Analysis of Milk Composition and Establishment of a Milk Energy Prediction Model for Yunnan Water Buffalo Postprint

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Abstract

This study was conducted to investigate the contents of milk fat, milk protein, lactose, and milk urea nitrogen (MUN) in buffalo milk and their correlations with milk energy, develop a prediction model for net energy for lactation, and facilitate scientific feeding management of dairy buffaloes during lactation. A total of 304 raw milk samples were collected from major dairy buffalo farms and breeding communities in Yunnan Province and delivered to the Kunming Dairy Production Performance Testing Center during September–December 2014–2015. Milk composition was determined by the Kunming Dairy Production Performance Testing Center using a MilkoScan FT+FC milk composition and somatic cell analyzer and a MilkoScan FT 120 milk composition analyzer. Fresh milk was dried at constant temperature in a vacuum drying oven, and milk energy was measured using an oxygen bomb calorimeter. The results indicated that milk fat, milk protein, lactose, MUN, total milk solids contents, and milk energy were 6.45%, 4.55%, 5.31%, 13.60 mg/dL, 18.77%, and 4.02 MJ/kg, respectively. Milk energy exhibited highly significant positive correlations with milk fat and milk protein contents ($r = 0.8960$, $r = 0.5630$, $P < 0.01$), and was strongly influenced by milk fat and milk protein contents. Using milk fat (F), milk fat and protein (F and P), milk fat, protein, and lactose (F, P, and La), and milk fat, protein, lactose, and MUN (F, P, La, and MUN) as predictors, the goodness of fit for the simple linear, two-predictor, three-predictor, and four-predictor regression equations for predicting milk energy (E) all exceeded 0.90. The equations were: $E = 0.388F + 1.540$ ($R2 = 0.9336$, $P < 0.01$); $E = 0.373F + 0.221P + 0.460$ ($R2 = 0.9267$, $P < 0.01$); $E = 0.396F + 0.186P + 0.105La - 0.104$ ($R2 = 0.9540$, $P < 0.01$); $E = 0.397F + 0.187P + 0.106La + 0.002MUN - 0.146$ ($R2 = 0.9580$, $P < 0.01$). These results demonstrate that the net energy for lactation of buffalo milk can be predicted from the contents of milk fat, milk protein, lactose, and MUN.

Full Text

Analysis of Milk Composition and Establishment of a Milk Energy Prediction Model for Yunnan Buffalo

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Abstract: This study analyzed the contents of milk fat, protein, lactose, and milk urea nitrogen (MUN) in buffalo milk and their correlations with milk energy to establish a prediction model for net energy of lactation, which can be used for scientific feeding management of dairy buffaloes during lactation. A total of 304 raw milk samples were collected from major dairy buffalo farms and farming communities in Yunnan Province between September and December 2014–2015 and submitted to the Kunming Dairy Production Performance Measurement Center. Milk composition was analyzed using a MilkoScan FT+FC milk composition and somatic cell analyzer and a MilkoScan FT 120 milk composition analyzer. Milk energy was determined using an oxygen bomb calorimeter after vacuum drying at constant temperature.

The results showed that milk fat, protein, lactose, MUN, total solids, and milk energy averaged 6.45%, 4.55%, 5.31%, 13.60 mg/dL, 18.77%, and 4.02 MJ/kg, respectively. Milk energy exhibited extremely significant positive correlations with milk fat and protein content ($r = 0.8960$ and $r = 0.5630$, respectively, $P < 0.01$), indicating that milk energy was strongly influenced by fat and protein content. Using milk fat (F), fat and protein (F and P), fat, protein, and lactose (F, P, and La), and fat, protein, lactose, and MUN (F, P, La, and MUN) as predictors, the fitting degrees of the one-, two-, three-, and four-variable regression equations for predicting milk energy (E) all exceeded 0.90. The equations were: $E = 0.388F + 1.540$ ($R^2 = 0.9336$, $P < 0.01$); $E = 0.373F + 0.221P + 0.460$ ($R^2 = 0.9267$, $P < 0.01$); $E = 0.396F + 0.186P + 0.105La - 0.104$ ($R^2 = 0.9540$, $P < 0.01$); and $E = 0.397F + 0.187P + 0.106La + 0.002MUN - 0.146$ ($R^2 = 0.9580$, $P < 0.01$). These results demonstrate that net energy of lactation in buffalo milk can be predicted from its fat, protein, lactose, and MUN contents.

Keywords: buffalo milk; milk composition; milk energy; prediction model

Energy is essential for animal growth, reproduction, and production. For lactating cows, milk energy represents net energy for lactation (NEL). Currently, countries adopting a net energy system uniformly calculate the energy requirements of lactating cows based on net energy for lactation, 4% fat-corrected milk, and milk yield [1-6]. Milk energy is derived from milk components such as fat, protein, and lactose. In practice, however, direct measurement of milk energy is relatively complex, whereas milk nutrients are routinely measured monthly on dairy farms and are readily available. Milk fat and protein content can not only predict milk energy values but also assess energy balance in lactating cows [7-8]. The American, British, and Chinese dairy feeding standards all include prediction models for calculating net energy for lactation from milk nutrient contents to guide production [4-6]. The milk samples used in these prediction models were all from Holstein cows, whose milk fat, protein, lactose, and MUN contents are lower than those of buffalo milk. The compositional differences between the two are substantial, particularly the higher MUN content in buffalo milk—whether this contributes to milk energy and whether Holstein-based prediction models can be applied to dairy buffaloes warrant careful consideration.

According to 2014 statistics from the Food and Agriculture Organization (FAO), the global dairy buffalo population reached 195.0983 million head, with fresh buffalo milk accounting for 12.92% of total milk production [9]. However, milk yield per buffalo remains low. In addition to genetic improvement through breeding, rational feeding and scientific management are core strategies for increasing yield [10]. Currently, no established nutritional requirements exist for dairy buffaloes, making it crucial to determine these requirements. This study used MilkoScan FT+FC and MilkoScan FT 120 analyzers to measure buffalo milk composition and an oxygen bomb calorimeter to determine milk energy. We investigated the correlation between buffalo milk energy and milk components and established prediction models for milk energy through regression analysis to determine the energy requirements for lactation in dairy buffaloes, providing foundational data for formulating buffalo feeding standards.

1.1 Experimental Materials

To ensure sample representativeness, three buffalo herds from different feeding systems and dietary levels in major dairy buffalo-producing regions of Yunnan Province were selected for analysis. Animals in all three herds were fed twice daily with ad libitum water access and milked twice daily.

Milk from Tengchong Binlangjiang buffaloes was obtained from the core breeding farm in Tengchong. The experimental animals consisted of 184 healthy Binlangjiang buffaloes in early to mid-lactation, primarily in parities 2-4. The diet consisted of self-formulated concentrate, whole-plant corn silage, and rice straw, with daily intake of 3-4 kg concentrate (adjusted based on milk yield), 20-25 kg corn silage, and ad libitum rice straw (4-5 kg intake), at a protein level of 12%.

Milk from Dali hybrid buffaloes was sourced from a farming community in Dali, comprising 56 healthy hybrid buffaloes in early to mid-lactation, primarily in parities 1-5. The diet included commercial concentrate, corn stover silage, rice straw, alfalfa hay, and ryegrass hay, with daily intake of 3–4 kg concentrate (adjusted based on milk yield), 15–20 kg corn stover silage, ad libitum rice straw (4–5 kg intake), and a protein level of 14%; during mid-lactation, 2 kg of alfalfa and ryegrass hay was added.

Milk from Dehong hybrid buffaloes came from a farming community in Dehong, consisting of 64 healthy hybrid buffaloes in early to mid-lactation, primarily in parities 1–5. The diet comprised self-formulated concentrate, sugarcane tops, rice straw, brewers' grains, and king grass, with daily intake of 1.5–2.0 kg concentrate (adjusted based on milk yield), 20 kg sugarcane tops, and ad libitum rice straw and king grass (4–5 kg intake), at a protein level of 11%; during mid-lactation, 2.0 kg of brewers' grains was added.

1.2 Sample Collection and Measurement

A total of 304 milk samples were collected from the three major buffalo farms and communities in Tengchong, Dali, and Dehong between September and December 2014–2015 for determination of milk composition and energy. Sampling occurred at the end of each month. Milk from the two daily milkings was mixed at a 1:1 ratio, poured into sample bottles, and shaken to ensure preservative dissolution. Each cow contributed 40–50 mL of sample.

1.3 Determination of Milk Composition and Energy

Milk composition was determined by the Kunming Dairy Production Performance Measurement Center using MilkoScan FT+FC and MilkoScan FT 120 analyzers. Milk energy was measured at Yunnan Agricultural University using a BH- S oxygen bomb calorimeter: 5 mL of milk sample was placed in a preweighed self-sealing bag (in a Gooch crucible), dried in a vacuum oven at (60 ± 5) °C, and then analyzed with the oxygen bomb calorimeter.

1.4 Data Processing

Data were organized and plotted using Excel 2016 and SPSS 22.0. Significance testing was performed using one-way ANOVA and Duncan' s multiple comparison tests based on the model Yi = µ + Ti + i (where Yi is the observed value, µ is the overall mean, Ti is the treatment effect, and i is random error). Results are expressed as mean ± standard deviation. Correlation analysis used Pearson correlation coefficients for bivariate analysis. The prediction model was developed using multiple linear regression: E = + F + P + La + MUN [where is the constant; , , , are partial regression coefficients; F is milk fat (%); P is milk protein (%); La is lactose (%); MUN is milk urea nitrogen (mg/dL); and E is milk energy (MJ/kg)].

2.1 Comparison of Buffalo Milk Composition

As shown in Table 1 , the mean values for milk fat, protein, lactose, MUN, total solids, and milk energy were 6.45%, 4.55%, 5.31%, 13.60 mg/dL, 18.77%, and 4.02 MJ/kg, respectively. Milk fat content ranged from 3.30% to 11.10% and increased with lactation stage. Protein content ranged from 2.84% to 6.58%; lactose from 3.09% to 5.99%; MUN showed a wide range of 1.40–26.60 mg/dL; and total solids ranged from 11.42% to 25.93%.

Comparisons within the same breed across months revealed no significant differences in fat, protein, lactose, MUN, total solids, or milk energy for Tengchong Binlangjiang buffalo milk ($P > 0.05$). For Dali and Dehong hybrid buffalo milk, no significant differences were observed in protein, lactose, total solids, or milk energy ($P > 0.05$), but both fat and MUN contents in September differed significantly from those in December ($P < 0.05$).

Table 2 shows that no significant differences existed among the three buffalo breeds in fat, protein, lactose, total solids, or milk energy ($P > 0.05$). However, MUN content in Dali hybrid buffalo milk differed significantly from that in both Tengchong Binlangjiang and Dehong hybrid buffalo milk ($P < 0.05$), while MUN content between the latter two groups showed no significant difference ($P > 0.05$).

Figure 1 [Figure 1: see original paper] demonstrates that across all 304 samples from the three herds, no significant monthly differences were observed in fat, protein, lactose, MUN, total solids, or milk energy ($P > 0.05$).

2.2 Correlation Analysis Between Milk Energy and Composition

Table 3 reveals that milk energy was extremely significantly positively correlated with milk fat and protein content ($r = 0.8960$ and $r = 0.5630$, respectively, $P < 0.01$). Figure 2 [Figure 2: see original paper] illustrates that milk energy was substantially influenced by fat and protein content.

2.3 Significance and Goodness-of-Fit Analysis of Regression Equations for Predicting Milk Energy

Table 4 shows that the calculated milk energy values from our buffalo milk equations were similar to those from the Chinese Dairy Feeding Standard and the Chinese Dairy Feeding Standard Research Collaborative Group equations [3–4], and were only 0.0826, 0.0410, and 0.0356 MJ/kg lower than the results from Musgrave et al. [1], AFRC [5], and NRC [6], respectively—differences that are not substantial.

Figures 3 [Figure 3: see original paper] through 6 [Figure 6: see original paper] demonstrate that all regression equations reached extremely significant statistical levels ($P < 0.01$). Using milk fat (F), fat and protein (F and P), fat, protein, and lactose (F, P, and La), and fat, protein, lactose, and MUN (F, P, La, and MUN) as predictors, the one-, two-, three-, and four-variable regression equations for predicting milk energy (E) all achieved R^2 values above 0.90:

E = 0.388F + 1.540 (R^2 = 0.9336, residual range: -1.1136 to 1.4660, $P < 0.01$, n = 304)
E = 0.373F + 0.221P + 0.460 (R^2 = 0.9267, residual range: -1.1250 to 1.5741, $P < 0.01$, n = 304)
E = 0.396F + 0.186P + 0.105La -0.104 (R^2 = 0.9540, residual range: -0.9981 to 1.5609, $P < 0.01$, n = 304)
E = 0.397F + 0.187P + 0.106La + 0.002MUN -0.146 (R^2 = 0.9580, residual range: -1.0070 to 1.5472, $P < 0.01$, n = 304)

Based on goodness-of-fit and milk energy residuals (observed -predicted values), the three- and four-variable regression equations using fat, protein, and lactose, and fat, protein, lactose, and MUN as predictors showed better fit and more accurate predictions. However, when only fat and protein data are available, the regression equation can still be used to predict net energy for lactation.

3.1 Comparison of Milk Composition and Influencing Factors

No significant monthly differences in fat, protein, lactose, MUN, total solids, or milk energy were observed for Tengchong Binlangjiang buffalo milk, which is consistent with unchanged dietary composition and feeding levels throughout the sampling period. Similarly, Dali and Dehong hybrid buffalo milk showed no significant differences in protein, lactose, total solids, or milk energy, as these components are minimally affected by season [5, 11-15]. No significant differences in fat, protein, lactose, or total solids were found among breeds, as breed has minimal influence on lactose and protein content [5, 11-15].

The mean fat content of 6.45% was lower than values reported by Xie et al. [16], Sun et al. [17], and Rafiq et al. [18] but higher than those from Islam et al. [19] and Javed et al. [20], likely related to dietary crude fat content and whether samples were collected from fore, mid, and late milking stages or as composite samples [21-22]. The fat content range of 3.30%-11.10% falls within previously reported ranges [11, 20, 23-25]. The mean protein content of 4.55% aligns with reports from Xie et al. [16], Javed et al. [20], and Zou et al. [23], with the range of 2.84%-6.58% matching reported values [11, 20, 23-25]. Lactose content ranged from 3.09%-5.99%, consistent with studies by Xie et al. [16] and Zou et al. [23]. MUN showed a wide range of 1.40-26.60 mg/dL, with Dali and Dehong hybrid buffalo milk exhibiting similar patterns of fat and MUN content, consistent with Rajala-Schultz et al. [26], who reported a positive correlation between MUN and fat content. The significant differences between September and December, as well as the significant difference in MUN between Dali hybrid buffalo milk and the other two breeds, are closely related to dietary composition and individual animal nitrogen metabolism [27]. Total solids ranged from 11.42%-25.93%, within the range reported by Nasr [11] and Rafiq et al. [18]. Milk energy ranged from 2.01-6.16 MJ/kg, with a mean of 4.02 MJ/kg—higher than the 2.84 MJ/kg reported for Holstein milk but lower than the 4.24–4.78 MJ/kg and 4.20–4.70 MJ/kg reported by Zou et al. [23] and Claeys et al. [24], respectively. These variations are associated with breed, animal size, temperature, environmental conditions, management, and hygiene [11, 28–30].

3.2 Correlation Analysis Between Milk Components and Energy

Milk energy was extremely significantly positively correlated with fat and protein content, consistent with Musgrave et al. [1], who reported a significant linear relationship between fat content and milk energy values. However, the correlation between fat content and milk energy was lower than that reported by Tyrrell et al. [2], possibly due to breed differences and the contribution of high MUN content in buffalo milk to energy, which reduces the correlation between fat and energy. Milk energy was substantially influenced by fat and protein content, as net energy for lactation equals the sum of the combustion heat values of all milk components. Reported combustion heats for fat, protein, and lactose are 38.87, 23.89, and 16.53 MJ/kg, respectively, with lactose showing minimal variation [5].

3.3 Analysis of Regression Equations for Predicting Milk Energy

In 1928, Gaines proposed using 4% fat-corrected milk to avoid interference from compositional changes on the relationship between milk components and energy. Musgrave et al. [1] suggested that the 4% fat-corrected milk equation adjusts for constant energy values in milk yield. The energy of buffalo milk with 4% fat content in this study was 3.0920 MJ/kg, within the reported range of 3.05–3.14 MJ/kg for 4% fat-corrected milk [3] and close to values calculated using the Chinese Dairy Feeding Standard and the Chinese Dairy Feeding Standard Research Collaborative Group equations [3–4].

The two-, three-, and four-variable regression equations using fat and protein; fat, protein, and lactose; and fat, protein, lactose, and MUN as predictors showed coefficients similar to those in the UK AFRC (1993) [6], NRC (2001) [5], Chinese Dairy Feeding Standard (2004) [4], and Chinese Dairy Feeding Standard Research Collaborative Group (1986) [3] equations. The coefficients were close to the reported combustion value of fat (0.388 MJ/kg) but differed from those for protein and lactose (0.238 and 0.165 MJ/kg), reflecting the combined influence of fat, protein, and lactose on milk energy [5]. The coefficients for fat, protein, and lactose differed substantially from those in Zou et al. [23], warranting further investigation.

The residual ranges for the prediction equations were -1.1136 to 1.4660, -1.1250 to 1.5741, -0.9981 to 1.5609, and -1.0070 to 1.5472, respectively. The three- and four-variable regression equations using fat, protein, and lactose, and fat, protein, lactose, and MUN as predictors had smaller residual ranges, indicating greater model validity and data reliability [31].

Net energy for lactation (E) in buffalo milk can be predicted from its fat (F), protein (P), lactose (La), and MUN content using the following equations:

E = 0.388F + 1.540 ($R^2 = 0.9336$, $P < 0.01$)
E = 0.373F + 0.221P + 0.460 ($R^2 = 0.9267$, $P < 0.01$)
E = 0.396F + 0.186P + 0.105La -0.104 ($R^2 = 0.9540$, $P < 0.01$)
E = 0.397F + 0.187P + 0.106La + 0.002MUN -0.146 ($R^2 = 0.9580$, $P < 0.01$)

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