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## Effects of trace elements applied to cows in transition period on serum fatty acid profile

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**ABSTRACT:** The aim of this study was to investigate the effects of trace elements applied to cows during the transition period on serum nonesterified fatty acid profile. The material of this study was consisted of 20 clinically healthy 3-5 year old Simmental cows kept under the same care and feeding conditions. Pregnant cows enrolled in the study were divided into two equal groups with 10 cows in each group. A single dose of 20 ml of the trace element solution was administered intramuscularly to the cows in the study group three weeks before the parturition. Blood samples were collected for analysis at three different periods: trace element administration time, parturition time and three weeks after parturition. Serum samples were analyzed on the gas chromatography-mass spectrophotometry (GC-MS) device to determine the levels of nonesterified fatty acids. The results showed that; the fatty acid levels determined three weeks before the parturition in the group treated with trace element solution were higher than those detected at the parturition time and three weeks after parturition. On the other hand, the fatty acid levels determined three weeks before the parturition increased at the time of parturition, and remained at high level three weeks after the parturition in the control group. It was therefore concluded that the trace element supplementation applied to pregnant cows during the transition period prevented excessive increase in serum nonesterified fatty acid levels.

**Key words:** Transition period, cows, fatty acid profile, trace element.

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## INTRODUCTION

The transition period is extremely important in determining future health, milk production, and reproductive success of the dairy cow. This period is from three weeks before calving to three weeks after calving as the cow transitions from the dry period to the milking herd (Goof and Horst, 1997). As stated by Goff and Horst, "The transition from the pregnant, nonlactating stage to the nonpregnant, lactating stage is too often a disastrous experience for the cow. The well-being and profitability of the cow could be greatly enhanced by understanding those factors that account for the high disease incidence in periparturient cows." A key area of the biology of transition cows relates to lipid metabolism. Excessive lipid mobilization from adipose tissue is linked with greater incidences of peri-parturient health problems (Roberts et al., 1981). Nonesterified fatty acids (NEFAs) released into the blood as a result of increased lipolysis are transported to the liver intensively, but the liver is not able to process these lipids at the same rate and the eventually conditions such as ketosis or fatty liver occur (Dyk et al., 1995; Van Saun, 2004).

Because of their important effects on animal health, trace elements must be present in the organism at a sufficient level (Spears and Weiss, 2008; Linn et al., 2011). Trace minerals such as zinc (Zn), copper (Cu), manganese (Mn) and selenium (Se) are essential with classically defined roles as components of enzymes, proteins and energy metabolism in ruminants (Overton and Waldron, 2004). The absorption of microelements is mainly related to their dietary levels (Knowles et al., 1999; Cag et al., 2000; Kuricova et al., 2003). Microelements is transported to the fetus via the placenta and ensuring the adequate level of these microelements in pregnant animals is very important in meeting the needs of their offspring (Hostetler et al., 2003; Pavlata et al., 2004; Andrieu, 2008). Excessive NEFAs reaching the liver due to increased lipolysis, which is common in dairy cows in the early lactation period, result in the production of hydrogen peroxide, which will lead to oxidative stress in hepatocytes. The most important active components of enzyme systems that represent antioxidant capacity are trace minerals. Especially in transition period rations, having sufficient levels of trace mineral is important in the adequacy of antioxidant capacity despite excessive oxidative response. Lipid metabolism is a key aspect of the physiology and energy metabolism of transition cows (Drackley, 1999).

The aim of this study was to investigate the effects of trace elements applied to cows during the transition period on serum NEFAs.

## MATERIALS AND METHODS

The material of the study was consisted of 20 healthy, 3-5 years old Simmental cows, that were kept under the same care and feeding conditions. A ration containing 4 kg of hay, 10 kg of corn silage, 2 kg of wheat straw, 1 kg of dried alfalfa and 3 kg concentrated feed (18% HP) was given to the cows during the transition period. In the first 3 weeks of postpartum, the cows were given a ration consisting of 22 kg corn silage, 4.5 kg alfalfa grass, 5.5 kg concentrated feed, 3 kg cotton seed meal (HP 32%). Body condition scores (BCS) of pregnant cows were determined during the transition period (5-points scale) and, recorded 3 times individually; 3 weeks before parturition, day of parturition, and 3rd week after parturition. BCS of the cows were determined by subcutaneous fat deposits in the back, waist, and pelvic region, and bone protrusions in the pelvic region. All pregnant cows used in the study were transferred to the dry period 2 months before calving. The normal gestation period of the cows was calculated as 285 days and by examining the artificial insemination records, those with 3 weeks left to parturition were included in the study. Pregnant cows enrolled in the study were divided into 2 equal groups with 10 cows in each group. A mineral solution (Activate, ALKE) containing 2.5 mg copper gluconate, 1.25 mg sodium selenite, 5 mg manganese and 5 mg zinc gluconate per ml was once administered intramuscularly to the cows in the study group 3 weeks before calving, at a total dose of 20 ml. Blood samples were taken for NEFA analysis at the 3 weeks before calving, at the day of calving, and at the 3rd week after calving. The blood samples were centrifuged at 5000 rpm for 5 minutes, and the obtained serum was stored at -20 °C until analysis.

Fatty acid standards: decanoic acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, cis-11 henoic acid and heneic acid were purchased from ZIVAK Technologies (Kocaeli / TURKEY). Standard stock solution of fatty acids was prepared in heptane at a concentration of 300 nmol/mL. For the calibration, 100 µg/mL (R1), 50 µg/mL (R2), 25 µg/mL (R3) and 5 µg/mL (R4) fatty acid solutions and 50 µg/mL (R5) internal standard were prepared. Then, samples were analyzed using Shimadzu GC-MS Plus gas chroma-

tography-mass spectrophotometry (GC-MS) device.

One of the prerequisites of parametric tests for data, the homogeneity of variances was checked with the “Levene” test, while the assumption of normality was examined with the “ShapiroWilk” test. Repeated measures method was used in the General Linear Model (GLM) procedure to examine the changes of the groups according to measurement time. Mauchly’s test of sphericity “Mauchly’s Test of Sphericity” method was used to determine the change according to the measurement time. Greenhouse-Geisser, Huynh-Feldt and Lower Bound tests were taken into consideration in cases where the assumption of sphericity was not met. Dependent Sample t-test “Paired Samples-t” was used for paired comparisons of measurement times within groups. Independent samples t-test was used for comparisons of control and experimental groups for each time. The data were presented as mean and standard deviation for groups. Statistical significance level was accepted as  $P < 0.05$  (IBM SPSS, 2013).

The study was approved by the Ethical Committee of the Firat University Animal Experiments Local Ethics Committee Presidency and informed consent was obtained from all subjects.

## RESULTS

The mean values, standard deviations and statistical significance levels of the NEFAs between and within the groups were presented in Table 1.

As shown in Table 1, initial values of decanoic acid, andecanoic acid, lauric acid, tridecanoic acid, pentadecanoic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid levels were determined to decrease at the day of calving in the experimental group, and these decrease seemed to continue in the 3rd week after calving. The differences between the days were determined to be statistically significant for decanoic acid ( $P < 0.01$ ), andecanoic acid ( $P < 0.05$ ), lauric acid ( $P < 0.05$ ), tridecanoic acid ( $P < 0.01$ ), pentadecanoic acid ( $P < 0.01$ ), palmitoleic acid ( $P < 0.05$ ), stearic acid ( $P < 0.01$ ) and linoleic acid ( $P < 0.05$ ), but no significant difference was observed for oleic acid ( $P < 0.395$ ). On the other hand, it was detected that though the initial values of myristic acid and arachidic acid obtained 3 weeks before calving decreased at the time of calving, they showed increases in the 3rd week after calving. However the changes in the levels of myristic acid ( $P < 0.09$ ) and arachidic acid ( $P < 0.490$ ) were not sta-

tistically significant.

In the control group, the fatty acid levels were observed to increase at the time of calving and the values obtained 3 weeks after calving were still higher than those determined 3 weeks before the calving, though they were lower than those at the moment of calving. The differences between the days in the control group were detected to be statistically significant for decanoic acid ( $P < 0.001$ ), andecanoic acid ( $P < 0.001$ ), lauric acid ( $P < 0.01$ ), myristic acid ( $P < 0.01$ ), pentadecanoic acid ( $P < 0.001$ ), palmitoleic acid ( $P < 0.001$ ), stearic acid ( $P < 0.01$ ), oleic acid ( $P < 0.01$ ), linoleic acid ( $P < 0.01$ ) and arachidic acid ( $P < 0.001$ ), but no significant difference was noted for tridecanoic acid ( $P < 0.395$ ).

BCS levels in the control group were determined as  $3.15 \pm 0.24$ ,  $3.35 \pm 0.24$ , and  $2.95 \pm 0.28$ , at 3 weeks prior to calving, the day of calving and 3 weeks after calving, respectively. On the other hand, these levels in the experimental group were found to be  $3.25 \pm 0.26$ ,  $3.35 \pm 0.24$ , and  $3.15 \pm 0.22$ , respectively. While there was no statistically significant difference between the days in terms of BCS levels in the experimental group, the only significant difference ( $P < 0.05$ ) was obtained between 3 weeks after calving in the control group. The difference between the days of control and experimental groups was not significant in terms of BCS levels.

In this study, automatic milking machines were not used to record the daily milk yield of the cows where the cows were located. For this reason, daily milk yield records of each cow could not be obtained. However, the information that the cows gave an average of 17 kg of milk per day was obtained from the breeder.

## DISCUSSION

The transition period is characterized by the mobilization of body fat, protein and mineral stores to satisfy the foetal demand for nutrient and the requirements for milk production and maintenance (Van Dorland et al., 2009). In dairy cows, the main changes in metabolic pathways begin approximately 3 weeks prior to calving, reach maximal at calving and last until 3 weeks after calving (Piccione et al., 2011). Hormonal changes occurring in the last stages of pregnancy and the decrease in feed consumption affect the metabolism negatively that mostly result in negative energy balance. The body fat stores mobilize as the nega-

**Table 1.** The mean values, standard deviations and statistical significance levels of nonesterified fatty acids determined in the experimental and control cows during the transition period

Item	Group	Time			P value for Time
		1	2	3	
Decanoic acid (nmol/ml)	Experimental	2017.20±214.56 <sup>a</sup>	1982.06±153.40 <sup>a</sup>	1726.94±210.72 <sup>b</sup>	<0.01
	Control	979.09±147.11 <sup>b</sup>	2006.15±250.18 <sup>a</sup>	1082.89±135.75 <sup>b</sup>	<0.001
<i>p</i> value for groups		<0.001	0.798	<0.001	
Andecanoic acid (nmol/ml)	Experimental	1168.67±176.04 <sup>a</sup>	1116.39±164.79 <sup>a</sup>	933.47±99.61 <sup>b</sup>	<0.05
	Control	610.99±61.21 <sup>b</sup>	1064.82±107.73 <sup>a</sup>	668.60±37.10 <sup>b</sup>	<0.001
<i>p</i> value for groups		<0.001	0.418	<0.001	
Lauric acid (nmol/ml)	Experimental	305.09±110.04 <sup>a</sup>	189.13±96.63 <sup>b</sup>	185.46±48.02 <sup>b</sup>	<0.05
	Control	93.98±12.82 <sup>b</sup>	207.41±100.75 <sup>a</sup>	102.28±31.28 <sup>b</sup>	<0.01
<i>p</i> value for groups		<0.001	0.684	<0.001	
Tridecanoic acid (nmol/ml)	Experimental	694.33±60.53 <sup>a</sup>	648.78±84.06 <sup>b</sup>	581.00±92.57 <sup>c</sup>	<0.01
	Control	674.32±88.57	721.88±102.07	679.07±42.34	0.395
<i>p</i> value for groups		0.522	0.097	<0.05	
Myristic acid (nmol/ml)	Experimental	319.86±77.27	251.59±88.92	313.43±94.19	0.09
	Control	308.59±42.95 <sup>b</sup>	400.17±54.32 <sup>a</sup>	372.33±20.48 <sup>a</sup>	<0.01
<i>p</i> value for groups		0.692	<0.05	0.068	
Pentadecanoic acid (nmol/ml)	Experimental	437.60±75.75 <sup>a</sup>	410.78±90.65 <sup>a</sup>	325.65±102.41 <sup>b</sup>	<0.01
	Control	282.97±55.77 <sup>b</sup>	468.05±43.26 <sup>a</sup>	295.12±70.60 <sup>b</sup>	<0.001
<i>p</i> value for groups		<0.001	0.88	0.262	
Palmitoleic acid (nmol/ml)	Experimental	789.57±165.13 <sup>a</sup>	755.93±188.98 <sup>a</sup>	616.58±104.19 <sup>b</sup>	<0.05
	Control	805.28±68.78 <sup>a</sup>	933.26±20.63 <sup>b</sup>	915.91±58.75 <sup>b</sup>	<0.001
<i>p</i> value for groups		<0.05	<0.01	<0.001	
Stearic acid (nmol/ml)	Experimental	1344.75±151.81 <sup>a</sup>	1228.63±138.75 <sup>a</sup>	1072.50±193.72 <sup>b</sup>	<0.01
	Control	1310.84±45.69 <sup>b</sup>	1442.43±74.01 <sup>a</sup>	1382.41±142.87 <sup>b</sup>	<0.01
<i>p</i> value for groups		0.507	<0.001	0.166	
Oleic acid (nmol/ml)	Experimental	99.20±26.33	89.90±19.81	87.78±20.02	0.395
	Control	39.85±17.07 <sup>b</sup>	80.67±37.92 <sup>a</sup>	47.34±17.53 <sup>b</sup>	<0.01
<i>p</i> value for groups		<0.001	0.606	<0.001	
Linoleic acid (nmol/ml)	Experimental	870.39±255.31 <sup>a</sup>	672.27±124.50 <sup>b</sup>	638.07±111.35 <sup>b</sup>	<0.05
	Control	393.27±59.69 <sup>b</sup>	654.21±144.07 <sup>a</sup>	487.53±155.28 <sup>b</sup>	<0.01
<i>p</i> value for groups		<0.001	0.768	<0.05	
Arachidic acid (nmol/ml)	Experimental	1104.3±481.72	969.98±303.86	990.54±189.71	0.490
	Control	237.18±71.25 <sup>b</sup>	764.45±144.17 <sup>a</sup>	593.66±192.58 <sup>a</sup>	<0.001
<i>p</i> value for groups		<0.001	<0.05	<0.001	

1: 3 weeks before parturition; 2: day of parturition; 3: 3rd week after parturition,

a-c: The difference between groups containing different letters on the same line is statistically significant (The change over time for each group was determined by Mauchly's test of sphericity). Greenhouse-Geisser, Huynh-Feldt and Lower Bound tests were used in cases where the assumption of sphericity could not be achieved. Paired samples t-test was used to compare the inter-group changes ( $P < 0.05$ ). Independent samples t test (independent t-test) was used for comparisons of control and experimental groups for each time ( $P < 0.05$ ).

**Table 2.** The mean values, standard deviations and statistical significance levels of body condition scores determined in the experimental and control cows during the transition period

Item	Group	Time			P value for Time
		1	2	3	
Body Condition Score	Experimental	3.25 ± 0.26	3.35 ± 0.24	3.15 ± 0.22	0.218
	Control	3.15 ± 0.24 <sup>abc</sup>	3.35 ± 0.24 <sup>b</sup>	2.95 ± 0.28 <sup>c</sup>	<0.05
<i>p</i> value for groups		0.338	1.000	0.107	

1: 3 weeks before parturition; 2: day of parturition; 3: 3rd week after parturition,

a-c: The difference between group days with different letters on the same line is statistically significant.



tive energy balance becomes more severe. Increased concentration of NEFAs in plasma is a risk factor in the postpartum period for the development of various diseases, especially fatty liver and ketosis (Grummer, 1993; Vazquez-Anon et al., 1994; Grum et al., 1996; Drackley, 1999; Reynolds et al., 2003). During the transition period, dairy cattle become extremely susceptible to metabolic and infectious diseases that deteriorate quality of life and productivity due to sudden feed transition, weakened immune system and severe negative energy balance (Sundrum, 2015).

Increases in plasma concentrations of NEFAs during transition period have been reported in many previous studies. These increased levels usually consisted of a mixture of different NEFAs released into plasma as a result of lipolysis (Contreras et al., 2010; Ospina et al., 2010; Mann et al., 2016). Fatty acid profiling has important potential application as a diagnostic tool across the species especially in cases where preclinical symptoms are difficult to observe (Serisier et al., 2006). By comparing Holstein, Brown Swiss, Simmental and crossbreed HolsteinxSimmental cows, Blum et al. (1993) observed higher NEFA values in Holstein cows. They attributed this observation to the comparably higher milk yield and consequently increased mobilization of body reserves at the beginning of the lactation caused by the high milk yield. Simmental cattle experienced less loss in body weight and back fat than Holstein breeds in the postnatal period (Aline et al., 2021). Furthermore, Sgorlon et al. (2015) reported no difference for NEFA and BHBA concentrations in the comparison of Holstein and Simmental cows after lactation peak.

Body condition score (BCS) is a simple method to assess the energy status in dairy cows due to the fact that there is a strong relationship between BCS and energy balance. The trace element supplementation was showed to have no effect on BCS (Sales et al. 2011, Bicalho et al. 2014, Machado et al. 2013). Although the ideal live weight varies from cow to cow, the ideal BCS is the same for all cows (Edmonson et al., 1989). Ferguson et al., (1994) observed that 3.0-3.75 scores are the most appropriate values. Busato et al., (2002) reported that dry period cows can be protected from postnatal risks when they have 3.25-3.50 points of BCS. Furthermore, it is considered that the ideal BCS was 3.0-3.5 at the time of parturition (Pryce et al., 2002). It has been reported that there is a linear relationship between BCS at parturition and BCS loss in early lactation, and the higher BCS at parturi-

tion time, the more BCS loss occurs in early lactation (Dechow et al., 2002).

In the present study, the BCS detected at the beginning of the transition period in cows in the experimental group increased at the day of parturition, but decreased in the 3rd week after calving. However, the BCS decreases detected in the 3rd week after calving in the experimental group were less than the control group. In the control group, the decrease in the BCS values in 3 weeks after the calving was higher when compared to the values of cows at the time of calving in the experimental group. It was observed that while cows in the control group lost approximately 0.20 BCS point in the 3<sup>rd</sup> week after calving, the loss in the experimental group remained as approximately 0.10 BCS point. As an indicator of the decrease in BCS in the control group, the levels of NEFAs were determined to be higher in the 3<sup>rd</sup> week after calving in comparison to the day of calving. Although the exact mechanism of how trace elements affected lipid metabolism in transitional cows was not determined in this study, the lesser decrease in BCS in the experimental group can be explained by the possible reflection of the positive effects of trace elements applied to the experimental group at the beginning of the transition period.

The appropriate intake of Se, Mn, Cu and Zn in cattle diets is important for optimising the health of lactating and periparturient cows (Andrieu, 2008). Different degrees of deficiency in these trace elements can cause clinical and subclinical symptoms in dairy cows, as well as reduced milk productivity and reproductive performance (Ballantine et al., 2002; Andrieu, 2008). Trace elements can cross the placental barrier and udder tissue. For this reason, ensuring adequate levels in pregnant animals is very important for calves to maintain a healthy life in the intrauterine and postnatal period (Hostetler et al., 2003; Overton and Waldron, 2004). Among the trace elements, especially Cu, Mn and Zn have important roles in carbohydrate and lipid metabolism (Andrieu, 2008). Zinc, also plays important role in insulin action (Chausmer, 1998).

It is clear that Cu plays a role in lipid metabolism in nonruminants, but there is a paucity of information on the role of this element in ruminants (Engle, 2011). However, some experiments have suggested that Cu supplementation can affect lipid metabolism in ruminants, as well (Engle and Spears, 2000; Engle et al., 2000a; Engle and Spears, 2001) Cu deficiency has been reported to cause an increase in plasma cholesterol-

ol (Engle et al., 2001). It was showed that the addition of 125 mg or 250 mg Cu reduced plasma cholesterol levels in chickens (Pesti et al., 1996). It has also been reported that the total lipid and cholesterol levels in the mammary muscle are significantly suppressed by Cu (Skrivan et al., 2002). Although it is yet to be clarified how Cu affects the profile of fatty acids, it has been indicated to play role on biohydrogenation, esterification and mobilisation of triglycerides (Netto et al., 2014). The Cu deficiency has been reported to cause hypercholesterolemia owing to the increase in hepatic GSH together with an increase in HMG-CoA reductase activity, which is the main enzyme that regulates the synthesis of cholesterol (Kim et al., 1992). Cu supplementation has also been found to reduce total cholesterol in rats (Galhardi et al., 2005). Moreover feeding rats with Cu deficient diets resulted in hypercholesterolemia (Al-Otman et al., 1994; Carr and Lei, 1990). Absolute Cu deficiency could play a role in the etiology of cardiovascular diseases in humans by altering the lipid metabolism (Thuiller-Jutau et al., 1987). One of the proposed mechanisms of action between reduced serum Cu concentration and hypercholesterolemia is that Cu deficiency increases B-hydroxyl-B-methyl-glutaryl CoA (HMG-CoA) reductase activity. This enzyme catalyzes the rate limiting step in the biosynthetic pathway of cholesterol from acetyl-CoA. Increased activity of this enzyme in rats with Cu deficiency results in increased cholesterologenesis (Valzala and Kurup, 1987).

Mangan deficiency causes abnormal lipid metabolism. It has a lipotropic effect and chronic or severe manganese deficiency leads to fatty liver, hypocholesterolemia and low HDL cholesterol (Dawis et al., 1990). Zinc enters into the structure of many enzymes in the body that are responsible for protein, carbohydrate, lipid, nucleic acid, HEM synthesis, gene expression, reproduction and embryogenesis (Rostan et al., 2002). Ranasinghe et al., (2015) demonstrated that Zn supplementation has favourable effects on the plasma lipid parameters in humans. They reported that Zn supplementation reduced the total cholesterol, LDL cholesterol and tryglicerides significantly.

The current study was conducted to investigate the effects of trace element solution administered to the cattle at the beginning of the transition period on serum NEFA levels. Statistically significant differences

were detected between the groups in terms of the levels of decanoic acid, andecanoic acid, lauric acid, penta decanoic acid, palmitoleic acid, oleic acid, linoleic acid and arachidic acid in the 3<sup>rd</sup> week before calving. Also, myristic acid, palmitoleic acid, stearic acid and arachidic acid levels were significantly different between the groups at the day of calving. In addition, the differences for all the values with the exception of myristic acid, pentadecanoic acid and stearic acid were significant in the 3<sup>rd</sup> week after calving. Netto et al., (2014) reported that there was no significant difference in the proportion of saturated and unsaturated acids with Cu, Se and Se/Cu supplementation. However, the decrease in linoleic and palmitic acid with the Se/Cu treatment in Brangus Bulls in relation to the control cannot be explained biologically. In the present study, the comparison of the levels of NEFAs between 3 weeks before calving and 3 weeks after calving revealed that all NEFA levels decreased at the end of the transition period in the experimental group, but they were higher than the baseline levels in the control group.

In short, the NEFAs remained at lower levels in the experimental group when compared with the control group. This can be explained by the positive effects of the trace elements on lipid metabolism. The findings of the current study were found to be consistent with the reports of Drackley and Andersen (2006) that trace element and vitamin injection during the transition period could suppress the negative effect of prepartum lipomobilization and reduce the incidence of peripartum problems.

## CONCLUSIONS

This is the first study that investigated changes in NEFA levels in Simmental cattle at the transition period. We also tried to reveal the types of different NEFA's and the level of changes in each fatty acid depending on trace element applications during the transition period. In the light of the findings of this study, it was concluded that trace element supplementation applied to pregnant Simmental cows during the transition period could prevent excessive increase in serum NEFAs.

## CONFLICT OF INTEREST

None declared by the authors.

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