

Lactation Stage Modulates Milk Citrate and Fatty Acid Synthesis via Acetate Chain Elongation (ACE) in Dairy Cows

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Abstract

This study investigated how lactation stage influences milk citrate concentration, fatty acid (FA) synthesis, and the utilization of acetate for chain elongation (ACE) in dairy cows. A total of 219 cows were grouped by lactation stage—early (1–100 DIM), mid (101–200 DIM), and late (201–305 DIM)—and fed a same diet. Milk citrate concentrations were significantly higher in early lactation compared to late lactation (148.0 vs. 136.6 mg/dL, $P < 0.05$), while β -hydroxybutyrate levels did not differ among stages ($P > 0.05$). De novo FA synthesis (DN FA, g/100g milk) increased across lactation stages (0.740, 0.834, and 0.927; $P < 0.01$), with the ratio of DN to preformed FA (PF FA) peaking during mid-lactation ($P < 0.01$). ACE in DN FA synthesis (mol/mol FA) was highest in mid-lactation (2.919, $P < 0.01$), corresponding to increased daily acetate utilization for FA synthesis (21.420 mol/d, $P < 0.01$). Regression analysis showed no significant relationship between ACE per mole of FA (mol/mol FA) and 305-day milk yield (305 MY, $P = 0.681$, $R^2 = 0.001$.) Conversely, daily acetate utilization for FA synthesis (ACE mol/d) was positive significantly associated with 305 MY (slope = 176.47 ± 17.22 , $P < 0.001$, $R^2 = 0.326$), indicating that milk yield depends more on total FA synthesis than on synthesis efficiency, highlighting the role of total mammary FA synthesis in driving milk production. Enhanced daily ACE is positively correlated with milk yield, emphasizing the importance of acetate availability for supporting mammary FA synthesis and overall lactational performance. In conclusion, lactation stage modulates milk citrate levels and FA synthesis. This study confirms that the variation in 305-day milk yield is associated with daily acetate utilization for fatty acid chain elongation (ACE, mol/d) in the mammary gland.

Material and methods

Animals

A total of 219 Holstein cows were categorized into early (1–100 DIM), mid (101–200 DIM), and late (201–305 DIM) lactation. All cows received a uniform total mixed ration (TMR) diet (forage-to-concentrate ratio: 52:48) and were housed in a free-stall facility without pasture access.

Milk Composition Analysis

Milk samples were analyzed for citrate, β -hydroxybutyrate (BHBA), and fatty acid (FA) composition using MilkoScan FT+ 300 (FOSS, Denmark).

ACE Calculations

ACE during de novo FA (DN FA) synthesis was estimated based on acetate and NADPH utilization. Chain elongation calculations followed Moore & Christie (1981) and Garnsworthy et al. (2006), considering FA synthesis pathways from C4:0 to C16:0.

Statistical Analysis

ANOVA tested differences in milk citrate, BHBA, FA composition, and ACE across lactation stages. Regression models evaluated relationships among milk citrate, FA synthesis, and milk yield.

Table 1. Milk citrate, β -hydroxybutyrate (BHBA), de novo (DN) or preformed (PF) fatty acids (FA) and their ratio, and acetate used for chain elongation (ACE) during de novo synthesis in groups of cows at 3 stages of lactation (n=219); all cows were fed the same diet.

Item	Stage of lactation ¹			SED	P
	Early	Mid	Late		
Citrate mg/dL	148.0 ^a	137.4 ^{ab}	136.6 ^b	1.9	<0.05
BHBA, mmol/L	0.021	0.026	0.030	0.004	NS
Denovo FA, g/100g milk ²	0.740 ^c	0.834 ^b	0.927 ^a	0.015	<0.01
Preformed FA, g/100g milk ³	1.211 ^b	1.126 ^b	1.395 ^a	0.021	<0.01
Ratio Denovo FA: Preformed FA	0.651 ^b	0.748 ^a	0.671 ^b	0.011	<0.01
ACE, ⁴ mol/mol of FA	2.781 ^c	2.919 ^a	2.886 ^b	0.007	<0.01
ACE, ⁵ mol/d	18.161 ^b	21.420 ^a	18.380 ^b	0.400	<0.01

a–c Means within row with different superscripts differ ($P < 0.05$).

1 Days in milk for Early: 1-100; Mid: 101-200; Late: 201-305.

2 De novo FA: C4 to C14.

3 Preformed FA: Greater than or equal to C18.

4 ACE in de novo synthesis of fatty acids calculated as (chain length/2–1.5) mol/mol for fatty acids C4 to C14 and (chain length/2–1.5)×0.6 mol/mol for C16.

5 For cows in early lactation with milk fat content > 40 g/kg, ACE calculated as (chain length/2–1.75) mol/mol for fatty acids C4 to C14 and (chain length/2–1.75)×0.1 mol/mol for C16.

NS $P > 0.05$

Discussion

The variations in milk citrate concentration across early, mid, and late lactation stages in this study are probably associated with changes in intramammary energy metabolism and FA synthesis. ACE was calculated based on the molar proportions of de novo synthesized fatty acids (DN FA). Unlike FA concentration ratios or yields, the stoichiometric calculation of acetate required for chain elongation (ACE) provides a more precise measure of synthase enzyme activity. Fig. 1 shows that ACE per mole of FA synthesized (ACE, mol/FA) remains stable across lactation stages, indicating that FA synthesis efficiency does not significantly influence 305-day milk yield.

However, as shown in Fig. 2, daily ACE utilization (mol/d) is strongly correlated with milk yield, with an increase of 1 mol/d in ACE leading to an additional 176 kg of milk over a 305-day lactation. Mid-lactation cows exhibited the highest ACE values, while early-lactation cows had the lowest, likely due to metabolic adaptations. Daily ACE utilization (mol/d) serves as a key predictor of milk yield, reflecting acetate metabolism efficiency and FA synthesis capacity. Integrating ACE analysis into dairy management can optimize feed efficiency and milk production.

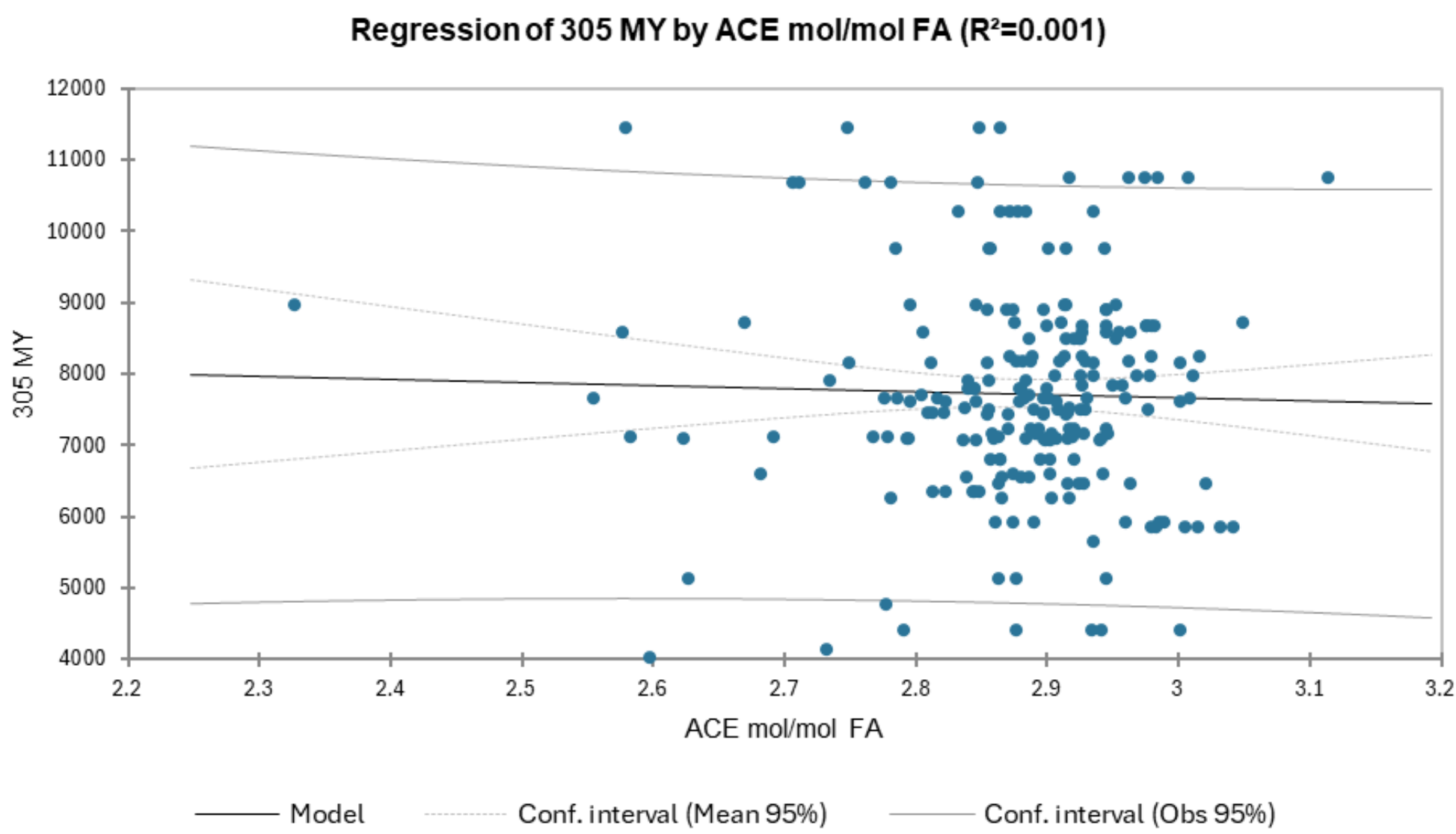


Fig. 1. Regression of 305-day milk yield (305 MY) by acetate used for chain elongation per mole of fatty acid (ACE, mol/mol FA). No significant relationship was observed between ACE (mol/mol FA) and 305 MY (slope = -432.20 ± 1050.50 , $P = 0.681$), with negligible explanatory power ($R^2 = 0.001$). The intercept was 8962.91 ($P = 0.003$), but the predictor variable (ACE/mol FA) lacked statistical significance. Shaded regions denote 95% confidence intervals for the mean and observations.

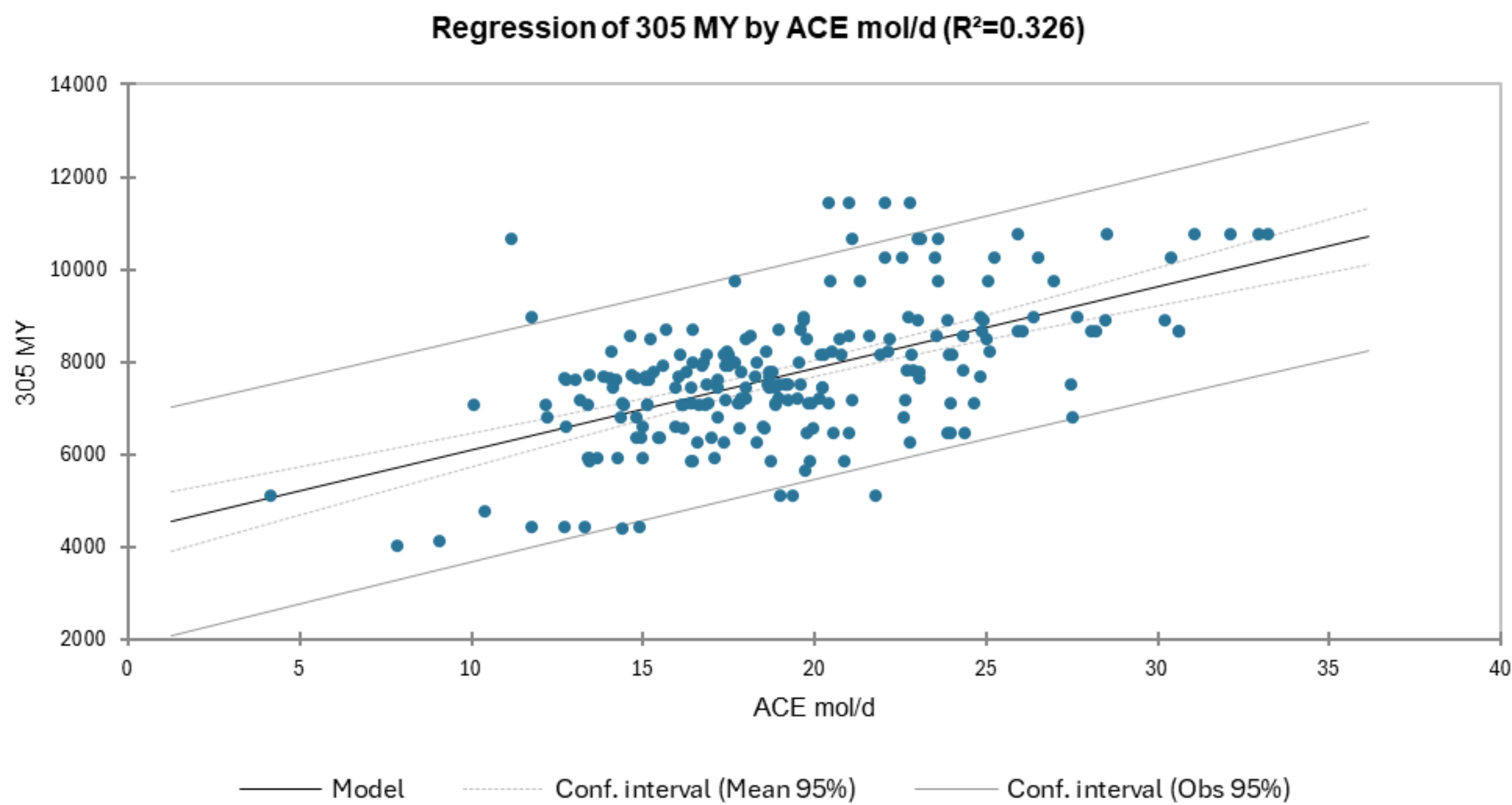


Fig. 2. Regression of 305-day milk yield (305 MY) by acetate used for chain elongation (ACE, mol/d). The regression analysis revealed a significant positive relationship between ACE (mol/d) and 305 MY (slope = 176.47 ± 17.22 , $P < 0.001$), with an explanatory power of $R^2 = 0.326$. The intercept was 4334.89 ($P < 0.001$), and the overall model was statistically significant ($P < 0.001$). Shaded regions represent 95% confidence intervals for the mean and individual observations.

