

Research on Muscle Protein Mobilization in Lactating Sows Based on the Tracer Kinetics of 3-[Methyl- ^3H] histidine

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Abstract. This study investigated the effects of dietary protein levels on muscle protein breakdown (MPB) in lactating sows using 3-[methyl- $^2\text{H}_3$]histidine (3MH) as an isotopic tracer. Objectives included the evaluation of candidate exponential models and a comparison between nonlinear regression and the traditional curve peeling method for analyzing 3MH dynamics. Eight sows were randomly assigned to a standard protein (SP; 18.0% CP) or a low-protein diet (LP; 14.0% CP + crystalline amino acids). Plasma 3MH kinetics were modeled using single-, two-, and three-exponential models. The three-exponential model (three-pool model) coupled with a nonlinear regression approach provided the most accurate characterization of 3MH dynamics. Model parameters derived from nonlinear regression and curve peeling were comparable, with the former being more objective and precise. ANOVA results indicated that the LP diet significantly increased MPB rates ($P < 0.05$), suggesting escalated mobilization of muscle reserves to sustain lactation. These findings highlight the trade-off between reduced dietary protein supply and increased maternal muscle catabolism, underscoring the necessity for precision diet formulation in lactating sows.

Keywords: Exponential Model, Non-linear Regression, Curve Peeling Method, Muscle Protein Breakdown.

1. Introduction

As the world's largest pig producer, China's production system efficiency is crucial to national food security, with the nutritional management of lactating sows being a key link affecting overall production efficiency and sustainability [1]. Against this background, the low-protein diet technique—reducing dietary crude protein levels while supplementing crystalline amino acids—has emerged as an important solution. It aims to decrease dependence on imported protein ingredients such as soybean meals, thereby lowering feed costs and mitigating environmental nitrogen pollution [2, 3]. However, the lactation period imposes extreme metabolic stress on sows. When dietary protein supply is inadequate, sows mobilize body reserves—primarily skeletal muscle protein—to support milk protein synthesis, a process known as muscle protein breakdown (MPB) [4, 5]. Although this is a physiological adaptation, excessive or sustained MPB can lead to severe body depletion, impairing long-term sow condition, welfare, and subsequent reproductive performance [6]. Therefore, the accurate quantification of MPB is essential for achieving precision nutrition and safeguarding sow herd health.

Accurate assessment of MPB requires specific biomarkers and appropriate kinetic modeling approaches. The isotopic tracer 3-[methyl- $^2\text{H}_3$] histidine (3MH) offers unique advantages in this regard. Incorporated into actin and myosin, 3MH is released upon their degradation and is not reutilized for protein synthesis, making it a specific marker for myofibrillar protein degradation [7, 8]. However, the plasma kinetics of 3MH are complex, reflecting its release from physiological compartments with different turnover rates, necessitating the use of multi-exponential models for accurate interpretation [9]. Traditionally, the curve peeling method has been used for such analyses, but its inherent subjectivity and risk of error propagation are widely recognized limitations [10]. In contrast, modern nonlinear regression provides a more objective and robust statistical framework for parameter estimation. Despite its advantages, a systematic comparison and evaluation of these two methods in the context of 3MH kinetics in lactating sows remains a notable methodological gap.

This study addresses this gap by integrating methodological innovation with practical application. The primary innovation lies in the systematic development, comparison, and validation of a series of compartmental models (single-, two-, and three-pool exponential models) to identify the most physiologically appropriate structure for characterizing 3MH kinetics. A rigorous, head-to-head comparison between the traditional curve peeling method and modern nonlinear regression will be conducted to objectively evaluate their performance in parameter estimation and establish a more reliable and standardized analytical protocol for future research. Building on this optimized modeling framework, the second core objective is to quantitatively investigate a question of immediate practical relevance: the impact of a low-protein diet on MPB dynamics in lactating sows. The findings are expected to yield critical data-driven insights that extend beyond methodological contributions. By precisely defining the metabolic trade-off between reduced dietary protein and maternal tissue mobilization, this research will provide a scientific basis for developing precision feeding strategies. Ultimately, it aims to guide industry in harnessing the economic and environmental benefits of low-protein diets while effectively protecting sow health and productivity, thereby promoting a more sustainable and resilient pig production system.

2. Methodology

2.1. Dietary treatments and animal management

Eight gestating sows (parity 2-4) were randomly assigned to one of two dietary treatments from parturition until the end of lactation: a standard protein diet (SP; 18% CP) or a low-protein diet (LP; 15% CP + CAAs). Diets were formulated to meet NRC (2012) nutrient requirements. The major ingredients and calculated nutrient content are presented in Table 1. Sows were fed three equal meals daily. On day 15-16 of lactation, ear vein catheters were surgically inserted for isotope infusion and blood sampling.

Table 1. Feed composition and nutrient content of diets (as-fed).

Item	Standard Protein Diet (SP)	Low Protein Diet (LP)
Major ingredient, %		
Corn, yellow dent	59.17	61.45
Soybean meal, 48 % CP	30.00	14.00
Sugar food product	5.00	5.00
Beef tallow	3.35	5.02
L-Lys·HCl	0	0.47
Limestone	1.18	0.93
Dicalcium phosphate	0.45	0.78
Calculated nutrient level		
Energy, kcal/kg	2,580	2,580
CP, %	18	14

2.2. Isotope Infusion and Blood Sampling

A bolus dose of 3-[methyl-²H₃] histidine (3MH), calculated based on 20% of the assumed extracellular free 3MH pool, was administered via the ear vein catheter. Blood samples were collected at 0, 0.08, 0.16, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 24, 34, 48, 58, and 72 hours post-infusion. Plasma was separated and analyzed for 3MH enrichment using HESI LC-MS.

2.3. Kinetic Modeling and Calculation

The tracer-to-tracee ratio (TTR) data was modeled using three candidate models:
Single-pool model:

$$y = A_1 e^{-k_1 t} \tag{1}$$

Two-pool model:

$$y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad (2)$$

Three-pool model:

$$y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t} \quad (3)$$

Two fitting methodologies were employed: 1) Curve Peeling: A sequential graphical method performed in Microsoft Excel to resolve the multi-exponential curve into its components (Figure 1). 2) Nonlinear Regression: A direct numerical optimization using the nls function in R software (version 4.5.0) to fit the models to the raw TTR data by minimizing the residual sum of squares. Key kinetic parameters, including the area under the curve (AUC), rate of appearance (Ra), and half-life, were calculated. The MPB rate (g/day) and fractional MPB rate (%/day) were derived from Ra and the estimated total protein-bound 3MH pool.

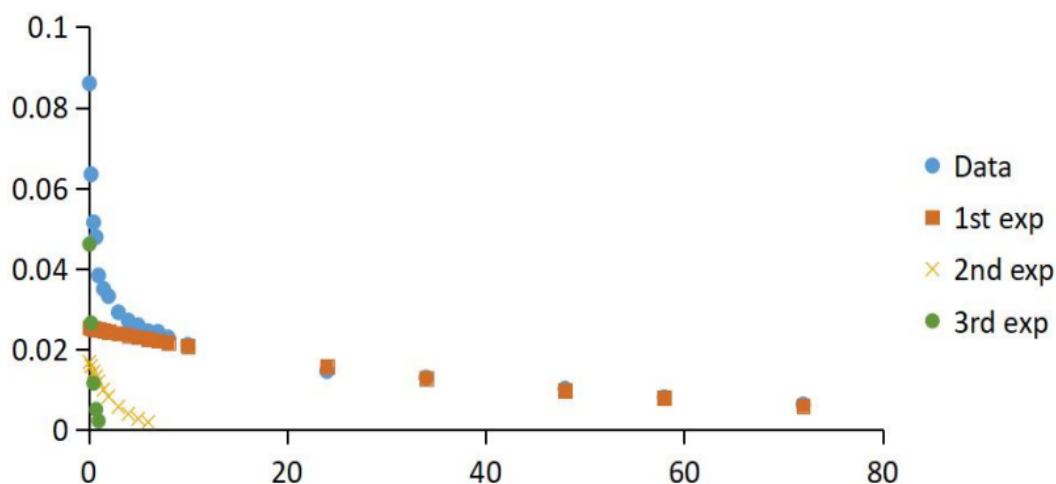


Figure 1. Procedures of curve peeling method.

Variance analysis (ANOVA) was performed in R to compare MPB indices between the SP and LP groups. Statistical significance was set at $P < 0.05$, and tendencies were noted at $0.05 < P \leq 0.1$.

3. Results

3.1. Model Comparison: One-Pool vs. Two-Pool vs. Three-Pool Model

Visual and quantitative assessment of model fits revealed that the three-pool model (three-exponential function) provided the best representation of the 3MH kinetic data. As shown in Figure 2, the three-pool model curve closely followed the raw data points across the entire time course, while the single-pool and two-pool models showed systematic deviations, particularly in the early and late phases. The three-pool model effectively captured the rapid initial distribution, intermediate transition, and slow terminal elimination phases of 3MH, which correspond to different physiological pools and turnover rates. The results are shown in Figure 2.

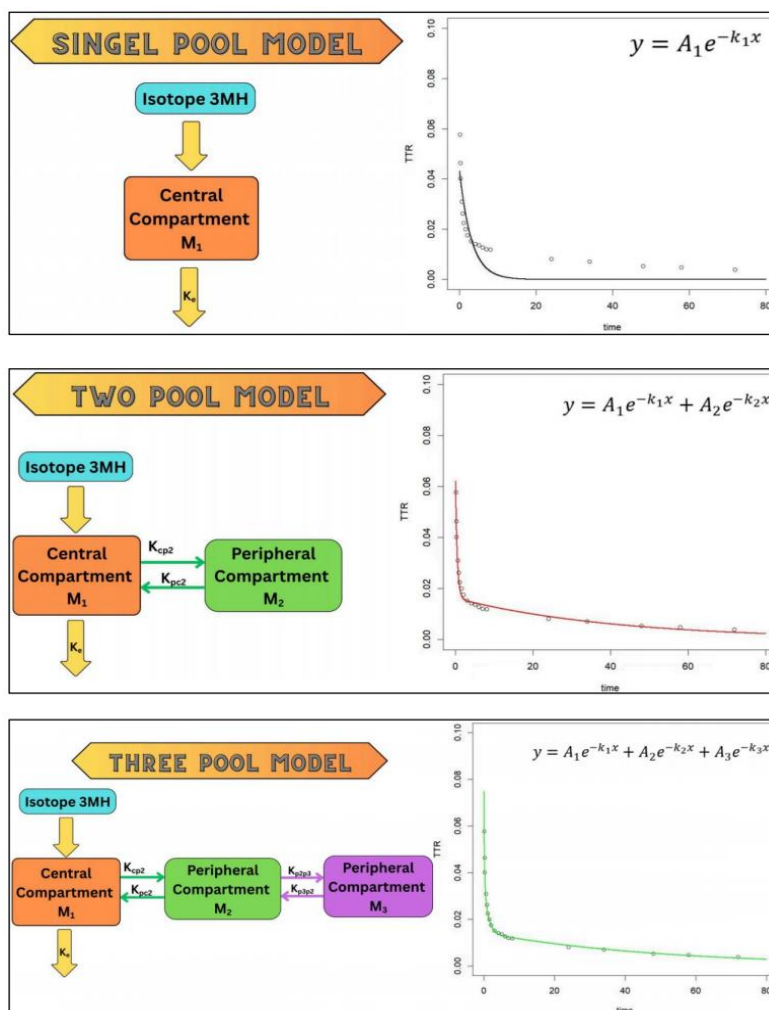


Figure 2. Comparison among single pool models, two pool models and three pool models.

3.2. Nonlinear Regression vs. Curve Peeling

The six parameters (A_1 , A_2 , A_3 , k_1 , k_2 , k_3) of the three-exponential model estimated by nonlinear regression and curve peeling were comparable. Figure 3 shows that the fitted curves generated from both sets of parameters were visually similar and close to the scatter plot of the raw data. However, nonlinear regression produced a slightly better fit with a lower residual standard error. The primary advantage of nonlinear regression was its objectivity, as it eliminated the subjective visual extrapolation required in curve peeling, which can introduce error, especially when pool turnover rates overlap. The results are shown in Figure 3.

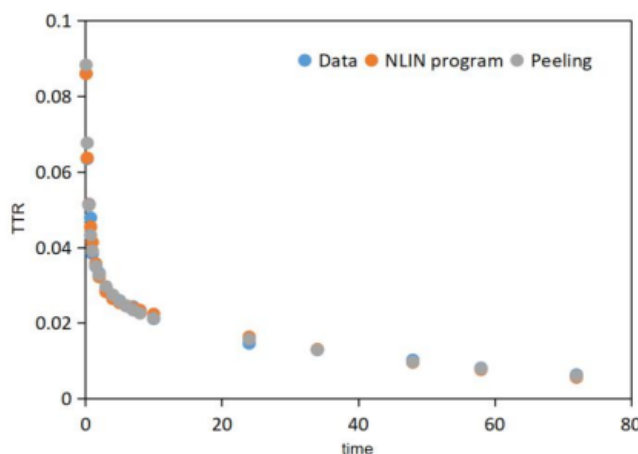


Figure 3. Comparison between non-linear model and curve peeling.

3.3. Muscle Protein Breakdown

The dynamics of 3MH and calculated MPB parameters are presented in Table 2. Sows fed the LP diet had a significantly higher ($P < 0.05$) rate of appearance (Ra) of 3MH compared to the SP group. Consequently, both the absolute MPB (g/day) and the fractional MPB (%/day) were significantly greater ($P < 0.05$) in the LP group. This indicates a higher rate of muscle tissue mobilization in sows subjected to the low-protein dietary regimen.

Table 2. Dynamics of 3-[methyl- $^2\text{H}_3$] histidine and muscle protein breakdown in sows fed standard protein (SP) or low protein (LP) diets.

Item	Standard protein	Low protein	SEM ²	P-value
No. of sows	4	4		
Parity	3.00	3.25	0.44	0.705
Body weight, kg	247.25	263.00	13.89	0.453
Total protein-bound 3MH pool ³ , mmol	78.64	83.65	4.42	0.453
Rate of appearance (Ra) ⁴ , $\mu\text{mol/h}$	141.12	217.13	15.15	0.012
Area under the curve (AUC) ⁵ , h	1.33	0.85	0.10	0.014
Half life ⁶ , h	31.74	32.88	1.86	0.680
Muscle protein breakdown ⁷ , g/d	874.32	1344.96	93.84	0.012
Fractional muscle protein breakdown ⁸ , %/d	4.32	6.24	0.48	0.040

4. Conclusions

This study establishes a robust methodological framework for quantifying muscle protein breakdown (MPB) in lactating sows through compartmental modeling of 3-[methyl- $^2\text{H}_3$] histidine (3MH) kinetics. The central problem addressed was the lack of a physiologically grounded and analytically optimized approach for interpreting complex 3MH tracer data. In response, this work delivers two primary innovations: first, the systematic evaluation and validation of a three-pool exponential model as the most appropriate structure for capturing the multi-compartmental nature of muscle protein turnover, outperforming simpler models by accounting for the rapid-exchange peripheral tissues and the slow-releasing myofibrillar protein pool; second, a rigorous comparison of parameter estimation methods, confirming nonlinear regression as the superior technique in terms of objectivity and precision, while also defining the role of curve peeling as a practical tool for generating initial estimates.

The key findings unequivocally demonstrate that a low-protein diet (14% CP), despite amino acid supplementation, significantly elevates MPB compared to a standard protein diet (18% CP). This result quantitatively confirms that the 3-percentage-point reduction in dietary crude protein exceeds the metabolic adaptive capacity of lactating sows, necessitating heightened mobilization of maternal muscle reserves and pushing them toward a negative protein balance. Thus, while low-protein strategies hold economic and environmental appeal, this study reveals their potential physiological cost when implemented aggressively.

In summary, this research resolves the methodological challenge of accurately modeling 3MH kinetics by establishing the three-pool model fitted via nonlinear regression as the recommended protocol. It further delivers the critical practical insight that dietary protein levels for lactating sows cannot be reduced to 14% CP without incurring substantial muscle catabolism. Future work should focus on identifying the optimal dietary protein threshold that minimizes tissue mobilization while preserving the benefits of low-protein feeding, thereby enabling truly precision nutrition in swine production.

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