J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 43(2): 147-153, 2019
Doi: 10.33483/jfpau.538179
ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECT OF PREPARTUM ANTIOXIDANT ADMINISTRATIONS ON BLOOD BHBA CONCENTRATION IN COWS

İNEKLERDE PREPARTUM ANTİOKSİDAN UYGULAMALARININ KAN BHBA KONSANTRASYONU ÜZERİNE ETKİSİ

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ABSTRACT

Objective: *The aim of this study was to investigate the effect of prepartum antioxidant administrations on blood Beta-hydroxybutyric acid (BHBA) concentration on day 7 postpartum in Holstein dairy cows. Antioxidants are defined as substances that delay or inhibit oxidative damage. Oxidants, such as reactive oxygen species (ROS) are produced as a consequence of adenosine triphosphate (ATP) production in mitochondria. Oxidants can cause tissue damage by damaging cells' macromolecules, such as deoxyribonucleic acid, proteins, and lipids. Lipids are the most sensitive macromolecules against oxidative damage. Oxidative stress can both disrupt the mechanism of insulin and induce more lipolysis. Therefore, ROS overproduction can enhance non-esterified fatty acids (NEFA) and BHBA production.*

Material and Method: *Eighty pregnant multiparous Holstein dairy cows were used in this study. The cows were divided into treatment (G1; n=40) and control groups (G2; n=40). Solutions containing vitamins (A, D, E) and trace elements (Cu, Se, Mn, Zn) were administered intramuscularly to G1 on 21±5 and 10±5 days before the expected delivery time (280 days). G2 was given the same amount of saline injections as the placebo.*

Result and Discussion: *It was found that blood BHBA concentrations were lower in the G1 (0.73±0.20 mmol/L) compared to the G2 (0.84±0.29 mmol/L). In conclusion, it was determined that prepartum antioxidant administrations decrease blood BHBA concentration in the postpartum period in dairy cows.*

Keywords: *antioxidant; BHBA; cow; oxidative stress*

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** This article was produced from the Ph.D. thesis/Bu makale doktora tezinden üretilmiştir

Submitted/Gönderilme: 11.03.2019 **Accepted/Kabul:** 19.04.2019

ÖZ

Amaç: *Sunulan çalışmada, Holstein sütçü ineklerde prepartum antioksidan uygulamalarının postpartum 7. günde kan Beta-hidroksibütirik asid (BHBA) konsantrasyonu üzerine etkisinin araştırılması amaçlandı. Antioksidanlar oksidatif hasarı geciktiren veya engelleyen maddeler olarak tanımlanmaktadır. Reaktif oksijen türleri (ROS) gibi oksidan maddeler mitokondrilerde adenozin trifosfat (ATP) üretiminin bir sonucu olarak oluşturulur. Oksidan maddeler hücrelerin dezoksiribonükleik asit, proteinler ve lipidler gibi makromoleküllerine zarar vererek doku hasarına neden olabilir. Lipitler oksidatif hasara karşı en hassas makromoleküllerdir. Oksidatif stres insülin mekanizmasını bozarak daha fazla lipolize neden olabilir. Böylece, aşırı derecede ROS üretimi esterleşmemiş yağ asitlerini (NEFA) ve BHBA üretimini artırabilir.*

Gereç ve Yöntem: *Bu çalışmada 80 adet multipar Holstein ırkı gebe sütçü inek kullanıldı. İnekler, tedavi (G1; n = 40) ve kontrol grubu (G2; n = 40) olmak üzere iki gruba ayrıldı. G1'e vitamin (A, D, E) ve iz element (Cu, Se, Mn, Zn) içeren solüsyonlar beklenen doğum tarihinden (280 gün) önce 21±5 ve 10±5 günlerde intramuskuler yolla uygulandı. G2'ye, plasebo ile aynı miktarda serum fizyolojik enjeksiyonu yapıldı.*

Sonuç ve Tartışma: *Kan BHBA konsantrasyonları G2'ye (0.84±0.29 mmol/L) göre G1'de (0.73±0.20 mmol/L) daha düşük bulundu. Sonuç olarak, sütçü ineklerde prepartum antioksidan uygulamalarının, postpartum dönemde kan BHBA konsantrasyonunu azalttığı tespit edildi.*

Anahtar Kelimeler: *antioksidan; BHBA; inek; oksidatif stres*

INTRODUCTION

Antioxidants are defined as substances that delay or inhibit oxidative damage [1,2]. These substances, are obtained in two different ways as endogenous (enzymatic (such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase), and non-enzymatic (such as glutathione, uric acid, melatonin, bilirubin, albumin)) and exogenous (such as Vitamins of A, E, D and C, selenium (Se), copper (Cu), manganese (Mn), zinc (Zn)) [3, 4]. Enzymatic and non-enzymatic antioxidants work in concert [5] and protect the body against oxidative damage [5, 6]. Moreover, exogenous antioxidants are used in the structure of endogenous antioxidants [4]. For example, SOD enzymes depend on Cu and Mn to provide the dismutation of O_2^- to H_2O_2. Later on, the reduction of H_2O_2 to H_2O and O_2 is ensured by catalases or Se-dependent GPx and thioredoxin reductase (Trx) enzymes [4]. However, when the production of oxidant substances exceeds the body's antioxidant capacity, oxidative stress occurs [7]. Free radicals are produced during the conversion of foods into a form of ATP using O_2 in mitochondria [8, 9]. And, reactive oxygen species (ROS) such as H_2O_2, HO., HO_2, O_2^-, $ROO^{\bullet}$ [9] are the most abundant free radicals in biological systems. ROS can cause tissue damage by damaging cells' macromolecules, such as deoxyribonucleic acid, proteins, and lipids [2]. It was reported that lipids are the most sensitive macromolecules against to oxidative damage [10]. Oxidative stress can induce more lipolysis [2] and disrupt the mechanism of insulin in cows [11]. Therefore, ROS overproduction enhances non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) production and results in metabolic stress in dairy cows [2]. Furthermore, because it is difficult to maintain an energy balance due to increased metabolic activities and nutritional needs in the periparturient period, dairy cows already experience a negative energy balance (NEB) [12]. NEB also induces the mobilization of body fat in the form of NEFA and subsequently it results in the increase in blood BHBA concentrations [13]. High levels of BHBA impair hepatic function and adversely affect health in many ways [14]. Antioxidants improve the mechanism of glucose and insulin in dairy cows [11]. But, the production of oxidants and the use of antioxidants are significantly increased due to increased metabolic activity during this period. For these reasons, the need for antioxidants increases and antioxidant therapy can be necessary in periparturient cows [5, 15]

The aim of this study was to investigate the effect of prepartum vitamins (A, D, E) and trace element (Cu, Zn, Se, Mn) administration on blood BHBA concentrations on day 7 postpartum in Holstein dairy cows.

MATERIAL AND METHOD

Animals

Eighty pregnant multiparous Holstein dairy cows from a commercial dairy herd in Aksaray, Turkey were used in this study. The cows had a milk yield of about 10,000 kg (per cow, per lactation), were milked twice daily, received periodic systematic vaccination, were fed with a total mix ration (TMR), were managed in free-stall barns and were clinically healthy. And the cows had a similar body condition score. The cows were divided into treatment (G1; n=40) and control group (G2; n=40) by the randomized grouping method.

Experimental Design

Eight ml of solutions (Ademin®, Ceva) containing vitamins A, D and E (500,000 I.U. of vitamin A, 75,000 I.U. of vitamin D_3, and 50 mg of vitamin E per ml) and 10 ml of solutions (Activate®, Alke) containing Cu, Se, Mn and Zn trace elements (2.5 mg of copper gluconate, 1.25 mg of sodium selenite, 5 mg of manganese gluconate, and 5 mg of zinc gluconate per ml) were administered intramuscularly using a 18-G cannula to each cow into G1 on 21±5 and 10±5 days before expected delivery time (280 days). G2 was given in the same amounts of saline injections as a placebo to each cow.

Analysis

Blood samples were collected from the coccygeal vein into vacutainer tubes using 18-G needles. Before clotting, blood BHBA concentrations were measured using a hand-held meter Vet TD-4235 β-ketone monitoring system (Hasvet, Turkey) and commercial kits (LOT: WK18D923-B0E, Hasvet, Turkey) within 1 minute after sampling on day 7±3 postpartum.

Statistical analysis

Individual cow data were exported from farms' herd management software (Dairy Plan, GEA Farm Technologies, ABD) to Microsoft Excel. Statistical analysis was performed with SPSS software (Version 14.01). The conformity of the data to the normal distribution was evaluated with the shapiro-wilk test. And the distribution was found as normal. For this reason, the relationship between blood BHBA concentrations and groups (G1 and G2) was performed with an Independent Sample t-Test. The statistical analysis limit was accepted as $p < 0.05$.

RESULT AND DISCUSSION

In the results of this study, it was found that blood BHBA concentrations were lower in the G1 (0.73±0.20 mmol/L) compared to the G2 (0.84±0.29 mmol/L). In addition, BHBA results were significant because of the p-value < 0.05 (Table 1).

Table 1. Comparison of the blood BHBA concentrations in G1 and G2.

	G1	G2	p
BHBA (mmol/L)	0.73±0.20	0.84±0.29	0.046

It was also detected that G1 and G2 had different BHBA distributions. And blood BHBA concentrations were lower in G1 compared to G2. But, the lowest BHBA values were measured to be 0.2 mmol/l and 0.4 mmol/l in G2 and in G1, respectively (Figure 1). At the end of this study, it was thought that the lipolysis rate was lower in G1 than G2.

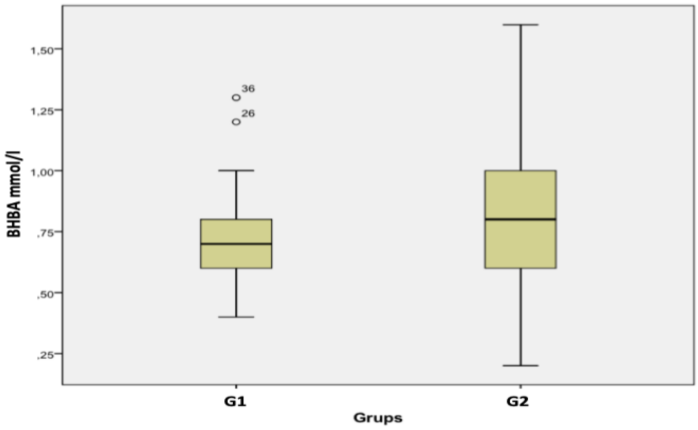

Figure 1. Blood BHBA distributions in G1 and G2 on day 7 postpartum in Holstein dairy cows.

Moderate levels of ROS are required for physiological processes like cell differentiation or proliferation and host immune response [2]. The overproduction of free radicals has an important roll in the development of ailments such as cancer, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [9]. For these reasons, antioxidants are indispensable substances for the body [3]. Antioxidant administrations before calving can have a positive effect on the mechanism of glucose and insulin in dairy cows [11]. Furthermore, insulin has an antilipolytic effect on fat tissue [16]. Oxidative stress can cause insulin resistance [11], and when the antilipolytic effect of insulin deteriorates, lipolysis is stimulated [16].

It was reported that lipids are the most sensitive macromolecules against oxidative damage of ROS in the body [10]. Another study showed that lipolysis increased ROS production [17]. Some researchers declared that overproduction of ROS increases NEFA and BHBA concentrations in the blood of dairy cows [2]. Moreover, inadequate levels of essential vitamins and trace elements result in diminished functional capabilities of immune cells. If ROS cannot be neutralized, its rate increases in the body [5]. Thus, the BHBA concentration increases due to lipolysis and then diseases enhance. These cases are more complex in the periparturient period because cows are already in NEB and oxidative stress. Therefore, the supplementation of antioxidants is very important during this period [2].

In another study, vitamin E and Se were administered to Holstein cows intramuscularly at 15±2 days before expected calving. It was found that blood BHBA levels were lower in the treatment group (10.31 mg/dL) compared to the control group (13.81 mg/dL) on days 3-7 postpartum (p-value nonsignificant). Moreover, an intravenous glucose tolerance test was performed on these cows on days 3-7 postpartum and better results were obtained in the treatment group compared to the control group [11].

It was reported that the blood BHBA normal level was less than 0.6 mmol/l in dairy cows [18]. In another study, it was suggested that the threshold value of BHBA was 1.200 μmol/L [19]. But, blood BHBA levels increase due to increased metabolic demands and NEB in the periparturient period [2]. In our study, the level of BHBA was found to be 0.73±0.20 mmol/l and 0.84±0.29 mmol/l on the 7th day postpartum in G1 and G2, respectively.

In conclusion, it was determined that prepartum antioxidant administrations decrease blood BHBA concentration in the postpartum period in dairy cows. Moreover, low levels of BHBA concentration in G1 showed the efficacy of antioxidants on lipolysis when compared to G2. Furthermore, it is thought that antioxidant administrations caused this effect by reducing the harmful effects of free radicals and improving the mechanism of insulin and glucose.

ACKNOWLEDGEMENT

This article was produced from the Ph.D. thesis titled 'Investigation Effect of Prepartum Antioxidant Administration on Oxidative Stress Energy Metabolism and Colostrum Quality in Cows' which belongs to Serdal Kurt. Ankara University-Scientific Research Projects (BAP) Coordinator project number: 18L0239022.

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