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Effects of Ketoprofen Administration on Relation between Acute Phase Proteins and Metabolic Parameters in Cows during Early Lactation

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ABSTRACT

***Background*:** Acute phase proteins (APPs) are clinically useful parameters for measuring the occurrence and severity of inflammatory responses in cattle. As large group of proteins, APPs are mainly secreted by hepatocytes whose concentration increase or decrease as response to tissue injury, inflammation or infection. The transition from late pregnancy to early lactation is associated with a compromised immune status coupled with increased acute phase response. Non-steroidal anti-inflammatory drugs (NSAIDs) are used in treatment of inflammatory conditions. The main objective of the present study was to analyze the effects of a preventive administration of the NSAID (ketoprofen) in post-calving dairy cows on concentration of APPs (haptoglobin and fibrinogen) and their relationship with indicators of liver function.

***Materials, Methods & Results*:** The treatment group (n = 15) was given an intramuscular injection of ketoprofen in the concentration of 3 mg/kg during three consecutive days after calving. The control group (n = 15) was not treated with ketoprofen. Blood samples were taken from coccygeal vein, on the first day of treatment and in the first and second week postpartum and they were analyzed for metabolic parameters (albumin and aspartat aminotransferaze) and APPs such as haptoglobin and fibrinogen. Compared with control, ketoprofen administration decrease the levels of haptoglobin and fibrinogen and AST activity. Increase in albumin concentration was recorded in experimental group of cows compared with control. Negative correlations ($P < 0.01$) were found between the haptoglobin concentration and albumin concentration and aspartate aminotransferaze activity.

***Discussion*:** As inflammatory indicator in dairy cows, it is recorded that serum concentrations of haptoglobin is increased during mastitis. Beside haptoglobin, fibrinogen represents one of the APPs whose serum concentration increases during response in the acute phase. During the first and second week after partus, in cows with acute puerperal metritis, an increase in fibrinogen concentration was observed in relation to clinically healthy animals. Based on our results, it can be concluded that an intramuscular injection of ketoprofen (3 mg × kg. bw.-1) administered in cows in the first days following parturition reduces the concentration of some APPs (haptoglobin and fibrinogen). In regard to Hp concentration, the treatment success agreed with previous results using i.m. acetyl-lysine salicylate during the first 5 days of lactation. Reduction in APPs concentration in our research results could be attributed to the anti-inflammatory effect of ketoprofen. In addition, use of ketoprofen reduces the intensity of relationship between inflammatory markers and indicators of liver function. Impaired functional capacity of the liver may be associated with reduced albumin concentration in the peripartal period, and during inflammatory or infectious diseases. Increase in albumin concentration in experimental group of cows compared to the control in our research could be attributed to the anti-inflammatory effect of ketoprofen. Hence it can be concluded that the use of ketoprofen immediately after calving reduces the intensity of relationship between inflammatory marker liver function and that NSAID treatment could be used in in improving animal well-being and controlling of the occurence and intesity of postpartum diseases.

Keywords: NSAID, haptoglobin, fibrinogen, BHBA, NEFA.

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INTRODUCTION

During transition period cows enter a period of negative energy balance when nutrient demands for lactation exceed nutrient intake [7] with high levels of non-esterified fatty acids (NEFA) derived from adipose tissue triacylglycerol catabolism [4,21,27]. State of reduced function and fat accumulation in the liver experiences is coupled with increased acute phase response during peripartal period characterized with secretion of a large group of proteins, called acute phase proteins (APPs) [1] whose plasma concentration increase or decrease as response to tissue injury, inflammation, stress, trauma or infection [24]. Haptoglobin (Hp) represents the most studied APP in detection of diseases in cattle as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses [8,12,26]. Beside Hp, fibrinogen represents one of the APPs whose serum concentration increases during response in the acute phase [15].

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used in human and veterinary medicine in treatment of pain and wide range of indications such as inflammatory conditions [13,18,22]. Use of ketoprofen is currently licensed for cows in the European Union at a dose rate of 3 mg/kg body weight [10]. Although ketoprofen has been indicated in cows for almost 20 years, there have been few reports on its clinical efficacy [3,6,19].

The aim of the present study was to analyze the effects of a preventive administration of ketoprofen in post-calving dairy cows. This analysis may contribute to a better understanding of the changes in transition period including correlation between APPs and indicators of liver function.

MATERIALS AND METHODS

Experimental animals, drug administration and sample collection

The study was conducted on 30 Holstein-Friesian multiparous dairy cows in second and third lactation (3 - 4-year-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 experimental cows (n = 15) were treated with ketoprofen whereas control cows (n = 15) were not treated. Experimental cows were treated with ketoprofen from the first day after calving for another three consecutive days with 3 mg × kg. bw.-1 ketoprofen given with IM injection. 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. 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[1] containing EDTA for biochemical analyses.

Measurement of metabolic parameters and acute phase proteins

Metabolic parameters (albumin and AST) were determined by using the colorimetric reaction according to the manufacturer's instructions using Colorimetric Kits[2], they were measured by means of the semi-automatic biochemistry analyzer[3]. The concentrations of acute phase proteins (haptoglobin and fibrinogen) were determined by the ELISA method, according to the standard manufacturer's instructions using Colorimetric Kits[4].

Statistical analysis

The difference in the concentration of the metabolic parameters and acute phase proteins (mean ± SD) in ketoprofen treated cows, in comparison to the control group, was determined using *t*-test. The statistical comparison of the groups was carried out by Statgraphics® Centurion XVII software and Microsoft Excel. The data analysis was performed using SPSS, version 19.0, software package for Microsoft Windows[5]. Compared results with $P < 0.05$ were considered as statistically significant. Differences in the correlation test between acute phase proteins and metabolic 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).

RESULTS

profen in the first days following parturition was efficient in a reduction in the concentration of Hp ($P < 0.05$) in the experimental group of cows compared to the control, in the first and second week after the partus (Figure 1). Fibrinogen concentration was significantly ($P < 0.05$) lower in the second week after partus in experimental group of cows compared to the control (Figure 2). Our results showed increase in albumin concentration in experimental group of cows compared to the control, in the first and second week after the partus (Figure 3). We found that AST activity was lower in ketoprofen treated cows than in control group (Figure 4). In the assessment of correlations between the haptoglobin, significant negative correlation ($P < 0.01$) were found between the haptoglobin concentration and albumin concentration and aspartate aminotransferaze activity (Table 1). This correlation was lower in ketoprofen treated cows.

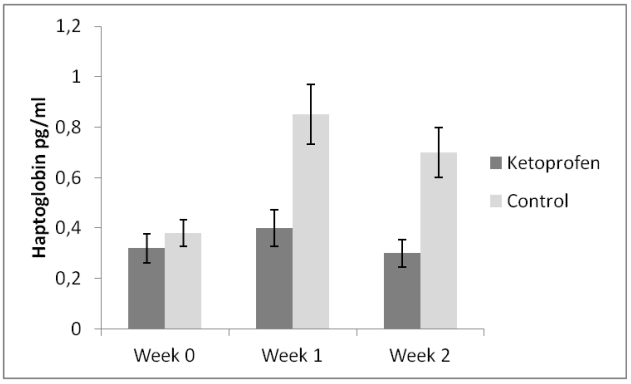

Figure 1. Influence of ketoprofen application on haptoglobin concentration.

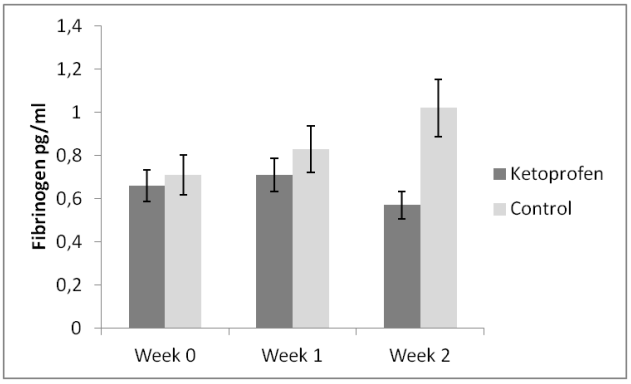

Figure 2. Influence of ketoprofen application on fibrinogen concentration.

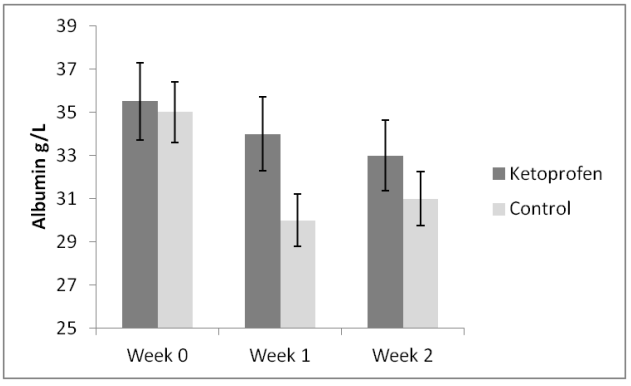

Figure 3. Influence of ketoprofen application on albumin concentration.

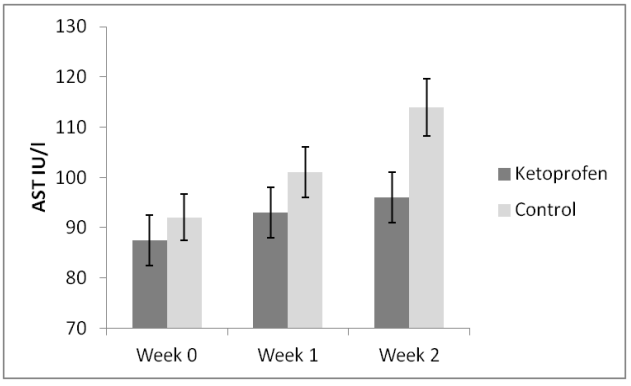

Figure 4. Influence of ketoprofen application on AST activity.

Table 1. The impact of ketoprofen application on the intensity of correlation between acute-phase proteins and biochemical parameters.

		Correlation	
		Haptoglobin	Fibrinogen
Albumin	Ketoprofen	- 0.44*	-0.29
	Control	- 0.69*	-0.30
	P	< 0.01	NS
AST	Ketoprofen	0.78*	0.28
	Control	0.56*	0.28
	P	< 0.01	NS

*Correlation is statistically significant minimum $P < 0.01$.

DISCUSSION

As inflammatory indicator in dairy cows, it is recorded that serum concentrations of Hp is increased during mastitis [14,20]. In regard to Hp concentration, the treatment success agreed with previous results using i.m. acetyl-lysine salicylate during the first 5 days of lactation [5,30]. According to Jeremejeva *et al*. [16], during the first and second week after partus, in cows with acute puerperal metritis, an increase in fibrinogen concentration was observed in relation to clinically healthy animals. Reduction in APPs concentration in our research results could be attributed to the anti-inflammatory effect of ketoprofen.

As one of the major APPs, Hp synthesis and secretion by the liver is regulated by the combined effect of cytokines and glucocorticoids [2]. Actually, one of the effects of cytokines is to trigger the synthesis of positive APPs (Hp, fibrinogen, C-reactive protein and serum amyloid A), and to reduce the synthesis of negative APPs (albumin, prealbumin, retinol binding protein and transferin) [9,23]. Our results are in accordance with this findings because we found negative correlation between Hp and albumin, significantly lower in ketoprofen treated cows than in control group. Besides, impaired functional capacity of the liver may be associated with reduced albumin concentration in the peripartal period [29]. In addition, the secretion of albumin was reduced during inflammatory or infectious diseases [11]. Increase in albumin concentration in experimental group of cows compared to the control in our research could be attributed to the anti-inflammatory effect of ketoprofen since inflammation is the principal cause of a decrease in serum albumin concentration.

Sun *et al*. [28] found that high AST was associated with impaired liver function in postpartum dairy cows. Lower AST activity in ketoprofen treated cows than in control group may be related with anti-inflammatory efects of NSAID used in experiment. High Hp concentration was associated with AST enzyme activity as marker of impaired liver function [25]. Hp serum concentrations and activity of AST tended to be greater in cows with left displaced abomasum compared to their healthy counterparts [17]. Besides, high Hp concentrations 1 week after calving might also impared liver function, reflected by changes in enzyme concentration and associated with a higher incidence of peri- and postpartum disorders [28]. These findings comply with our findings where we found significantly stronger decrease correlation between Hp concentration and AST activity in ketoprofen-treated cows compared to the control group.

CONCLUSIONS

Based on our results, it can be concluded that an intramuscular injection of ketoprofen (3 mg × kg. bw.$^{-1}$) administered in cows in the first days following parturition reduces the concentration of some APPs (haptoglobin and fibrinogen) and that use of ketoprofen reduces the intensity of relationship between inflammatory markers and indicators of liver funcition. Additionally, NSAID treatment could be used in in improving animal well-being and controlling of the occurence of postpartum diseases, but further investigations regarding ketoprofen influence on proinflammatory cytokine concentration are needed.

MANUFACTURERS

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***Ethical approval*.** All procedures were 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.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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