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THE EFFECT OF A BYPASS N-3 FATTY-ACID SUPPLEMENTATION ON THE REPRODUCTIVE RESUMPTION IN DAIRY COWS AFTER CALVING

UČINAK DODAVANJA BYPASSA N-3 MASNIH KISELINA NA NASTAVAK REPRODUKCIJE MLIJEČNIH KRAVA NAKON TELJENJA

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SUMMARY

This study evaluated the effects of the n-3 fatty-acid supplementation on a reproductive and productive performance of dairy cattle, focusing on a successful early insemination. A total of 61 cows were assigned to either the control (control, n = 31) or Hi-Flax (HF, n = 30) group on a commercial farm located in Normandy (Manche, France). Both groups received the same basal diet, and the animals in the HF group were supplemented with 250 g/day rumen protected source of n-3 fatty acids during the first 55 days of lactation. The productive parameters included the milk yield, fat, and protein content, feed intake time, and rumination time during the first 100 days of lactation. The reproductive parameters analyzed were as follows: calving interval (i.e., the intervals from calving to first estrus), first AI, and a successful AI; the number of AIs, and the fertility at the first AI. The ANOVA and the linear mixed models were applied for the productive traits, with a treatment, parity, and days in milk as the fixed effects, and cow as a random effect. The reproductive parameters were analyzed using the linear and the generalized linear mixed models, assuming a binomial distribution for fertility. The results indicated no significant differences in a productive performance. However, the HF group had reduced the intervals from calving to the first AI (-24 days, 18%, p = 0.06) and a successful AI (-31.94 days, 19%, p = 0.01), leading to a shorter calving interval (-31.94 days, 7%, p = 0.02). These findings showed that the supplementation with a rumen-protected source of n-3 fatty acids improved a reproductive performance in dairy cattle, with a subsequent reduction of non-productive periods. The results highlighted a beneficial impact on the resumption to a reproductive activity, with a reduction of an interval from calving to the first estrous (heat), an earlier first successful artificial insemination, and a resulting calving-interval decrease.

Keywords: Omega 3, reproductive performance, dairy cattle, productivity.

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INTRODUCTION

An excellent reproductive performance in both males and females is essential for a profitable dairy- and beef-production systems (Berry et al., 2014). A poor reproductive performance reduces both the herd and an individual cow profitability González-Recio et al. (2004) estimated an economic impact of fertility traits, having reported that each additional insemination costs -$67.32/cow/year due to an increased semen use, hormonal treatments, and culling expenses. Furthermore, the cows with a poor fertility have a lower lifetime production, shorter productive lives, and a reduced profitability. Similarly, each additional day in the calving interval results in a loss of -$4.90/cow/year due to a prolonged dry period and a decreased milk production in a late lactation (CONAFE, 2024). To optimize reproduction and minimize the non-productive periods—thereby enhancing profitability—nutritional, genetic/selection, and management strategies must be carefully designed. From a nutritional perspective, in recent decades a particular attention has been dedicated to a transitional period, during which the cows may experience a negative energy balance, impairing the metabolic functions and exacerbating inflammation. Focusing on the inflammation, an n-3 anti-inflammatory activity can diminish the improvement of the status of an animal, especially in a postpartum period (Van Winters, 2023). Roche (2006) described the causes of the problems associated with a transitional period as multifactorial, primarily linked to an inadequate nutrition during the transitional period, leading to a reduced glucose, insulin, insulin-like growth factor, and a low LH-pulse frequency. Several metabolic disorders negatively affect fertility, including ketosis, hypocalcemia, acidosis, fatty liver, metabolic acidosis, tetany, and a displaced abomasum (Sundrum, 2015., Ruiz Rodriguez et al., 2019). To mitigate these metabolic disorders and their effects on fertility, the targeted nutritional strategies have been developed, including an energy supplementation through the bypass fats, rumen-protected amino acids and vitamins, and the rumen-fermentation modulators such as the organic acids, yeasts, and other bioactive compounds, particularly following a ban on the antibiotic use in Europe. Additionally, a regulation of physiological and inflammatory status during insemination is crucial for the improvement of conception rates and overall fertility. In this context, the omega-3 fatty acids (n-3s) have been proven to play a significant role in a reproductive performance (Bork et al., 2010) and inflammation control. These fatty acids promote the PG3α production, enhancing follicular maturation and increasing the embryo survival by preventing a luteal regression. They also positively influence the LH and progesterone secretion (Staples et al., 1998; Petit et al., 2004; Gulliver et al., 2012). Moreover, the n-3s modulate an immune function by promoting a lymphocyte proliferation and inhibiting the pro-inflammatory cytokines (Serhan et al., 2015). This study aimed to evaluate the impact of a n-3 fatty-acid supplementation on a reproductive and productive performance of dairy cattle, with a particular focus on the achievement of an early and successful insemination.

MATERIALS AND METHODS

The data from 61 cows from a commercial farm in Bourgvallées (Manche, France) were analyzed to evaluate the differences between the two treatments: a control (n = 30) and an HF (n = 31) one. The cows were randomly assigned to the treatment groups, with both groups having an average parity of 1.9. The average milk yield amounted to 26 kg in the control group and 27.8 kg in the HF group. While the control group received a standard commercial diet, the HF group was supplemented with 250 g/day of Hi-Flax® (a rumen-protected source of n-3 from the flaxseed oil, with a hydrogenated stearin protection) during the first 55 days of lactation.

A productive performance was monitored during the first 100 days of lactation, measuring a daily milk yield (MY, kg), fat content (FC, %), protein content (PC, %), fat yield (FY, kg), protein yield (PY, kg), feed intake time (FIT, min), and rumination time (RT, min), which were measured using the NEDAP CowContol collars (nedap-livestockmanagement.com). The reproductive parameters included a calving interval (CI, days), an interval from calving to the first estrus (CII, days), an interval from calving to the first artificial insemination (CIAII, days), an interval from calving to a successful AI (CAII, days), a number of AIs (n.AI), and a conception rate in the first AI (CR, %).

The statistical analyses of the productive traits were performed applying the ANOVA, followed by a post hoc Tukey's test with Bonferroni's corrections, applying a linear mixed model. The model included parity, days in milk, and treatment (control, CTRL,

and HF) as the fixed effects, with a cow treated as a random effect. The reproductive parameters were analyzed applying the linear models that accounted for a treatment and parity as the fixed effects, except for fertility, which was analyzed using a generalized linear mixed model. The fertility model included a treatment, a number of artificial inseminations, and parity as the fixed effects, with a cow being treated as a random effect, assuming a binomial distribution for a response variable with a logit-link function. All statistical analyses were conducted using the lme4, emmeans, multcomp, and car packages in the *R* software (R Core Team, 2021).

RESULTS AND DISCUSSION

Table 1 presents the least square means and the contrasts assessing the differences among the treatments for both the productive and reproductive parameters. The absence of significant differences in the MY, FC, PC, FIT, and the RT indicates that a dietary supplementation with the n-3 fatty acids did not exert a detrimental effect on an overall productivity. These results are consistent with the previous findings reported by Gonthier et al. (2005), Bork et al. (2010), and Nanas et al. (2023), who similarly observed no significant modifications in milk production following an n-3 fatty-acid supplementation.

In our study, the non-differences in the FC among the treatments were estimated according to Nanas et al. (2023). This observation may be explained by a rumen-protected nature of the supplemented fatty acids, which likely reduced their susceptibility to biohydrogenation in the rumen fluid. This ruminal biohydrogenation generates the specific trans-fatty acid intermediates, such as the trans-10 and the cis-12 conjugated linoleic acid (CLA), which have been identified as the potent inhibitors of a de novo fatty acid synthesis in the mammary gland and are associated with a milk-fat depression (Jenkins and Harvatine, 2014). Moallem (2009) reported a decrease when supplementing with the extruded flaxseed (40 g/kg of DM) from 3.6 to 3.4% ($p < 0.05$) in the high-yielding cows, but with a non-effect on

Table 1 The least square means (s. e.) and a contrast of differences (s. e.) of the control (CTRL) and HF treatments regarding the productive and reproductive traits

Tablica 1. Srednje vrijednosti najmanjih kvadrata (s. e.) i kontrast razlika (s. e.) kontrolne (CTRL) i HF skupine u pogledu produktivnih i reproduktivnih svojstava

Productive traits / Produktivne osobine	Diet / Dijeta		Difference / Razlika	
	Control	HF	HF-CTRL	P Value
Milk Production (MY, kg) / Prinos mlijeka (PM, kg)	40.2(0.76)	39.5(0.77)	-0.67(1.05)	P=0.52
Fat content (FC, %) / Sadržaj masti (SM, %)	4.06(0.06)	4.04(0.06)	-0.02(0.07)	P=0.79
Protein content (PC, %) / Sadržaj bjelančevina (SB, %)	3.38(0.03)	3.33(0.03)	-0.05(0.037)	P=0.14
FC/PC / SM/SB	1.20(0.02)	1.22(0.02)	0.014(0.03)	P=0.53
Fat Yield (FY, kg) / Prinos masti (PM, kg)	1.62(0.04)	1.57(0.04)	-0.05(0.05)	P=0.28
Protein Yield (PY, kg) / Prinos bjelančevina (PB, kg)	1.30(0.03)	1.34(0.03)	0.04(0.04)	P=0.27
Intake Time (FIT, min) / Vrijeme unosa hrane (VUH, min)	482(21.4)	480(21.4)	-2.94(29.39)	P=0.92
Rumination Time, (RT, min) / Vrijeme preživanja, (VP, min)	368(23.7)	384(23.7)	15.89(0.32)	P=0.63
Consumption / Konzumacija	6.54(0.11)	6.77(0.11)	0.23(0.15)	P=0.12
Reproductive traits / Reproduktivne osobine				
Calving - First Oestrous (C1E, days) / Teljenje – Prvi estrus (T1E, dani)	68.3(10.1)	48.4(10.1)	-19.93(13.92)	P=0.15
Calving - First AI (C1AI, days) / Teljenje – Prvi UO (T1UO, dani)	136(8.39)	112(8.74)	-24.00(11.32)	P=0.06
Calving - Successful AI (CAI, days) / Teljenje – Uspješan UO (TUO, dani)	163(8.95)	131(9.58)	-31.94(13.1)	P=0.01
Calving Interval (CI, days) / Interval teljenja (IT, dani)	446(8.95)	414(9.58)	-31.94(13.03)	P=0.02
Number of AI (n.AI, n) / Broj UO (b.UO, n)	1.46(0.18)	1.56(0.18)	0.09(0.24)	P=0.68
Fertility (CR) / Plodnost (TR)	0.56(0.09)	0.59(0.10)	0.03(0.10)	P=0.85

P<0.05 was considered statistically significant, p<0.10 was considered a tendency / P<0.05 smatralo se statistički značajnim, p<0,10 smatralo se tendencijom

the fat yield (FY). Jahani-Moghadam et al. (2015) reported a decrease from 4.17 to 3.55% ($p < 0.05$) but with a non-compromising FY ($p > 0.05$) in the cows supplemented during the first 40 days post calving. In our study, the encapsulation of the n-3 fatty acids may have mitigated this inhibitory effect. The previous studies reported slight reductions in the milk-fat percentage when the whole flaxseed was used as an n-3 fatty-acid source, if compared with the rumen-protected sources (Petit, 2002; Petit et al., 2002). The results support a hypothesis that the effective protection strategies can enhance the utilization of n-3 fatty acids in the lactating dairy cows without the adverse effects on a productive performance or a milk-fat synthesis.

With regard to an effect exerted on the fertility parameters, notable improvements were observed in a reproductive performance. The cows in the HF group exhibited a faster resumption of a reproductive activity, as evidenced by an earlier first estrus, occurring 19.9 days sooner than in the control group ($p = 0.15$). This reduction was greater than a 12-day decrease and a 3.2% increase in the pregnancy rate, reported by Nanas et al. (2023) in the multiparous cows supplemented with flaxseed and lupins. These values also exceeded a 6.5-day reduction reported by Jahani-Moghadam et al. (2015). Moreover, an interval from calving to the first artificial insemination (CIAI) was significantly reduced by 24 days ($p = 0.02$), leading to an earlier conception. A time from calving to a successful AI was shortened by 31.9 days ($p = 0.01$), which directly contributed to a 31.9-day reduction in a calving interval (CI;$p = 0.01$). In a previous study, following the same supplementation protocol, López-Paredes et al. (2023) reported a similar reduction in a calving interval (33 days, $p < 0.05$), along with a decrease of 0.5 in the number of AIs required for pregnancy.

With a focus on the underlying mechanisms behind these improvements, the positive effects of n-3 fatty acids on a reproductive function are linked to their role in the follicular development, embryo quality, and luteal function. The n-3s contribute to the regulation of prostaglandin synthesis, particularly of the PG3α, which plays a crucial role in the maintenance of the luteal phase and the prevention of a premature luteolysis, ultimately enhancing the pregnancy rates (Madureira; Petit et al., 2002; Dirandeh et al., 2013; Grossi et al., 2013; Bragaglio et al., 2015). Additionally, the n-3 fatty acids have been proven to influence cholesterol metabolism, a key precursor to a steroid-hormone synthesis. Cholesterol serves as a substrate for progesterone production, which is essential for the corpus luteum maintenance and early pregnancy establishment. This mechanism further reinforces a beneficial impact of an n-3 supplementation concerning the fertility outcomes, contributing to an improved reproductive management and farm profitability.

CONCLUSIONS

The supplementation with rumen protected source of n-3 fatty acids improves the reproductive performance in dairy cattle with the subsequent reduction of the non-productive periods. The results highlighted the beneficial impact on the resumption to the reproductive activity, with a reduction of the interval from calving to first estrous (heat), and the interval from calving to first successful artificial insemination, and the resulting calving interval decrease.

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SAŽETAK

Ova je studija ispitivala učinke dodatka n-3 masnih kiselina na reproduktivne i proizvodne parametre mliječnih krava, s naglaskom na rano uspješno osjemenjivanje. Ukupno 61 krava bila je raspoređena u kontrolnu skupinu (kontrolna n = 31) ili Hi-Flax (HF, n = 30) skupinu na komercijalnoj farmi u Normandiji (Manche, Francuska). HF skupina primala je 250 g/dan zaštićenog izvora n-3 tijekom prvih 55 dana laktacije, dok su obje skupine bile hranjene istim obrokom. Proizvodni parametri uključivali su količinu mlijeka, udio masti i bjelančevina, vrijeme unosa hrane i vrijeme preživanja tijekom prvih 100 dana laktacije. Analizirani reproduktivni parametri obuhvaćali su interval od teljenja do prvoga estrusa, prvoga umjetnog osjemenjivanja (UO) i uspješnoga UO-a, broj osjemenjivanja te plodnost pri prvome UO-u. ANOVA i linearni mješoviti modeli korišteni su za analizu proizvodnih parametara, pri čemu su tretman, redoslijed laktacije i dani u laktaciji bili fiksni efekti, a krava je bila nasumični efekt. Reproduktivni parametri analizirani su linearnim i generaliziranim linearnim mješovitim modelima, pri čemu je pretpostavljena binomska distribucija za plodnost. Rezultati nisu pokazali značajne razlike u proizvodnim parametrima. Međutim, HF skupina imala je kraće intervale od teljenja do prvoga UO-a (-24 dana, 18 %, p = 0.06) i uspješnoga UO-a (-31.94 dana, 19 %, p = 0.01), što je dovelo do skraćenja intervala između teljenja (-31.94 dana, 7 %, p = 0.02). Ovi su rezultati pokazali da suplementacija zaštićenim izvorom n-3 masnih kiselina poboljšava reproduktivnu sposobnost mliječnih goveda, uz naknadno smanjenje neproduktivnih proizvoda. Rezultati su istaknuli povoljan učinak na nastavak reproduktivne aktivnosti, sa smanjenjem intervala od teljenja do prvoga estrusa (tjeranja) i prvoga uspješnog umjetnog osjemenjivanja, te rezultirajućim smanjenjem intervala teljenja.

Ključne riječi: Omega 3, reproduktivni parametri, plodnost