inflammatory and IR parameters. TNF- α concentrations in the control group gradually increased from a baseline value of 0.38 ± 0.05 ng/mL to 0.69 ± 0.06 ng/mL by week 2, whereas in the ketoprofen-treated group, a continuous decrease was observed from 0.35 ± 0.05 to 0.21 ± 0.05 ng/mL, with significant effects of week, treatment, and their interaction ($p < 0.05$ and $p < 0.01$, respectively). In the control group, IL-1 β values increased from approximately 0.38 ng/mL at week 0 to around 0.45 ng/mL in week 1 ($p < 0.05$), before returning to near baseline levels (~ 0.37 ng/mL), suggesting spontaneous modulation of inflammatory activity in the absence of therapeutic intervention. In the ketoprofen-treated group, IL-1 β levels remained lower throughout the period. Baseline values at week 0 were similar to those of controls (~ 0.36 ng/mL), but unlike controls, concentrations did not significantly increase during week 1 and significantly decreased to ~ 0.29 ng/mL by week 2 ($p < 0.05$). Statistical analysis indicated significant effects of treatment and week ($p < 0.05$), whereas the treatment $\times$ week interaction was not significant (NS). Haptoglobin levels in the control group increased significantly (0.41 ± 0.12 to 0.90 ± 0.09 g/L), whereas a decrease was observed in the ketoprofen-treated group (0.36 ± 0.08 to 0.24 ± 0.09 g/L), with significant effects of week, treatment, and their interaction ($p < 0.01$). Fibrinogen levels increased in the control group (6.61 ± 1.22 to 9.86 ± 1.61 g/L), while the treated group exhibited lower values (5.50 ± 1.22 to 5.08 ± 1.18 g/L), with significant differences between treatment and time ($p < 0.05$).

Table 1. Influence of week after calving, treatment and treatment $\times$ week interaction on inflammatory and IR parameters.

Blood Parameters	Control			Ketoprofen			Treatment	Week	Treatment $\times$ Week
	Week 0	Week 1	Week 2	Week 0	Week 1	Week 2			
TNF- α (ng/mL)	0.38 $\pm$ 0.05 ^a	0.54 $\pm$ 0.04 ^b	0.69 $\pm$ 0.06 ^c	0.35 $\pm$ 0.05 ^a	0.31 $\pm$ 0.06 ^a	0.21 $\pm$ 0.05 ^d	<0.01	<0.01	<0.05
IL-1 β (ng/mL)	0.38 $\pm$ 0.04 ^a	0.45 $\pm$ 0.05 ^b	0.37 $\pm$ 0.04 ^a	0.36 $\pm$ 0.04 ^a	0.39 $\pm$ 0.04 ^a	0.29 $\pm$ 0.03 ^c	<0.05	<0.05	NS
Haptoglobin (g/L)	0.41 $\pm$ 0.12 ^a	0.84 $\pm$ 0.11 ^b	0.90 $\pm$ 0.09 ^c	0.36 $\pm$ 0.08 ^a	0.49 $\pm$ 0.8 ^a	0.24 $\pm$ 0.09 ^d	<0.01	<0.01	<0.01
Fibrinogen (g/L)	6.61 $\pm$ 1.22 ^a	8.69 $\pm$ 1.51 ^b	9.86 $\pm$ 1.61 ^b	5.50 $\pm$ 1.22 ^a	7.53 $\pm$ 1.25 ^c	5.08 $\pm$ 1.18 ^a	<0.05	<0.05	<0.05
NEFA (mmol/L)	0.94 $\pm$ 0.11 ^a	0.82 $\pm$ 0.09 ^b	0.73 $\pm$ 0.09 ^b	0.92 $\pm$ 0.1 ^a	0.61 $\pm$ 0.09 ^c	0.51 $\pm$ 0.7 ^d	<0.01	<0.01	<0.01
Glucose (mmol/L)	2.29 $\pm$ 0.26 ^a	2.00 $\pm$ 0.25 ^a	2.55 $\pm$ 0.21 ^b	2.16 $\pm$ 0.23 ^a	2.04 $\pm$ 0.25 ^a	2.64 $\pm$ 0.26 ^b	NS	<0.05	NS
Insulin (mU/L)	6.10 $\pm$ 0.51 ^a	5.14 $\pm$ 0.43 ^b	4.50 $\pm$ 0.48 ^c	5.83 $\pm$ 0.41 ^a	5.23 $\pm$ 0.43 ^b	5.09 $\pm$ 0.55 ^b	NS	<0.05	NS
RQUICKI	0.48 $\pm$ 0.02 ^a	0.46 $\pm$ 0.01 ^b	0.45 $\pm$ 0.01 ^b	0.45 $\pm$ 0.01 ^b	0.49 $\pm$ 0.02 ^c	0.49 $\pm$ 0.01 ^c	<0.01	<0.01	<0.05
Adipo-IR	5.51 $\pm$ 0.52 ^a	4.40 $\pm$ 0.59 ^b	3.81 $\pm$ 0.54 ^c	4.99 $\pm$ 0.53 ^a	3.17 $\pm$ 0.48 ^d	2.35 $\pm$ 0.41 ^e	<0.01	<0.01	<0.01
HOMA-IR	0.61 $\pm$ 0.08 ^a	0.46 $\pm$ 0.07 ^b	0.51 $\pm$ 0.09 ^b	0.57 $\pm$ 0.08 ^a	0.47 $\pm$ 0.07 ^b	0.60 $\pm$ 0.08 ^a	NS	<0.05	NS
QUICKI	0.55 $\pm$ 0.03 ^a	0.53 $\pm$ 0.02 ^a	0.52 $\pm$ 0.03 ^a	0.53 $\pm$ 0.03 ^a	0.55 $\pm$ 0.03 ^a	0.54 $\pm$ 0.02 ^a	NS	NS	NS

^{a,b,c,d,e} Different superscripts mean significant differences between values.

NEFA levels slightly decreased in the control group, whereas the ketoprofen group showed a pronounced reduction (0.92 ± 0.10 to 0.46 ± 0.07 mmol/L; $p < 0.01$). Glucose concentrations in the control group increased in week 2 (2.55 ± 0.21 mmol/L), while values in the treated group remained similar, showing a significant effect of time but not treatment ($p < 0.05$). Insulin levels decreased in both groups over the study period ($p < 0.05$), but remained more stable and slightly higher in the ketoprofen-treated group, without reaching statistical significance. The RQUICKI index increased in both groups, with higher values in the treated group (0.49 ± 0.01), indicating improved insulin sensitivity under ketoprofen administration ($p < 0.01$). Adipo-IR values showed a significant decline in both groups, particularly in the treated group (4.99 ± 0.53 to 2.35 ± 0.41 ; $p < 0.01$), further confirming the positive effect of the drug on metabolic balance. HOMA-IR decreased in the control group but slightly increased in the treated group during week 2, with significant effects of time and interaction ($p < 0.05$). QUICKI showed no significant changes or effects of the experimental factors. Overall, the results indicate that ketoprofen significantly reduces inflammatory markers and improves insulin sensitivity parameters during the experimental period, particularly adipose tissue insulin sensitivity.

In the next step, we examined the linear relationships between inflammatory markers and indicators of IR. All inflammatory markers showed positive correlations with NEFA ($R^2 = 0.09$ to 0.26 , $p < 0.01$) (Figure 1). TNF- α and fibrinogen exhibited non-significant positive correlations, whereas IL-1 β and haptoglobin displayed statistically significant negative correlations with glucose ($R^2 = 0.18$ and 0.24 , $p < 0.01$, respectively) (Figure 2). TNF- α and fibrinogen demonstrated significant negative correlations with insulin ($R^2 = 0.24$ and 0.15 , $p < 0.01$, respectively), while the other two inflammatory parameters did not show statistically significant linear associations with this hormone (Figure 3). TNF- α exhibited a statistically significant negative correlation with the RQUICKI index ($R^2 = 0.13$, $p < 0.01$), whereas the other inflammatory markers showed the same trend but did not reach statistical significance (Figure 4). TNF- α and IL-1 β were significantly positively correlated with Adipo-IR ($R^2 = 0.05$, $p < 0.05$ and $R^2 = 0.08$, $p < 0.01$, respectively), whereas acute-phase proteins did not show a linear relationship with this adipose tissue IR marker (Figure 5). All inflammatory markers were negatively correlated with the HOMA-IR index ($R^2 = 0.06$ to 0.17) (Figure 6). TNF- α and fibrinogen did not show statistically significant

associations, while IL-1 β and haptoglobin demonstrated positive correlations with the QUICKI ($R^2 = 0.13$ and 0.17 , $p < 0.01$, respectively) index (Figure 7).

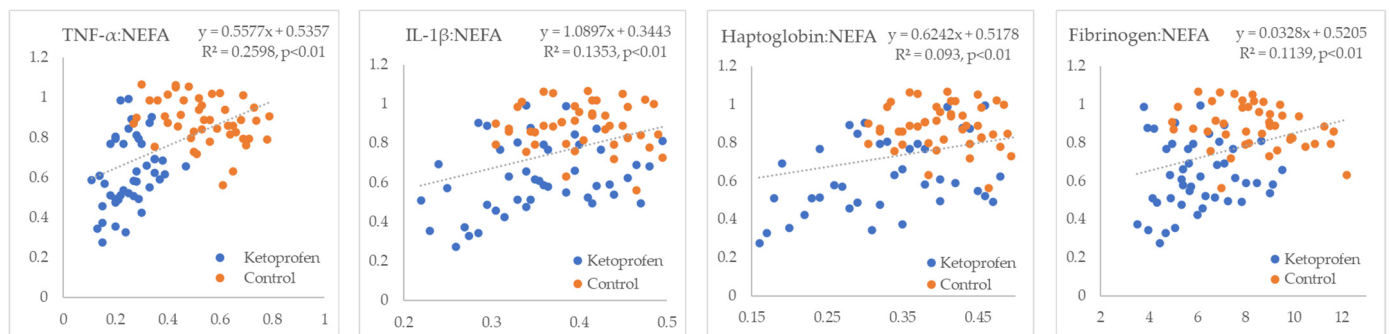


Figure 1. Linear regression and correlation between blood inflammatory parameters (X-axis) and NEFA (Y-axis) in cows during early lactation.

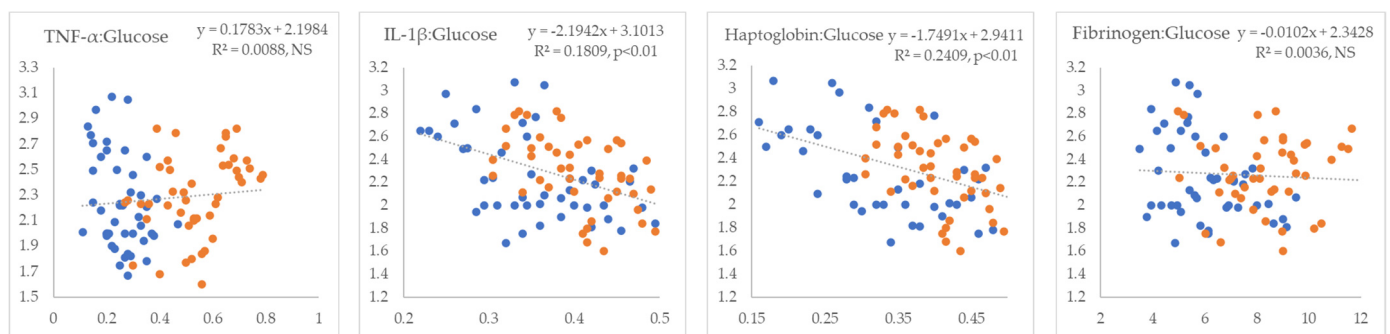


Figure 2. Linear regression and correlation between blood inflammatory parameters (X-axis) and glucose (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

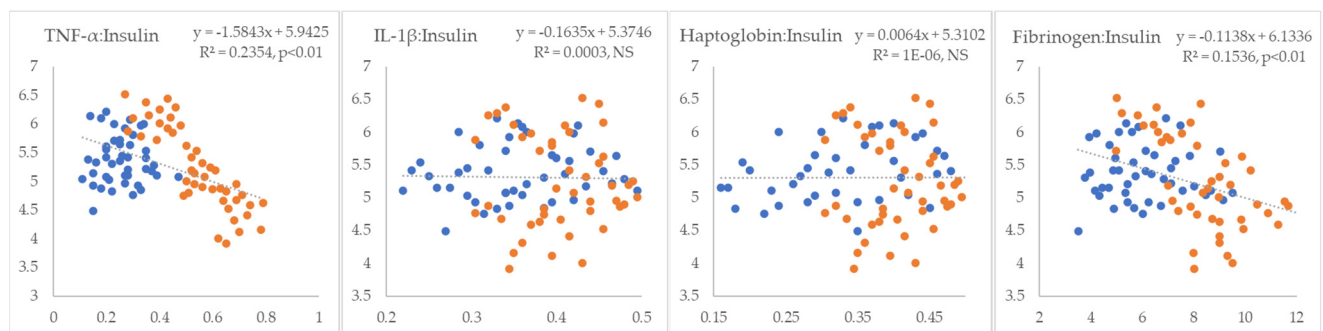


Figure 3. Linear regression and correlation between blood inflammatory parameters (X-axis) and insulin (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

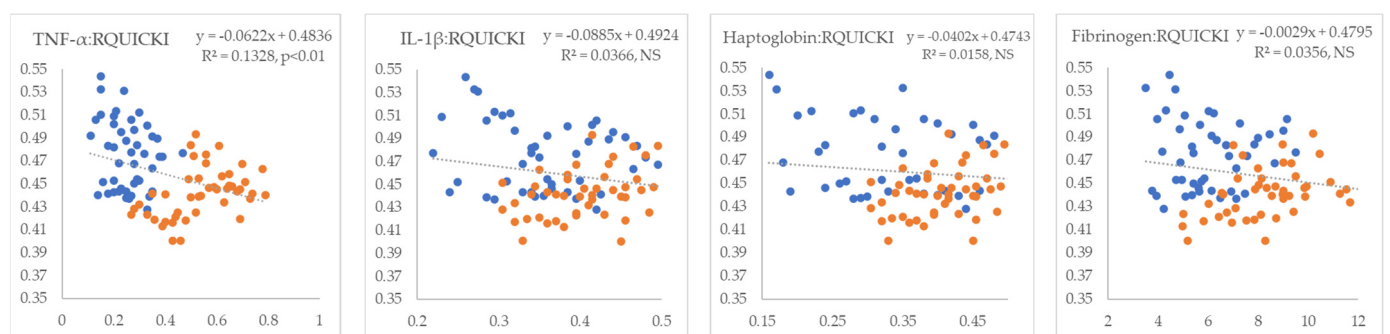


Figure 4. Linear regression and correlation between blood inflammatory parameters (X-axis) and RQUICKI index of IR (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

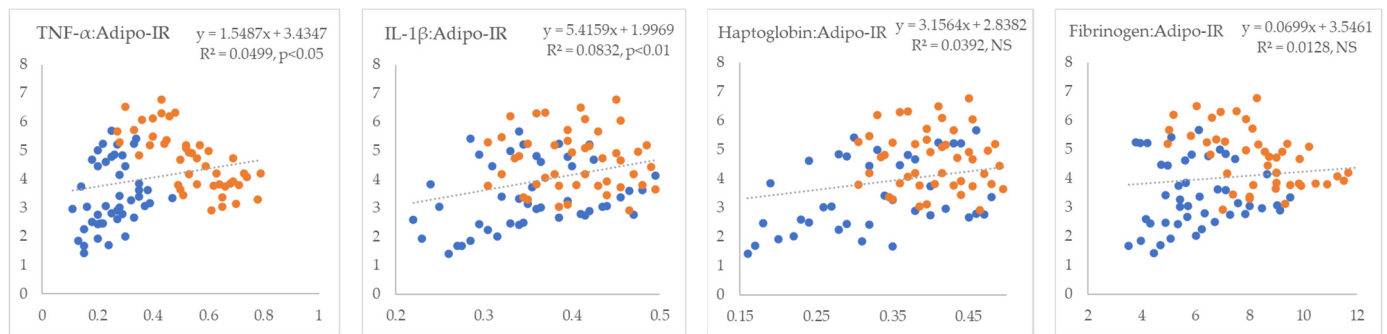


Figure 5. Linear regression and correlation between blood inflammatory parameters (X-axis) and Adipo-IR index of IR (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

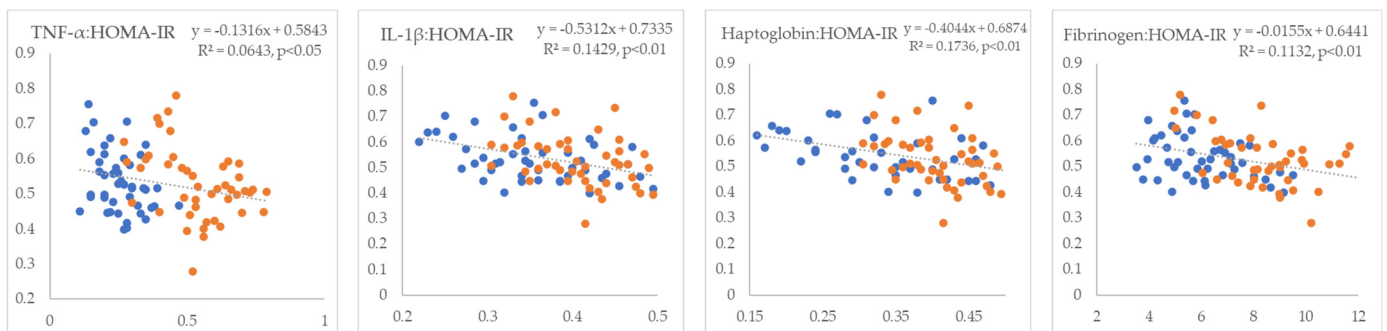


Figure 6. Linear regression and correlation between blood inflammatory parameters (X-axis) and HOMA-IR index of IR (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

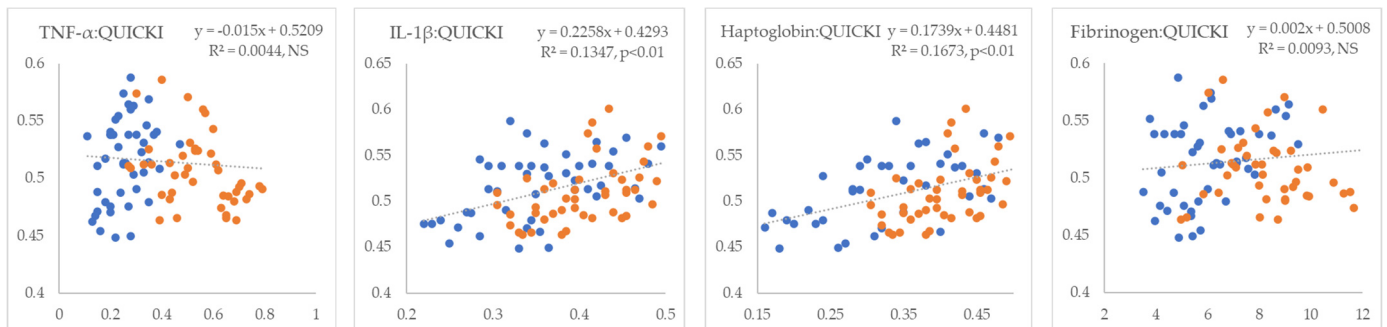


Figure 7. Linear regression and correlation between blood inflammatory parameters (X-axis) and QUICKI index of IR (Y-axis) in cows during early lactation (legend of the circle colors in Figure 1).

4. Discussion

Concentrations of pro-inflammatory cytokines and acute-phase proteins in the control group of cows increased during the first two weeks postpartum. TNF- α and acute-phase proteins showed a sustained elevation, whereas IL-1 β peaked during the first week after parturition. These concentration patterns are consistent with previous studies [24–26]. These cytokines stimulate immune cells and hepatocytes to synthesize acute-phase proteins, which play protective and reparative roles [27]. Therefore, measuring their concentrations is important for assessing the immune responsiveness of cows in the early postpartum period. A significant increase in haptoglobin was observed compared to later lactation stages, aligning with earlier reports [28,29]. Haptoglobin is a dynamic marker, with consistently higher values in cows with inflammatory conditions than in healthy animals [30]. Fibrinogen concentrations remained within established reference ranges [31] and were stable in clinically healthy cows during the first two weeks of lactation [32], which agrees with our

results. Inflammatory processes represent part of the metabolic adaptation during early lactation and occur in clinically healthy individuals [33]. They are initiated by activation of intracellular signaling pathways and the release of TNF- α , IL-1 β , and IL-6 [34]. In this study, the observed changes in TNF- α and IL-1 β concentrations indicate the presence of systemic inflammation.

Administration of ketoprofen resulted in reductions in all measured inflammatory parameters, with their temporal changes exhibiting patterns opposite to those observed in the control group. The anti-inflammatory effects of ketoprofen and flunixin meglumine are associated with the inhibition of cytokine synthesis and reversible blockade of COX enzyme isoforms, predominantly COX-1 [35]. Furthermore, previous studies have reported decreased plasma TNF- α concentrations following administration of salicylates during the early postpartum period [36]. Additionally, lower haptoglobin concentrations have been observed in cows treated with lysine-acetylsalicylate [37]. Ketoprofen has also been shown to reduce TNF- α production in the mammary gland following lipopolysaccharide stimulation, further confirming its anti-inflammatory properties [38].

During early lactation, it is common to observe increased NEFA levels alongside decreases in glucose and insulin, reflecting NEB and the redirection of glucose to the mammary gland [39–41]. This pattern was confirmed in the present study. The insulin, glucose and NEFA concentrations were comparable to previously published results from cows in similar geographic regions [2,4,5,12,21], with no extreme values were detected within the population. Analysis of IR indices indicated the development of adipose tissue IR, as evidenced by decreased RQUICKI and increased Adipo-IR values. The HOMA-IR index showed a reduction, which could paradoxically suggest higher insulin sensitivity, while QUICKI did not change significantly over the course of the study. RQUICKI, a frequently used indicator of metabolic adaptation [42], exhibited values consistent with prior research. Although Adipo-IR has not been widely applied in cows previously, it proved useful for assessing adipose tissue IR and is a well-validated surrogate index [23]. HOMA-IR offers a simple and validated method based on the assumption of feedback regulation between the liver and pancreatic β -cells [43]. Glucose concentration is regulated through insulin-dependent hepatic glucose production, while insulin levels depend on β -cell responsiveness to glucose. In early lactation, this relationship is disrupted due to reduced feed intake and glucose redirection toward the mammary gland [44,45], so the decline in HOMA-IR represents a numerical artifact arising from the combination of low glucose and insulin values. The decreases in glucose and insulin also reflect reduced feed intake typical of early lactation, i.e., the period during which pro-inflammatory cytokines exert central effects that contribute to decreased feed consumption [46].

Ketoprofen administration resulted in reduced NEFA levels and had no effect on glucose concentrations, while insulin was non-significantly higher in treated cows during weeks 1 and 2 compared to the control group. The RQUICKI index was significantly higher, and Adipo-IR lower, indicating improved adipose tissue insulin sensitivity. HOMA-IR and QUICKI did not show significant changes. Previous studies have confirmed that NSAIDs can reduce fat mobilization [47] and inhibit lipolysis in adipocytes [48]. However, the effects of NSAIDs on lipid metabolism are not always consistent and depend on the type of drug, dose, and metabolic status of the animal. Lower NEFA concentrations following sodium salicylate administration have been reported in some studies [36], whereas other authors observed only a trend toward reduced lipolysis without statistical significance [49]. It has also been shown that salicylates can decrease circulating NEFA, particularly in multiparous cows [50], whereas selective COX-2 inhibitors, such as meloxicam, do not consistently affect lipid metabolism in healthy cows [41].

The effects of NSAIDs on glucose and insulin concentrations vary depending on the type of drug, dosage, and duration of treatment. In some studies, administration of sodium salicylate during the first days postpartum led to reductions in glucose and insulin levels, accompanied by an increase in the RQUICKI index of insulin sensitivity [36]. Other studies reported no significant changes [49], and selective COX-2 inhibitors had only minimal effects [41]. The most pronounced changes were observed during the first week of lactation, when salicylates increased peripheral insulin sensitivity, whereas other NSAIDs generally did not induce significant alterations [36,41,49]. The mechanism of action of these drugs is associated with inhibition of COX and NF- κ B pathways, leading to reduced concentrations of pro-inflammatory cytokines that negatively impact insulin signaling, as well as potential alterations in hepatic gluconeogenesis [41,49].

In this study, inflammatory parameters showed positive correlations with NEFA levels and negative correlations with glucose, insulin, and insulin sensitivity indices (RQUICKI, Adipo-IR). This pattern indicates that inflammation influences the redirection of metabolic fluxes during early lactation [51]. Systemic inflammation in the peripartum period results from changes occurring before and after calving, including remodeling of the mammary gland, uterus, and digestive organs, NEB, lipolysis, and oxidative stress [52]. Elevated NEFA concentrations arising from NEB trigger so-called meta-inflammation. Saturated fatty acids, particularly palmitic acid, directly activate the TLR4 signaling pathway and the NLRP3 inflammasome [53]. Increased β -oxidation and lipid accumulation generate reactive oxygen species (ROS) and activate endoplasmic reticulum stress, which enhances NF- κ B signaling and leads to apoptosis and the release of damage-associated molecular patterns (DAMPs), thereby establishing a vicious cycle between lipotoxicity and inflammation [54]. During ketosis induced by enhanced lipid mobilization, TNF- α , IL-1 β , and haptoglobin concentrations increase [55], whereas administration of niacin, which has an antilipolytic effect, attenuates the inflammatory response [56]. Haptoglobin, in addition to its anti-inflammatory role, is involved in the regulation of lipid metabolism [57], which may explain its association with lipomobilization.

Inflammatory markers negatively correlate with RQUICKI and positively with Adipo-IR, confirming the association between inflammation and IR. The mechanisms linking these processes include activation of serine kinases by pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β), which phosphorylate IRS and reduce PI3K/AKT signaling, thereby limiting glucose uptake and decreasing insulin sensitivity. In addition, NEFA and bioactive lipids such as ceramides directly inhibit insulin signaling and activate the TLR4/NF- κ B pathway in the liver and adipocytes, further enhancing the inflammatory response and acute-phase protein synthesis [58]. These processes fully support the results and correlations observed in the present study.

5. Conclusions

In early-lactation cows, systemic inflammation is associated with increased lipid mobilization and IR. The present results indicate that ketoprofen effectively modulates the inflammatory response, contributing to improved insulin sensitivity. Reductions in pro-inflammatory marker concentrations were accompanied by favorable changes in metabolic parameters and IR indices. Correlation analysis confirms a close association between inflammation and metabolic disturbances, with more pronounced inflammatory processes linked to decreased insulin sensitivity and increased adipose tissue IR. These findings suggest that pharmacological modulation of inflammatory activity in early-lactation cows may represent an effective approach for preventing and mitigating IR. The use of inflammatory cytokines and adipose tissue IR indices provides reliable parameters for monitoring the interplay between inflammation and IR, as well as the effects of NSAID administration, in cows during early lactation.

Author Contributions: Conceptualization, M.C. and Z.K.; methodology, M.C., R.D. and D.S.; software, M.C.; validation, Z.K., M.M. and M.P.; formal analysis, Z.K.; investigation, M.C. and J.S.; resources, D.S.; data curation, M.C.; writing—original draft preparation, M.C.; writing—review and editing, J.S., D.S. and Z.K.; visualization, M.C. and M.M.; supervision, R.D. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of science of the Republic of Serbia, grant number 451-03-137/2025-03/200117.

Institutional Review Board Statement: The animal study protocol was approved by the Ethical Committee of the University of Novi Sad (01-90/11-4, 06 July 2015).

Informed Consent Statement: Not applicable.

Data Availability Statement: Original data can be available after personal communication with first author.

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

Abbreviations

The following abbreviations are used in this manuscript:

IR	Insulin resistance
NEB	Negative Energy Balance
TNF- α	Tumor Necrosis Factor Alpha
IL-1 β	Interleukin 1 Beta
NEFA	Non-Esterified Fatty Acids
RQUICKI	Revised Quantitative Insulin Sensitivity Check Index
Adipo-IR	Adipose Tissue Insulin Resistance Index
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
QUICKI	Quantitative Insulin Sensitivity Check Index
NSAID	Nonsteroidal Anti-Inflammatory Drugs
COX-1 or 2	Cyclooxygenase-1 or 2
LPS	Lipopolysaccharide
NF- κ B	Nuclear Factor kappa-light-chain-enhancer of activated B cells
TLR4	Toll-Like Receptor 4
ROS	Reactive Oxygen Species
DAMP	Damage-Associated Molecular Patterns
PI3K/AKT	Phosphoinositide 3-Kinase / Protein Kinase B (AKT) signaling pathway
IRS	Insulin Receptor Substrate
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3

References

1. Mezzetti, M.; Cattaneo, L.; Passamonti, M.M.; Lopreiato, V.; Minuti, A.; Trevisi, E. The transition period updated: A review of the new insights into the adaptation of dairy cows to the new lactation. *Dairy* **2021**, *2*, 617–636. [\[CrossRef\]](#)
2. Krnjaić, S.; Cincović, M.; Djoković, R.; Belić, B.; Ježek, J.; Starič, J. The influence of energy balance, lipolysis and ketogenesis on metabolic adaptation in cows milked twice and three times daily. *Metabolites* **2022**, *12*, 1090. [\[CrossRef\]](#)
3. Habel, J.; Chapoutot, P.; Koch, C.; Sundrum, A. Estimation of individual glucose reserves in high-yielding dairy cows. *Dairy* **2022**, *3*, 438–464. [\[CrossRef\]](#)
4. Djoković, R.; Dosković, V.; Cincović, M.; Belić, B.; Fratrić, N.; Jašović, B.; Lalović, M. Estimation of Insulin Resistance in Healthy and Ketotic Cows during an Intravenous Glucose Tolerance Test. *Pak. Vet. J.* **2017**, *37*, 387–392.
5. Delić, B.; Belić, B.; Cincović, M.R.; Djoković, R.; Lakić, I. Metabolic adaptation in first week after calving and early prediction of ketosis type I and II in dairy cows. *Large Anim. Rev.* **2020**, *26*, 51–55.
6. Cincović, M.; Kirovski, D.; Vujanac, I.; Belić, B.; Đoković, R. Relationship between the indexes of insulin resistance and metabolic status in dairy cows during early lactation. *Acta Vet.-Beogr.* **2017**, *67*, 57–70. [\[CrossRef\]](#)
7. Youssef, M.A.; El-Ashker, M.R.; Younis, M.S. The effect of subclinical ketosis on indices of insulin sensitivity and selected metabolic variables in transition dairy cattle. *Comp. Clin. Pathol.* **2017**, *26*, 329–334. [\[CrossRef\]](#)

8. Omid, A.; Mohebbi-Fani, M.; Nazifi, S.; Mirzaei, A.; Seirafinia, M. The effects of post-partum drops in body condition on indices of energy metabolism in mid-lactation Holstein cows. *Iran. J. Vet. Res.* **2019**, *20*, 180.
9. Guyot, H.; Dettileux, J.; Lebreton, P.; Garnier, C.; Bonvoisin, M.; Rollin, F.; Sandersen, C. Comparison of various indices of energy metabolism in recumbent and healthy dairy cows. *PLoS ONE* **2017**, *12*, e0169716. [[CrossRef](#)] [[PubMed](#)]
10. Khodadadi, M.; Jafari-Gharabaghlo, D.; Zarghami, N. An update on mode of action of metformin in modulation of meta-inflammation and inflammaging. *Pharmacol. Rep.* **2022**, *74*, 310–322. [[CrossRef](#)] [[PubMed](#)]
11. Blond, B.; Majkić, M.; Spasojević, J.; Hristov, S.; Radinović, M.; Nikolić, S.; Anđušić, L.; Čukić, A.; Došenović Marinković, M.; Vujanović, B.D.; et al. Influence of Heat Stress on Body Surface Temperature and Blood Metabolic, Endocrine, and Inflammatory Parameters and Their Correlation in Cows. *Metabolites* **2024**, *14*, 104. [[CrossRef](#)]
12. Petrović, M.Ž.; Cincović, M.; Starić, J.; Djoković, R.; Belić, B.; Radinović, M.; Majkić, M.; Ilić, Z.Ž. The Correlation between Extracellular Heat Shock Protein 70 and Lipid Metabolism in a Ruminant Model. *Metabolites* **2022**, *12*, 19. [[CrossRef](#)]
13. Al-Mansoori, L.; Al-Jaber, H.; Prince, M.S.; Elrayess, M.A. Role of inflammatory cytokines, growth factors and adipokines in adipogenesis and insulin resistance. *Inflammation* **2022**, *45*, 31–44. [[CrossRef](#)]
14. Leguisamo, N.M.; Lehnen, A.M.; Machado, U.F.; Okamoto, M.M.; Markoski, M.M.; Pinto, G.H.; Schaan, B.D. GLUT4 content decreases along with insulin resistance and high levels of inflammatory markers in rats with metabolic syndrome. *Cardiovasc. Diabetol.* **2012**, *11*, 100. [[CrossRef](#)] [[PubMed](#)]
15. Camell, C.D. Adipose tissue microenvironments during aging: Effects on stimulated lipolysis. *Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids* **2022**, *1867*, 159118. [[CrossRef](#)] [[PubMed](#)]
16. Brodzki, P.; Marczuk, J.; Lisiecka, U.; Szczubiał, M.; Brodzki, A.; Gorzkoś, H.; Kulpa, K. Comparative evaluation of cytokine and acute-phase protein concentrations in sera of dairy cows with subclinical and clinical ketosis as a different view of the causes of the disease. *Vet. World* **2021**, *14*, 1572. [[CrossRef](#)] [[PubMed](#)]
17. Seminara, J.A.; Seely, C.R.; McArt, J.A.A. Acute phase responses in clinically healthy multiparous Holsteins with and without calcium dysregulation during the early postpartum period. *J. Dairy Sci.* **2025**, *108*, 1930–1939. [[CrossRef](#)]
18. Kumar, B.; Sahani, V.; Patil, S. Review on Ketoprofen (Anti-Inflammatory Drug). *J. Res. Appl. Sci. Biotechnol.* **2024**, *3*, 41–50. [[CrossRef](#)]
19. Lora, I.; Massignani, M.; Stefani, A.; Gottardo, F. Potential benefits to dairy cow welfare of using a ceftiofur–ketoprofen combination drug for the treatment of inflammatory disease associated with pyrexia: A field clinical trial on acute puerperal metritis. *Animals* **2021**, *11*, 1597. [[CrossRef](#)]
20. Barragan, A.A.; Hovingh, E.; Bas, S.; Lakritz, J.; Byler, L.; Ludwikowski, A.; Takitch, S.; Zug, J.; Hann, S. Effects of postpartum acetylsalicylic acid on metabolic status, health, and production in lactating dairy cattle. *J. Dairy Sci.* **2020**, *103*, 8443–8452. [[CrossRef](#)]
21. Kovačević, Z.; Stojanović, D.; Cincović, M.; Belić, B.; Davidov, I.; Plavša, N.; Radinović, M. Association of metabolic and inflammatory markers with milk yield in postpartum dairy cows treated with ketoprofen. *Pol. J. Vet. Sci.* **2018**, *21*, 325–331. [[CrossRef](#)]
22. National Research Council; Committee on Animal Nutrition; Subcommittee on Dairy Cattle Nutrition. *Nutrient Requirements of Dairy Cattle: 2001*; National Academies Press: Washington, DC, USA, 2001.
23. Ryden, M.; Andersson, D.P.; Arner, P. Usefulness of surrogate markers to determine insulin action in fat cells. *Int. J. Obes.* **2020**, *44*, 2436–2443. [[CrossRef](#)]
24. Brodzki, P.; Kostro, K.; Brodzki, A.; Wawron, W.; Marczuk, J. Inflammatory cytokines and acute-phase proteins concentrations in the peripheral blood and uterus of cows that developed endometritis during early postpartum. *Theriogenology* **2015**, *84*, 11–18. [[CrossRef](#)]
25. Chitko-McKown, C.G.; Bierman, S.L.; Kuehn, L.A.; Bennett, G.L.; DeDonder, K.D.; Apley, M.D.; Harhay, G.P.; Clawson, M.L.; White, B.J.; Larson, R.L.; et al. Detection of bovine inflammatory cytokines IL-1 β , IL-6, and TNF- α with a multiplex electrochemiluminescent assay platform. *Vet. Immunol. Immunopathol.* **2021**, *237*, 110274. [[CrossRef](#)]
26. Peker, C.; Musal, B. Assessment of inflammatory cytokine concentrations during diagnosis and after treatment of postpartum dairy cows with clinical and subclinical endometritis. *Large Anim. Rev.* **2022**, *28*, 213–220.
27. Cecilian, F.; Ceron, J.J.; Eckersall, P.D.; Sauerwein, H. Acute Phase Proteins in Ruminants. *J. Proteom.* **2012**, *75*, 4207–4231. [[CrossRef](#)] [[PubMed](#)]
28. Ametaj, B.N.; Bradford, B.J.; Bobe, G.; Nafikov, R.A.; Lu, Y.; Young, J.W.; Beitz, D.C. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* **2005**, *85*, 165–175. [[CrossRef](#)]
29. Kováč, G.; Tóthová, C.; Nagy, O.; Seidel, H.; Konvičná, J. Acute phase proteins and their relation to energy metabolites in dairy cows during the pre-and postpartal period. *Acta Vet. Brno* **2009**, *78*, 441–447. [[CrossRef](#)]
30. Nazifi, S.; Rezakhani, A.; Koohimoghadam, M.; Ansari-Lari, M.; Esmailnezhad, Z. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in Shiraz, a tropical area in Southern Iran. *Bulg. J. Vet. Med.* **2008**, *11*, 95–101.

31. Milczak, A.; Abramowicz, B.; Szczepanik, M.; Madany, J.; Wrześniewska, K.; Buczek, K.; Staniec, M.; Żółkiewski, P.; Kurek, Ł. Could Fibrinogen Concentration Be a Useful Indicator of Cattle Herd Health Status? Approaches to Setting Reference Values. *Agriculture* **2023**, *13*, 1224. [\[CrossRef\]](#)
32. Jeremejeva, J.; Orro, T.; Kask, K. Relationship between acute phase proteins and subsequent fertility of dairy cows after postpartum uterine inflammation and healthy cows. *Vet. Med. Zoot* **2015**, *70*, 37–41.
33. Zhang, M.Q.; Heirbaut, S.; Jing, X.P.; Stefańska, B.; Vandaele, L.; De Neve, N.; Fievez, V. Systemic inflammation in early lactation and its relation to the cows' oxidative and metabolic status, productive and reproductive performance, and activity. *J. Dairy Sci.* **2024**, *107*, 7121–7137. [\[CrossRef\]](#)
34. Newton, K.; Dixit, V.M. Signaling in Innate Immunity and Inflammation. *Cold Spring Harb. Perspect. Biol.* **2012**, *4*, a006049. [\[CrossRef\]](#)
35. Donalizio, C.; Barbero, R.; Cuniberti, B.; Vercelli, C.; Casalone, M.; Re, G. Effects of flunixin meglumine and ketoprofen on mediator production in ex vivo and in vitro models of inflammation in healthy dairy cows. *J. Vet. Pharmacol. Therapeutics* **2013**, *36*, 130–139. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Farney, J.K.; Mamedova, L.K.; Coetzee, J.F.; KuKanich, B.; Sordillo, L.M.; Stoakes, S.K.; Minton, J.E.; Hollis, L.C.; Bradford, B.J. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **2013**, *305*, R110–R117. [\[CrossRef\]](#)
37. Bertoni, G.; Trevisi, E.; Piccioli-Cappelli, F. Effects of acetyl-salicylate used in postcalving of dairy cows. *Vet. Res. Commun.* **2004**, *28* (Suppl. 1), 217–219. [\[CrossRef\]](#)
38. Dan, D.; Bruckmaier, R.M.; Wellnitz, O. Ketoprofen affects the mammary immune response in dairy cows in vivo and in vitro. *J. Dairy Sci.* **2018**, *101*, 11321–11329. [\[CrossRef\]](#)
39. Gross, J.J. Dairy cow physiology and production limits. *Anim. Front. Rev. Mag. Anim. Agric.* **2023**, *13*, 44. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Martens, H. Invited Review: Increasing Milk Yield and Negative Energy Balance: A Gordian Knot for Dairy Cows? *Animals* **2023**, *13*, 3097. [\[CrossRef\]](#)
41. Pascottini, O.B.; Van Schyndel, S.J.; Spricigo, J.F.W.; Carvalho, M.R.; Mion, B.; Ribeiro, E.S.; LeBlanc, S.J. Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. *Sci. Rep.* **2020**, *10*, 5236. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Cincović, M.R.; Đoković, R.; Belić, B.; Lakić, I.; Stojanac, N.; Stevančević, O.; Staničkov, N. Insulin resistance in cows during the periparturient period. *Acta Agric. Serbica* **2018**, *23*, 233–245. [\[CrossRef\]](#)
43. Tahapary, D.L.; Pratisthita, L.B.; Fitri, N.A.; Marcella, C.; Wafa, S.; Kurniawan, F.; Rizka, A.; Tarigan, T.J.E.; Saksono Harbunono, S.; Purnamasari, D.; et al. Challenges in the diagnosis of insulin resistance: Focusing on the role of HOMA-IR and Tryglyceride/glucose index. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2022**, *16*, 102581. [\[CrossRef\]](#)
44. Baumgard, L.H.; Rhoads Jr, R.P. Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.* **2013**, *1*, 311–337. [\[CrossRef\]](#)
45. Baumgard, L.H.; Collier, R.J.; Bauman, D.E. A 100-Year Review: Regulation of nutrient partitioning to support lactation. *J. Dairy Sci.* **2017**, *100*, 10353–10366. [\[CrossRef\]](#)
46. Kuhla, B. Pro-inflammatory cytokines and hypothalamic inflammation: Implications for insufficient feed intake of transition dairy cows. *Animal* **2020**, *14*, s65–s77. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Trevisi, E.; Ferrari, A.; Archetti, I.; Bertoni, G. Anti-inflammatory treatments in calving dairy cows: Effects on haematological and metabolic profiles. *Ital. J. Anim. Sci.* **2005**, *4*, 203–205. [\[CrossRef\]](#)
48. De Zentella, P.M.; Vázquez-Meza, H.; Piña-Zentella, G.; Pimentel, L.; Piña, E. Non-steroidal anti-inflammatory drugs inhibit epinephrine- and cAMP-mediated lipolysis in isolated rat adipocytes. *J. Pharm. Pharmacol.* **2002**, *54*, 577–582. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Montgomery, S.R.; Mamedova, L.K.; Zachut, M.; Kra, G.; Häussler, S.; Vaughn, M.; Gonzales, J.; Bradford, B.J. Effects of sodium salicylate on glucose kinetics and insulin signaling in postpartum dairy cows. *J. Dairy Sci.* **2019**, *102*, 1617–1629. [\[CrossRef\]](#)
50. Carpenter, A.J.; Ylloja, C.M.; Mamedova, L.K.; Olagaray, K.E.; Bradford, B.J. Effects of early postpartum sodium salicylate treatment on long-term milk, intake, and blood parameters of dairy cows. *J. Dairy Sci.* **2018**, *101*, 1437–1447. [\[CrossRef\]](#)
51. McGuckin, M.M.; Giesy, S.L.; Overton, T.R.; Boisclair, Y.R. Inflammatory tone in liver and adipose tissue in dairy cows experiencing a healthy transition from late pregnancy to early lactation. *J. Dairy Sci.* **2023**, *106*, 8122–8132. [\[CrossRef\]](#)
52. Trevisi, E.; Jahan, N.; Bertoni, G.; Ferrari, A.; Minuti, A. Pro-inflammatory cytokine profile in dairy cows: Consequences for new lactation. *Ital. J. Anim. Sci.* **2015**, *14*, 3862. [\[CrossRef\]](#)
53. Dong, Z.; Zhuang, Q.; Ning, M.; Wu, S.; Lu, L.; Wan, X. Palmitic acid stimulates NLRP3 inflammasome activation through TLR4-NF-κB signal pathway in hepatic stellate cells. *Ann. Transl. Med.* **2020**, *8*, 168. [\[CrossRef\]](#)
54. Sun, X.; Tang, Y.; Jiang, C.; Luo, S.; Jia, H.; Xu, Q.; Zhao, C.; Liang, Y.; Cao, Z.; Shao, G.; et al. Oxidative stress, NF-κB signaling, NLRP3 inflammasome, and caspase apoptotic pathways are activated in mammary gland of ketotic Holstein cows. *J. Dairy Sci.* **2021**, *104*, 849–861. [\[CrossRef\]](#) [\[PubMed\]](#)

55. El-Deeb, W.M.; El-Bahr, S.M. Biomarkers of ketosis in dairy cows at postparturient period: Acute phase proteins and pro-inflammatory cytokines. *Vet. Arh.* **2017**, *87*, 431–440. [[CrossRef](#)]
56. Petrović, K.; Stojanović, D.; Cincović, M.R.; Belić, B.; Lakić, I.; Đoković, R. Influence of niacin application on inflammatory parameters, non-esterified fatty acids and functional status of liver in cows during early lactation. *Large Anim. Rev.* **2021**, *27*, 17–21.
57. Wan, B.N.; Zhou, S.G.; Wang, M.; Zhang, X.; Ji, G. Progress on haptoglobin and metabolic diseases. *World J. Diabetes* **2021**, *12*, 206. [[CrossRef](#)]
58. Qiao, K.; Jiang, R.; Contreras, G.A.; Xie, L.; Pascottini, O.B.; Opsomer, G.; Dong, Q. The Complex Interplay of Insulin Resistance and Metabolic Inflammation in Transition Dairy Cows. *Animals* **2024**, *14*, 832. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.