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Original article

Effect of postpartum administration of ketoprofen on proinflammatory cytokine concentration and their correlation with lipogenesis and ketogenesis in Holstein dairy cows

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Abstract

Early lactation period in dairy cows could be harmful to their health since it is challenging and demanding. Proinflammatory cytokine concentrations are increased in the early phase of the inflammatory response and during the early lactation period in cows. The aim of this study was to determine if ketoprofen treatment in the first days following parturition would decrease proinflammatory cytokine concentration and their correlation between lipid mobilization, ketogenesis and metabolic parameters in cows. The study was conducted on 30 cows divided into two groups of 15 cows each. The experimental group was treated with 3 mg × kg.bw.$^{-1}$ ketoprofen for three consecutive days after parturition. The blood samples were collected on the first day of treatment and in the first and second week postpartum and they were analyzed for biochemical parameters such as non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), glucose, cholesterol and total bilirubine and inflammatory parameters such as *tumour necrosis factor*-α (TNF-α), interleukin-1α (IL-1α) and interferon-γ (IFN-γ). The results suggested that ketoprofen-treated cows had a significantly lower concentration of TNF-α, IL-1α, IFN-γ, NEFA and BHBA in the first and second postpartum week compared to the control group. Ketoprofen administration increased glucose levels (the first week, $p<0.05$), increased cholesterol levels (the second week, $p<0.01$) and decreased serum total bilirubin levels (second week, $p<0.01$) compared to the control group of cows. A positive correlation was found between TNF-α and NEFA and total bilirubin, significantly more expressed in the control than in experimental group of cows ($p<0.01$) and it was also found between IL-1α and NEFA ($p<0.01$). A negative correlation was found between TNF-α and glucose and cholesterol, significantly more expressed in the control than in experimental group of cows ($p<0.01$). A positive correlation was also found between IL-1α and glucose ($p<0.01$). Ketoprofen given parenterally to Holstein cows immediately after calving could reduce inflammation and decrease the relation between inflammatory response and lipogenesis and ketogenesis in postpartum cows.

Key words: ketoprofen, TNF-α, IL-1α, IFN-γ, cow

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Introduction

Recent studies have confirmed the association between inflammatory mediators and metabolic disorders. In humans, high non eserifed fatty acids (NEFA) levels in the blood have been found to result in a lower incidence of inflammation. They may also affect the impaired function of the immune system (Harford et al. 2011, Linegaard et al. 2013). Similar results were found in dairy cows (Sordillo et al. 2009, Contreras et al. 2010, Mirzadeh et al. 2010) since they are at an increased risk of health disorders during the first 2 weeks of lactation (Goff and Horst 1997), which is characterized by one of the dramatic changes in the efficiency of the bovine immune system, since significant increases in lipid mobilization predispose dairy cows to health disorders, especially inflammatory diseases (Bernabucci et al. 2005, Hotamisligil 2006, Calder 2008, Sordillo et al. 2009). According to Bradford (2015) nearly all cows experience inflammation due to calving.

Fatty acids can be used as precursors of inflammatory eicosanoids and this is one of the reasons why the increased NEFA concentration in early lactation can lead to inflammation and affect its intensity and duration. High levels of these fatty acids can trigger an inflammatory pathway of the nucleus-κB (NF-kB), activate Toll-like receptors (TLRs) and cyclooxygenase pathways, resulting in the release of proinflammatory cytokines (Baker et al. 2011), which are indicators of inflammatory changes in the organism (Ishikawa et al. 2004). Increased concentrations of proinflammatory cytokines can significantly affect metabolic adaptation in early lactation, thus potentiating the onset of metabolic stress and lipid mobilization and ketogenesis (Sordillo et al. 2009).

Proinflammatory cytokine concentrations, such as TNF-α and IL-1 (Herath et al. 2009) and IFN-γ (Hisaeda et al. 2001, Sacchini et al. 2012) are increased in the early phase of the inflammatory response, and during the early lactation period in cows (Schroder et al. 2004). Heiser et al. (2015) reported that during the lactation period there was increased gene expression of TNF-α and IFN-γ gene. Some authors monitored the effect of inflammatory mediators on the development of metabolic changes. Trevisi et al. (2009) confirmed that oral administration of low-dose interferon-α (cytokine) during the last 2 weeks of gestation lead to inflammatory liver changes, including increased release of acute phase proteins.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in veterinary medicine because of their analgesic, anti-inflammatory, anti-endotoxic and anti-pyretic effects. As such, they could be expected to have significant effects on the metabolic and inflammatory changes in postpartum cows. As a NSAID, ketoprofen has antipyretic, anti-inflammatory and analgesic effects and has been approved for use in cows (EMEA/CVMP/MRL/ Summary Report of Ketoprofen 1995). The analgesic effects of ketoprofen have been explained by a decrease in the biosynthesis of prostaglandins induced by inhibiting cycleoxygenase enzymes (Kantor 1986). In addition, ketoprofen reduces inflammation by decreasing the release of granulocyte, basophils and mastocyte inflammation mediators, thus decreasing the sensitivity of blood vessels to bradykinin and histamine, which affect the production of lymphokines from T-lymphocytes and consequently vasodilation (Katzung 1995). The use of various NSAIDs leads to a decrease in lipid mobilization and ketogenesis due to a reduction in the concentration of proinflammatory cytokines (Kushibiki et al. 2001, Bertoni et al. 2004, Medzhitov 2008). According to the results of our previous research, ketoprofen treatment immediately after calving reduces metabolic adaptation dependence on the intensity of lipid mobilization and ketogenesis during early lactation (Kovacevic et al. 2016a) and hematological parameters dependence on the intensity of these two processes (Kovacevic et al. 2016b).

So far, no information appears to be available in the literature on inflammatory response associated with lipid mobilization and ketogenesis in ketoprofen treated postpartum cows. Therefore, our study investigated the effect of postpartum (p.p.) administration of ketoprofen on the inflammatory response and the correlation between this process and metabolic parameters in cows. We studied proinflammatory cytokine concentration (TNF-α, IL-1α and IFN-γ) as markers of inflammatory response. Our hypothesis was that ketoprofen, when administered p.p. to cows, would reduce the correlation between the inflammation and lipid mobilization and ketogenesis and improve their performance. Hence, the question was would the application of this drug reduce the interconnectedness of these two processes in the organism, and therefore decrease the risk of the development of various postpartal diseases.

Materials and Methods

Experimental Animals, Housing and Medication

The study sample consisted of 30 Holstein-Friesian dairy cows from a commercial dairy farm in Banatska Topola, Serbia. The selected cows were in the postpartum period, clinically healthy and in a normal body condition (scored from 3.25 to 3.50). The cows were randomly allocated to two groups, experimental and con-

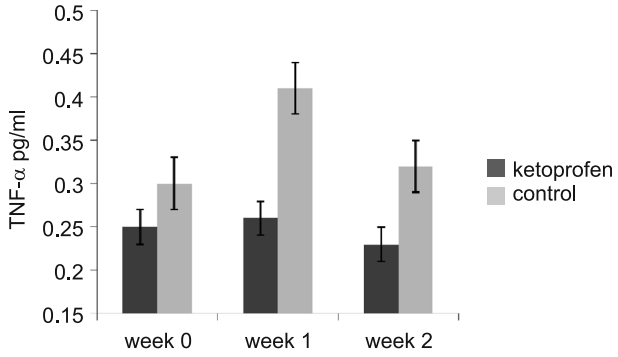

Fig. 1. Cytokine concentration (TNF-α) at 0, 1 and 2 postpartum weeks in ketoprofen treated and non-treated cows.

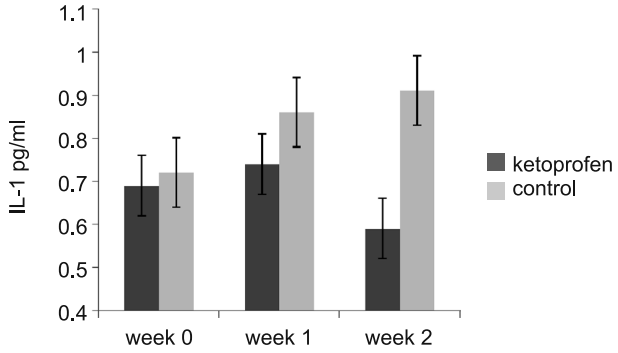

Fig. 2. Cytokine concentration (IL-1α) at 0, 1 and 2 postpartum week in ketoprofen treated and non-treated cows.

trol, both containing 15 animals. The experimental group (KET) received 3 mg × kg.bw.$^{-1}$ ketoprofen (Meloxifen®, Vetmedic, Serbia) IM and the placebo group (PLAC) received an equal volume of isotonic saline which were given on the first day postpartum, and repeated once daily for a further 2 consecutive days.

Blood Sampling and Laboratory Analyses

Blood samples were taken from the coccygeal vein using sterile vacuum tubes containing EDTA for biochemical analyses (BD Vacutainer® EDTA, BD Plymouth, UK). They were taken three times: on the day of calving, at the end of the first week (on day 8) and the end of the second week (on day 15) after parturition. Serum concentrations of proinflammatory cytokines (TNF-α, IL-1α and IFN-γ) were measured using a commercially available ELISA kit (Cloud-Clone Corp., Houston, USA), according to the standard manufacturer's instructions for cows. Biochemcal parameters such as NEFA, BHBA, glucose, cholesterol and total bilirubin were determined using a colorimetric reaction in accordance with the manufacturer's instructions (Randox, UK and Point Scientific, USA). A semi-automatic biochemistry analyser was used to measure them (AnalyzerRayto RT-1904cv, RaytoL.L.C. Rayto Electronics Inc., China).

Statistical methods

The statistical comparison of the groups was carried out using Statgraphics® Centurion XVII software and Microsoft Excel. Student's t-test was used to determine the ketoprofen application influence on the proinflammatory cytokine and biochemical parameters concentration. The results of comparison with $p<0.05$ were considered to represent statistically significant differences. The difference in the correlation test between proinflammatory cytokine concentration and other biochemical parameters in the experimental and control group of cows was determined using the Fischer r-to-z transformation. Linearity was tested in all 45 samples of the experimental and control group (15 cows x 3 weeks). The data analysis was performed using SPSS, version 19.0, software package for Microsoft Windows (IBM, Armonk, NY, USA). Compared results with $p<0.05$ were considered as statistically significant.

Results

Ketoprofen given to Holstein cows immediately after calving showed a significantly ($p<0.05$) lower concentration of TNF-α (Fig. 1), IL-1α (Fig. 2) and IFN-γ (Fig. 3) in contrast to the control group in the first and second week after the partus. Ketoprofen administration decreased the NEFA and BHBA levels in the first and second week after calving, ($p<0.01$), increased

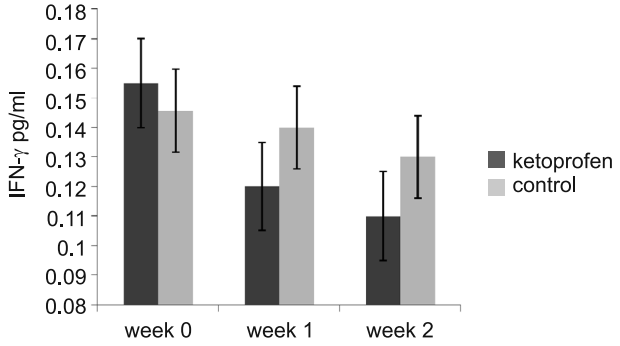

Fig. 3. Cytokine concentration (IFN-γ) at 0, 1 and 2 postpartum week in ketoprofen treated and non-treated cows.

Table 1. Influence of ketoprofen application on biochemical parameters in cows in early lactationat 0, 1 and 2 postpartum week.

Biochemical parameter	Group	Week 0	Week 1	Week 2
NEFA	Ketoprofen	0.32 ± 0.11	0.58 ± 0.08	0.49 ± 0.09
Mmol/l	Control	0.38 ± 0.13	0.76 ± 0.1**	0.6 ± 0.12**
BHB	Ketoprofen	0.49 ± 0.18	0.63 ± 0.15	0.86 ± 0.14
Mmol/l	Control	0.55 ± 0.16	0.88 ± 0.12**	1.05 ± 0.13**
Glucose	Ketoprofen	3.06 ± 0.4	2.65 ± 0.49	2.45 ± 0.42
Mmol/l	Control	2.9 ± 0.32	2.15 ± 0.43*	2.2 ± 0.39
Cholesterol Mmol/l	Ketoprofen	2.2 ± 0.4	2.5 ± 0.2	2.6 ± 0.3
	Control	2.5 ± 0.3	2.4 ± 0.2	1.9 ± 0.2**
Total bilir.	Ketoprofen	5.9 ± 1.7	6.2 ± 1.8	7.1 ± 1.6
µmol/l	Control	5.1 ± 1.6	7.6 ± 2.0	9.5 ± 1.8**

Significance of the differences in the parameters tested the blood of ketoprofen treated and control group of cows *p<0.05; **p<0.01

glucose levels (first week, p<0.05), increased cholesterol levels (the second week, p<0.01) and decreased serum total bilirubin levels (second week, p<0.01) compared to the control group of cows (Table 1). No significant difference was found in correlation between the concentration of IFN-γ and biochemical parameters (glucose, NEFA, BHBA, cholesterol and total bilirubin) (Table 2), nor between concentration of IL-1α and some biochemical parameters such as BHBA, cholesterol and total bilirubin (Table 2 and Table 3). A positive correlation was found between TNF-αconcentration and biochemical parameters concentration such as NEFA and total bilirubin, significantly more expressed in the control than in the experimantal group of cows (p<0.01) (Table 2 and Table 3). In addition, a positive correlation, significantly more expressed in the control than in the ketoprofen treated group of cows (p<0.01), was found between IL-1α and NEFA. A correlation between TNF-α and BHBA was positive in the control group, while in the experimental group this correlation was negative. A negative correlation was found between TNF-α concentration and glucose and cholesterol concentration, significantly more expressed in the control than in the experimantal group (p<0.01). A positve correlation was found between IL-1α and glucose, significantly more expressed in the control than in the ketoprofen treated group (p<0.01) (Table 2).

Discussion

Ketoprofen was shown to have anti-inflammatory effects in cattle in the treatment of acute clinical mastitis (Sordillo and Raphael 2013). Some authors reported (Shpigel et al. 1994, Drackley 1999) that significantly improved recovery in clinical mastitis was recorded after ketoprofen treatment, indicating that this NSAID suppresses inflammatory processes in dairy cows. It was also shown that orally and parenteraly administered ketoprofen has antipyretic effects in cows (Banting et al. 2008). Moreover, a recent study of Zecconi et al. (2018) indicated that chronic mastitis has a significant effect on the milk ejection curve and that anti-inflammatory ketoprofen treatment was shown to be efficacious in reducing these effects. Furthermore, anti-inflammatory effects of ketoprofen was shown in cattle by regulating cytokines that are involved in the immune response. Recent research has shown that intramammary administration of ketoprofen for mastitis treatment could reduce inflammation of the udder and could support a fast return to a normal milk composition. Actually, *in vitro* study of the effects of ketoprofen

Table 2. Influence of ketoprofen application on the correlation between cytokine concentration and biochemical parameters (glucose, NEFA and BHBA).

	Correlation coefficient								
	Glucose			NEFA			BHBA		
	Ketoprofen	Control	p°	Ketoprofen	Control	p	Ketoprofen	Control	p
IL-1α	-0.49	-0.31	<0.05	0.33	0.35	NS	0.28*	0.30*	NS
TNF-α	-0.34	-0,52	<0.05	0.74	0.55	<0.01	-0.81	0.62	<0.01
IFN-γ	-0.11*	-0,17*	NS	0.36	0.35	NS	0.21*	0.27*	NS

°difference between ketoprofen and control group; * non-significant correlation

Table 3. Influence of ketoprofen application on the correlation between cytokine concentration and biochemical parameters (cholesterol and total bilirubin).

	Correlation coefficient					
	Cholesterol			Total bilirubin		
	Ketoprofen	Control	p°	Ketoprofen	Control	p
IL-1α	-0.19*	-0.18*	NS	0.26*	0.22*	NS
TNF-α	-0.71	-0.51	<0.01	0.77	0.52	<0.01
IFN-γ	0.11*	0.13*	NS	0.26*	0.25*	NS

°difference between ketoprofen and control group; * non-significant correlation

on the mRNA abundance of selected immune factors of mammary epithelial cells it showed that ketoprofen reduced the LPS-induced increase in mRNA abundance of tumor necrosis factor α (TNF-α), interleukin-8 (IL-8), serum amyloid A (SAA) and cyclooxygenase-2 (Dan et al. 2018). In addition, it was reported that ketoprofen reduces the production of TNF-α in in calves that were challenged with LPS (Donalisio et al. 2013, Plessers et al. 2016). It is important to note that serum TNF-α increases during many inflammatory diseases in postpartum cows (Hisaeda et al. 2001, Kim et al. 2005, Sacchini et al. 2012, Kasimanickam et al. 2013).

Previous research in cows (Faraney et al. 2013a,b) demonstrated that oral administration of aspirin at a dose of 1.95 g/L, seven days after partus, resulted in a decrease in TNF-α concentration. This is in accordance with our study where ketoprofen-treated cows had a significantly lower concentration of TNF-α in contrast to the control group in the first and second postpartum week, suggesting that a short duration of anti-inflammatory therapy may decrease the inflammatory response in dairy cows early in the post-partum period.

In addition to an increase in TNF-α concentration, an increase in IL-1β concentration was also reported in cows with various inflammatory disorders (Kasimanickam et al. 2013). It has also been demonstrated by Herath et al. (2009) that there is an increase in proinflammatory cytokine concentrations (IL-1α or IL-1β) in the initial phase of inflammation. We found significantly higher IL-1β concentrations in the control group, compared to the treated group in the first and second week after partus, confirming anti-inflammatory effect ofketoprofen. Accordingly, reduced IFN-γ concentration in the first and second week after the partus in the experimental group of cows in our research has confirmed the effect of ketoprofen anti-inflammatory therapy, since in the early phase of the inflammatory response, in addition to increased TNF-α and IL-1 concentrations (Herath et al. 2009), increased IFN-γ concentrations were also determined (Hisaeda et al. 2001, Sacchini et al. 2012) including the period of early lactation in cows (Schroder et al. 2004). NSAID treatment dramatically affected lipid metabolism in early-lactation cows resulting in elevation in plasma NEFA and BHBA concentrations (Farney et al. 2013). Kushibiki et al. (2002) reported that an inflammatory agent such as TNF-α treatment can stimulate adipose tissue lipolysis, increasing plasma NEFA concentrations and providing a substrate for hepatic ketogenesis. Therefore, higher plasma NEFA concentration could be associated with immune system activation. This observation is in accordance with our study, where a positive correlation was determined between TNF-α and NEFA concentration in the experimental and control group. This correlation is stronger in ketoprofen-treated cows than in the control group. According to the Khovidhunkit et al. (2004), TNF-α can lead to a decrease in BHBA concentration in the postpartum period in cows. This negative correlation among TNF-α and BHBA was confirmed in our study in the experimental group of cows, also confirming the anti-inflammatory effect of the ketoprofen.

Reduced synthesis of IFN-γ has been demonstrated in cows with intense lipid mobilization (Szuster-

-Ciesielska et al. 1995, Lacetera et al. 2005). Elevated NEFA concentrations may have an effect on the lymphocyte function, i.e., the reduced synthesis of IgM and IFN-γ in the peripartal period in cows (Lacetera et al. 2005). In our research, it was found that in the experimental group of cows there was a slight positive correlation between the NEFA and IFN-γ concentrations. Also, significant correlations of the mentioned parameters were not established between the experimental and control group. A positive correlation between TNF-α and total biluribine concentrations was recorded in humans with diagnosed hepatitis C (Ren et al. 2009) and hepatitis B (Wang et al. 1999). This is in accordance with our research, where a positive correlation between TNF-α and total bilirubin was more pronounced in ketoprofen treated cows.

During an acute inflammatory response, short-term hyperglycaemia occurs, followed by long-term hypoglycaemia (Kushibiki et al. 2000). There is reduced expression of the gene responsible for glucose synthesis in cows with diagnosed mastitis (Jiang et al. 2008). According to Kettelhut et al. (1987) TNF-α has been shown to have direct effects on metabolic processes in the liver, primarily on reduced glucose production. Heiser et al. (2015) have indicated that during the lactation period there is an increased gene expression for both TNF-α and IFN-γ. In our research, a negative correlation between the concentration of glucose and proinflammatory cytokines (IL-1 and TNF-α) was established. However, the intensity of this correlation is weakened by the use of ketoprofen.

In conclusion, based on the results obtained in this study, it was found that parenteral administration of ketoprofen could reduce inflammation and decrease the relation between inflamatory response and lipogenesis and ketogenesis in postpartum cows. In addition, health events during the postpartum period produce important economic losses on dairy farms, and affect the welfare of dairy cows. Hence, a reduction in the occurrence of inflammatory disorders can improve animal welfare and wellbeing.

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