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# The effect of co-supplementation of fish oil and vitamin E on reproductive performance and metabolic profile during the transitional period in dairy cows

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**ABSTRACT**

The objective of the present study was to determine the effect of oral supplementation of dairy cows with a combination of fish oil and vitamin E (Vit E) from approximately 7 days (d) before the expected calving date up to 21 days postpartum, on their reproductive performance and metabolic profile. Eighty Holstein multiparous cows were randomly assigned into one of two ration groups during the transitional period. The treatment group cows (n=40) received a transitional diet (pre-and post-partum based on the NRC 2001) supplemented with fish oil (FO, 100 g/ once per day +Vit E (8000IU/kg/d); and the control cows (n=40) received the same concentrate without FO. Blood samples were collected at 1 week before the expected calving date, and 1, 2 and 3 weeks postpartum. After a voluntary waiting period, all cows received timed artificial insemination (TAI) at 76-81 days in milk (DIM) following the Presynch-OvSynch protocol. Pregnancy diagnosis was performed 30-40 days after TAI using a transrectal ultrasonography. Our results showed that the cows fed the FO+Vit E diet had a statistically higher first service pregnancy rate (46.3 % vs. 39.6%, $P<0.001$) at 30-40 days after TAI than those fed the control diet. Also, cows that consumed the FO+Vit E treatment diet had lower late embryo loss at 40-70 days post insemination than the control. Plasma concentrations of triglycerides (d7, 14, and 21), cholesterol (d7 and 14), glucose (d14), insulin (d-7) progesterone (d14 and 21) in the treatment group were higher ($P<0.05$) compared to the cows fed control diets. Non-esterified fatty acid (NEFA) levels were not significantly affected by the dietary treatments pre-partum, while β –hydroxybutirate acids (BHBA) levels on day 21 ($P<0.05$) were higher in the control group. The results indicated that supplementation of the diet with fatty acid and high doses of vitamin E could improve reproductive performance in dairy cattle.

**Key words:** fish oil; vitamin E; metabolic profile; reproductive performance; dairy cows

## Introduction

The transition period in dairy cows is a critical period from 2-4 weeks prior to calving to 2-4 weeks after calving (LEBLANC, 2010) associated with metabolic adaptations and physiological changes (GOFF and HORST, 1997). At present, selection of dairy cows intended for high milk production has a negative effect on the animals' ability for hormonal regulation of metabolic processes (KOČILA et al., 2013). During the transitional period the effects of negative energy balance (NEB) are not limited to endocrinological impact, as fertility may be affected by oocyte quality (LEROY et al., 2018). NEB in the

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early post-partum period, that disrupts endocrine signals, leads to delayed ovulation, decreased conception rates, and increasing embryonic mortality (STAPLES et al., 1998). Moreover, high levels of NEFA and BHBA were associated with increased peri-partum complications and, as a result, infertility problems (ĐURIČIĆ et al., 2020). Reproductive performance in dairy cattle is affected by multiple factors, including nutritional and metabolic status during the transitional period (MICHAEL et al., 2019). Thus, various additives, such as fatty acids (FA) and polyunsaturated FA (PUFA) are used in dairy cow rations during the periparturient period to enhance their reproductive status. It is well established that dietary fats can affect whole body metabolism, as well as having direct effects on the reproductive tract (HAYIRLI et al., 2011). Earlier information mainly focused on the effect of *omega-6* (*n*-6) and especially *omega-3* (*n*-3) FA. The (n-3) PUFA is known to be an important FA in mammals, particularly ruminants, which are not capable of synthesizing this FA and must have it supplied through feeding. Evidence shows the key role of FA and PUFA in various reproductive processes, particularly serving as precursors of prostaglandin and steroid hormones (SANTOS et al., 2008).

Fish oil (FO) contains relatively high concentrations of *n*-3 long-chain PUFAs, such as docosahexaenoic acid (DHA, C22:6), and eicosapentaenoic acid (EPA, C20:5). They are involved in the decrease of PGF2α production through the uterus (MATTOS et al., 2004; ELIS et al., 2016). Several reports in dairy cows have demonstrated the positive effects of *n*-3 PUFA supplementation on reproductive variables, including a higher pregnancy rate due to a decrease in embryo mortality and a tendency to increase fertility rates (DIRANDEH et al., 2013; PETIT and TWAGIRAMUNGU, 2006).

Vitamin E (Vit E) is a lipid-soluble cellular antioxidant that plays critical roles in the fertility, immunity, and preservation of cellular membranes, with declines in lipid peroxidation (RAEDERSTORFF et al., 2015 ). Based on the nutrient requirements of dairy cattle (NRC), Vit E supplementation levels are 0.8 and 1.6 IU/kg of body weight for lactating and pregnant cows, respectively. These levels reflect a basic threshold to prevent deficiency symptoms, and guarantee reasonable animal efficiency. However, dietary vitamin E levels of 1000 IU are suggested as they were shown to reduce the incidence of placental retention, as well as to improve the immune defenses of dairy cows (ALLISON and LAVEN, 2000). In addition, it has been suggested that dietary supplementation dietary with Vit E during transitional period was associated with fewer days to first observed estrus, days to first artificial insemination (AI), days open, and the number of AI per conception (MOGHIMI-KANDELOUSI et al., 2020). Supplementation of dietary fat might increase intestinal absorption of vitamin E and its transport capability, and thus enhance the energy condition of dairy cows around parturition (KARIMIAN et al., 2015). However, AMIRIFARD et al. (2016) found that there was no evidence of any positive effect on lactation performance correlated with 3000IU /day of vitamin E and dietary fat supplements in the transition period in cows.

According to previous studies, dietary fat consumption alone did not have any positive effect on reproduction performance (SWANEPOEL and ROBINSON, 2020). Therefore, it has been hypothesized that feeding dairy cows with a combination of FO and higher doses of Vit E (4000IU/day) from approximately 1 week before the expected calving date and until 3 weeks postpartum might reduce early lactation NEB and enhance the reproductive performance of multiparous Holstein cows. Therefore, this study aimed to evaluate the effects of oral supplementation of FO (n-3 PUFA source) and Vit E on the reproductive performance and metabolic profiles of dairy cows in the transitional period.

## Materials and methods

*Animals and experimental design.* This study was conducted during June 2014 to December 2015 on a large commercial dairy farm (AzarNegin Agro Ind. & Husbandry Co., Tabriz, Iran). The cows were kept in two free-stall barns, and housing and feeding management were the same for all the cows. All animals were cared for according to the guidelines established by the Iranian Council on Animal Care. For this study, 80 Holstein multiparous cows were randomly assigned into two groups (n= 40 cows per treatment), so that the mean of parity number (Parity=3.1±1.4, mean ± SD) and the average (±SEM) daily milk yield per cow (32.84±3.3) were similar in the two groups. Cows in each group received one of two rations during the transitional period. The treatments were: 1) treatment cows received a transitional diet daily (pre-and post-partum based on the NRC 2001) supplemented with fish oil (FO, 100 g/ once per day +Vit E (4000IU/ kg/d; Aras E-Vimix®, Pharmaceutical Co., Amol, Iran); and 2) control cows received the same concentrate without FO from 1 week antepartum up to 3 weeks postpartum. The rations were formulated according to NRC guidelines for dairy cows during the pre- and postpartum period, and were isocaloric and isoitrgenous (Table 1). The cows were fed the total mixed-ration (TMR) twice daily with no limitation in access to water. Table 2 shows the analysis of fatty acid (FA) profiles of FO using gas chromatography, as previously reported by NATEGHI et al. (2019).

Table 1. Ingredients and nutrient composition (DM basis) of pre-partum and post-partum diet

| Item | Pre-partum diet | Post-partum diet |
|---|---|---|
| Ingredient, % | | |
| Alfalfa hay | 23 | 24.8 |
| Corn silage | 28.3 | 15.3 |
| Beet sugar pulp | - | 5.5 |
| Wheat straw | 5.2 | 0.5 |
| Coarse ground barley | 15.1 | 8.2 |
| Coarse ground corn | 9 | 20.2 |
| Soybean meal | 6.1 | 9.0 |
| Whole linted cottonseed | 1.0 | 2.7 |
| Full-fat soybean | 2.2 | 3.3 |
| Canola meal | 2.2 | 2.4 |
| Corn gluten meal | 1.7 | 3 |
| Fat supplement | - | 1.2 |
| Calcium carbonate | 1.4 | 1.0 |
| Salt | - | 0.3 |
| Sodium bicarbonate | - | 0.9 |
| Magnesium oxide | - | 0.2 |
| Vitamin and mineral mix 1[1] | 4.8 | - |
| Vitamin and mineral mix 2[2] | - | 1.5 |
| Chemical composition | | |
| $NE_L$, Mcal/kg | 1.49 | 1.68 |
| Dry matter | 45.5 | 56.8 |
| Organic matter | 89.0 | 91.2 |
| Crude protein | 14.2 | 17.7 |
| Neutral detergent fiber | 38.9 | 32.1 |

Table 1. Ingredients and nutrient composition (DM basis) of pre-partum and post-partum diet (continued)

| Item | Pre-partum diet | Post-partum diet |
|---|---|---|
| Acid detergent fiber | 24.8 | 21.0 |
| Ether extract | 3.2 | 3.3 |
| Ca | 1.60 | 1.27 |
| P | 0.40 | 0.38 |

[1]Contained (DM basis): 15.2% Ca, 2.44% Mg, 10.46% Cl, 0.04% K, 0.82% Na, 3.5% S, 1,400 mg/kg Fe, 288.2 mg/kg of Mn, 302.5 mg/kg of Cu, 12.7 mg/kg of I, 504 mg/kg of Zn, 150 kIU/kg of vitamin A, 50 kIU/kg of vitamin D3, 4,000 IU/kg of vitamin E.
[2]Contained: 12% Ca, 2% P, 2.05% Mg, 18.6% Na, 0.3% S, 1,250 mg/kg of Fe, 2,250 mg/kg of Mn, 7,700 mg/kg of Zn, 14 mg/kg of Co, 1,250 mg/kg of Cu, 56 mg/kg of I, 10 mg/kg of Se, 250 kIU/kg of vitamin A, 50 kIU/kg of vitamin D3.

Table 2. The major fatty acid profiles of fish oil (% of total fatty acids).

| Fatty acid | Fish oil | Fatty acid | Fish oil |
|---|---|---|---|
| C14:0 | 2.58 | C18:3 n-3 | 2.12 |
| C14:1 | 0.29 | C20:0 | 0.33 |
| C15:0 | 0.63 | C20:1 | 2.64 |
| C15:1 | 0.15 | C21:0 | - |
| C16:0 | 18.07 | C20:2 | 0.10 |
| C16:1 | 6.31 | C22:0 | 0.33 |
| C17:0 | 1.47 | C20:3 n-3 | 0.78 |
| C17:1 | 1.02 | C20:4 n-6 | 0.07 |
| C18:0 | 5.46 | C22:2 | 1.04 |
| C18:1t | 0.23 | C24:0 | 0.13 |
| C18:1c | 29.65 | C24:1 | 1.56 |
| C18:2t | 0.22 | C20:5 n-3 EPA | 6.22 |
| C18:2c | 2.94 | C22:6 n-3 DHA | 14.27 |
| C18:3 n-6 | 0.20 | Other fatty acids | 1.19 |

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

*Sample collection and determination of hormones and metabolites.* Two hours after feeding, blood samples (10mL) were taken from the tail coccygeal veins using evacuated tubes containing anti-coagulant EDTA, 1week before the expected calving, and 1, 2 and 3 weeks postpartum. Plasma samples were collected by centrifuging the blood at 3000 *g* at 4°C for 10 min, and kept frozen at −20°C for further analysis. Commercial kits were used to determine the concentrations of glucose (kit no. 2135, Pars Azmoon Co., Tehran, Iran), cholesterol (kit no. 5412, Pars Azmoon Co.), triglycerides (TG; kit no. 6752, Pars Azmoon Co.), plasma BHBA concentrations were assessed using the Ranbut d-3-hydroxybutyrate (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK), using a kinetic enzymatic reaction with a sensitivity of 0.1 nmol/L. Plasma NEFA was measured using a biotin-labeled commercial ELISA kit (Bioassay Technology Laboratory, Shanghai, China). Insulin and progesterone concentrations were assessed using commercial ELISA kits (DRG Instruments GmbH, Marburg, Germany). The sensitivity levels of assay detection for progesterone and insulin were 0.1ng /ml and 0.75μIU/ml, respectively. The assay methods followed the manufacturers' instructions.

*Ultrasound examination and evaluation of reproductive performances.* The ovaries and uteri were examined by transrectal ultrasonography (SIUI- CTV-200V, with a 7.5-MHz linear-array transducer, China). The ovaries' follicular and corpus luteum (CL) dynamics were examined by ultrasonography every week between day 20 and 40 DIM, to look for the presence of a corpus luteum (CL), and to determine the time of postpartum cyclicity resumption. Ovulation was proved by the lack of a dominant (diameter≥10mm) follicle that was detected at the previous examination, and consequent corpus luteum formation ( COLAZO et al., 2009 ). On day 40 (± 5) after calving, the animals were assigned to Presynch-OvSynch: the cows received 2 injections of PGF2α analog, cloprostenol sodium (150 μg, i.m., D-Cloprostenol; Vetaglandin®; Aburaihan Pharmaceutical Company; Iran) 14 days apart, for presynchronization, and the Ovsynch-56 TAI protocol began 12 days later, with an injection of a GnRH analog, (25 μg, i.m., Alarelin Acetate; Vetarolin®; Aburaihan Pharmaceutical Company, Iran) on day 66, an injection of PGF2α analog on day 73, a second injection of GnRH analog 56 hours after PGF2 α , and TAI 12-14 hours later at 76±5 DIM (HERLIHY et al., 2012).

Pregnancy diagnosis was performed 30-40 days after TAI using transrectal ultrasonography (SIUI- CTV-200V, with a 7.5-MHz linear-array transducer, China) and pregnancy status was reconfirmed 60-70 days after TAI by palpation of the uterus (BARLETTA et al., 2017). Late embryonic loss (d 40 to 70 after TAI) was confirmed on the basis of non-observation of heart rate, and signs of embryo degeneration. Thus, pregnancy loss included both early and late embryonic loss in this study (GÁBOR et al., 2016). Reproductive performance, such as first service pregnancy rate, intervals from calving to first insemination, was determined in the studied groups.

*Statistical analyses.* Data were analyzed using a multivariable logistic model (GLIMMIX procedure of SAS version 9.3). The data were checked for normality before analysis using the Shapiro-Wilk test. When the data were not normally distributed, they were transformed using a $\log_{10}$ transformation before analysis. Analyses of covariance were performed through repeated measures with a first-order autoregressive covariance structure. The model included the effects of treatment and time, and the interaction of treatment × time. The Tukey-Kramer adjustment was applied for multiple comparisons. Also, reproductive performance parameters were analyzed by survival analysis using the product limit method of the Kaplan-Meier model by the LIFETEST procedure of SAS. The threshold of significance was set at $P \leq 0.05$, and $0.05 \leq P \leq 0.1$ was considered as a tendency.

## Results

*Reproductive performances.* Our results showed that there were no differences in intervals from calving to first insemination between the supplemented and control groups (43 vs. 46 DIM; $P = 0.1$). Cows fed the FO+Vit E diet had a statistically higher first service pregnancy rate (46.3% vs. 39.6%, $P<0.001$) at 30-40 days after TAI than those fed the control diet. Furthermore, cows that consumed FO+VitE had a lower late embryo loss at 40-70 days post insemination than those fed the control diet (5.5% vs. 8.5 %, $P<0.001$).

*Plasma metabolites and hormones findings.* Plasma concentrations of metabolites and hormones are presented in Fig.1-3. During the postpartum period (days 7, 14 and 21), the concentrations of triglycerides were greater ($P<0.001$, $P<0.001$ and $P<0.01$, respectively) in the cows that consumed the FO+Vit E diet than those fed the control diet (Fig.1). Plasma concentrations of cholesterol on days 7 and 14 after parturition were significantly higher ($P<0.001$ and $P<0.05$, respectively) in the cows fed the treatment diet than those fed the control diet (Fig. 1). Plasma glucose concentrations on day 14 in the cows fed the FO+Vit E diet were dramatically higher ($P<0.001$) but on day 7 they were significantly lower ($P<0.05$) than those of the control group. Plasma concentrations of insulin decreased from days 7 to day 14 postpartum in both groups, while they significantly (P<0.001) increased in control cows during 21 days postpartum (Fig.1). Furthermore, plasma progesterone concentrations in the cows fed the FO+Vit E diet on day 14 (P<0.01) and day 21 (P<0.05) postpartum were drastically higher compared with the control group (Fig. 2). NEFA levels were not significantly affected by the FO+Vit E supplemented diet during the transitional period. However, BHBA levels on day 21 in cows fed FO+Vit E were significantly lower (P<0.05) compared to the control group (Fig.3).

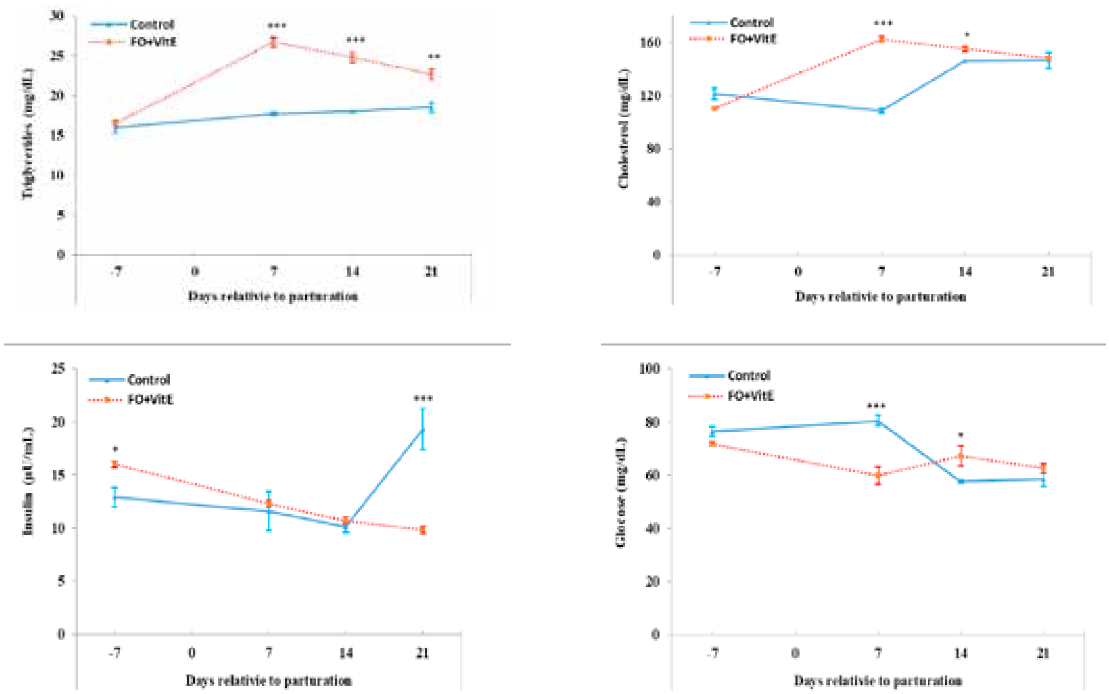


Fig.1. Time course of the plasma concentrations (means± SEM) of triglycerides, cholesterol, insulin and glucose in dairy cows supplemented with (FO+VitE) or without (Control) fish oil combined with vitamin E during late gestation and early lactation. * (P<0.05), ** (P<0.01) and *** (P<0.001) indicate a significant difference between Control and FO+Vit E at a given time-point.

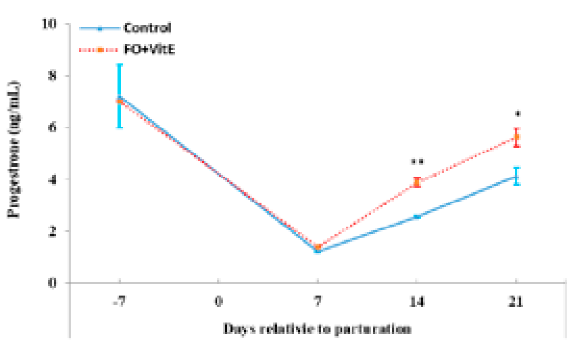


Fig. 2. Time course of the plasma (means ± SEM) progesterone concentration in dairy cows supplemented with (FO+VitE) or without (Control) fish oil combined with vitamin E during late gestation and early lactation. *(P<0.05) and **(P<0.01) indicate a significant difference between Control and FO+Vit E at a given time-point.

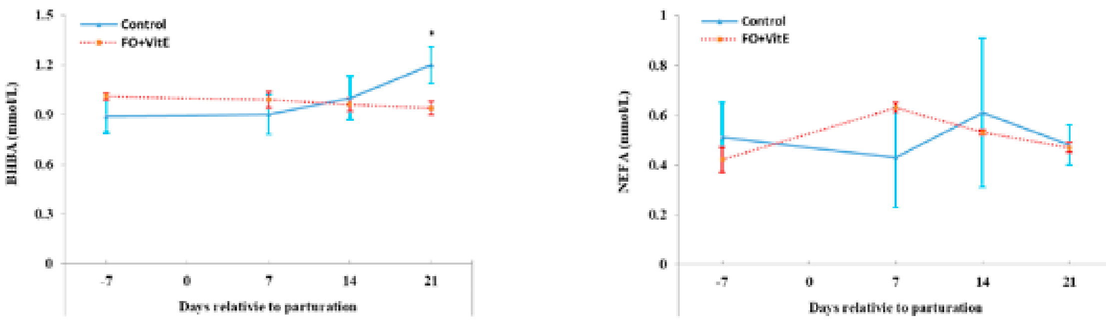


Fig. 3. Time course of the plasma (means ± SEM) non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) concentration in dairy cows supplemented with (FO+VitE) or without (Control) fish oil combined with vitamin E during late gestation and early lactation.
*(P<0.05) and **(P<0.01) indicate a significant difference between Control and FO+Vit E at a given time-point.

## Discussion

During the transitional period, feed intake often does not supply the energy needed for maintaining basal metabolism and milk production (GRUMMER et al., 2004; FOLNOŽIĆ et al., 2015), which leads to the development of a NEB (KOČILA et al., 2009). It has already been shown that a NEB can influence the timing of the first postpartum ovulation and progesterone levels in dairy cows (SZENCI et al., 2018). Previous studies have suggested that dietary fat supplementation may improve energy status and reproductive performance during the postpartum period (DIRANDEH et al., 2013; RODNEY et al., 2015). Our results showed that dietary supplementation with a combination of FO and Vit E improved pregnancy rates at 30-40 days after TAI and reduced the rate of late embryo loss at 40-70 days postpartum . This effect may be the result of improving oocyte quality (BURKE et al., 1997). In agreement with our results, BURKE et al. (1997) reported that there was an improvement in pregnancy rate in cows fed fishmeal compared with control cows. In addition, SILVESTRE et al. (2011) showed that pregnancy per AI was not affected by dietary conjugated linoleic acid (CLA) supplementation during the postpartum period, but pregnancy loss was lower in supplemented cows.

In this experiment, we measured the effects of FO and high doses of vitamin E in the transition diet on reproductive efficiency. Our results showed that supplementation of the transition diet with FO plus vitamin E had no effect on the first service pregnancy rate, or intervals from calving to first insemination. In agreement with our results SWANEPOE and ROBINSON (2020) suggested that Optomega as a source of FO could not improve the first service pregnancy rate in dairy cattle. Previous studies have shown that increased feeding of fat during the transitional period was related to negative impacts on rumen fermentation and dry matter (DM) intake, without any positive effect on reproductive efficiency (SWANEPOEL and ROBINSON, 2020). Contrary to our results MCNAMARA et al. (2003) found that feeding fats increased first service pregnancy, but did not change the overall percentage of pregnant cows. RODNEY et al. (2015) stated that the overall effects of feeding fat enhanced the proportion of cows pregnant to service, and tended to reduce the interval from calving to pregnancy in treated cattle.

The results of the present study showed that supplementation FO+VitE in the diet of cows during the transition period reduced embryonic death and increased pregnancy at 30-40 days after the TAI. In agreement with our results, AMBROSE et al. (2006) emphasized that fatty acid could increase the size of the dominant follicle and reduce pregnancy loss.

In this experiment, we did not measure progesterone and metabolites of PGF2α a few weeks after TAI, but MATTOS et al. (2004) reported that FO could improve pregnancy rates primarily by reducing embryonic losses by alterations in the endometrial expression of the genes regulating PGF2α synthesis, potentially due to the reduction in uterine PGF2α , which might delay the regression of the corpus luteum (CL), and hence improve embryo survival. BADIEI et al. (2014) demonstrated that plasma progesterone concentrations increased in cows fed diets enriched with n-3 or n-6 FA, which is in agreement with the present study.

In the present study, the effects of FO and a high dose of vitamin E on the amount of metabolic and hormonal factors in plasma were measured. Similarly to previous studies, total cholesterol in the control cows decreased during late pregnancy up to parturition (FOLNOŽIĆ et al., 2015). However, cows on a diet supplemented with a combination of FO and VitE, had increased cholesterol levels after calving. MOSSA et al. (2012) suggested that a high level of cholesterol could improve pregnancy rates. Furthermore, WESTWOOD et al. (2002) reports that higher concentrations of plasma cholesterol were associated with a shorter interval from calving to pregnancy, with a greater probability of conception and successful pregnancy by day 150 of lactation. The higher pregnancy rates in the supplemented cows may be, at least in part, due to higher cholesterol concentrations as a progesterone precursor, which is an important hormone for the conception and nourishment of a newly formed embryo in mammals (STAPLES et al., 1998; BUTLER, 2001).

In this study, the cows that did not receive FO+VitE had low concentrations of triglycerides at calving and they remained at lower levels until day 21. Similar results were obtained in previous studies (TURK et al., 2013). MANTOVANI et al. (2010) reported that the mammary gland took up the triglycerides for milk fat synthesis (BERNARD et al., 2008) or triglyceride accumulation in the liver (RUKKWAMSUK et al., 1998; TURK et al., 2005). However, in this study, the cows that received fish oil plus vitamin E in their diet had higher triglyceride levels. This is in agreement with the report by YADAV et al. (2019) that triglyceride and cholesterol levels increased from 7 - 21 days after calving. Previous studies have shown that high cholesterol levels in plasma leads to increased ovarian activity in animals (GUEDON et al., 1999). Therefore, changes in lipid metabolism during the transitional period tend to be related to the resumption of cyclicity during the early postpartum period.

In the present study, FO provided a higher plasma glucose concentration two weeks after calving. According to GROSSI et al. (2013) decreasing lipomobilization in cows receiving omega-3 FA was followed by the reduced concentration of BHB and increased plasma glucose concentration. It has been shown that, increased glucose at calving may be due to the release of glucocorticoids before calving, which promote glycogenolysis and gluconeogenesis, whereas the lower concentration of glucose during the post-partum period relative to the pre-partum period is presumably related to DMI, which is inadequate to fulfill the glucose requirements for milk synthesis (LEURY et al., 2003). Accordingly, supplementation of meals with glucose precursors leads to improvements in productivity and animal health (ALADROVIĆ et al., 2018)

It has been indicated that ovarian follicular development is influenced by plasma insulin concentrations, as it stimulates steroidogenesis and the proliferation of granulosa cells (CHILDS et al., 2008; FRANCISCO et al., 2003). Our results revealed that three weeks after calving, plasma insulin concentrations decreased in cows fed diets enriched with fish oil plus vitamin E. Also, similar results were reported by BILBY et al. (2006) who found lower plasma insulin concentrations in cows supplemented with FO than in those supplemented with whole cottonseed (BILBY et al., 2006). Contrary to our results, it has been confirmed that unsaturation of FA has an effect on insulin release in animals, and a higher insulin response to rumen-protected, unsaturated, long-chain FA is expected (MOALLEM et al., 2007).

In the present study, progesterone concentrations were very low until the resumption of cyclic ovarian activity. The release of progesterone 14 days after calving may be related to the first ovulation, and this indicates that the FO+ vitamin E accelerates the time of the first ovulation in cows. A previous study showed that normal cows had less plasma progesterone (P4) and smaller corpora lutea than cows receiving the Trans-10, cis-12 conjugated linoleic acid (CLA) (HUTCHINSON et al., 2012).

Multiple studies have shown a negative association between of NEFA and fertility (BUTLER, 2001; SZENCI et al., 2018). One trial found that increased NEFA concentrations during the transition period were associated with a decrease in pregnancy rates at 70 days after the VWP (OSPINA et al., 2010). Our results showed that NEFA levels were not affected in cows fed FO in the transition period. The results showed that serum BHB concentrations on day 21 postpartum were significantly higher in the control group compared to the FO treatment. Results obtained in studies by other authors (TURK et al., 2016; FOLNOŽIĆ et al., 2015) indicated that serum NEFA and BHBA enhanced significantly from parturition until day 19 after calving. These changes indicate fat mobilisation from adipose tissue due to the energy deficit during the transition period (FOLNOŽIĆ et al., 2015).

The results of this study showed that plasma NEFA concentrations decreased from days 14 to 21 after calving compared to the control group; however, these differences were not significant. In line with our results, BALLOU et al. (2009) indicated that cows fed FO supplement in the first two weeks of lactation had decreased NEFA and BHBA concentrations. It has been shown that lower concentrations of NEFA in the cows were due to the resumption of ovarian cyclicity during the puerperium period (FOLNOŽIĆ et al., 2016). These findings revealed that the cows in the control group might have a longer NEB than in the FO+VitE group.

## Conclusion

It may be assumed that supplementing dairy cows with a mixture of fish oil and a high dose of vitamin E during the transitional period could change their metabolic profile, pregnancy rate, and improve the reproductive efficiency of dairy cows.

**Conflict of interest**

All authors of this study declare that they have no conflict of interest.

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**ASADPOUR, R., H. SADRI, R. JAFRI-JOOZANI, M. TOLOUEI: Učinak dodanog ribljeg ulja i vitamina E na reproduktivnu sposobnost i metabolički profil mliječnih krava tijekom prijelaznog razdoblja. Vet. arhiv 93, 17-30, 2023.**

**SAŽETAK**

Cilj istraživanja bio je utvrditi učinak peroralnog dodavanja kombinacije ribljeg ulja i vitamina E (VIT E) u obroke mliječnih krava. Dodavanje je započelo približno 7 dana (d) prije očekivanog teljenja i trajalo do 21. dana nakon teljenja, a učinci su procijenjeni s obzirom na reproduktivnu sposobnost i metabolički profil krava. Osamdeset višeteltki pasmine holštajn nasumično je raspoređeno u dvije brojčano ujednačene skupine tijekom prijelaznog razdoblja. Eksperimentalna skupina (n = 40) primila je hranu za prijelazno razdoblje (prije i poslije teljenja na temelju NRC 2001) dopunjenu (FO) ribljim uljem 100 g/jednom dnevno i vitaminom E 8000IU/kg/d. Krave iz kontrolne skupine (n = 40) primile su istu hranu za prijelazno razdoblje bez FO. Uzorci krvi krava prikupljeni su 1 tjedan prije očekivanog datuma teljenja te 1, 2 i 3 tjedna nakon teljenja. Servisno razdoblje svih krava završilo je umjetnim osjemenjivanjem (TAI) koje je prema protokolu Presynch-OvSynch provedeno od 76. do 81. dana laktacije (DIM). Provjera gravidnosti provedena je transrektalnim ultrazvukom 30-40 dana nakon umjetnog osjemenjivanja. Naši rezultati pokazuju da su nakon 1. pripusta 30.-40. dana nakon umjetnog osjemenjivanja krave eksperimentalne skupine (FO + VIT E dodatak) imale statistički višu stopu gravidnosti u odnosu na krave kontrolne skupine (46,3% prema 39,6%, P<0,001). Također, krave koje su primile FO + VIT E dodatak prehrani imale su u odnosu na krave kontrolne skupine manje kasne gubitke embrija 40-70 dana nakon osjemenjivanja. Koncentracije triglicerida (dani 7., 14. i 21.), kolesterola (dani 7. i 14.), glukoze (dan 14.), inzulina (dan 7.) progesterona (dani 14. i 21.) u plazmi krava iz eksperimentalne skupine bile su više (P<0,05) u usporedbi s plazmom krava kontrolne skupine. Prenatalni dodaci prehrani nisu znakovito utjecali na razine neesterificiranih masnih kiselina (NEFA), a razine β hidroksibutirat kiselina (BHBA) 21. dana bile su više (P<0,05) u kontrolnoj skupini. Rezultati su pokazali da bi dopuna prehrane masnim kiselinama i visokim dozama vitamina E mogla poboljšati reproduktivnu sposobnost mliječnih krava.

**Ključne riječi**: riblje ulje; vitamin E; metabolički profil; reproduktivna svojstva; mliječne krave