Relationship between Milk Fat Odd- and Branched-Chain Fatty Acid Content and Rumen Fluid Volatile Fatty Acid Content Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to determine the relationship between the content of odd and branched-chain fatty acids (OBCFA) in milk fat and the content of volatile fatty acids (VFA) in rumen fluid. Nine healthy Holstein dairy cows of the same parity with similar body weight were selected, with three cows each in early, mid, and late lactation stages (cows within the same stage had similar milk yield). Rumen fluid and milk samples were collected within a 12-hour period, and the contents of VFA in rumen fluid and OBCFA in milk fat were measured, respectively. The results indicated that for each type of VFA content in rumen fluid, a corresponding significantly or highly significantly correlated OBCFA content could be identified (P$ 0.05*orP* $0.01). The root mean square errors of the regression equations for predicting acetate, propionate, and butyrate contents based on milk fat OBCFA content were 1.57, 1.06, and 0.33, respectively, while those for predicting isobutyrate, isovalerate, and valerate were 0.13. The results suggest that regression equations for predicting various VFA contents in rumen fluid based on milk fat OBCFA content can be applied to predict VFA contents in the rumen.

Full Text

Relationship between Contents of Milk Fat Odd- and Branched-Chain Fatty Acids and Rumen Fluid Volatile Fatty Acids

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Abstract: This experiment aimed to determine the relationship between volatile fatty acid (VFA) contents in rumen fluid and odd- and branched-chain fatty acid (OBCFA) contents in milk fat. Nine healthy lactating Holstein dairy cows of the same parity with similar body weight were selected, with three cows each at early, middle, and late lactation stages (cows within the same stage had similar milk production). Rumen fluid and milk samples were collected over a 12-hour period to determine VFA contents in rumen fluid and OBCFA contents in milk fat. The results showed that significant or highly significant correlations could be found between individual VFA contents and specific OBCFA contents (P$ 0.05*orP* $0.01). The root mean square errors of regression equations for predicting acetate, propionate, and butyrate contents from milk fat OBCFA were 1.57, 1.06, and 0.33, respectively, while those for isobutyrate, isovalerate, and valerate were 0.13. These findings suggest that regression equations based on milk fat OBCFA content can be applied to predict various VFA contents in rumen fluid.

Key words: milk odd- and branched-chain fatty acids; rumen fermentation; volatile fatty acids

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It is estimated that 70%–80% of absorbed energy in ruminants is provided by volatile fatty acids (VFAs) [1]. Research indicates that acetate and butyrate in the rumen are primarily used for fatty acid synthesis, while propionate serves as a precursor for glucose synthesis. Therefore, the content and composition of various VFAs in the rumen are crucial for evaluating energy utilization in ruminants [2]. Dijkstra et al. [3] developed a dynamic model simulating rumen digestion and absorption in ruminants, but improving prediction accuracy requires understanding nutrient digestion and absorption within the rumen. Friggens et al. [4] investigated the relationship between rumen fermentation parameters and dietary chemical composition in sheep, obtaining equations to predict rumen fluid VFA content. They found that rumen VFA content was related to feed chemical composition, but incorporating dietary factors into regression equations did not improve prediction accuracy. Rymer et al. [5] discovered that in vitro gas production rate was negatively correlated with rumen acetate and butyrate contents but positively correlated with propionate and valerate contents. However, Brown et al. [6] found that in vitro gas production rate was negatively correlated with acetate and butyrate contents but positively correlated with propionate content. Currently, accurate determination of rumen VFA content requires surgical cannulation or esophageal intubation, which causes stress and harm to animals, introduces experimental errors, and violates animal welfare principles. Therefore, developing a new non-invasive method to study rumen fermentation is essential. Milk fat, as an easily obtainable sample, has been used in animal health and nutrition research [7-8]. Odd- and branched-chain fatty acids (OBCFAs) in milk fat primarily originate from lipids of bacteria leaving the rumen, with limited endogenous synthesis [9]. Research shows that different rumen bacteria have distinct OBCFA compositions, which can be used to assess rumen

microbial population composition and changes. Vlaeminck et al. [10] found high correlations between rumen fluid OBCFA content and acetate, propionate, and butyrate contents through in vitro experiments, and later obtained similar results when studying the relationship between milk fat OBCFA and rumen fluid VFA content [11]. To eliminate the influence of different lactation stages, this experiment collected samples from cows at various lactation stages. The objective was to investigate the relationship between milk fat OBCFA content and rumen fluid VFA content and to develop regression equations for predicting rumen fluid VFA content from milk fat OBCFA.

1.1 Experimental Design

The experiment was conducted at the Wandashan Dairy Farm in Duiqingshan Town, Harbin, Heilongjiang Province. Nine healthy Holstein dairy cows of first parity with similar body weight $[(650\$\pm\$33)kg]wereselected, withthreecowseachatearly, middle, andlatelactatio$ kg/d, respectively. Cows were managed according to conventional high-yielding dairy cow practices, receiving total mixed ration (TMR) at 06:00 and 18:00. The TMR was a conventional diet used at the farm, with free access to feed and water. Its composition and nutrient levels are shown in Table 1 .

The main nutrient levels of TMR were determined as follows: samples were dried at (60$\pm$5) ℃ to determine dry matter (DM) content [12]; nitrogen content was determined using the Kjeldahl method [12], with crude protein content calculated as nitrogen content × 6.25; acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined according to Van Soest et al. [13] using heat-stable α-amylase with an Ankom 220 fiber analyzer; calcium (Ca) and total phosphorus (TP) contents were determined according to Yang [14]; net energy for lactation was calculated according to methods in *Dairy Cow Nutrition Requirements and Feed Composition* [15].

Milk and rumen fluid samples (approximately 100 mL each) were collected and stored at -20 ℃. Milk samples were collected using an Afikim automatic milking system. Approximately 200 mL was sampled from each cow at 02:00, 10:00, and 16:00 daily, mixed at a 4:3:3 ratio, and 5 mL was taken and stored at -20 ℃, yielding 27 samples total. Rumen fluid was collected using a stomach-tube sampler (Wuhan Kelibo Equipment Co., Ltd.). To avoid causing harm and stress to the animals, samples were collected every 2 h from 08:00 to 20:00 over 3 days at the following time points: Day 1 at 08:00, 14:00, and 20:00; Day 2 at 12:00 and 18:00; Day 3 at 10:00 and 16:00. The collected rumen fluid was filtered through 4 layers of gauze, and 5 mL was mixed with 15% (m/V) metaphosphoric acid preservative solution at a 20% ratio, then stored at -20 ℃, yielding 63 samples total.

1.2 Sample Processing and Analysis

Rumen fluid samples were processed according to the method provided by Li et al. [16]. After thawing, rumen fluid was centrifuged

at 1,000×g for 15 min, the supernatant was collected and then centrifuged at 12,000×g for 15 min, filtered through a 0.22 m membrane, and analyzed for individual VFA content using a gas chromatograph (GC-2010, Tokyo, Japan) with an FFAP column (HP-INNOWAX, 30 m$\times 0.25 mm \times 0.2 \mu m$). $Milk fat processing followed the method of Vlaeminck et al.$ [7], $with OBCFA content deter$ $2010, Tokyo, Japan) and an SP-2560 TM column (100 m \times 0.25 mm \times 0.2 \mu m). The specific procedure was as follows$ $2 mL of milk sample was added to a 25 mL screw-cap heat-resistant centrifuge tube, followed by 2.5 mL methanola$ for 3 min at room temperature, and the upper layer was filtered through a 0.22 m membrane for analysis. Gas chromatography conditions were: injector temperature 240 ℃, pressure 266.9 kPa, carrier gas high-purity nitrogen. The column oven used a stepwise temperature program: initial temperature 170 ℃ held for 30 min, then increased at 1.5 ℃/min to 200 ℃ held for 20 min, and finally increased at 5 ℃/min to 230 ℃ held for 5 min.

1.3 Data Analysis

Rumen fluid VFA content was expressed as the proportion (%) of each VFA to total VFA, with total VFA concentration being the sum of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate concentrations. Milk fat OBCFA content was expressed as the proportion (%) of each OBCFA to total fatty acids. All data analyses were performed using SAS 9.2 software. For correlation and regression analyses, milk fat OBCFA content served as the independent variable and rumen fluid VFA content as the dependent variable, with each rumen fluid VFA data point from individual cows at each time point matched with the corresponding milk fat OBCFA data collected on the same day. Correlation analysis used the PROC CORR procedure with Pearson correlation testing, where 0.05<P$ 0.10 $indicated a tendency to correlate, P$ 0.05 $indicated significant correlation, and P$ 0.01 $indicated high$ The RMSE was calculated as follows:

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

where n is sample size, y and ŷ are observed and predicted values, respectively.

Predicted rumen fluid VFA contents were obtained by substituting measured milk fat OBCFA contents into the regression equations. Correlation analysis between predicted and measured results was performed using the CORR procedure with Pearson testing, and relative deviation was calculated to generate residual plots.

2.1 Relationship between Individual Rumen VFA Contents and Milk Fat OBCFA

As shown in Table 2 , acetate, propionate, and butyrate were the major VFAs with relatively small coefficients of variation, while isobutyrate, isovalerate, and valerate were present in smaller amounts with larger coefficients of variation.

Acetate was the most abundant VFA in rumen fluid, followed by propionate and butyrate, with other acids present in smaller quantities. Various OBCFAs were present at different levels in milk fat, with linear odd-chain fatty acids being more abundant than trans-isomer branched-chain fatty acids. Among OBCFAs, anteiso-C17:0 and C15:0 were the most abundant, followed by iso-C17:0, C17:0, and anteiso-C15:0. Trans-isomer and linear odd-chain fatty acids showed relatively small variation, while iso-fatty acids had larger coefficients of variation.

Table 3 presents the correlation results between individual VFA contents and OBCFA contents. Significant or highly significant correlations could be found between each VFA content and specific OBCFA contents (P$ 0.05orP 0.01).$C11 : 0contentshowedhighcorrelationswithacetateandvaleratecontents.Iso−C15 : 0contenttendedtocorrelatewithisovaleratecontent(0.05 < P$0.10). C15:0 content showed high correlations with acetate and propionate contents. Iso-C16:0 content showed high correlations with all VFAs except propionate and butyrate. Iso-C17:0 content showed high correlations with isovalerate and valerate contents. C17:0 content showed high correlations with all VFA contents, being positively correlated with acetate content but negatively correlated with other VFA contents.

2.2 Multiple Regression Equations

As shown in Table 4 , all parameter tests had P-values less than 0.05. The RMSE values for regression equations predicting acetate, propionate, and butyrate were 1.57, 1.06, and 0.33, respectively, while those for isobutyrate, isovalerate, and valerate were all 0.13. All equations had coefficients of determination above 0.4, with those for acetate and butyrate above 0.6.

2.3 Comparison of Estimated and Measured Values from Multiple Regression Equations

Table 5 shows that correlation analysis between measured and regression-estimated values revealed highly significant correlations (P$ $0.01) for all VFAs. Isobutyrate and isovalerate showed relatively high correlations (r>0.5) between predicted and measured values, while others were lower. For relative deviations within $ $10%, the proportions of data points were 90.48% for acetate, 85.71% for propionate, 55.56% for isobutyrate, 90.48% for butyrate, 68.25% for isovalerate, and 71.43% for valerate.

The residual plot (Figure 1 [Figure 1: see original paper]) with predicted values from the regression equations as the x-axis and residuals between measured and predicted values as the y-axis showed data points randomly distributed above and below the x-axis.

3.1 Relationship between Rumen VFA Content and Milk Fat OBCFA Content

Sutton et al. [18] found that VFA proportions in rumen fluid are more valuable and accurate for evaluating VFA absorption. Therefore, rumen VFA content provides more valuable information for animal nutrient utilization. This experiment used the proportions of individual VFAs to total VFA in rumen fluid. The contents of various VFAs in rumen fluid and OBCFAs in milk fat were similar to results from other studies [15].

Rumen microorganisms synthesize saturated fatty acids from VFAs as precursors under the action of fatty acid synthase. Propionate and valerate are elongated to form odd-chain linear fatty acids [19], while branched-chain fatty acids are synthesized from branched-chain amino acids (leucine, isoleucine, and valine) and short branched VFAs (isobutyrate and isovalerate) as precursors [20]. This experiment found large correlation coefficients between milk fat C15:0 content and rumen acetate and propionate contents, and between milk fat C17:0 content and rumen acetate, propionate, and butyrate contents, consistent with results from Vlaeminck et al. [11] on the relationship between milk fat OBCFA content and rumen fermentation parameters. Additionally, Vlaeminck et al. [11] found positive correlations between milk fat iso-C14:0 and iso-C15:0 contents and rumen acetate content, while this experiment found a negative correlation between milk fat iso-C16:0 content and rumen acetate content. This discrepancy may be due to different synthesis processes for various iso-fatty acids by rumen bacteria [19]. Furthermore, Dijkstra [21] noted that rumen-fermentable fiber produces more acetate and less propionate and butyrate compared to starch, and different fermentation patterns of substrates may also contribute. This experiment showed a positive correlation between rumen acetate content and milk fat C15:0 content, similar to findings by Vlaeminck et al. [11]. Rumen propionate content was negatively correlated with milk fat C15:0 and C17:0 contents. Research indicates that mammary tissue can synthesize propionyl-CoA for odd-chain linear fatty acid synthesis. French et al. [22] infused propionate into the rumen and found no significant increase in rumen propionate content but a significant increase in milk fat C15:0 and C17:0 contents. The rapid absorption rate of propionate in the rumen [23] may explain these results. This experiment also found a negative correlation between butyrate content and milk fat C17:0 content. Like acetate, butyrate can be used for fatty acid synthesis in adipose and mammary tissues, and acetate and butyrate can interconvert [18]. Additionally, butyrate differs from acetate in absorption rate [23] and metabolic pathways [24] in the rumen, leading to different relationships with milk fat fatty acids.

Isoacids are essential nutritional factors for some rumen bacteria, particularly fiber-degrading bacteria, for protein digestion and synthesis [25]. Liu et al. [26] found that dietary isoacid supplementation benefits rumen microbial protein synthesis, plant cell wall fermentation, and dry matter digestion. This experiment found positive correlations between isobutyrate, isovalerate, and valerate contents and milk fat iso-C16:0 content, negative correlations with milk fat

C17:0 content, and a positive correlation between rumen isovalerate content and milk fat iso-C15:0 content. Rumen microorganisms both degrade dietary branched-chain amino acids to produce isoacids and utilize isoacids to synthesize microbial branched-chain amino acids [27]. Therefore, rumen isoacid content affects microbial growth and is also related to dietary protein. Cabrita et al. [28] found that anteiso-C17:0 content correlated with rumen ammonia nitrogen content and suggested it could serve as a marker for early protein availability or deficiency in the rumen.

3.2 Establishment of Multiple Regression Equations and Comparison of Estimated and Measured Values

The regression equations for predicting rumen VFA content from milk fat OBCFA content shown in Table 4 are reliable. RMSE indicates the reliability of prediction equations, with lower values indicating greater reliability. Bannink et al. [29] summarized RMSE values for various rumen VFA prediction models, with acetate ranging from 0.89–5.00, propionate from 4.55–9.70, and butyrate from 1.50–5.32. Vlaeminck et al. [11] obtained RMSE values of 2.05, 1.77, and 1.07 for acetate, propionate, and butyrate prediction equations, respectively. The RMSE values obtained in this experiment were relatively small, indicating high reliability of the regression equations for practical application. Generally, when the coefficient of determination is less than 0.5, the regression line poorly explains the dependency between variables. In this experiment, the prediction equations for acetate and butyrate had high coefficients of determination (>0.5) with small coefficients of variation for estimated values, indicating high reliability.

3.3 Comparison of Predicted and Measured Values for Rumen VFA from Multiple Regression Equations

The differences between predicted and measured values for each regression equation were not significant, and the two sets of values were highly correlated, with more than 50% of data points having relative errors within $ $10%. These results demonstrate that the equations for predicting rumen VFA content have certain accuracy. For acetate and butyrate, over 90% of data points had relative errors within $ $10%, indicating these two prediction equations provide more accurate evaluations.

Since rumen VFA content is influenced by rumen fermentation patterns, dietary forage-to-concentrate ratios and oil supplementation can affect rumen fermentation and cause deviations in regression equation predictions. Studies have shown that high levels of rumen-degradable starch in diets can lead to underestimated acetate and overestimated propionate predictions [30]. High-forage diets for dairy cows can result in overestimated acetate and slightly underestimated propionate predictions [31]. Linseed oil digestion in the rumen produces acetate, affecting the accuracy of acetate content prediction [32]. Therefore, to improve prediction accuracy, dietary factors should be incorporated into re-

gression equations. Additionally, further research is needed on the transport processes of various OBCFAs in the body and their contribution to milk fat OBCFA, particularly regarding negative energy balance.

In conclusion, milk fat OBCFA content correlates with various rumen VFA contents, and regression equations established from milk fat OBCFA show high accuracy for predicting rumen VFA contents, particularly for acetate and propionate, and can be applied in experimental research and production practice.

References

[1] VAN HOUTERT M. Challenging the retinal for altering VFA ratios in growing ruminates[J]. Feed Mix, 1996, 4(1): 514-525.

[2] SUTTON J D. Digestion and absorption of energy substrates in the lactating cow[J]. Journal of Dairy Science, 1985, 68(12): 3376-3393.

[3] DIJKSTRA J, NEALH D S C, BEEVER D E, et al. Simulation of nutrient digestion, absorption and outflow in the rumen: model description[J]. The Journal of Nutrition, 1992, 122(11): 2239-

[4] FRIGGENS N C, OLDHAM J D, DEWHURST R J, et al. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage[J]. Journal of Dairy Science, 1998, 81(5): 1331-1344.

[5] RYMER C, GIVENS D I. Relationships between patterns of rumen fermentation measured in sheep and in situ degradability and the in vitro gas production profile of the diet[J]. Animal Feed Science and Technology, 2002, 101(1/2/3/4): 31-44.

[6] BROWN V E, RYMER C, AGNEW R E, et al. Relationship between in vitro gas production profiles of forages and in vivo rumen fermentation patterns in beef steers fed those forages[J]. Animal Feed Science and Technology, 2002, 98(1/2): 13-24.

[7] VLAEMINCK B, DUFOUR C, VAN VUUREN A M, et al. Use of odd and branched-chain fatty acids in rumen contents and milk as a potential microbial marker[J]. Journal of Dairy Science, 2005, 88(3): 1031-1042.

[8] MOTTRAM T. Automatic monitoring of the health and metabolic status of dairy cows[J]. Livestock Production Science, 1997, 48(3): 209-217.

[9] KEENEY M, KATZ I, ALLISON M J. On the probable origin of some milk fat acids in rumen microbial lipids[J]. Journal of the American Oil Chemists Society, 1962, 39(4): 198-201.

[10] VLAEMINCK B, FIEVEZ V, VAN LAAR H, et al. Rumen odd and branched chain fatty acids in relation to in vitro rumen volatile fatty acid productions and dietary characteristics of incubated substrates[J]. Journal Animal Physiology Animal Nutrition, 2004, 88(11/12): 401-411.

[11] VLAEMINCK B, FIEVEZ V, TAMMINGA S, et al. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern[J]. Journal of Dairy Science, 2006, 89(10): 3954-

[12] AOAC. Official methods of analysis[M]. 15th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists, 1990.

[13] VAN SOEST P J, ROBERTSON J B, LEWIS B A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition[J]. Journal of Dairy Science, 1991, 74(10): 3583-3597.

[14] YANG S. Feed analysis and feed quality detection technology[M]. Beijing: China Agricultural University Press, 1993: 28-32.

[15] FENG Y L, LU Z N. Dairy cow nutrition requirements and feed composition[M]. Beijing: China Agriculture Press, 2007: 1-2.

[16] LI Y, MENG Q. Effect of different types of fibre supplemented with sunflower oil on ruminal fermentation and production of conjugated linoleic acids in vitro[J]. Archives of Animal Nutrition, 2006, 60(5): 402-411.

[17] ST-PIERRE N R. Invited review: integrating quantitative findings from multiple studies using mixed model methodology[J]. Journal of Dairy Science, 2001, 84(4): 741-755.

[18] SUTTON J D, DHANOA M S, MORANT S V, et al. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets[J]. Journal of Dairy Science, 2003, 86(11): 3620-3633.

[19] VLAEMINCK B, FIEVEZ V, CABRITA A R J, et al. Factors affecting odd- and branched-chain fatty acids milk: a review[J]. Animal Science Technology, 2006, 131(3/4): 389-417.

[20] KANEDA T. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance[J]. Microbiology and Molecular Biology Reviews, 1991, 55(2): 288-302.

[21] DIJKSTRA J. Production and absorption of volatile fatty acids in the rumen[J]. Livestock Production Science, 1994, 39(1): 61-69.

[22] FRENCH E A, BERTICS S J, ARMENTANO L E. Rumen and milk odd- and branched-chain fatty acid proportions are minimally influenced by ruminal volatile fatty acid infusions[J]. Journal of Dairy Science, 2012, 95(4): 2015-2026.

[23] DIJKSTRA J, BOER H, VAN BRUCHEM J, et al. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acids concentration, pH and rumen liquid volume[J]. British Journal of Nutrition, 1993, 69(2): 385-396.

[24] DU R P. Patterns of acetate and butyrate production, absorption and utilization in sheep rumen and determination of metabolizable lipogenic substances

(MLS)[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2006.

[25] ALLISON M J, BRYANT M P. Biosynthesis of branched-chain amino acids from branched-chain fatty acids rumen bacteria[J]. Archives of Biochemistry Biophysics, 1963, 101(2): 269-277.

[26] LIU Q, WANG C, YANG W Z, et al. Effects of isobutyrate on rumen fermentation, lactation performance and plasma characteristics in dairy cows[J]. Animal Feed Science and Technology, 2009, 154(1/2): 58-67.

[27] ANDRIES J I, BUYSSE F X, DE BRABANDER D L, et al. Isoacids in ruminant nutrition: their role in ruminal and intermediary metabolism and possible influences on performances—a review[J]. Animal Feed Science and Technology, 1987, 18(3): 169-180.

[28] CABRITA A R J, FONSECA A J M, DEWHURST R J, et al. Nitrogen supplementation of corn silages. 2. Assessing rumen function using fatty acid profiles of bovine milk[J]. Journal of Dairy Science, 2003, 86(12): 4020-4032.

[29] BANNINK A, DE VISSER H, VAN VUUREN A M. Comparison and evaluation of mechanistic rumen models[J]. The British Journal of Nutrition, 1997, 78(4): 563-581.

[30] VAN NESPEN T, VLAEMINCK B, WANZELE W, et al. Use of specific milk fatty acids as diagnostic tool for rumen acidosis in dairy cows[J]. Communications in Agricultural and Applied Biological Sciences, 2005, 70(2): 277-280.

[31] KRAFT J, COLLOMB M, MÖCKEL P, et al. Differences in CLA isomer distribution of cow' s milk lipids[J]. Lipids, 2003, 38(6): 657-664.

[32] LOOR J J, FERLAY A, OLLIER A, et al. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil[J]. Journal of Dairy Science, 2005, 88(2): 726-740.

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