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Original article

Association of metabolic and inflammatory markers with milk yield in postpartum dairy cows treated with ketoprofen

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Abstract

Inflammation together with lipolysis and ketogenesis in early lactation can cause low productivity and may be harmful to the cow health. The objective of the study was to determine if ketoprofen treatment in the first days following parturition would positively affect the milk production and whether it was associated with the metabolic and inflammatory response. 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 non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), tumour necrosis factor- α (TNF- α) and haptoglobin. The results suggested that ketoprofen-treated cows with a higher milk production had a significantly lower concentration of NEFA, BHB, TNF- α and haptoglobin in the first and second week postpartum. No differences were found in the control group in metabolic status regardless of the achieved level of milk production. Ketoprofen administration in postpartum cows can enhance the milk yield. The higher milk yield in the experimental group might be associated with a lower degree of lipolysis, ketogenesis and reduced inflammatory response in the first two weeks postpartum.

Key words: ketoprofen, TNF- α , haptoglobin, NEFA, BHB, cow, milk yield

Introduction

The regulation and coordination of lipid metabolism between adipose tissue, liver, gut and mammary gland in peripartum period are the key components of the adaptations to lactation (Drackley 1999). A metabolic disorder, such as ketosis and fatty liver along with metritis and mastitis during the period of lactation may limit the nutrient supply of the mammary gland, reduce

the milk yield and affect the productivity of cows throughout the lactation period. During the period of early lactation an increase in non-esterified fatty acids (NEFA) and cytokines in the plasma was observed in dairy cows, which can be correlated with the development of an inflammatory response in the liver. Accordingly, the increased fatty acid synthesis is directed towards the triglyceride synthesis and may result in a reduction in gluconeogenesis and continued mobiliza-

tion of adipose tissue (Bradford et al. 2009). Metabolic disorders in dairy cows increase the incidence of oxidative stress as a reduction in the antioxidant capacity of an organism in the early lactation (Turk et al. 2005). Oxidative stress represents a link between nutrient metabolism and inflammation during this period (Sordillo and Mavangira 2014). The main task of the oxidative stress prevention should be to regenerate previously damaged cellular structures, which will lead to the weakening of immunodeficiency of the organism (Wojtas et al. 2017). Hence, the production of acute phase protein haptoglobin, an inflammatory marker, is activated by cytokines in the cows in early lactation (Ametaj et al. 2005).

Besides, the impact of inflammation on hepatic function reduction during the early lactation, and thus the reduction of productive and reproductive performance in high-yielding dairy cows was reported by Bertoni et al. (2008). A characteristic expression pattern for inflammatory mediators such as interferon- γ (IFN- γ), *tumour necrosis factor- α* (TNF- α), interleukin-17 (IL-17) and IL-10 suggests that the animals are immunosuppressed after calving (Heiser et al. 2015). Inflammatory mediators influence the development of the mammary gland and can lead to a reduction in the synthesis of milk during lactation (Cao et al. 2001).

The occurrence of inflammation is common in the post-parturient period and it is associated with the failure of successful transition. Thus, early postpartum treatment of cows with non-steroidal anti-inflammatory drugs (NSAID) aimed at blocking endogenous inflammation has been studied by various researchers, who often obtained different results (Bertoni et al. 2004, McDougall et al. 2009, Farney et al. 2013, Priest et al. 2013). Ketoprofen (KTP) or 2-(phenyl3-benzoyl) propionic acid is a nonsteroidal anti-inflammatory drug (NSAID) that is commonly used in a wide range of indications, both in human and veterinary medicine as an anti-inflammatory, antipyretic and analgesic medicine (Lees et al. 2004, Pardon et al. 2012). Our previous study has confirmed that ketoprofen applied in the first days after calving reduces the metabolic adaptation dependence on the intensity of lipid mobilization and ketogenesis during early lactation (Kovacevic et al. 2016), but we have not examined milk production and its association with metabolic and inflammatory parameters.

Therefore, the present study was aimed at determining whether ketoprofen therapy applied immediately after calving would positively affect milk production in standard lactation and whether milk yield was associated with metabolic and inflammatory response in the cows in early lactation.

Materials and Methods

Experimental Animals and Drug Administration

This research was approved by the decision, number 01-90/11-4, of the Ethical Committee of the University of Novi Sad, in order to safeguard the welfare of experimental animals. The study was carried out on 30 Holstein-Friesian multiparous dairy cows in second and third lactation (3-4 years old), located at the commercial dairy farm in Banatska Topola, Serbia.

The selected cows were in the postpartum period, in a normal body condition (scored from 3.25 to 3.50), clinically healthy and with no clinical symptoms of any metabolic disorders, mastitis and retention of fetal membrane and remain clinically healthy throughout the study period. Cows were daily monitored by veterinarians in first and second week of lactation. The control cows (n=15) were not treated, whereas the experimental ones (n=15) were treated from the first day after calving for another three consecutive days with 3 mg \times kg.bw.⁻¹ ketoprofen given with IM injection (Mediprofen®, Vetmedic, Serbia). On the basis of milk production the experimental and control group of cows were divided into two subgroups as follows: the cows with a higher milk production (the milk yield above the median ME +) and the cows with a lower milk production (the milk yield below the median ME -).

Sample Collection and Preparation

The blood samples were taken from the coccygeal vein three times: on the day of calving, at the end of the first week (on day 7) and the second week (on day 14) after parturition. The samples were collected using sterile vacuum tubes containing EDTA for biochemical analyses (BD Vacutainer® EDTA, BD Plymouth, UK). Metabolic parameters such as NEFA and BHB were determined using the colorimetric reaction in accordance with the manufacturer's instructions (Randox, UK and Point Scientific, USA), they were measured by means of the semi-automatic biochemistry analyzer (Analyzer Rayto RT-1904cv, Rayto L.L.C. Rayto Electronics Inc., China). The concentrations of acute phase protein (haptoglobin) and inflammatory cytokine (TNF- α) were determined by the ELISA method, according to the standard manufacturer's instructions (Cloud-Clone Corp., Houston, USA). The milk yield was observed in the experimental and control group of cows during the standard lactation period (305 days of lactation) using the computer software available at the farm.

Table 1. The impact of ketoprofen application on the milk yield in the cows in standard lactation.

	Ketoprofen	Control	<i>P</i> value
Total milk yield production	7960±470 L	7350±480 L	> 0.05 ^{NS}
Below the median	7700±219 L	7295±211 L	< 0.01 ^{**}
Above the median	8151±218 L	7853±207 L	< 0.01 ^{**}

NS = not significant

** = Highly significant

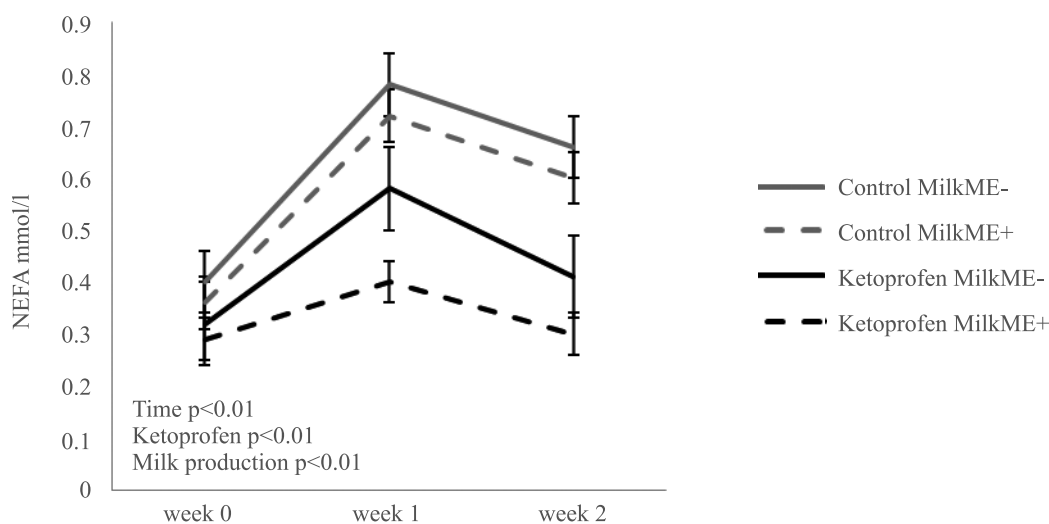


Fig. 1. NEFA concentration linked to the ketoprofen administration and milk yield.

Statistical Analysis

The statistical comparison of the groups was carried out by Statgraphics® Centurion XVII software and Microsoft Excel. The difference in the value of NEFA, BHB, TNF- α and haptoglobin in early lactation was determined using ANOVA analysis and LSD test in all four groups of cows (on the day of calving and in the first and second week after calving). The regression parameter *b* in the linear equation $Y = a + BX$ was determined, where *X*: represented the week of calving. It was determined to establish how the value of the metabolic parameters changed from the time of calving to the second week after calving. The difference in the value of the parameter *b* in the experimental and control group of cows was determined (*t*-test). Finally, the correlation between the achieved milk yield in lactation and total parameter *b* for all cows was determined (Pearson correlation). The difference in the milk yield between the experimental and control group was determined by *t*-test, being significant at $p < 0.05$, highly significant at $p < 0.01$, and non-significant at $p > 0.05$.

Results

A propionic acid-derived NSAID (i.e. ketoprofen) given to Holstein cows immediately after calving improved the standard 305-day milk yield and had a significant positive impact on the milk production parameters.

Ketoprofen-treated cows produced 7960 L of milk in the 305-days standard lactation, while the control group of cows produced 7350 L ($P = 0.23$, NS). Thus, as shown in Table 1, ketoprofen-treated cows produced 8.3% more milk in the standard lactation compared to the controls. A significant difference in the cow metabolic adaptation in function of ketoprofen application and milk production was achieved in standard lactation. Ketoprofen-treated cows with larger milk production (ME +) had significantly lower concentration of NEFA, BHB, TNF- α and haptoglobin in comparison to the control group in the first and second week after calving. No difference in the metabolic status, regardless of the achieved level of milk production, was found in the control group (Figs. 1-4).

The dynamic changes in the parameter values from 0 up to 2 postpartum weeks were also significant. A significantly smaller increase in the examined parameters compared to the controls was observed in ketoprofen-treated cows. The larger increase in the value of

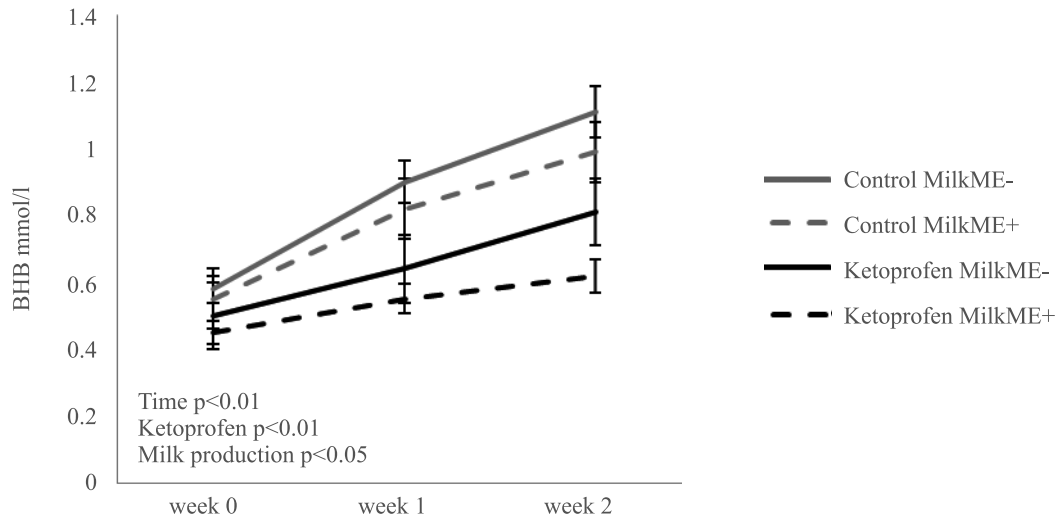


Fig. 2. BHB concentration linked to the ketoprofen administration and milk yield.

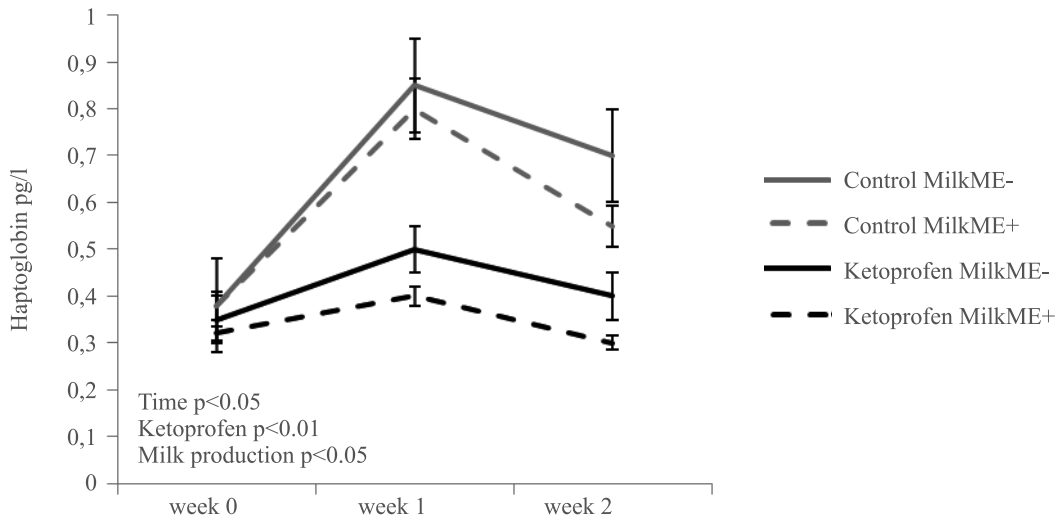


Fig. 3. Haptoglobin concentration linked to the ketoprofen administration and milk yield.

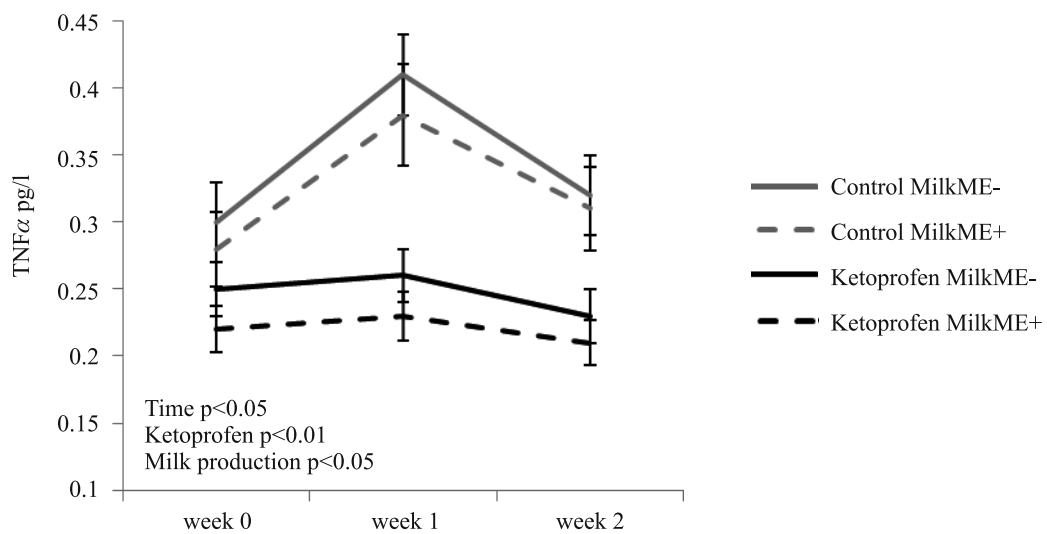


Fig. 4. TNF- α concentration linked to the of ketoprofen administration and milk yield.

Table 2. Correlation of milk yield with change of the value of parameters (NEFA, BHB, TNF- α and haptoglobin).

Regression parameter b (change from 0 to 2 week)	Impact of ketoprofen application to the value of changes in metabolic parameters during week			Correlation with milk production	
	Ketoprofen	Control	<i>P</i> value	Correlation with milk production	<i>P</i> value
NEFA	0.05 \pm 0.03	0.12 \pm 0.04	< 0.05*	- 0.38	< 0.05*
BHB	0.08 \pm 0.02	0.25 \pm 0.02	< 0.01**	- 0.42	< 0.05*
Haptoglobin	0.023 \pm 0.04	0.087 \pm 0.05	< 0.01**	- 0.4	< 0.05*
TNF- α	-0.01 \pm 0.01	0.015 \pm 0.01	< 0.05*	- 0.52	< 0.01*

* Significant; ** high significant

NEFA, BHB, TNF- α and haptoglobin in the period from the partum to the second postpartum week indicated a lower milk production (Table 2).

Discussion

Literature offers different results of NSAID application on milk production in dairy cows regarding the NSAID class of drugs, the timing of application and the length of exposure. Two studies dealt with the effects of flunixin meglumine, a commonly used anti-inflammatory drug in the veterinary medicine, on milk production. Duffield et al. (2009) treated the cows 2 and 24 hours after parturition, while Shwartz et al. (2009) treated them with this NSAID during the first three days after parturition, and they concluded that flunixin meglumine had no influence on the increase in milk production regardless of the duration of therapy.

A recent study indicated that the parenteral administration of another class of NSAIDs, e.g. oxicam (meloxicam), after parturition did not improve the milk yield. Neither did this study demonstrate any significant effect of meloxicam on the acute phase response of haptoglobin (Mainau et al. 2014). In addition, the results after the treatment with salicylic acid-derived NSAIDs in postpartum period were inconsistent. Bertoni et al. (2004) treated the cows with acetyl-lysine salicylate during the first 5 days of lactation, which increased the production of milk compared to the control group. Contrary to these findings, sodium salicylate applied in the first week of lactation did not increase the milk production in the period of seven days. The increased milk production was recorded on day 21 of the lactation (Farney et al. 2013).

Several researchers treated postpartum cows with propionic acid-derived NSAIDs, carprofen and ketoprofen. Meier et al. (2014) treated one group of cows in the first week after calving (on day 1, 3 and 5) and another one in the third week after calving (on day 19, 21

and 23) with carprofen. There was no increase in milk production in these two groups of cows regardless of the time of drug administration. Neither was the milk production affected by the application of 3 injections at intervals of 3 days at the dose of 1.4 mg of carprofen / kg. bw, between day 21 and 31 postpartum. The authors correlated this change with a delay in the NSAID application until 3 weeks after calving (Priest et al. 2013). Recently, carprofen administered on day 1, 3 and 5 post calving (i.e. early after calving), did not affect the cytokine expression at any recorded time point (Heiser et al. 2015). Richards et al. (2009) followed the impact of ketoprofen on the milk yield and fertility, where ketoprofen was administered at a dose of 3 mg \times kg. bw.⁻¹ immediately and 24 hours after parturition. The authors concluded that ketoprofen reduced the risk of retention of placenta after parturition, but it did not affect the increase in the milk yield. Contrary to these results, Newby et al. (2014) recorded a higher milk yield in cows treated with 3 mg/kg. bw. of ketoprofen in the transition period which could be explained with the longer treatment window. These findings are consistent with our results, where the cows treated with ketoprofen for three consecutive days after parturition gave 8.3% more milk compared to the control group in the standard lactation (Table 1). In addition, our results are in consonance with the results obtained by Carpenter et al. (2016), where the total milk yield increased from 7 to 9% after three day treatment with two different classes of NSAIDs (sodium salicylate and meloxicam).

There was an increase in NEFA during the postpartum period in the cows, which could represent a risk of the development of many inflammatory diseases (Calder 2008). Actually, the increased accumulation of fat in the liver activated the acute phase proteins response during the early postpartum period (Ametaj et al. 2005). It has also been shown that a higher concentration of NEFA is associated with increased haptoglobin plasma concentrations in the cows during this peri-

od (Stengärde et al. 2008, Kovac et al. 2009). Besides, there is a positive correlation between the concentrations of BHB and haptoglobin in dairy cows during the period of early lactation (Kovac et al. 2009). Haptoglobin, as an inflammatory marker in cows is increased during mastitis (Grönlund et al 2005, Huzzey et al 2015). Haptoglobin is also increased significantly during the early postpartum period in cows (Ametaj et al 2005, Kovac et al. 2009). Bertoni et al. (2004) reported that the cows treated with lysine acetyl-salicylate intramuscularly in the first 5 days of lactation had significantly lower concentrations of haptoglobin in comparison to the control group. This observation is in accordance with our study, where ketoprofen-treated cows with larger milk production have had a significantly lower concentration of NEFA, BHB and haptoglobin in contrast to the control group of cows in the first and second postpartum week. Grönlund et al. (2005) suggested that the increased concentration of haptoglobin during the postpartum period was associated with a lower milk yield due to its positive correlation with mastitis. An association between the increased concentration of haptoglobin and lower milk yield during the postpartum period was noticed by Huzzey et al. (2015). In addition, recombinant bovine TNF- α administration in dairy cows reduced the milk yield thus supporting the possibility that TNF- α was responsible for the changes in milk production and inflammatory parameters observed during coliform mastitis (Kushibiki et al. 2003).

Inflammatory mediators affect the development of the mammary gland and can lead to a reduction in the milk synthesis during lactation (Cao et al. 2001). It was also reported that NEFA and BHB concentrations around calving were associated with the reduced milk yield (Ospina et al. 2010, Huzzy et al. 2015). A possible reason could be that the lipid accumulation in the liver may contribute to health disorders and decrease the milk production (Drackley et al. 1999). Our analysis has demonstrated that a greater increase in the values of NEFA, BHB, haptoglobin and TNF- α in the period from calving to the second week after calving signifies a lower milk production.

In conclusion, ketoprofen applied in the first days following parturition had positive effects on the milk production in standard lactation. In fact, a higher milk yield in ketoprofen-treated cows could be associated with a lower degree of lipolysis and ketogenesis and reduced inflammatory response in the first two weeks after calving. To summarise, our results provide evidence for the presence of negative correlation between the milk yield and haptoglobin and TNF- α , being a component of acute phase response and NEFA and BHB, being metabolic indicators, in the first and second week after parturition.

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