Meta-Analysis of the Effects of Dietary Neutral Detergent Fiber to Starch Ratio on Production Performance and Milk Component Synthesis in Dairy Cows: Post-Print

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Abstract

The objective of this study was to investigate the effects of dietary neutral detergent fiber (NDF) to starch ratio on dairy cow production performance and milk composition synthesis using Meta-analysis. A total of 27 research articles on dairy cow carbohydrate nutrition were compiled, encompassing 123 treatments. Meta-analysis was employed to examine the correlations between dietary NDF, starch content, and NDF to starch ratio with dairy cow dry matter intake, milk yield, and milk composition, and corresponding regression analyses were conducted. The results demonstrated that compared with dietary NDF and starch content, dietary NDF to starch ratio exhibited stronger correlations with dairy cow dry matter intake ($R2 = -0.799$, $P < 0.01$), milk yield ($R2 = -0.730$, $P < 0.01$), milk fat percentage ($R2 = 0.664$, $P < 0.01$), and milk protein percentage ($R2 = -0.788$, $P < 0.01$). Significant simple linear regression relationships were identified between dietary NDF to starch ratio and dairy cow dry matter intake ($P = 0.02$), milk yield ($P < 0.01$), milk fat percentage ($P < 0.01$), milk protein percentage ($P < 0.01$), milk protein yield ($P < 0.01$), and lactose yield ($P < 0.01$). For each unit increase in dietary NDF to starch ratio, dairy cow dry matter intake, milk yield, milk protein yield, and lactose yield decreased by 0.81, 1.36, 0.06, and 1.50 kg, respectively, while milk fat percentage and milk protein percentage increased by 0.11% and 0.07%, respectively. Meta-analysis results indicated that dietary NDF to starch ratio can serve as a nutritional evaluation index reflecting carbohydrate composition of dairy cow diets, which can be used to guide dairy cow production and regulate milk composition.

Full Text

Meta-Analysis of the Effects of Dietary Neutral Detergent Fiber to Starch Ratio on Performance and Milk Component Synthesis in Dairy Cows

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Abstract: This study employed meta-analysis to investigate the effects of dietary neutral detergent fiber (NDF) to starch ratio on dairy cow performance and milk component synthesis. Twenty-seven publications on dairy cow carbohydrate nutrition, encompassing 123 treatment groups, were compiled and analyzed. Meta-analysis was used to examine correlations between dietary NDF content, starch content, and NDF:starch ratio with dry matter intake, milk yield, and milk composition, followed by regression analysis. The results demonstrated that, compared with dietary NDF or starch content alone, the NDF:starch ratio exhibited stronger correlations with dry matter intake (R^2 = -0.799, $P < 0.01$), milk yield (R^2 = -0.730, $P < 0.01$), milk fat percentage (R^2 = 0.664, $P < 0.01$), and milk protein percentage (R^2 = -0.788, $P < 0.01$). Significant linear regression relationships were identified between dietary NDF:starch ratio and dry matter intake ($P = 0.02$), milk yield ($P < 0.01$), milk fat percentage ($P < 0.01$), milk protein percentage ($P < 0.01$), milk protein yield ($P < 0.01$), and lactose yield ($P < 0.01$). For each unit increase in dietary NDF:starch ratio, dry matter intake, milk yield, milk protein yield, and lactose yield decreased by 0.81 kg, 1.36 kg, 0.06 kg, and 1.50 kg, respectively, while milk fat percentage and milk protein percentage increased by 0.11% and 0.07%, respectively. These meta-analysis results indicate that dietary NDF:starch ratio can serve as a nutritional evaluation index reflecting dietary carbohydrate composition and may be used to guide dairy production and milk component regulation.

Keywords: dairy cows; carbohydrate composition; performance; milk components; meta-analysis

Introduction

Carbohydrates constitute a critical component of dairy cow diets, accounting for 60–80% of dietary dry matter. Following digestion in the rumen and hindgut, carbohydrates provide energy for rumen microorganisms and host metabolism. Rumen acetate serves as an important substrate for de novo milk fat synthesis, while propionate is the primary substrate for gluconeogenesis in dairy cows. Dietary carbohydrate composition can alter rumen fermentation patterns by changing the acetate-to-propionate ratio or affecting gene expression related to volatile fatty acid absorption and metabolism in rumen epithelial cells, thereby influencing milk fat and lactose content. Additionally, microbial protein and metabolizable protein synthesis capacity are crucial for milk protein synthesis, representing the hub linking dietary protein to milk protein. Carbohydrate degradation products in the rumen provide energy for microbial proliferation and facilitate the conversion of metabolizable protein to microbial protein, making carbohydrates a key factor affecting microbial protein synthesis and the mammary gland' s ability to uptake blood free amino acids. The extent and rate of carbohydrate degradation in the rumen directly affect rumen and host health; diets high in grains degrade rapidly, potentially causing accumulation of volatile fatty acids or lactic acid, reducing rumen pH and leading to subacute or acute rumen acidosis. Forage particle length also significantly affects rumen pH—overly short forage reduces rumen stimulation, affecting rumination and chewing behavior, decreasing saliva secretion, and consequently reducing the buffering capacity for rumen volatile fatty acids, which lowers rumen pH. Therefore, balancing carbohydrate composition is essential for maintaining rumen health.

Dietary carbohydrate composition is closely related to milk yield, milk composition, cow health, and feed efficiency. Carbohydrates are broadly classified as structural or non-structural carbohydrates. Chemically, neutral detergent fiber (NDF) and starch represent the primary components of structural and non-structural carbohydrates, respectively. Beckman et al. first used the NDF:starch ratio to study the effects of dietary carbohydrates on nutrient digestibility. In recent years, numerous researchers have investigated how dietary NDF:starch ratio affects dairy cow performance and milk composition. To further explore whether this ratio can serve as a comprehensive evaluation index for dietary carbohydrate composition, this study employed meta-analysis to examine the feasibility of using NDF:starch ratio as an evaluation metric and its correlation with dairy cow performance and milk composition.

1.1 Literature Search and Screening

Literature search: Relevant studies were retrieved from Web of Science, Science Direct, PubMed, and NCBI databases using keyword combinations including "dairy cows," "milk production," "milk composition," "carbohydrate," "starch," and "forage and concentrate ratio."

Data selection criteria: (1) Holstein lactating cows as experimental animals; (2) complete dietary information including crude protein (CP), NDF, and starch content; (3) complete performance data including intake, milk yield, and milk composition. Following literature search and data screening, 27 publications with 123 treatment groups were included. The experimental designs of all included studies were analyzed, specific treatment groups were selected, and a comprehensive database was established (Table 1).

Table 1 Publications and data used in the meta-analysis

No.	References (published year)	Treatment No.
1	Abrahamse et al. [15] (2008)	4
2	Hristov et al. [16] (2003)	3
3	Aguerre et al. [17] (2011)	4
4	Kendall et al. [18] (2009)	4
5	Alstrup et al. [19] (2014)	4
6	Krause et al. [20] (2002)	4
7	Alzahal et al. [21] (2009)	4
8	Krause et al. [22] (2003)	4
9	Benchaar et al. [28] (2014)	4
10	San Emeterio et al. [29] (2000)	3
11	Boivin et al. [30] (2013)	3
12	Sterk et al. [31] (2011)	4
13	Cabrita et al. [32] (2007)	4
14	Whelan et al. [33] (2014)	3
15	Eriksson et al. [34] (2004)	4
16	Zhang et al. [35] (2010)	4
17	Gozho et al. [36] (2008)	4
18	Zhao et al. [37] (2016)	4
19	Hall et al. [38] (2010)	4
20	Le et al. [39] (1998)	3
21	Hassanat et al. [40] (2013)	4
22	Arndt et al. [23] (2014)	4
23	Miyaji et al. [24] (2012)	3
24	Beauchemin et al. [25] (2003)	4
25	Mullins et al. [26] (2010)	4
26	Beckman et al. [1] (2005)	4
27	Ranathunga et al. [27] (2010)	4

1.2 Statistical Analysis

Meta-analysis of multi-level experimental data was performed using the Mixed procedure in SAS 9.1 software. Heterogeneity among studies was assumed (due to differences in diets, special additives, animal body condition, statistical methods, etc.), and study effects were corrected to remove between-study variation, thereby integrating all studies into a unified analysis framework. The general model was:

$$Y_{ij} = B_0 + B_i + B_{1i}X_{ij} + e_{ij}$$

where: i represents study number (i = 1, 2, 3, ···, n); j represents observation number within each study (j = 1, 2, 3, ···, n); B_0 is the overall intercept (fixed effect); B is the random effect of the ith study; B_1 is the linear regression coefficient for the ith study; and e is the residual error, normally distributed as $N(0, \sigma^2)$ (random effect).

SAS mixed model code:

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PROC MIXED data = temp;
CLASS Study;
MODEL Y = X Z / Solution OUTP = Predictionset OUTPM = PredY;
RANDOM intercept X / TYPE = UN SUBJECT = Study;
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The independent variable (dietary NDF:starch ratio) corrected for random effects was plotted on the Y-axis against dry matter intake, milk yield, milk fat percentage, milk protein percentage, lactose percentage, milk fat yield, milk protein yield, or lactose yield on the X-axis for curve fitting. SAS 9.1 software was used to calculate regression coefficient P-values, root mean square error (RMSE), F-values, and correlation coefficients (R^2).

RMSE calculation formula:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}}$$

where y is the observed value, $\hat{y}_i$ is the predicted value, and n is the number of observations.

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2 Results and Analysis

2.1 Description of Dietary and Cow Production Information

Dietary nutrient composition and cow information are presented in Table 2 . Across the meta-analysis, dietary CP, NDF, and starch contents averaged 17.1%, 33.2%, and 21.7% (DM basis), respectively, with maximum-minimum differences of 40.8%, 57.8%, and 89.4%. The average NDF:starch ratio was 1.92.

For production parameters, analysis of 123 treatments from 27 studies revealed average dry matter intake, milk yield, milk fat percentage, milk fat yield, milk protein percentage, and milk protein yield of 23.3 kg/d, 35.7 kg/d, 3.78%, 1.32 kg/d, 3.17%, and 1.14 kg/d, respectively. Analysis of 96 treatments from 21 studies showed average lactose percentage and lactose yield of 4.72% and 1.69 kg/d, respectively.

Table 2 Database of diets and dairy production in meta-analysis

Item	Treatment No.	Trial No.	Mean	Minimum	Maximum
Diet parameters					
Crude protein, % DM	123	27	17.1	14.2	20.0
NDF, % DM	123	27	33.2	23.5	37.1
Starch, % DM	123	27	21.7	13.5	25.6
NDF:starch ratio	123	27	1.92	1.03	2.74
Production parameters					
Dry matter intake, kg/d	123	27	23.3	18.5	27.8
Milk yield, kg/d	123	27	35.7	26.4	44.2
Milk composition, %					
Milk fat percentage	123	27	3.78	2.85	4.65
Milk protein percentage	123	27	3.17	2.68	3.89
Lactose percentage	96	21	4.72	4.35	5.08
Milk component yield, kg/d					
Milk fat yield	123	27	1.32	0.89	1.85
Milk protein yield	123	27	1.14	0.84	1.56
Lactose yield	96	21	1.69	1.24	2.18

2.2 Linear Correlation Analysis Between Dietary NDF:Starch Ratio and Dry Matter Intake, Milk Yield, and Milk Components

As shown in Table 3 , dietary NDF content exhibited significant positive linear correlation with milk fat percentage (R^2 = 0.567, P < 0.01) and significant negative linear correlations with dry matter intake (R^2 = -0.678, P < 0.01), milk yield (R^2 = -0.517, P < 0.01), milk protein percentage (R^2 = -0.704, P < 0.01), milk fat yield (R^2 = -0.345, P < 0.01), milk protein yield (R^2 = -0.298, P < 0.01), and lactose yield (R^2 = -0.429, P < 0.01). Dietary starch content showed significant positive linear correlations with dry matter intake (R^2 = 0.699, P < 0.01), milk yield (R^2 = 0.650, P < 0.01), milk protein percentage (R^2 = 0.732, P < 0.01), and lactose yield (R^2 = 0.489, P < 0.01), and a significant negative correlation with milk fat percentage (R^2 = -0.658, P < 0.01). Dietary NDF:starch ratio demonstrated significant positive linear correlation with milk fat percentage (R^2 = 0.664, P < 0.01) and lactose yield (R^2 = -0.355, P < 0.01), and significant negative correlations with dry matter intake (R^2 = -0.799, P < 0.01), milk yield (R^2 = -0.730, P < 0.01), milk protein percentage (R^2

= -0.788, P < 0.01), and milk protein yield (R^2 = -0.567, P < 0.01). Dry matter intake exhibited the strongest correlations with production performance, showing significant positive linear relationships with milk yield (R^2 = 0.820, P < 0.01), milk protein percentage (R^2 = 0.756, P < 0.01), milk fat yield (R^2 = 0.394, P < 0.01), milk protein yield (R^2 = 0.923, P < 0.01), and lactose yield (R^2 = 0.730, P < 0.01), and a significant negative correlation with milk fat percentage (R^2 = -0.644, P < 0.01).

Table 3 Linear correlation analysis of diet nutritional composition with DMI, MY and milk components

Variable	DMI	Milk yield	Milk fat %	Milk protein %	Lactose %	Milk fat yield	Milk protein yield	Lactose yield
NDF	-0.678**	-0.517**	0.567**	-0.704**	-0.146 (P=0.17)	-0.345**	-0.298**	-0.429**
Starch	0.699**	0.650**	-0.658**	0.732**	-0.122 (P=0.25)	-0.034 (P=0.65)	0.149 (P=0.12)	0.489**
NDF:starch	-0.799**	-0.730**	0.664**	-0.788**	0.020 (P=0.84)	-0.126 (P=0.18)	-0.567**	-0.355**
DMI	1.000	0.820**	-0.644**	0.756**	0.064 (P=0.53)	0.394**	0.923**	0.730**

Note: P > 0.05 indicates no significant correlation; P < 0.05 indicates significant correlation. **P < 0.01.

2.3 Simple Linear Regression Analysis Between Dietary NDF:Starch Ratio and Dry Matter Intake, Milk Yield, and Milk Components

The simple linear regression relationships between dietary NDF:starch ratio and dry matter intake, milk yield, and milk components are presented in Table 4 . Meta-analysis results indicated that simple linear regression equations for dietary NDF:starch ratio against dry matter intake (P < 0.01), milk yield (P < 0.01), milk fat percentage (P < 0.01), milk protein percentage (P < 0.01), milk protein yield (P < 0.01), and lactose yield (P < 0.01) all reached significance. For each unit increase in dietary NDF:starch ratio, dry matter intake and milk yield decreased by 0.81 kg and 1.36 kg, respectively (Figure 1 [Figure 1: see original paper]). Milk fat percentage and milk protein percentage increased by 0.11% and 0.07%, respectively (Figure 2 [Figure 2: see original paper]). Milk protein yield and lactose yield decreased by 0.06 kg and 1.50 kg, respectively (Figure 3 [Figure 3: see original paper]).

Table 4 Linear regression relationship analysis of dietary NDF:starch with DMI, MY and milk components

Item	Treatments	Intercept	Linear term (X)	RMSE	F-value	P-value
Dry matter intake, kg/d	123	27.86	-2.13	1.42	215.6	<0.01
Milk yield, kg/d	123	48.23	-6.53	2.85	168.3	<0.01
Milk fat percentage, %	123	1.65	1.11	0.28	95.8	<0.01
Milk protein percentage, %	123	4.51	-0.70	0.15	189.7	<0.01
Lactose percentage, %	96	4.73	-0.03	0.18	0.3	0.61
Milk fat yield, kg/d	123	1.58	-0.13	0.15	1.8	0.18
Milk protein yield, kg/d	123	1.71	-0.30	0.09	58.2	<0.01
Lactose yield, kg/d	96	2.51	-0.43	0.21	16.4	<0.01

Note: $P > 0.05$ indicates no significant linear regression relationship; $P < 0.05$ indicates significant linear regression relationship.

Figure 1 Relationship between dietary NDF:starch and dry matter intake or milk yield
Figure 2 Relationship between dietary NDF:starch and milk fat percentage or milk protein percentage
Figure 3 Relationship between dietary NDF:starch and milk protein yield or lactose yield

3 Discussion

Carbohydrates play an extremely important role in ruminant nutrition by maintaining normal rumen fermentation, ensuring gastrointestinal health, and serving as an energy carrier for rumen microorganisms and host metabolism. Therefore, developing comprehensive nutritional evaluation indices to optimize dietary carbohydrate composition is particularly important. The forage-to-concentrate ratio only distinguishes between plant stems/leaves and seeds, reflecting merely the difference in fiber and non-fiber content. The non-fibrous carbohydrate to NDF ratio emphasizes the proportion of carbohydrates to fiber, but NDF is the main component of structural carbohydrates while starch exceeds 80% of non-structural carbohydrates. Consequently, the NDF:starch ratio can more accurately reflect dietary carbohydrate composition.

Dry matter intake is fundamental for providing nutrients to maintain dairy cow health and production, with dietary NDF content and forage-to-concentrate ratio being the main nutritional factors affecting intake. Previous studies have reported significant negative correlations between dietary NDF content and dry matter intake in dairy cows, as increased NDF reduces digesta passage rate through the rumen, thereby decreasing intake. This study found a significant negative linear correlation between dietary NDF content and dry matter intake ($R^2 = 0.678$). Meta-analysis results also showed a significant positive linear correlation between dietary starch content and dry matter intake ($R^2 = 0.699$), likely because changes in forage-to-concentrate ratio typically alter starch content. Easily fermentable carbohydrates like starch can increase dry matter intake due to their higher degradation and passage rates in the rumen compared to structural carbohydrates. The NDF:starch ratio exhibited a significant negative linear correlation with dry matter intake ($R^2 = 0.799$), with stronger correlation than either NDF or starch content alone, suggesting it better predicts and evaluates dry matter intake.

Milk yield is primarily influenced by dry matter intake, as higher intake provides more nutritional substrates for production and milk component synthesis. This meta-analysis supports this concept, showing the strongest correlation between dry matter intake and milk yield ($R^2 = 0.820$). Among carbohydrate composition indicators, correlations with milk yield from strongest to weakest were NDF:starch ratio (R^2 = -0.730), starch content (R^2 = -0.650), and NDF content (R^2 = -0.517). Thus, as an indicator of dietary carbohydrate composition, the NDF:starch ratio more accurately reflects carbohydrate composition and predicts milk yield.

Dietary carbohydrates affect milk component synthesis: NDF provides substrates for milk fat synthesis, easily fermentable starch-type carbohydrates promote lactose synthesis, and rumen-fermentable carbohydrates influence microbial protein synthesis to provide quality protein for milk protein synthesis. Meta-analysis revealed that among indicators reflecting dietary carbohydrate composition, correlations with milk fat percentage from strongest to weakest were NDF:starch ratio (R^2 = 0.664), starch content (R^2 = -0.658), and NDF content (R^2 = 0.567). Correlations with milk protein percentage from strongest to weakest were NDF:starch ratio (R^2 = -0.788), starch content (R^2 = 0.732), and NDF content (R^2 = -0.704). These results indicate that the NDF:starch ratio shows the strongest correlations with milk fat and protein percentages, enabling more accurate prediction of these components. However, no correlations were observed between NDF:starch ratio, starch content, or NDF content with lactose percentage, likely because lactose percentage remains relatively stable in milk compared to fat and protein percentages.

Milk component yields depend on both milk yield and component concentration. In this study, milk fat yield showed positive linear correlation with dry matter intake (R^2 = 0.394). Although dry matter intake was negatively correlated with milk fat percentage (R^2 = -0.644), the strong correlation between dry matter intake and milk yield resulted in a positive correlation with milk fat yield. Milk fat yield was negatively correlated with dietary NDF content (R^2 = -0.345), while dietary NDF content was positively correlated with milk fat percentage—this apparent contradiction likely stems from the negative correlation between NDF content and milk yield. No correlations were found between NDF:starch ratio or starch content with milk fat yield, suggesting the NDF:starch ratio may not reflect milk fat synthesis capacity.

For milk protein yield, dry matter intake showed an extremely strong positive linear correlation (R^2 = 0.923). Hristov et al. used meta-analysis to examine relationships between dietary carbohydrates and milk protein yield, concluding that dry matter intake is the determining factor. This study found a negative linear correlation between NDF:starch ratio and milk protein yield (R^2 = -0.567), while dietary NDF content showed weaker correlation and starch content showed no correlation, indicating that the NDF:starch ratio can partially reflect milk protein synthesis capacity. For lactose yield, dry matter intake showed the strongest correlation (R^2 = 0.730), primarily because intake provides sufficient energy and nutrients. Dietary starch content also showed positive linear correlation with lactose yield (R^2 = 0.489), as lactose synthesis substrates originate directly or indirectly from dietary starch.

4 Conclusions

1. Through data compilation from 123 treatments across 27 publications, meta-analysis revealed significant linear correlations and simple linear regression relationships between dietary NDF:starch ratio and dairy cow dry matter intake, milk yield, milk fat percentage, and milk protein percentage.
2. Compared with dietary NDF and starch contents alone, the NDF:starch ratio more scientifically, accurately, and comprehensively reflects dietary carbohydrate composition and can be used to predict dairy cow performance and milk composition.

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Note: Figure translations are in progress. See original paper for figures.