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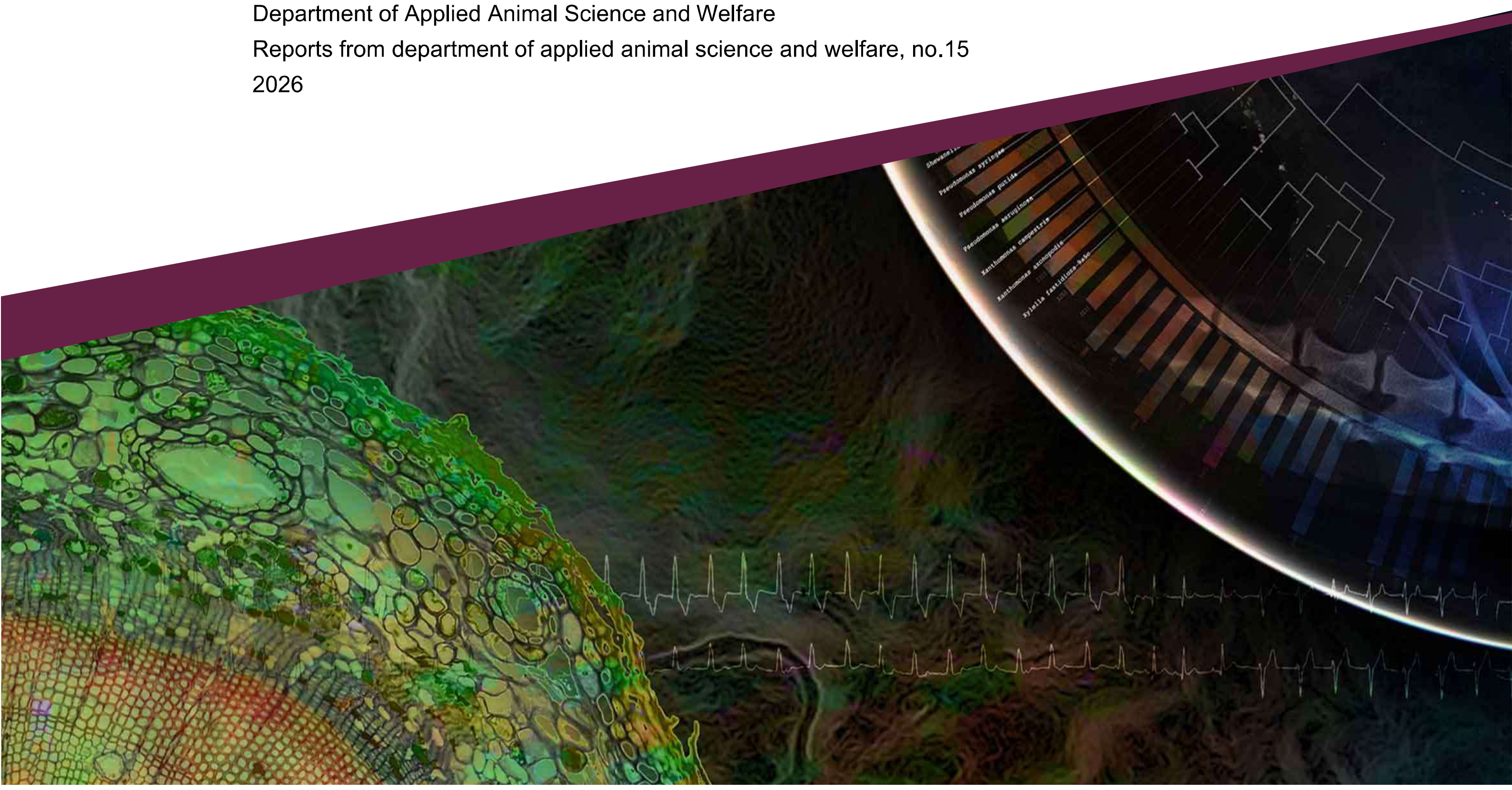

Dietary AAT calculated in NorFor and its effects on responses in energy-corrected milk and milk protein yields in dairy cows

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Introduction

Increased demand for milk has led dairy farmers to consider strategies to increase milk yield per cow. One proposed measure has been to increase the dietary AAT supply, based on the assumption that this may promote milk yield (Benchaar *et al.*, 2023). However, increased dietary crude protein (CP) supply reduces nitrogen use efficiency in dairy cows and increases nitrogen losses to the environment (Katongole & Yan, 2020; Hristov *et al.*, 2026). To evaluate the effects of increased dietary AAT supply, a NorFor task group has collected data from recent Nordic and international feeding experiments with the aim of evaluating NorFor's current AAT recommendations and assessing whether there is a need for revision.

Materials and Methods

<u>Data:</u> A literature search was conducted using Google Scholar and other scientific databases, applying index terms related to dietary protein supply and production response, including level of CP and/or metabolizable protein (AAT equivalent), milk yield, protein yield, and effects of feed ration. Studies were eligible for inclusion if experimental data were available on animal characteristics and key production variables, including days in milk (DIM), diet composition, dry matter intake (DMI), milk yield, and milk composition. In addition, studies were required to report a measure of variability (SEM or equivalent) for milk and/or protein yield. Furthermore, studies comparing different levels of CP and/or AAT between treatments, involving partial or complete replacement of protein sources, or evaluating responses to different CP levels across stages of lactation, were considered for inclusion. Based on these criteria, the energy-corrected milk (ECM) model included 132 observations from 45 independent studies (random-effect units) derived from 26 papers, whereas the corresponding protein yield model included 120 observations from 40 studies derived from 23 papers due to data availability (Suppl. Materials 1).

<u>Statistic modelling:</u> Models for ECM and milk protein production responses included the variables MJ net energy for lactation per kg DM (NEL), days in milk (DIM), content of NDF, starch (ST), and crude fat (CFat) as g/kg DM, in addition to protein supply variables (AAT/NEL[1], or AAT, CP, and PBV as g/kg DM). AAT and PBV were calculated according to the latest version of the NorFor model (Volden, 2011). All variables were included as second-order polynomials, without interaction effects. Protein supply variables (the selected protein measure and PBV) were forced into the model as second-order terms. Models were fitted using weighted regression with study (as defined above) included as a random effect to

[1] Grams AAT available for milk production/MJ NEL requirement for ECM production

account for between-study heterogeneity, and weights defined as the inverse of the variance in milk yield or protein yield, to account for heterogeneity in precision among experiments. Backward elimination with $P \leq 0.05$ was applied for model reduction. Prediction error was assessed using leave-one-study-out cross validation. Normality of residuals was assumed, acknowledging that this assumption may not be fully met in all cases.

Results and Discussion

The included parameters and their estimates for the selected models for ECM and protein production are shown in Table 1. Both the first and second order term for AAT/NEL and PBV were highly significant, as well as the first order term for dietary constituents. Figure 1 show the predicted responses of ECM and protein yield with varying levels of g AAT/MJ NEL. Responses in both ECM and protein yield exhibited a clear plateau around 16 g AAT/MJ NEL, with only marginal gains above the current recommended minimum level of 15 g AAT/MJ NEL in NorFor (Volden, 2011, Ch. 9). Increasing AAT/NEL from 15.0 to 16.0 g/MJ increases ECM by 0.1 kg and protein yield by 0.01 kg per day. Our findings are consistent with studies showing diminishing production responses once metabolizable protein requirements are met (Benchaar et al., 2023), and with CP-based studies indicating that dietary protein levels around 16-17% of DM are sufficient to maximize milk production (Olmos Colmenero & Broderick, 2006). Within the recommended PBV range (5-40 g/kg DM), predicted ECM responses were small ($\leq$ 0.5 kg), with a maximum around 25 g PBV and little net difference between low and high PBV levels, indicating that PBV had a secondary impact relative to AAT/NEL within the operational range.

Table 1 Included parameters ($P \leq 0.05$) and their estimates for the selected models for responses in ECM and protein production.

Parameters, g/kg DM, unless otherwise stated	Energy-corrected milk		Protein production	
	Estimate	Sign. level[1]	Estimate	Sign. level
Intercept	74.86		2158	
g AAT/MJ NEL	3.618	***	147.7	***
g AAT/MJ NEL (quadratic)	-0.1134	***	-4.424	***
PBV	0.05964	**	4.345	***
PBV (quadratic)	-0.00112	***	-0.07786	***
NEL, MJ/kg DM	-5.592	***	-170	***
NDF	-0.08212	***	-2.954	***
Starch	-0.02683	**	-0.8284	**

[1] * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

The present findings, together with previous literature, suggest that milk production responses to protein supply are primarily driven by interactions between ruminal nitrogen availability and energy supply, whereas post-ruminal amino acid supply plays a secondary role once microbial protein synthesis and energy intake are unlikely to be limiting. This interpretation is consistent with previous work demonstrating that interactions between energy supply and metabolizable protein are key determinants of nitrogen efficiency and production responses in dairy cows (Rius et al., 2010). Even though models based on alternative protein descriptors (grams AAT and CP per kg DM) occasionally yielded slightly better information criteria (AIC/BIC), AAT relative to energy supply (AAT/NEL) was retained, as the results indicate that production responses are primarily driven by the balance between ruminal nitrogen availability and energy supply, rather than by absolute protein supply. Accordingly, providing AAT above the level required to maximize milk production is unlikely to elicit further production responses and may instead result in increased nitrogen losses rather than increased milk output.

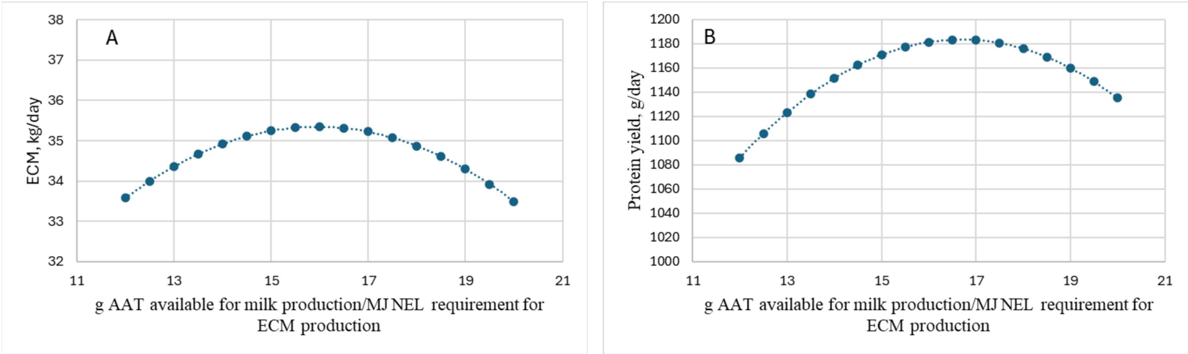

Figure 1 Predicted responses in (A) energy-corrected milk (ECM) and (B) protein yield with increasing AAT/NEL (grams AAT available for milk production/MJ NEL requirement for ECM production) when NEL per kg DM = 6.5 MJ, and PBV, NDF, and starch = 20, 350, and 150 grams per kg DM, respectively.

Conclusions

Given the predicted ECM and protein yield responses identified in this study, there are no scientific reasons for adjusting the AAT/MJ NEL recommendations in the NorFor model. At the same time, the shallow production response around current recommendation levels indicates limited sensitivity of milk yield to small changes in dietary AAT supply.

Supplementary Materials 1: List over studies included in the analysis.

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Supplementary materials 1

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