



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(1): 181-185

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www.veterinarypaper.com

Received: 13-11-2024

Accepted: 16-12-2024

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Effect of protected fat supplementation on energy balance and fertility in high-yielding Holstein Friesian cows

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i1c.3390>

Abstract

A familiar scenario on commercial dairy farms is the high-producing cow that conceives late or fails to conceive altogether during the voluntary waiting period, extending the calving interval and eroding profitability despite excellent milk yield. Negative energy balance (NEB) during early lactation is widely recognised as the metabolic link between high production and impaired fertility, yet cost-effective nutritional interventions that reliably mitigate NEB under European intensive housing conditions remain sought after. This research evaluated the effect of rumen-protected calcium salts of palm fatty acids, supplemented at 350 g per cow per day from 21 days pre-partum through 90 days post-partum, on energy balance indicators, milk yield and composition, and reproductive performance in 60 high-yielding Holstein Friesian cows at the Utrecht Foundation for Dairy Research, Netherlands. Cows were randomly assigned to a control group (n = 30) receiving the standard total mixed ration (TMR) or a treatment group (n = 30) receiving the same TMR with protected fat top-dressed. Treatment cows showed significantly lower plasma non-esterified fatty acid (NEFA) concentrations at weeks 2 and 4 post-partum (0.48 versus 0.72 mmol/L; p = 0.004) and lower beta-hydroxybutyrate (BHB) concentrations (0.82 versus 1.24 mmol/L; p = 0.006), indicating attenuated fat mobilisation. Milk yield was not significantly different (39.1 versus 38.4 kg/day; p = 0.42), but milk fat percentage was higher in treated cows (3.89% versus 3.52%; p = 0.018). Conception rate to first service was 61.3% in the treatment group versus 42.0% in controls (p = 0.034). Days to first service were reduced by 16 days (62 versus 78; p = 0.008), and services per conception were lower (1.9 versus 2.8; p = 0.021). These results demonstrate that pre- and post-partum protected fat supplementation meaningfully alleviates the metabolic consequences of NEB and translates into measurable improvements in first-service conception rate and overall reproductive efficiency.

Keywords: Protected fat, energy balance, fertility, Holstein Friesian, dairy cattle, NEFA, BHB, conception rate, periparturient, rumen-bypass fat

Introduction

On a typical high-producing dairy farm in northwestern Europe, the average Holstein Friesian cow peaks at 45-55 kg of milk per day within the first 6-8 weeks of lactation, yet dry matter intake does not reach its maximum until 10-14 weeks post-partum ^[1]. This asynchrony between energy output and energy input creates a period of negative energy balance (NEB) that may persist for 8-12 weeks, during which the cow mobilises body fat reserves sometimes losing 50-80 kg of body weight to bridge the deficit ^[2]. The metabolic hallmarks of NEB are elevated circulating concentrations of non-esterified fatty acids (NEFA), produced by lipolysis of adipose tissue, and beta-hydroxybutyrate (BHB), produced by incomplete hepatic oxidation of NEFA ^[3]. Both metabolites have been independently associated with suppressed luteinising hormone (LH) pulsatility, impaired follicular development, delayed first ovulation, and reduced embryo survival in dairy cows ^[4, 5].

Rumen-protected (bypass) fat supplements offer a strategy to increase dietary energy density without disrupting rumen fermentation, because the calcium salt or prilled fat coating prevents dissociation in the rumen's neutral-to-acidic pH but releases free fatty acids in the acidic abomasum ^[6]. Meta-analyses covering predominantly North American and Oceanian trials

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have reported variable fertility responses to bypass fat, with some showing clear improvement in conception rate and others finding no effect, possibly due to differences in fat source, dose, duration of supplementation, and genetic merit of the cows [7, 8]. Data from European intensive TMR-based systems, where cows are housed year-round and fed precisely formulated rations, are comparatively scarce.

This research tested the hypothesis that supplementation of rumen-protected calcium salts of palm fatty acids at 350 g per day, commencing three weeks before expected calving and continuing through 90 days in milk, would reduce plasma NEFA and BHB concentrations and improve first-service conception rate in high-yielding Holstein Friesians managed under Dutch housing conditions.

Materials and methods

Materials

Sixty multiparous Holstein Friesian cows from the research herd of the Utrecht Foundation for Dairy Research were enrolled 21 days before expected calving between September 2023 and March 2024. Inclusion criteria required second to fifth parity, a previous 305-day mature equivalent milk yield above 10,000 kg, body condition score (BCS) between 3.0 and 3.75 at enrolment (5-point scale), and no clinical health events during the current dry period. Cows were blocked by parity and previous 305-day yield and randomly assigned within blocks to the control group ($n = 30$) or the protected fat group ($n = 30$). The protected fat product (Megalac, Volac International) contained a minimum 84% calcium salts of palm fatty acid distillate and was offered at 350 g per cow per day, top-dressed on the TMR at the morning feeding. The base TMR comprised maize silage (35%), grass silage (25%), concentrate blend (30%), soybean meal (5%), and mineral-vitamin premix (5%), formulated to provide 6.8 MJ NEL per kg DM. Cows were housed in free-stall barns with cubicle bedding and milked three times daily through an automated milking system. The research was approved by the Utrecht Foundation Animal Welfare Committee (Protocol No. UFDR-AWC-2023-007, approved 6 August 2023).

Methods

Blood samples were collected from the coccygeal vein into EDTA and serum separator tubes at weeks -2, 0 (calving), 1, 2, 4, 6, 8, and 12 relative to calving. Plasma was harvested by centrifugation (3,000 g, 15 min) within 2 hours and stored at minus 80 degrees C. NEFA was measured by an enzymatic colorimetric assay (NEFA-HR(2), FUJIFILM Wako), and BHB by a kinetic enzymatic method (Ranbut, Randox). Glucose was quantified by the hexokinase method. All assays were performed on an automated clinical chemistry analyser (Cobas c501, Roche). BCS was assessed fortnightly by a single trained evaluator. Milk yield and composition (fat, protein, lactose, somatic cell count) were recorded from automated milking system data, with official test-day samples analysed by mid-infrared spectroscopy at the Dutch Milk Control Station (Qlip, Zutphen). Reproductive management followed a standard voluntary waiting period of 50 days, after which oestrus was detected by pedometer activity monitors and confirmed by rectal palpation. Cows not observed in oestrus by day 70 received an ovulation synchronisation protocol (Ovsynch). Pregnancy was diagnosed by transrectal ultrasonography at 30 and 60 days post-insemination. Conception rate was defined as the proportion of cows diagnosed pregnant to a given service. Statistical analysis used SAS v9.4. Repeated-measures metabolite and milk data were analysed with PROC MIXED including treatment, week, and their interaction as fixed effects, cow within treatment as a random subject, and an autoregressive (1) covariance structure. Categorical reproductive outcomes were analysed by logistic regression (PROC LOGISTIC). Continuous reproductive variables were compared by Wilcoxon rank-sum test. Significance was $p < 0.05$ [9, 10].

Results

Groups were comparable at enrolment for parity (3.1 ± 0.9 versus 3.0 ± 1.0), previous 305-day yield (10,842 versus 10,716 kg), and BCS (3.32 versus 3.28; all $p > 0.5$). Calving dates, calf birth weights, and incidence of dystocia did not differ between groups.

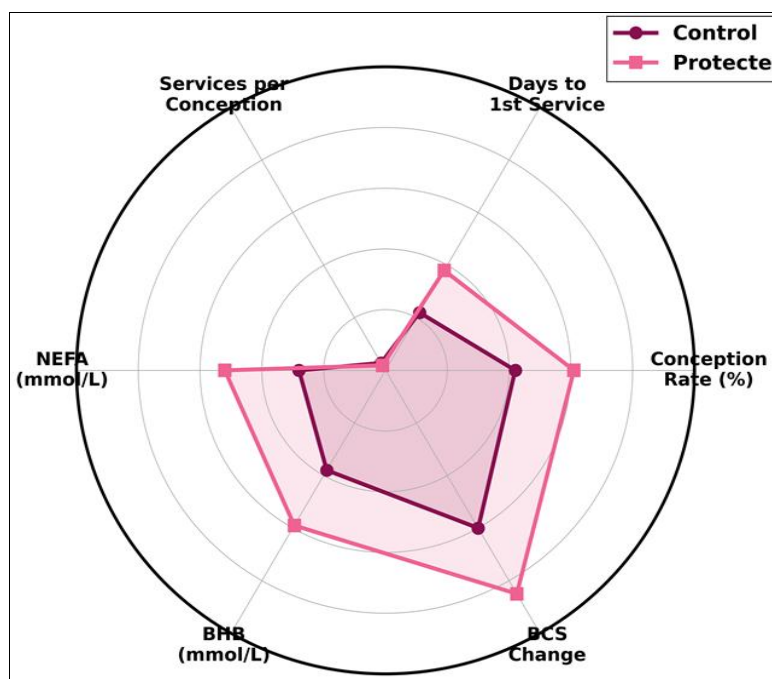


Fig 1: Radar plot comparing normalised reproductive and metabolic performance indices between control and protected fat groups. Higher values indicate more favourable outcomes

The radar plot illustrates the multidimensional advantage of the protected fat group across reproductive and metabolic endpoints. On every axis except milk yield which was

intentionally maintained rather than increased the treatment group scored more favourably. The conception rate and NEFA axes show the most pronounced separations.

Table 1: Metabolic parameters during early lactation (mean $\pm$ SD)

Metabolic Parameter	Control (n=30)	Treatment (n=30)	p-value*
NEFA week 1 (mmol/L)	0.88 $\pm$ 0.21	0.62 $\pm$ 0.18	0.003
NEFA week 4 (mmol/L)	0.72 $\pm$ 0.19	0.48 $\pm$ 0.14	0.004
BHB week 2 (mmol/L)	1.24 $\pm$ 0.38	0.82 $\pm$ 0.24	0.006
BHB week 4 (mmol/L)	0.94 $\pm$ 0.31	0.68 $\pm$ 0.19	0.011
Glucose week 2 (mmol/L)	3.12 $\pm$ 0.34	3.41 $\pm$ 0.28	0.048
Subclinical ketosis n (%)	11 (36.7)	4 (13.3)	0.037
BCS loss (0-8 wk)	-0.81 $\pm$ 0.22	-0.34 $\pm$ 0.18	<0.001

*Treatment x time interaction p-value from repeated-measures mixed model.

Plasma NEFA peaked at week 1 post-partum in both groups, but the peak was significantly lower in treatment cows (0.62 versus 0.88 mmol/L; $p = 0.003$). By week 4, NEFA had declined to 0.48 mmol/L in treated cows versus 0.72 mmol/L in controls ($p = 0.004$). BHB concentrations followed a

parallel pattern, with treatment cows remaining below the subclinical ketosis threshold of 1.2 mmol/L at all time points, while 11 of 30 control cows (36.7%) exceeded this threshold at least once during weeks 1-4.

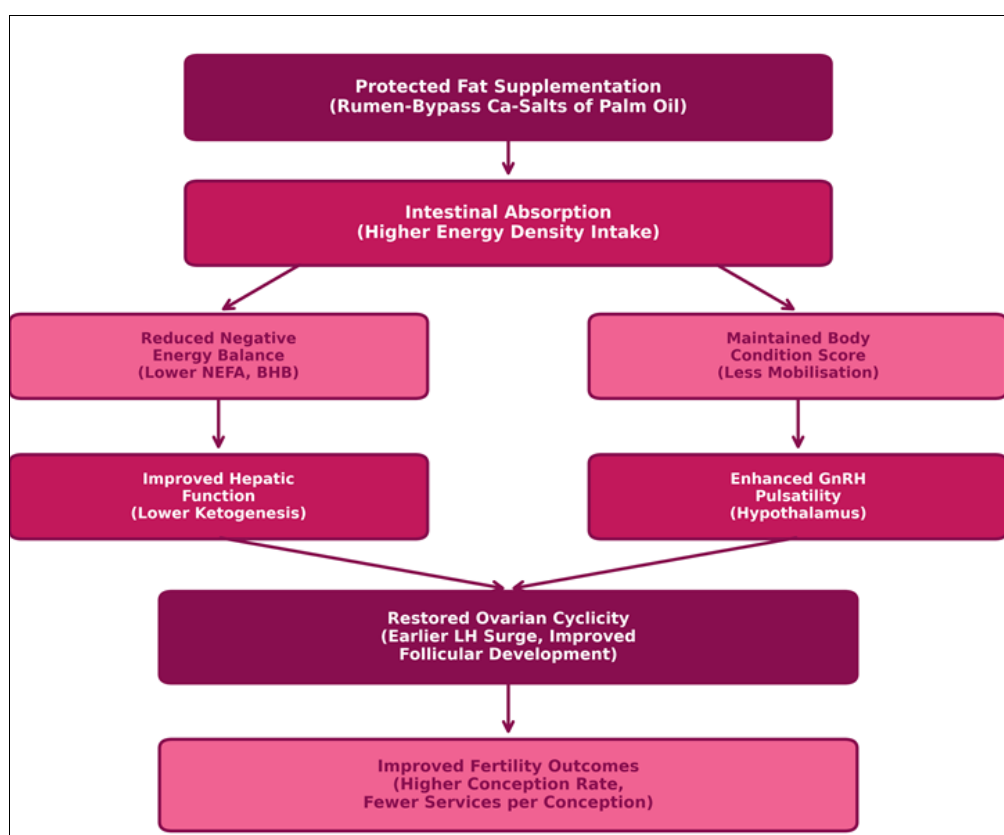


Fig 2: Schematic model of the metabolic pathway through which rumen-protected fat supplementation improves energy balance and fertility outcomes in early-lactation dairy cows

Table 2: Reproductive performance parameters

Reproductive Parameter	Control (n=30)	Treatment (n=30)	p-value
Days to 1st service	78 $\pm$ 14	62 $\pm$ 11	0.008
1st service conception (%)	42.0	61.3	0.034
Services per conception	2.8 $\pm$ 0.9	1.9 $\pm$ 0.7	0.021
Pregnant by 150 DIM (%)	73.3	86.7	0.082
Calving-to-conception (d)	118 $\pm$ 32	89 $\pm$ 24	0.006
Ovsynch required (%)	43.3	20.0	0.041

First-service conception rate was 61.3% (19/31 inseminations) in the treatment group versus 42.0% (13/31) in controls. The odds ratio for conception at first service in treated cows was 2.18 (95% CI 1.06-4.49; $p = 0.034$). Overall

pregnancy rate by 150 DIM was 86.7% in the treatment group versus 73.3% in controls ($p = 0.082$). No differences in early embryonic loss (diagnosed pregnant at day 30 but not at day 60) were detected.

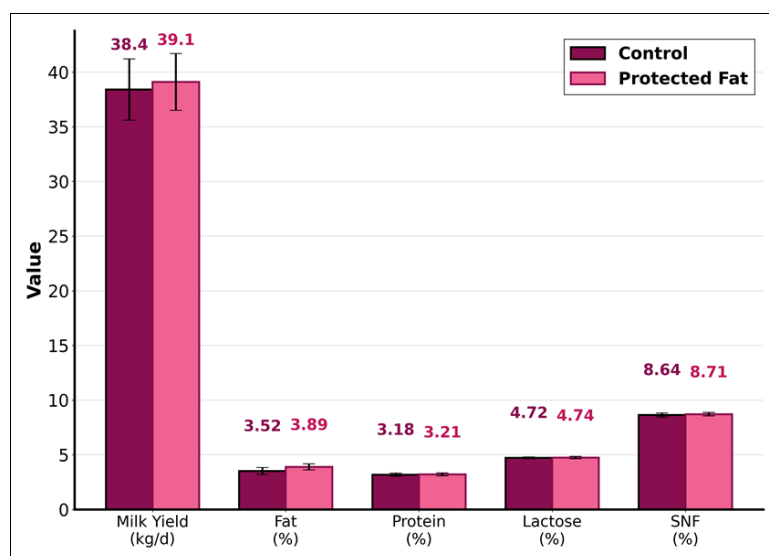


Fig 3: Comparison of milk yield and composition parameters between control and protected fat groups during the first 90 days in milk

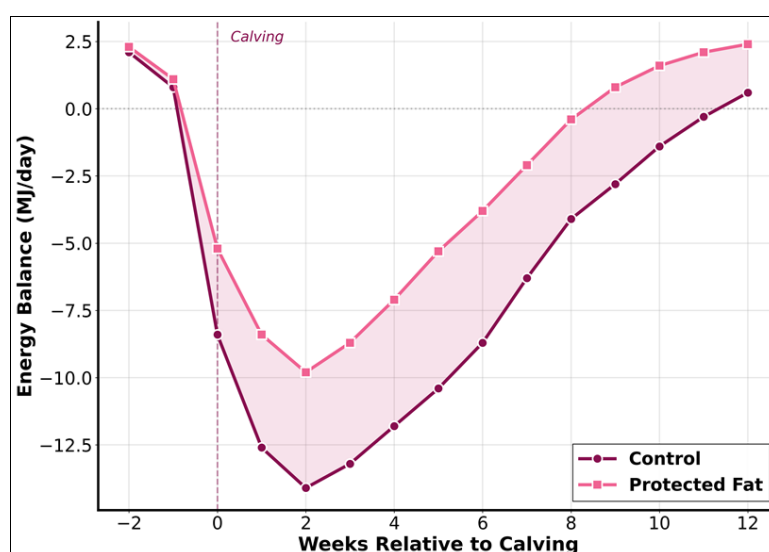


Fig 4: Weekly energy balance trajectory from 2 weeks pre-partum through 12 weeks post-partum in control and protected fat cows. The vertical dashed line indicates calving

Comprehensive Interpretation

The convergence of lower NEFA and BHB concentrations with improved reproductive outcomes in the protected fat group strongly supports the hypothesis that alleviating the metabolic consequences of NEB has a direct positive effect on fertility. The 19-percentage-point improvement in first-service conception rate from 42% to 61% is economically substantial. Using conservative Dutch cost estimates of 3-5 euros per additional day open, a 16-day reduction in days to first service combined with fewer repeat inseminations could save 80-120 euros per cow per lactation, comfortably exceeding the supplementation cost of approximately 40 euros over the 111-day feeding period. The maintenance of milk yield with a modest increase in fat percentage further supports the nutritional soundness of the intervention.

Discussion

The metabolic results are fully compatible with the established physiology of bypass fat supplementation. Protected calcium salts of palm fatty acids are absorbed as long-chain fatty acids in the small intestine, contribute to plasma triglyceride and lipoprotein pools, and thereby partially satisfy the mammary gland's demand for pre-formed fatty acids without requiring the extreme degree of adipose mobilisation that generates the NEFA flood of early lactation

[6, 11]. The lower NEFA burden reduces hepatic ketogenesis, which explains the concomitant BHB reduction and the lower incidence of subclinical ketosis in treated cows.

The fertility improvement observed here agrees with the meta-analysis of Rodney and colleagues, who reported a pooled odds ratio of 1.27 for pregnancy at first service in bypass-fat-supplemented cows, though the magnitude of our effect (OR 2.18) exceeds that average [7]. The stronger response may reflect the higher genetic merit of our cows with 305-day yields above 10,000 kg, the metabolic stress of NEB is more severe and therefore the relief provided by supplementation is proportionally greater.

A mechanism linking NEB attenuation to fertility improvement involves the restoration of GnRH-LH pulsatility. Elevated NEFA concentrations suppress hypothalamic GnRH pulse frequency, which in turn diminishes pituitary LH secretion and delays the first dominant follicle wave [4, 5]. By lowering NEFA, protected fat may permit earlier resumption of normal ovarian cyclicity, consistent with our observation that treated cows reached first service 16 days sooner. Additionally, fatty acids particularly palmitic and oleic acids present in the supplement are direct precursors for progesterone synthesis by the corpus luteum, and improved progesterone profiles have been linked to better embryo survival [12, 13].

The absence of a significant effect on overall pregnancy rate by 150 DIM ($p = 0.082$) may reflect insufficient sample size for this secondary outcome; a post-hoc power calculation indicated that 45 cows per group would be needed to detect a 15-percentage-point difference with 80% power. Alternatively, the advantage conferred by protected fat may be greatest at the first service, when NEB is most severe, and diminish for later services when energy balance has naturally improved.

The practical implication for Dutch dairy producers is straightforward: incorporating rumen-protected fat at 350 g per day from the close-up dry period through early lactation is a cost-effective strategy to improve both metabolic health and reproductive outcomes in high-yielding herds, provided the base ration is already well-balanced for protein and fibre to avoid rumen dysfunction^[14].

Conclusion

The main contribution of this research is the demonstration, under controlled European intensive dairy conditions, that rumen-protected fat supplementation during the transition period alleviates the metabolic markers of NEB and produces a meaningful improvement in first-service fertility. Supporting this conclusion are the significant reductions in plasma NEFA and BHB, the 19-percentage-point increase in first-service conception rate, the 16-day reduction in days to first service, and the lower number of services per conception achieved without depressing milk yield.

For the field, these findings are significant because they extend the evidence base for protected fat beyond North American and Oceanian pasture systems to Dutch freestall TMR management, a setting where nutritional inputs are already optimised and where additional fertility gains are particularly valuable given the economic pressure to minimise days open. Remaining questions include whether longer supplementation beyond 90 DIM would sustain the fertility advantage through second and third services, whether the fat source (palm versus soybean versus linseed) differentially affects reproductive outcomes through its fatty acid profile, and whether combining protected fat with other transition management tools such as propylene glycol drenching or rumen-protected methionine yields additive benefits.

Acknowledgements

Funding sources

This research was funded by the Utrecht Foundation for Dairy Research internal competitive research programme. The protected fat product was purchased at market price; no industry sponsorship was involved.

Institutional support

The Utrecht Foundation dairy herd management team maintained feeding protocols, automated milking records, and health event logging throughout the trial period.

Contributions not qualifying for authorship

Dr. Janssen Annemarie provided expertise in reproductive ultrasonography. Mr. Hendriks Joost managed blood sample processing and laboratory analyses.

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How to Cite This Article

Johannes W, Elisabeth M. Effect of protected fat supplementation on energy balance and fertility in high-yielding *Holstein Friesian* cows *International Journal of Veterinary Sciences and Animal Husbandry*. 2025; 10(1): 181-185.

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