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*Original article*

# Effects of antioxidant vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) on some metabolic and reproductive profiles in dairy cows during transition period

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## Abstract

The objective of this study was to determine the effects of some antioxidant vitamins and trace elements on some metabolic and postpartum reproductive profiles in dairy cows during transition period. In the study, altogether 20 clinically healthy Brown Swiss dairy cows (aged 4-5 years-old) under the same management and feeding conditions in periparturient period were used. The animals were divided into two equal groups: control (C) and treatment (T) group (n=10 for each group). Vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) were administered intramuscularly into the cows of the T group, while isotonic saline, as *placebo*, was injected subcutaneously into those in the C group. Blood samples were collected by venipuncture of the jugular vein at the beginning of transition period, parturition and 3-weeks after the parturition. The metabolic and reproductive parameters were determined. In the C group, statistically significant changes were observed in the levels of non-esterified fatty acids (NEFA), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein (TP) ($p<0.05$), glucose (GLU), progesterone ($P_4$) ($p<0.01$), total cholesterol (T.CHOL), triglycerides (TG), UREA, creatinine (CRSC) and total bilirubin (TBIL) ($p<0.001$). In the T group, significant changes in the levels of NEFA, TBIL ($p<0.05$), T.CHOL, HDL, LDL ($p<0.01$), TG, GLU, $P_4$, TAC and TOC ($p<0.001$) were observed. It was concluded that the administration of various vitamins and trace elements could be effective to improve some metabolic and reproductive profiles in dairy cows during the transition period.

**Key words**: dairy cow, metabolic profile, reproductive profile, trace element, transition period, vitamin

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## Introduction

Transition period includes the end of gestation and early lactation (Laporta and Hernandez 2015). In this period, rapid variation in physiologic reactions is critical (Basoglu and Sevinc 2004). The examination of blood provides important data on the metabolic profile (Pilar et al. 2014). This profile may vary according to area, animal's breed, milk yield, lactation period and nutrition (Taylor et al. 2003). Determination of the levels of non-esterified fatty acids (NEFA) and beta-hydroxy butyric acid (BHB) in circulation is the most important for evaluation of the energy balance status in periparturient cows. Low-density lipoprotein (LDL) and high density lipoprotein (HDL) are used in the diagnosis of metabolic diseases that can be configured in pre- and post-partum periods as well as the assessment of nutritional and health statues of the animals (Duffield 2000, Holtenius et al. 2003). In addition, for evaluating the metabolic profile in periparturient cows, some biochemical tests are scrutinized as aspartate amino transferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), glucose (GLU), total protein (TP), albumin (ALB), urea, creatinine (CRSC) and total bilirubin (TBIL) levels (Sevinc et al. 1999, Van Saun 2004).

The assessment of reproductive profile is important for normal fertility processes in dairy cows. In this sense, follicle stimulating hormone (FSH) and luteinising hormone (LH) are the two anterior pituitary hormones controlling gonadal function (Pierce and Parsons 1981). Progesterone ($P_4$) is responsible for maintaining pregnancy (Mann and Lamming 1999).

Oxidative stress leads to the degradation of reproductive, physiological and metabolic functions causing an increase in reactive oxygen species (ROS) and reduction in antioxidant protection mechanisms (Ulutas et al. 2005). Defence mechanisms against free radical-induced oxidative stress involve: preventative mechanisms, repair mechanisms, physical defences, and antioxidant defences (Valko et al. 2007). The total oxidant capacity (TOC) assay has been used in three species of scallops for quantifying their ability to neutralise peroxyl (ROO) and hydroxyl (OH) radicals, and peroxynitrite (HOONO) (Regoli et al. 2000). Total antioxidant capacity (TAC) measures the antioxidant capacity of all antioxidants in a biological sample (Kusano and Ferrari 2008).

Vitamins are necessary for growth, reproduction and health. Deficiencies lead to significant symptoms in the organism (Lee and Dabrowski 2004). Vitamin A has lipophilic compound and it is essential for health, fertility and the growth, and differentiation of epithelial tissues (Tous et al. 2014). Vitamin D contributes to bone formation, calcium balance, and other physiological processes critical for dairy cattle production and well-being. It also contributes to reproductive performance and mammary development (Panda et al. 2001, Kemmis et al. 2006, Hewison 2010). Vitamin E is an important lipid soluble antioxidant that protects against free radical-initiated lipid peroxidation and enhances the oxidative stability of organisms due to its ability to protect polyunsaturated fatty acids from peroxidation and to scavenge free radicals (Evstigneeva et al. 1998, Halliwell and Gutteridge 1999).

Farm animals should intake the trace elements sufficient for optimal performance (Avci et al. 2013). Selenium (Se) acts in the antioxidant system as an essential component of a family of glutathione peroxidase enzymes. These enzymes destroy hydrogen peroxide ($H_2O_2$) and lipid hydroperoxides (Mustacich and Powis 2000). Copper (Cu) is an essential component of several enzymes, various enzyme activities and the oxidation-reduction process (Reece 2004, Ahola et al. 2005). It is involved in the antioxidant system via its involvement in the enzymes of Cu-Zn superoxide dismutase (SOD). Cu-Zn SOD is responsible for the dismutation of superoxide radicals to hydrogen peroxide in the cytosol. Zinc (Zn) is another essential component of numerous enzymes including enzymes involved in the synthesis of DNA and RNA. In the antioxidant system, it is a component of Cu-Zn SOD. It also induces synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals (Prasad et al. 2004). Manganese (Mn) is an important co-factor in enzymatic reactions involved in metabolic regulation in animals. It is closely regulated by homeostatic mechanisms, but blood Mn concentrations increase throughout the pregnancy (Mora et al. 2014). By this way, microelements could pass across the placental barrier and breast tissue. Ensuring their adequate levels in pregnant animals is also very important for obtaining adequate mineral support of calves in intrauterine and postnatal periods (Abdelrahman and Kincaid 1993, Hostetler et al. 2003).

In the literature, only a few data exist on the metabolic and reproductive parameters during the transition period in dairy cows (Ucar et al. 2011, Avci and Kizil 2013). Therefore, the objective of the present study was to determine of effects of various exogenous vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) on metabolic and reproductive profile in transition dairy cows.

Table 1. Complementary diet used in the feeding of dairy cows.

| | | |
|---|---|---|
| Analytical Components (%) | Humidity | 12.9 |
| | Crude protein | 16.3 |
| | Crude fat | 2.8 |
| | Crude cellulose | 10.2 |
| | Crude ash | 7.8 |
| | Sodium | 0.40 |
| Additives (mg/kg) | Vitamin A | 1142.08 |
| | Vitamin E | 39.86 |
| Trace Elements (mg/kg) | Manganese sulphate | 43.70 |
| | Ferrous sulphate monohydrate | 99.58 |
| | Zinc oxide | 52.17 |
| | Copper sulphate pentahdyrate | 17.11 |
| | Cobalt carbonate | 0.21 |
| | Sodium selenite | 0.93 |
| | Anhydrous calcium iodate | 0.20 |

* Complementary Dairy Cattle Pellet Feed; Bayramoglu, Erzurum, Turkey.

## Materials and Methods

### Animals

The animal material composed of clinically healthy 20 Brown Swiss dairy cows (aged 4-5 years weighing 500-550 kg), kept under the same management and nutritional conditions in Ataturk University, Faculty of Veterinary Medicine Research and Application farm (Erzurum, TURKEY) as shown in Table 1. Herein, the cows identified in the last 3 weeks of gestation were used based on artificial insemination records.

### Experimental design and sample collection

Pregnant cows used were divided into two equal groups (n=10 for each group). Twenty ml of mineral solution, containing 2.5 mg of copper gluconate, 1.25 mg of sodium selenite, 5 mg of manganese gluconate, 5 mg of zinc gluconate per ml (Activate®, Alke, Istanbul-Turkey 50 ml, i.m.) and 10 ml of vitamin solution, containing 500 000 IU vitamin A, 75 000 IU vitamin $D_3$, 50 mg vitamin E per ml (Ademin®, Ceva Dif, Istanbul-Turkey, 50 ml, i.m.) were administered intramuscularly to each cow in the treatment (T) group 3 weeks prior to parturition. Twenty ml saline, as *placebo*, was administered subcutaneously to cows in control (C) group 3 weeks before the parturition. Blood samples were taken for biochemical analysis at the time of administration, at the moment of parturition and 3 weeks after the parturition. The samples were taken from the jugular vein into vacuum tubes (Vacutainer, BD, UK); the sera were extracted and stored at -80ºC until analyses.

Prior to the study, Ataturk University Board of Ethics Committee for Animal Experimentation approved the experimental protocol (The approval number: 2013/45).

### Biochemical Analyses

BHB and NEFA analyses from the sera samples were studied using diagnostic test kits (BHB: vetspec BHB Kit Ref No: C442-0 Lot: LD101401, NEFA: vetspec NEFA Reagent Kit Ref No: C514-0 Lot No: DC131401) (Catachem Inc., Oxford, CT, USA) running with the principle of enzymatic-colorimetric test. Tests were performed by an automatic biochemistry analyser (Tokyo Boeki TMS 1024, Japan) under the direction of the kit's manufacturer.

Serum liver enzyme (ALT, AST, ALP and GGT) activities, ALB, GLU, DBIL, TBIL, T.CHOL, LDL, HDL, TG, TP, CRSC and UREA concentrations were determined by commercial enzyme kits using a biochemistry auto-analyser (Beckman Coulter, AU5800, USA). Hormone levels (FSH, LH and $P_4$) were analysed by chemiluminescence method (Beckman Coulter DXI 800, USA).

For identifying the lipid profile of sera, sample tubes were taken out of the freezer a day before the analysis and they were kept out until they dissolved.

Table 2. The mean values, mean of standard error, and statistical differences of the metabolic and reproductive parameters in the control group cows in transition period (Mean ± SEM).

| Parameters | 3 weeks before parturition | Parturition | 3 weeks after parturition |
|---|---|---|---|
| NEFA mg/L | 408.6 ± 67.9$^{a}$ | 440.2 ± 51.3$^{ab}$ | 484.8 ± 65.4$^{b*}$ |
| BHB mmol/L | 0.877 ± 0.094 | 0.795 ± 0.067 | 1.081 ± 0.153 |
| GLU (mg/dl) | 58.1 ± 1.27$^{a}$ | 80.5 ± 5.96$^{b**}$ | 50.7 ± 3.71$^{a}$ |
| T.CHOL (mg/dl) | 112.0 ± 7.86$^{b*}$ | 94.0 ± 6.05$^{a}$ | 136.8 ± 11.9$^{b***}$ |
| HDL (mg/dl) | 70.3 ± 4.75$^{a}$ | 74.8 ± 4.33$^{ab}$ | 93.3 ± 8.00$^{b*}$ |
| LDL (mg/dl) | 43.3 ± 3.86$^{a}$ | 46.7 ± 4.10$^{ab}$ | 59.4 ± 6.69$^{b*}$ |
| TG (mg/dl) | 46.8 ± 5.14$^{b***}$ | 12.6 ± 1.27$^{a}$ | 13.9 ± 1.46$^{a}$ |
| AST U/L | 83.2 ± 3.6 | 104.4 ± 4.7 | 100.9 ± 5 |
| ALT U/L | 36.7 ± 1.24 | 25.7 ± 1.40 | 28.7 ± 1.50 |
| ALP U/L | 51.7 ± 7.2 | 51.8 ± 6.4 | 33.5 ± 2.9 |
| GGT U/L | 18.7 ± 1.6 | 25.2 ± 1.8 | 20.7 ± 1.1 |
| UREA (mg/dl) | 44.3 ± 2.58$^{b}$ | 46.8 ± 2.39$^{b}$ | 23.5 ± 1.65$^{a***}$ |
| CRSC (mg/dl) | 1.39 ± 0.078$^{b**}$ | 1.49 ± 0.082$^{b***}$ | 1.20 ± 0.0756$^{a}$ |
| TBIL (mg/dl) | 0.201 ± 0.017$^{a}$ | 0.298 ± 0.019$^{b***}$ | 0.217 ± 0.022$^{a}$ |
| DBIL (mg/dl) | 0.063 ± 0.018 | 0.057 ± 0.009 | 0.034 ± 0.007 |
| TP (g/dl) | 7.41 ± 0.08$^{b}$ | 6.85 ± 0.12$^{a*}$ | 7.65 ± 0.19$^{b}$ |
| ALB (g/dl) | 3.26 ± 0.064 | 2.96 ± 0.309 | 3.02 ± 0.087 |
| FSH (mIU/L) | 0.271 ± 0.112 | 0.452 ± 0.148 | 0.180 ± 0.046 |
| LH (mIU/L) | 0.949 ± 0.331 | 1.486 ± 0.618 | 1.119 ± 0.392 |
| $P_4$ (ng/ml) | 13.47 ± 3.08$^{c**}$ | 0.870 ± 0.205$^{a*}$ | 3.158 ± 0.983$^{b*}$ |
| TAC (mmolTroloxEquiv/L) | 1.768 ± 0.020 | 1.759 ± 0.030 | 1.756 ± 0.026 |
| TOC (μmol$H_2O_2$ Equiv/L) | 2.601 ± 0.040 | 2.591 ± 0.031 | 2.67 ± 0.023 |

$^{abc}$ There is statistical significance between the values represented by different letters. * p<0.05, ** p<0.01, *** p<0.001.

The analysis was made with High Performance Thin Layer Chromatography (HPTLC) method in Biochemistry Laboratory of Faculty of Veterinary Medicine at Ataturk University. This process was carried out using 20 x 10 cm Silica Gel 60 F254 HPTLC plate. After 0.5 ml n-hexane/izo-propanol (2:1 (h/h)) mixture was added into the samples (1 ml each) (Hara and Radin, 1978), the lids of the tubes were put on and they were vigorously vortexed. After they were kept immobile for ten min, they were vortexed again. This procedure was further repeated twice. The vortexed tubes were centrifuged at 5,000 *g* for 10 min and the supernatant (hexane phase) were loaded on the HPTLC plates. The lipid classes loaded on plates were run in the mixture of hexane: dietylether: formic acid (80:20:2 (h/h/h)) for 7 cm and dried at room temperature. 3% $CuSO_4$ in 8% $H_3PO_4$ was sprayed on those dried plates and it was burnt in a 180ºC oven for approximately 10 min to make serum lipid bands visible. After HPTLC plates were photographed with Epson Perfection V700 Photo Scanner, footprint of lipid bands for each sample was calculated by Phoretix 1D (TL120) software and it was presented in percentage in the total mixture (Kaynar et al. 2013).

Total antioxidant capacity (TAC) levels were determined using a novel automated colorimetric measurement method developed by Erel (2004). In this method, hydroxyl radical, the most potent biological one, is produced by the Fenton reaction and it reacts with the colourless substrate O-dianisidine to produce the dianisyl radical in bright yellowish-brown colour. Upon the addition of sample, oxidative reactions initiated by hydroxyl radicals present in the reaction mix are suppressed by antioxidant components of the sample, preventing the colour change and thereby providing an effective measure of total antioxidant capacity of the sample. The assay has excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equivalents/l for serum.

Total oxidant capacity (TOC) was determined using a novel automated measurement method, developed by Erel (2005). Oxidants present in the sample oxidise the ferrous ion-O-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol

Table 3. The mean values, mean of standard error, and statistical differences of the metabolic and reproductive parameters in the treatment group cows in transition period (Mean ± SEM).

| Parameters | 3 weeks before parturition | Parturition | 3 weeks after parturition |
|---|---|---|---|
| NEFA mg/L | $307.3 \pm 24.9^{a}$ | $310.5 \pm 25.2^{a}$ | $366.6 \pm 33.4^{b*}$ |
| BHB mmol/L | $0.66 \pm 0.06$ | $0.65 \pm 0.04$ | $0.67 \pm 0.07$ |
| GLU (mg/dl) | $65.5 \pm 5.8^{b}$ | $57.7 \pm 20.5^{ab}$ | $54.25 \pm 5.5^{a***}$ |
| T.CHOL (mg/dl) | $91.2 \pm 2.9^{b}$ | $71.1 \pm 4.7^{a**}$ | $113.0 \pm 9.3^{c*}$ |
| HDL (mg/dl) | $63.3 \pm 2.6^{b}$ | $50.8 \pm 2.7^{a**}$ | $70.9 \pm 5.4^{b}$ |
| LDL (mg/dl) | $50.2 \pm 1.9^{b}$ | $37.9 \pm 3.3^{a**}$ | $58.6 \pm 4.8^{b}$ |
| TG (mg/dl) | $27.6 \pm 1.9^{b}$ | $14.1 \pm 1.9^{a***}$ | $13.8 \pm 1.5^{a***}$ |
| AST U/L | $99.5 \pm 9.6$ | $102.6 \pm 3.5$ | $116.0 \pm 3.0$ |
| ALT U/L | $31.6 \pm 2.7$ | $28.1 \pm 1.2$ | $36.6 \pm 1.8$ |
| ALP U/L | $68.3 \pm 8.0$ | $87.6 \pm 19.9$ | $58.9 \pm 6.2$ |
| GGT U/L | $18.4 \pm 3.2$ | $22.9 \pm 2.9$ | $20.1 \pm 1.2$ |
| UREA (mg/dl) | $24.7 \pm 3.9$ | $32.5 \pm 3.9$ | $24.8 \pm 2.1$ |
| CRSC (mg/dl) | $1.17 \pm 0.045$ | $1.21 \pm 0.085$ | $1.23 \pm 0.073$ |
| TBIL (mg/dl) | $0.16 \pm 0.02^{a*}$ | $0.22 \pm 0.02^{b}$ | $0.20 \pm 0.02^{ab}$ |
| DBIL (mg/dl) | $0.033 \pm 0.007$ | $0.045 \pm 0.009$ | $0.051 \pm 0.013$ |
| TP (mg/dl) | $7.70 \pm 0.29$ | $6.93 \pm 0.21$ | $7.57 \pm 0.11$ |
| ALB (mg/dl) | $3.33 \pm 0.17$ | $3.27 \pm 0.11$ | $3.29 \pm 0.12$ |
| FSH (mIU/L) | $0.271 \pm 0.112$ | $0.169 \pm 0.063$ | $0.263 \pm 0.081$ |
| LH (mIU/L) | $0.949 \pm 0.331$ | $0.707 \pm 0.425$ | $0.528 \pm 0.291$ |
| $P_4$ (ng/ml) | $13.47 \pm 3.08^{b**}$ | $0.485 \pm 0.106^{a***}$ | $1.9745 \pm 0.987^{a***}$ |
| TAC (mmolTroloxEquiv/L) | $1.570 \pm 0.19^{a}$ | $1.751 \pm 0.22^{b***}$ | $2.084 \pm 0.50^{c***}$ |
| TOC (μmol$H_2O_2$ Equiv/L) | $2.508 \pm 0.028^{c***}$ | $2.259 \pm 0.033^{b***}$ | $1.986 \pm 0.030^{a}$ |

[abc] There is statistical significance between the values represented by different letters. * p<0.05, ** p<0.01, *** p<0.001.

molecules, abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, measurable spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with $H_20_2$ and the results are expressed in terms of mmol $H_2O_2$ equivalent/l for serum.

## Statistical analysis

Statistical comparisons of data were analysed using General Linear Model/Repeated Measures (SPSS, Version IBM 20.0 Microsoft, Chicago, IL, USA) in-group comparisons. Data were expressed as mean ± standard error of the mean (SEM). Differences were considered significant when p<0.05.

## Results

Statistical significances between metabolic, reproductive and oxidative parameters in control cows are presented in Table 2.

In the C group, there were significant changes in the levels of NEFA, HDL, LDL, TP (p<0.05), GLU, P4 (p<0.01), T.CHOL, TG, UREA, CRSC and TBIL (p<0.001), while the levels of BHB, AST, ALT, ALP, GGT, DBIL, ALB, FSH, LH, TAC and TOC remained unchanged (p>0.05).

At parturition, the increasing levels of HDL, LDL and NEFA continued to rise in the next 3 weeks after parturition. It was observed that although the levels of ALT, $P_4$, T.CHOL, TG, and TP decreased during the parturition, they increased again 3 weeks later. By contrast, the AST, ALP, GGT, GLU, TBIL, CRSC and UREA levels increased during parturition, but they decreased after 3 weeks.

Table 4. Lipid profile in the treatment and control group cows in transition period.

| Groups | Parameters | 3 weeks before parturition | Parturition | 3 weeks after parturition |
|---|---|---|---|---|
| Control (n=10) | HC + TAG (%) | 73.621 ± 0.81 | 73.798 ± 0.816 | 73.553 ± 0.889 |
| | PL (%) | 17.788 ± 0.599 | 18.013 ± 0.749 | 17.924 ± 0.693 |
| | STR (%) | 8.59 ± 0.0432 | 8.19 ± 0.465 | 8.524 ± 0.481 |
| Treatment (n=10) | HC + TAG (%) | 72.891 ± 0.772 | 76.225 ± 0.778 | 71.111 ± 0.847 |
| | PL (%) | 18.029 ± 0.425 | 16.125 ± 0.419 | 19.2 ± 0.308 |
| | STR (%) | 9.080 ± 0.329 | 7.649 ± 0.396 | 9.696 ± 0.0322 |

Significances between metabolic, reproductive and oxidative parameters in the treatment cows are presented in Table 3.

In the T group, significant changes in the levels of NEFA, TBIL ($p<0.05$), T.CHOL, HDL, LDL ($p<0.01$), TG, GLU, $P_4$, TAC and TOC ($p<0.001$) were observed, while there were no such changes in the levels of BHB, AST, ALT, ALP, GGT, UREA, CRSC, DBIL, TP, ALB, FSH and LH ($p>0.05$).

At parturition, increased levels of AST and NEFA continued to rise in the next 3 weeks after parturition. It was determined that increasing TAC level showed the antioxidant status ($p<0.001$). It was noted that ALT, LDL, HDL, T.CHOL and $P_4$ levels decreased at parturition and increased 3 weeks after parturition. The level of TBIL increased at parturition and decreased after 3 weeks. A continuous decline in the GLU, TG and TOC levels was observed.

The lipid profiles of cows in the treatment and control groups are given in Table 4. No increase was observed in the prepartum, partum and postpartum HC+TAG, PL and STR levels in cows of the treatment group. Although there was a decrease in PL and STR levels in the treatment group, the HC+TAG levels increased in the partum period.

## Discussion

Transition period is a very sensitive period that should be managed in terms of the energy balance. Therefore, we planned to investigate the efficiency of the antioxidant vitamins (A, D, E) and trace elements (Se, Cu, Mn, Zn) on some metabolic and reproductive profiles in dairy cows during transition period.

If animals are fed insufficiently in terms of energy during this period, the blood glucose level decreases and NEFA is released from the adipose tissue increases. The best indicator of NEB is its blood level during transition period (Civelek et al. 2011). NEB normally occurs in dairy cow as they pass from the late gestation to early lactation and thus an excessive energy demand for milk production occurs (Herdt 2000). The increased levels of NEFA are indicative of the increasing depletion of lipid reserves, as a response to growing energy demand (Fox et al. 1991). Prepartum blood NEFA level may be used for detecting cows at risk for severe NEB (LeBlanc 2006). Increased prepartum NEFAs have also been found to increase the risk of clinical ketosis and metritis (Ospina et al. 2010) as well as retained foetal membranes (Chapinal et al. 2011). Avci and Kizil (2013) stated that the administration of trace elements affected the NEFA level in transition cows. In our study, the NEFA level measured at the 3rd week prepartum in the control cows increased in the intrapartum period and it kept increasing postpartum. This finding could imply the fact that the NEB and lipomobilisation continue throughout the transition period. In the treatment group, on the other hand, though the prepartum NEFA level shows an increase in the intrapartum and postpartum periods, the rate of this increase is low as compared to the control group. Therefore, it may be stated that the trace element and vitamin combinations implemented in the transition period affected the NEB and lipomobilisation.

The serum BHB level is an indicator of energy metabolism failures and is in compliance with lipomobilisation. Cows in which the demand for glucose exceeds the gluconeogenic capacity of the liver typically develop increased BHB levels 3-6 weeks postpartum (Oetzel 2004, Chapinal et al. 2011). Herein, BHB levels were lower in the treatment group as compared to the controls. It means that the administration of antioxidant vitamins and trace elements affected the lipomobilisation.

In ruminants, ingested carbohydrates are fermented to short-chain fatty acids by rumen microbes and thus, the most of glucose must be synthesised by the liver (Reynolds et al. 1988). Glycaemia in pregnant females and puerperal cows ranged within the physiological limits, e.g. from 2.5 to 4.2 mmol/l (Radostits et al. 2010). In lactating cows, serum glucose levels were reported to be low in early lactation and

these levels increased towards the middle of lactation (Turk et al. 2008). In dairy animals, both early lactation and after parturition are the periods of severe NEB characterised by some changes such as reduced blood glucose and insulin levels and elevated blood growth factors levels (Celi et al. 2010). In addition, NEB has been related to altered hormonal levels in the ability of the hypothalamo-hypophyseal axis that does not support a functional reproductive system during early lactation (Butler et al. 2003). Besides, within 4 days postpartum, the demands for glucose, amino acids and fatty acids are 2-5 times higher than the prepartum requirements due to milk production (Bell 1995). Therefore, there exist few studies related to this process. Avci and Kizil (2013) pointed out that the administration of trace elements keeps the glucose concentrations stable throughout the transition period. Herein, we observed that the glucose level measured at the 3rd week prepartum in the treatment group decreased in the intrapartum and postpartum periods, while glucose levels remained within the normal reference range (Kaneko et al. 1997). It is thought that this situation in the treatment group results from the administration of minerals regarding carbohydrate metabolism such as mangan (Abdelrahman and Kincaid 1993). For the control group, high glucose levels measured during the intrapartum period, as compared to the prepartum and postpartum values, agree with the previous reports that blood glucose levels increase during the intrapartum period (Ghergariu et al. 1984, Park et al. 2010, Hesari et al. 2012).

Various studies have shown that lipoprotein (VLDL, HDL, LDL) levels in serum decrease in accordance with the liver steatosis that emerges in varying degrees during postpartum period (Basoglu et al. 1998, Sevinc et al. 1999). In this study, for both groups, the LDL, HDL and T.CHOL levels measured in the partum and prepartum periods were low as compared to the postpartum values. It is likely that the NEFA levels remained within the normal reference range; they do not cause damage in the liver, and the production of VLDL is insufficient because the decrease in VLDL synthesis results in a decrease in the LDL and HDL levels, originating from the VLDL. Herein, the lipoprotein levels decreased in the intrapartum period in the treatment group and the increased NEFAs drew close to the baseline values in the postpartum period, hence, it was considered that the damage in the liver is probably temporal. The decline in the T.CHOL levels in intrapartum period is an indication of a liver damage in both groups.

Enzymes such as the GGT, AST and ALT along with the TBIL levels are determinative for the evaluation of liver functions in transition animals (Van Saun 2004). We observed that the AST, GGT and TBIL levels in both groups were higher in the parturient period as compared to the prepartum period. Although Hafez et al. (1983) stated that the AST and ALT levels are higher in pregnant animals than non-pregnant animals, but in some other studies (Bogin et al. 1988, Bobe et al. 2004) the AST, ALT and TBIL levels were higher in the postpartum period as compared to the dry period. Ucar et al. (2011) emphasised that in cows receiving mineral solution, the ALT and LDH levels remained the same, while the AST tended to increase. In another study (Sevinc et al. 1999), there was an increase in the TBIL and DBIL levels postpartum indicating that this increase resulted from the deficiency in liver functions developed due to the NEB in that period. Elitok et al. (2006) detected a slight increase in blood bilirubin within the first week following the birth. Herein, the AST, ALT, GGT and TBIL levels increased in the intrapartum period as compared to the prepartum period in both groups. However, increases in the enzyme activities remained within the reference range (Kaneko et al. 1997). Such increases may have resulted from the minimal level of cellular damage emerged in the liver due to the lipomobilisation based on the energy deficit arising as the birth approaches.

A slight increase in the blood urea levels detected in postpartum period (measured at normal levels in pre- and postpartum periods as a result of the insufficient protein intake in transition cows) is thought to result from the decrease in glomerular filtration rate caused by the birth stress (Sevinc et al. 1999). Elitok et al. (2006) found low levels of urea in postpartum cows given a birth recently and considered that this situation resulted from the decrease in protein anabolism caused by fat infiltration during that period. Similarly, herein, while the UREA and CRSC levels remained unchanged in pre- and intrapartum periods for the control group, the levels decreased in postpartum period. In the treatment cows, the UREA and CRSC levels remained within the reference range in prepartum, parturient and postpartum periods. In addition, herein, it is argued that the decreases in UREA and CRSC levels in postpartum period in the control group resulted both from the possible damage in the liver and the decrease in protein anabolism caused by this damage.

During transition period, the ALB level in particular was associated with some postpartum diseases; hence, it could be used for the evaluation of disease risk in cows both in stables and on pasture (Van Saun 2004). Tothova et al. (2008) stated that in postpartum period, the serum TP and ALB levels were low as compared to the prepartum and parturient periods. Hesari et al. (2012) noted that copper additions did

not cause any damage in the ALB rates in lactating cows. Ucar et al. (2011) stated that CRSC and ALB levels remained unchanged in cows receiving mineral solution. Avci and Kizil (2013) noted that the intrapartum TP and ALB values were low in cows receiving trace elements as compared to the prepartum values, and revealed that although this decrease continued in the control group during postpartum period, they increased again in the treatment cows. We observed herein that the TP concentration in intrapartum period is low in the control cows, while it remained unchanged in the treatment group. Besides, the ALB levels remained the same over the study period. Hence, we considered that the administration of trace element and vitamin combinations had a positive effect on the protein metabolism.

The NEB may affect the ovarian activity by decreasing LH pulsatility that leads to delayed resumption of luteal activity (Opsomer et al. 2000). Herein, there was no marked effect of trace element and vitamin combination on the FSH and LH levels, and despite the rising levels of NEFA over the study periods, a continuous decline was observed in the LH levels. This finding supports the study of Opsomer et al. (2000). The $P_4$ is important for establishment and maintenance of pregnancy and ensuring proper embryo development (Lonergan et al. 2007). As expected, we observed a marked difference in $P_4$ levels in the control and treatment groups over the study periods.

Maternal fat and energy intake during lactation can play a vital role in the development of metabolic disorders observed in their offspring, and that maternal oxidative stress can be chosen as the factor involved (Bouanane et al. 2009). Dairy cows suffer more from oxidative stress and have low antioxidant defence just after the parturition than the advanced pregnancy. Some factors can lead to this situation because of increased susceptibility to diseases such as mastitis, metritis and retention of foetal membranes (Sharma et al. 2011). Metabolic demands associated with late pregnancy, parturition and initiation of lactation would be expected to increase the production of reactive oxygen species (ROS) (Sordillo 2005). Herein, plasma TOC levels in the early lactation period were markedly higher than late lactation period in goats, while the level of TAC was lower. In goats, the TAC levels were higher in the late lactation period compared to early lactation, and TOC levels were markedly lower (Karapehlivan et al. 2013). This situation was thought to be due to the decrease in the lipomobilisation occurring in adipose tissue due to the transition from the NEB to positive energy balance (PEB) in late lactation period. Lactation stages include the early and late lactation periods, since the increased catabolic reactions at the cellular level may have effect on some metabolic functions related with the level of free radicals (Bernabucci et al. 2005). A number of trace minerals are required for the functions of enzymes involved in the antioxidant defence system (Spears and Weiss 2008). Indeed, we observed herein that vitamin and trace element combinations caused a constant increase in the TAC level, while they led to a constant decrease in the TOC level. A decrease in the antioxidant capacity may occur depending on the secretory function and synthesis in the liver of ruminants. Indeed, hepatomegaly is seen in ruminants due to the elevated intracellular lipid rate resulting from the increased NEFA synthesis (Pedernera et al. 2010). Avci and Kizil (2012) observed a decrease in the oxidative parameters in postpartum period of cows in the group receiving trace element solution, along with an increase in antioxidant parameters. The NEFA level remaining at normal levels in treatment group shows parallelism with the TAC.

Finally, it has been observed herein that the administration of antioxidant vitamins and trace elements for managing the NEB, blood NEFA, and BHB concentrations around transition period could have a beneficial influence on the metabolism. By optimising diet ingredients and energy consumption in pre- and postpartum periods, this goal would be achievable for minimising the NEB during transition period. The provision of adequate amounts of dietary antioxidant micronutrients, such as vitamin E and selenium, proved to be an effective way of controlling the oxidative stress studied herein.

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