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Research Article

Influence of Ketoprofen Application on Lipid Mobilization, Ketogenesis and Metabolic Status in Cows during Early Lactation [1]

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Abstract

Changes in metabolic functions in transition dairy cows represent a result of negative energy balance. This leads to increased lipid mobilization and ketogenesis, followed by increased concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Hence, high lipid mobilization and ketogenesis modulate inflammation response and vice versa. The aim of this study was to investigate correlations between ketoprofen administration, high lipid mobilization, ketogenesis and characteristics of metabolic adaptation in cows. Ketoprofen was administered intramuscularly in the concentration of 3 mg/kg, during three consecutive days in 15 postpartum cows. The control group included 15 cows which were not treated with ketoprofen. Blood samples were taken from coccygeal vein, after calving, in the first and second week of the postpartum period. When compared with control, ketoprofen administration decrease the levels of NEFA, BHB and total bilirubin, increase levels of glucose, albumin and cholesterol. Our results showed decreased activity of AST in ketoprofen treated cows in comparison with control group. There was an increase in the intensity of lipolysis and ketogenesis in 66.7% of cows, with NEFA and BHB values over the optimal results, because ketoprofen was not applied to these animals. Cows in the control group were 2 or 2.4 times more likely to come to a state of increased lipid mobilization and ketogenesis. We have found high concordance between NEFA and BHB, and metabolic parameters. This correlation was lower in experimental group of cows hence we can conclude that the use of ketoprofen immediately after calving reduces lipid mobilization and ketogenesis during early lactation and the metabolic adaptation dependence on the intensity of these two processes.

Keywords: *Ketoprofen, Dairy cows, Metabolic status, Early lactation*

Ketoprofen Uygulamasının Süt İneklerindeki Erken Laktasyon Döneminde Lipit Mobilizasyon, Ketogenez ve Metabolik Adaptasyon Üzerine Etkisi

Özet

Geçiş dönemindeki süt ineklerinin metabolik fonksiyonlarında değişiklikler negatif enerji dengesinin sonucudur. Bu da esterleşmemiş yağ asitlerinin (NEFA) ve beta-hidroksibütirat (BHB) düzeylerindeki artışı takiben lipit mobilizasyonu ve ketogeneze yol açar. Bu yüzden yüksek lipit mobilizasyonu ve ketogenez yangısal tepkimeler ile düzenlenir ve (veya?) tersi de söz konusu. Bu çalışmanın amacı, ineklerde ketoprofen uygulanması ile yüksek lipit mobilizasyonu, ketogenez ve metabolik adaptasyonun özellikleri arasındaki ilişkileri incelemektir. Ketoprofen doğum sonrası dönemde ineklerde art arda üç gün, 3 mg/kg dozunda intramüsküler olarak uygulandı. Kontrol grubu ketoprofen uygulanmayan 15 inekten oluşturuldu. Kan örnekleri doğum sonrası birinci ve ikinci haftada coccygeal toplardamardan alındı. Kontrol grubu ile karşılaştırıldığında, ketoprofen uygulaması NEFA, BHB ve total bilirubin düzeylerini azaltırken, glikoz, albümin ve kolesterol düzeylerinde ise artışa neden oldu. Bizim sonuçlarımız, kontrol grubuyla karşılaştırıldığında ketoprofen uygulanmasının AST aktivitesinde azaldığını gösterdi. İneklerin %66.7'si normalin üzerinde NEFA ve BHB değerine sahip, yüksek lipoliz ve ketogenez yoğunluğunda artış mevcuttu; çünkü bu hayvanlara ketoprofen uygulanmadı. Kontrol grubundaki inekler kuvvetle muhtemel 2 ila 2.4 kat daha fazla yüksek lipid mobilizasyonu ve ketogenez durumuna sahipti. Biz NEFA ve BHB ile metabolik parametreler arasındaki yüksek düzeyde uyum olduğunu bulduk. Bu ilişki deney grubu hayvanlarında daha düşüktü ve doğum sonrasında hemen ketoprofenin kullanımının lipit mobilizasyon ve ketojenezi erken laktasyon döneminde düşürdüğü ve metabolik adaptasyonun bu iki sürecin yoğunluğuna bağlı olduğu sonucuna varabiliriz.

Anahtar sözcükler: *Ketoprofen, İnek, Metabolik durum, Erken laktasyon*

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INTRODUCTION

The transition period for dairy cows is from 3 to 2 weeks prepartum to 2 to 3 weeks postpartum, when cows are undergoing numerous physiological adaptations, including endocrine and metabolic changes in order to meet increase in energy requirements that are necessary for the synthesis of milk [1-3]. These adaptive processes have resulted in increased lipid mobilization and ketogenesis, with increased concentration of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Hence, it contributes to the deposition of excess energy in the form of fats, which introduces body into a state of metabolic realignment, oxidative stress, inflammation and immunosuppression [1], and entails a number of consequences.

The correlation between inflammatory and metabolic response in cows is the subject of much contemporary research. Although the mechanism of action by which lipids induce inflammatory response is not known, there are several ways in which lipids trigger this response. The importance of fatty acids as modulators of inflammatory reaction is confirmed in many studies on humans and animal models [4-6]. Also, many studies have confirmed that excessive amounts of fat and elevated NEFA concentrations represent positive risk factors for the development of many pro-inflammatory peripartum diseases in dairy cows, including mastitis and metritis [7-9]. There is another important way in which fatty acids can affect the immune and inflammatory response, and that is through the biosynthesis of lipid mediators, including eicosanoids, lyso-phospholipids, sphingolipids, diacylglycerol, phosphatidic acid and ceramide [6]. Among these lipid mediators, eicosanoids are the regulators of the inflammatory response and they play a key role in the regulation of acute and chronic inflammatory reactions. Eicosanoids are formed from polyunsaturated fatty acids, which are metabolized by cyclooxygenase (COX) or lipoxygenase pathway [4]. There are two isoforms of cyclooxygenase; COX-1 and COX-2. The COX-1 isoform is present in the most tissues and synthesized by the action of low concentrations of prostaglandins and COX-2, which is associated with the biosynthesis of inflammatory mediators [4,10].

The clinical setting has proved that cows after calving were burdened by metabolic and inflammatory changes. It is important to find solution to reduce the incidence of inflammation and metabolic stress. Non-steroidal anti-inflammatory drugs (NSAIDs) are pharmacological group of drugs that equally affects both processes [11,12]. The use of NSAIDs can lead to reduced lipid mobilization and ketogenesis in the liver by inhibiting the action of epinephrine [13] and potentiating insulin action [14], reducing the concentration of pro-inflammatory cytokinesis whose value rises during early lactation and in ketosis [15-18].

As a NSAID, the primary mechanism of action of ketoprofen is a reversible inhibition of the cyclooxygenase enzyme, and a reduced biosynthesis of thromboxane A_2 and prostaglandin from arachidonic acid [19-21]. Ketoprofen inhibits both isoforms of the COX enzyme, although it is considered as a COX-1 selective drug [22,23] and it is used extensively in human and veterinary medicine. This NSAID has powerful anti-inflammatory, analgesic and antipyretic properties [24]. In veterinary practice, ketoprofen is used in the treatment of inflammatory and painful conditions of the bones and joints and muscular-skeletal systems in cattle, horses, dogs and cats, and in symptomatic treatment of colic in horses and cattle [25].

The aim of this study was to investigate the correlations between ketoprofen administration, high lipid mobilization, ketogenesis and metabolic adaptation in cows treated with ketoprofen immediately after calving.

MATERIAL and METHODS

Animals and Blood Collection

This study included 30 Holstein-Friesian cows divided in two groups, 15 cows in experimental and 15 cows in control group. The first group of cows was treated with ketoprofen (experimental group) and the second one was not treated with ketoprofen (control group). Ketoprofen was administered in therapeutic dose, intramuscularly, 3 mg *per kg* of body weight in the period of three consecutive days after parturition, starting at the first day postpartum. The mixture of vitamin C (vol. 10 ml, dose 1.000 mg) and rehydration agent (Saline solution 500 ml) was applied to all cows by parenteral route (slow i.v.) and thus, all of them were exposed to the same stress, due to application of the drug, and there was no need to apply a placebo to the control group in order to ensure an identical impact of stress for both groups. Administered volume and dose of saline solution and vitamin C not affect the metabolic adaptation or blood volume of cows and there is not interaction with ketoprofen.

Blood samples were taken three times, at the day of calving and during first and the second week after parturition. They were collected from the *coccygeal* vein using sterile vacuum tubes containing EDTA for bio-chemical analyses (BD Vacutainer® EDTA, BD Plymouth, UK).

This research was approved by the decision, number 01-90/11-4, of Ethical Committee of the University of Novi Sad, in order to safeguard the welfare of experimental animals.

Measurement of Metabolic Parameters

Metabolic parameters such as NEFA, BHB, glucose, total protein, albumin, AST, cholesterol, total bilirubin and calcium were determined using colorimetric reaction according to the manufacturer's instructions colorimetric kits (Randox, UK and Pointe Scientific, USA) and were

measured using a semi-automatic biochemistry analyzer (Analyzer Rayto RT- 1904cv, Rayto L.L.C. Rayto Electronics Inc., China).

Statistical Analyses

The effect of ketoprofen on the metabolic adaptation of cows has been tested using several statistical methods. The difference in the concentration of the metabolite (mean ± SD) in cows which were treated by ketoprofen, in comparison to the control group, was determined by t-test. Application of ketoprofen and its influence on intensive lipid mobilization and ketogenesis were evaluated using Chi-square test. All samples of the experimental and control group (45 samples, 15 cows x 3 weeks) were classified as those in which an optimum lipid mobilization was found (NEFA <0.6 mmol/L) and ketogenesis (BHB <1.1 mmol/L), and those in which we have found increased lipid mobilization (NEFA ≥0.6 mmol/L) and ketogenesis (BHB ≥1.1 mmol/ L).

We have also calculated positive predictive value (PPV), in order to determine the percentage of high lipid mobilization and ketogenesis that can be attributed to the fact that ketoprofen is not applied to cows, as well as likelihood ratio (LR) to determine the extent of the risk of intensive lipid mobilization and ketogenesis development in cows which were not treated with ketoprofen. Changes in the value of the metabolites in cows depended on the intensity of lipid mobilization and ketogenesis, and often showed a linear dependence. Differences in the correlation test between NEFA and BHB, and other blood parameters in experimental and control group of cows were determined by the Fischer r-to-z transformation test. Linearity is 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

When compared with control, ketoprofen administration decrease the NEFA and BHB levels (in the first and second week after calving, P<0.01), increase glucose levels (first week, P<0.05), increase albumin levels (second week, P<0.05), decrease AST levels (first and second week, P<0.05), increase cholesterol levels (the second week, P<0.01), decrease serum total bilirubin levels (second week, P<0.01). Difference in total protein and calcium concentration did not find between control and ketoprofen group. These results are provided in *Table 1*.

There was a statistically significant relation between application of ketoprofen and proportions of blood samples in early lactation with high lipid mobilization

Table 1. *Influence of ketoprofen application on metabolic status in cows in early lactation*
Tablo 1. *Erken laktasyon ineklerde metabolik durumu hakkında ketoprofen uygulamasının etkisi*

Metabolite (Mean±SD)	Group	Week 0	Week 1	Week 2
NEFA mmol/L	Control Ketoprofen P	0.38±0.13 0.32±0.11 >0.05	0.76±0.1 0.58±0.08 <0.01	0.6±0.12 0.49±0.09 <0.01
BHB mmol/L	Control Ketoprofen P	0.55±0.16 0.49±0.18 >0.05	0.88±0.12 0.63±0.15 <0.01	1.05±0.13 0.86±0.14 <0.05
Glucose mmol/L	Control Ketoprofen P	2.9±0.32 3.06±0.4 >0.05	2.15±0.43 2.65±0.49 <0.05	2.2±0.39 2.45±0.42 >0.05
Total protein g/L	Control Ketoprofen P	73±3.1 75±2.9 >0.05	69±3.3 72±3.2 >0.05	68±3.3 71±3.5 >0.05
Albumin g/L	Control Ketoprofen P	35±1.6 36±1.9 >0.05	30±1.9 34±1.8 >0.05	31±1.7 32±1.8 <0.05
AST IU/L	Control Ketoprofen P	92.1±10.5 87.5±10.1 >0.05	105.9±9.5 96.1±9.8 <0.05	112.7±9.9 101.3±11.1 <0.05
Cholesterol mmol/L	Control Ketoprofen P	2.5±0.3 2.2±0.4 >0.05	2.4±0.2 2.5±0.2 >0.05	1.9±0.2 2.6±0.3 <0.01
Total bilir. µmol/L	Control Ketoprofen P	5.1±1.6 5.9±1.7 >0.05	7.6±2.0 6.2±1.8 >0.05	9.5±1.8 7.1±1.6 <0.01
Ca mmol/L	Control Ketoprofen P	2.3±0.1 2.2±0.09 >0.05	2.2±0.12 2.1±0.11 >0.05	2.3±0.12 2.1±0.14 >0.05

(χ^2 = 16.2, P<0.01) and ketogenesis (χ^2 = 10, P<0.01), hence the proportion of cows with high lipid mobilization and ketogenesis were lower in ketoprofen treated cows. In the group of cows that had increased lipid mobilization and ketogenesis, 66.4% were from the control group (PPV value). The risk to develop high lipid mobilization and ketogenesis, when we did not apply ketoprofen, was 2 or 2.4 times higher than in the group of ketoprofen treated cows (LR). These results are provided in *Table 2*.

Linear relationship was significant between NEFA or BHB with glucose, cholesterol, AST and total bilirubin. Albumin showed linear relationship only with BHB. Total protein and Ca were not showed linear relationship with NEFA or BHB. The correlation coefficients were significantly lower in the experimental-ketoprofen group, which means that metabolic changes are not strongly defined by NEFA and BHB as in control group of cows. The results are provided in *Table 3*.

DISCUSSION

Testing of anti-inflammatory action of ketoprofen was carried out in cows in different conditions. It was found that the application of ketoprofen during fistula surgery on cows positively influences the postoperative period [26]. Also, one study indicates that ketoprofen promoted the contractions of rumen and decreased inflammatory responses to mastitis [11]. In both cases, it is shown that ketoprofen has a positive effect on food intake in cows, which is important because during the

Table 2. *Influence of ketoprofen application on the intensity of lipolysis and ketogenesis in cows in early lactation*
Tablo 2. *Erken laktasyon ineklerde lipoliz ve ketogenez yoğunluğuna ketoprofen uygulamasının etkisi*

Intense of Lipid Mobilization and Ketogenesis	Ketoprofen Aplication		Chi-square (P)	PPV	LR
	Yes (Ketoprofen)	No (Control)			
High NEFA ≥0.6 mmol/l	11	21	16.2 (P<0.01)	66.7%	2.4
Normal NEFA <0.6 mmol/l	34	14			
High BHB ≥1.1 mmol/l	15	18	10.0 (P<0.01)	66.7%	2.0
Normal BHB <1.1 mmol/l	30	17			

Table 3. *Influence of the application of ketoprofen on correlations between NEFA or BHB with metabolic parameters in cows during early lactation*
Tablo 3. *Erken laktasyon döneminde ineklerde metabolik parametreleri ile NEFA veya BHB arasındaki korelasyon üzerine ketoprofenin uygulamasının etkisi*

Metabolite	Group	NEFA	BHB
BHB mmol/L	Ketoprofen Control P	0.62 0.86 <0.01	/ / /
Glucose mmol/L	Ketoprofen Control P	-0.52 -0.81 <0.01	-0.59 -0.84 <0.01
Total protein g/L	Ketoprofen Control P	0.19 0.25 >0.05	0.27 0.29 >0.05
Albumin g/L	Ketoprofen Control P	0.26 0.33 >0.05	0.43 0.64 <0.05
AST IU/L	Ketoprofen Control P	0.49 0.76 <0.05	0.51 0.81 <0,01
Cholesterol mmol/L	Ketoprofen Control P	-0.37 -0.56 <0.05	0.51 -0.74 <0.01
Total bilirubin µmol/L	Ketoprofen Control P	0.32 0.65 <0.01	0.49 0.82 <0.01
Ca mmol/L	Ketoprofen Control P	0.26 0.48 <0.05	0.26 0.33 >0.05

perinatal period, food intake is being reduced and we can also see a negative energy balance.

Increased NEFA and BHB represent characteristic changes in the period after calving in cows due to increased lipid mobilization and ketogenesis. Also, excessive elevations in BHB concentrations are associated with poor health and production outcomes in dairy cattle [27]. The increased lipid mobilization and ketogenesis lead to numerous changes in metabolic adaptation in cows and to the emergence of various diseases, such as ketosis. The results show different limits for NEFA (0.5 to 0.7) and BHB (0.9 to 1.2), above which the risk for poor metabolic adaptations or disease increases significantly [28-32].

It is well known that NEFA concentration are increased in the week after calving as a result of energy deficit and changes in hormonal status of cows [33,34]. These changes lead to increased lipogenesis and ketogenesis in the liver, while high concentrations of BHB decrease rates of β-oxidation, gluconeogenesis and the citric acid cycle [27]. In our study, we have found a significant increase in the concentration of NEFA and BHB in the weeks after parturition, but it is less conspicuous in cows which are treated with ketoprofen. It is known that lipid mobilization is the most intensive in the first week after calving, while ketogenesis is the most intensive in the second week after calving, and our findings have confirmed that [35-37].

Fatty acids can be used as precursors for inflammatory eicosanoids and that is the reason why increased NEFA concentration in early lactation may promote inflammation and why it has an impact on the duration and magnitude of it [4]. In another study [13], the NSAIDs in isolated rat adipocytes, inhibited stimulated lipolysis by reducing the release of fatty acids from adipose tissue to the liver by inhibiting the epinephrine-stimulated lipolysis. This, in part, explains the protective action of NSAIDs and potentially cause of decrease in NEFA and BHB concentration. There is a strong correlation between concentration of NEFA, BHB and acute phase proteins in cows after parturition [38]. Decrease in concentrations of total proteins and albumin levels could be linked to the decreased liver synthesis of albumin during inflammation [13]. In contrast to these findings, there is an increase of albumin levels in cows treated with ketoprofen. It could be potentially linked with anti-inflammatory influence of ketoprofen.

It is well known that peripartal period is characterized by increased bilirubin concentration [40] and decreased cholesterol concentration [41]. The bilirubin concentration is increased as a result of puerperal ketosis [39]. The cholesterol concentration is decreased as a result of metabolic disorder [40] and hepatic lipidosis [27]. Low concentrations of the AST in the blood are also present in the healthy animals, but AST rises in the cows around calving [42]. In this study, there was an increase in cholesterol concentrations and decrease in AST and bilirubin concentrations in ketoprofen treated cows, since there is smaller influx of NEFA and BHB in the liver as a result of a lower degree of fatty liver. It is probably because of influence of ketoprofen. Examining the effects of NSAIDs administration in experimentally induced hyperlipidemia in rats [43], it was concluded that these drugs significantly reduce the total cholesterol, triglycerides and LDL concentrations in the plasma of hyperlipidemic rats. Their results potentially link anti-inflammatory activity with hypolipidemia. Trevisi et al.[44] demonstrated that in high yielding cow with high IL-6 concentration as inflammatory marker there is lower liver functionality index. Correlation coefficients between determined metabolic parameters and the values of NEFA and BHB were decreased and significantly lower in the group of cows treated with ketoprofen. NEFA and BHB showed influence on metabolic adaptation in early lactation [28], and connection between metabolic adaptation and lipolisis/ketogenesis. It depends on energy balance and period of lactation [45].

In conclusion, the use of ketoprofen immediately after calving reduces lipid mobilization and ketogenesis during early lactation, as well as metabolic adaptation dependence on the intensity of these two processes. Accordingly, the use of ketoprofen could be recommended in the prevention of ill effects of intensive homeorhesis and the adjustments on milk production during early lactation.

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