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Relationships Between Precursors of Milk Components Concentration in Blood and Milk Fat and Protein Content

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Abstract: Low nutritional quality of cow's milk has increasingly become a severe challenge faced by the healthy development of dairy industry in China. Milk fat and protein are the main substances to determine the nutritional quality of cow's milk. To maximize milk fat and protein content we study the relationships between the concentration of milk fat precursors and milk protein precursors in the blood and fat and protein contents in cow's milk. The results showed that significant positive relationship ($p<0.05$) between acetic acid and milk fat content. The correlation between NEFA/MF was positive relationship ($p>0.05$). The correlations between BHBA/MF and BHBA/ACE were negative relationships ($p>0.05$). NEFA, BHBA and ACE were the main parameters in that affect milk fat content. The correlations between cysteine, phenylalanine, arginine, aspartic acid, leucine, isoleucine and milk protein content ($p>0.05$). The correlations between proline/MP, glycine/MP and methionine/MP were negative relationships ($p>0.05$). Arginine, histidine, methionine, tyrosine and serine were the main parameters in that affect milk protein content. Therefore, we hypothesize that increase dietary fiber level and regulation dietary amino acid composition are helpful for improvement raw milk nutrition quality.

INTRODUCTION

Good-quality raw milk is required to make good-quality dairy products. Milk fat and protein are the main substances to determine the nutritional quality of cow's raw milk[1, 2]. Normal values for milk fat typically range from 3.5-4.9%; milk protein ranges from 3.1-3.8%. Following an increased importance of the milk fat or protein concentration on milk pricing, a number of research projects have been conducted to study various factors that can affect milk fat and protein content. Factors which affect milk composition include genetics, stage of lactation, level of milk production and age of cow, environment, nutrition, neuroendocrine factor, dry matter intake and disease[3].

Milk components are for the most part formed in the mammary gland (the udder) of a cow, from precursors that are the results of digestion. From cellulose and other

carbohydrates, acetic, propionic, butyric and lactic acid are formed which are taken up in the blood[4]. In rumen, proteins are broken down into amino acids. The rumen flora uses these to make proteins but can also, synthesize amino acids from low-molar-mass nitrogenous components. Further on in the digestive tract the microbes are digested, liberating amino acids[5]. All these precursors can reach the mammary gland and the rate of milk fat or protein production by the mammary gland is dependent on the combined influence of changes in blood flow and the concentration of milk fat precursors and milk protein precursors in the blood[6]. Low nutritional quality of cow's milk has increasingly become a severe challenge faced by the healthy development of dairy industry in China. Ministry of Agriculture encourages dairy farmers or manufacturers to produce milk that are superior in quality, nutritional value and functionality. To achieve these goals, we study the relationships between the concentration of milk fat precursors and milk protein precursors in the blood and fat and protein contents in cow's milk.

MATERIALS AND METHODS

Animals: This study was approved by the Jilin University All-University Committee on Animal Use and Care. A total of 60 sec-lactation China Holstein cows in peak lactation (32±2 months old; 90±20 days in milk; 24±2.5 kg daily milk yield) were used at local dairy cattle farms in Shuangcheng, P.R. China. Cows were not pregnant and in healthy condition. All the cows were fed a Total Mixed Ration (TMR) consisting of corn silage, rice straw and soybean meal *ad libitum* thrice daily (5.00, 11.00 a.m. and 5.00 p.m.) with free access to water. The ingredient and nutrient composition of TMR according to NRC (Table 1). Cows were milked twice daily (4.30 a.m. and 4.00 p.m.).

Sample collection: Caudal vein blood samples were withdrawn in 10 mL tubes under the negative pressure before 5.00 a.m. Blood samples were standing at ambient temperature for 30 min to clot, then were centrifuged for 10 min at 2200 g. Serum were aliquoted of 1.0 mL in 1.5 mL epphendorf tubes directly after centrifugation and were stored at -80°C. Milk samples were collected at 4.30 a.m. and 4.00 p.m. according to DHI uniform data collection procedures, then samples from twice collection were mixed.

Analysis of the concentration of precursors of milk components in serum: Serum acetic acid (ACE) β-Hydroxybutyric Acid (BHBA) Triglyceride (TG) and Non-Esterified Fatty Acids (NEFA) were analyzed using photospectrometrical methods (UNICO, UV-2000, Japan). Briefly, ACE was measured by the acyl-CoA synthetase-based assay (Beijing Biostest Co., Beijing, China) BHBA was measured by the BHBA colorimetric assay (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) NEFA was measured by determination of Cu-ion with Cu-reagent (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and TG was measured by the triglyceride colorimetric assay kit (Beijing BHTK clinical reagent Co., Ltd, Beijing, China). Serum free amino acid analysis was carried out on a Hitachi L-8900 PH amino acid analyzer according to the instruction of manufacturer. Assay was repeated thrice.

Table 1: Diet ingredient and nutrients composition

Items	Content (%)	Nutrition composition	Values
Corn silage	35.5	DM (%)	55.38
Rice straw	14.0	CP (%)	15.02
Chinese wildrye	6.7	EE (%)	1.28
Corn	23.4	NDF (%)	57.43
Wheat bran	3	peNDF>1.18 (%)	47.78
Soybean meal	10.6	ADF (%)	19.66
Cottonseed fuzzy	4.0	Ca (%)	0.73
Calcium phosphate	0.4	P (%)	0.29
Limestone	1.3	NE_L (MJ Kg^{-1})	2.17
Salt	0.5		
Premix	0.6		
Total	100.0		

1 kg premix contain VA = 2,000,000 IU, VD = 600,000 IU, VE = 10,800 mg, Fe = 5,500 mg, Cu = 4,080 mg, Mn = 4,989 mg, Zn = 17,500 mg, I,180 mg, Se = 110 mg and Co = 8,805 mg

Analysis of milk fat and milk protein content: The fat and protein content of milk samples were measured using FOSS analyze-instruments according to the instruction of manufacturer. Assay was repeated thrice.

Statistical analysis: Data were analyzed by SPSS (version 13) and mean±SEM were determined for all parameters in the blood and milk samples. The correlation was subjected to analysis by linear regression to make up the suitable equations among blood and milk parameters. Then the data was classified into high milk fat group, low milk fat group, high milk protein group and low milk protein group (n = 10). Analysis of variance was carried out to reveal the differences among the parameters. Differences were considered significant at $p<0.05$ and extremely significant at $p<0.01$.

RESULTS AND DISCUSSION

Relationships between precursors of milk fat concentration in serum and milk fat content: The results of the correlations between precursors of milk concentration in serum and milk fat content Table 2 showed that significant positive relationship ($p<0.05$) between ACE and Milk Fat content (MF). The correlation between NEFA/MF was positive relationship ($p>0.05$). The correlations between BHBA/MF and BHBA/ACE were negative relationships ($p>0.05$). The correlations

Table 2: Correlations among precursors of milk concentration in serum and milk fat content (n = 60)

Variables	Items	Milk fat content	Acetic acid	BHBA	NEFA	TG
Milk fat content	Pearson correlation (r)	1.000	0.622*	-0.540	0.334	0.085
	Significant (P)	0.000	0.037	0.067	0.190	0.414
Acetic acid	Pearson correlation (r)	0.622*	1.000	-0.548	0.021	0.087
	Significant (P)	0.037	0.000	0.063	0.479	0.412
BHBA	Pearson correlation (r)	-0.540	-0.548	1.000	0.229	-0.127
	Significant (P)	0.067	0.063	0.000	0.277	0.372
NEFA	Pearson correlation (r)	0.334	0.021	0.229	1.000	-0.154
	Significant (P)	0.190	0.479	0.277	0.000	0.346
TG	Pearson correlation (r)	0.085	0.087	0.412	-0.154	1.000
	Significant (P)	0.414	0.412	-0.127	0.346	0.000

*indicates $p<0.05$

Table 3: Correlations among precursors of amino acid in serum and milk protein content (n = 60)

Items	Index	MP	Items	Index	MP	Items	Index	MP
Asp	r	0.512	Ala	r	-0.006	Leu	r	0.496
	P	0.150		P	0.496		P	0.361
Thr	r	-0.041	Cys	r	0.662	Tyr	r	-0.045
	P	0.469		P	0.060		P	0.466
Ser	r	0.016	Val	r	0.258	Phe	r	0.649
	P	0.488		P	0.311		P	0.081
Glu	r	0.030	Met	r	-0.366	Lys	r	-0.176
	P	0.477		P	0.238		P	0.370
Gly	r	-0.368	Ile	r	0.403	His	r	-0.101
	P	0.236		P	0.214		P	0.425
Pro	r	-0.478	Arg	r	0.541			
	P	0.169		P	0.134			

among other parameters were not noticeable ($|r|<0.3$). NEFA, BHBA and ACE were the main parameters in that the relationships between precursors of milk fat concentration in serum and milk fat content. The standardized coefficients (Beta) of NEFA (x1) BHBA (x2) ACE (x3) and TG (x4) was 0.434, -0.425, 0.374 and 0.065, respectively. The equation was:

$$Y (MF) = 1.011+0.191\ x1-1.831\ x2+3.875\ x3+3.24\ x4$$

Relationships between free amino acid concentration in serum and milk protein content: The results of the correlations between free amino acid concentration in serum and milk protein content Table 3 showed that positive relationships between cysteine, phenylalanine, arginine, aspartic acid, leucine, isoleucine and Milk Protein content (MP) ($p>0.05$). The correlations between proline/MP, glycine/MP and methionine/MP were negative relationships ($p>0.05$). The correlations among other parameters were not noticeable ($|r|<0.3$). Arginine, histidine, methionine, tyrosine and serine were the main parameters in that the relationships between precursors of milk protein concentration in serum and milk protein content. The standardized coefficients of Arginine (x1) Histidine (x2) Methionine (x3) Tyrosine (x4) and Serine (x5) was 1.237, -0.834, -0.685, 0.064, -0.056 and 0.065, respectively. The equation was:

$$Y (MP) = 2.162+0.090\ x1-0.129\ x2-0.428\ x3+ 0.023\ x4-0.014\ x5$$

In addition, the correlations between arginine/ phenylalanine, leucine/isoleucine, leucine/valine and isoleucine/isoleucine were extremely strong positive relationships ($r>0.8$, $p<0.01$). The correlations between arginine/isoleucine, arginine/valine and leucine/cysteine were strong positive relationships ($r>0.3$, $p<0.05$). The correlation between glycine/proline was extremely strong positive relationship ($r>0.8$, $p<0.01$). The correlation between aspartic acid/proline was strong negative relationship ($r<-0.8$, $p<0.05$).

Variation of precursors of milk fat concentration in serum between high milk fat group and low milk fat group: The data of milk fat content was classified into high milk fat group (>3.40) and low milk fat group (<3.10). As show in Fig. 1, milk fat content of high milk fat group was higher extremely significantly compared with low milk fat group ($p<0.01$). As show in Fig. 2, serum NEFA levels of high milk fat group were higher significantly compared with low milk fat group ($p<0.05$). The levels of serum TG, ACE and BHBA of high milk fat group were higher than low milk fat group but the differences among these parameters were not noticeable ($p>0.05$).

Variation of free amino acid concentration in serum between high milk protein group and low milk protein group: The data of milk protein content was classified into high milk protein group (>3.20) and low milk protein group (<2.90). As show in Fig. 3, milk protein content

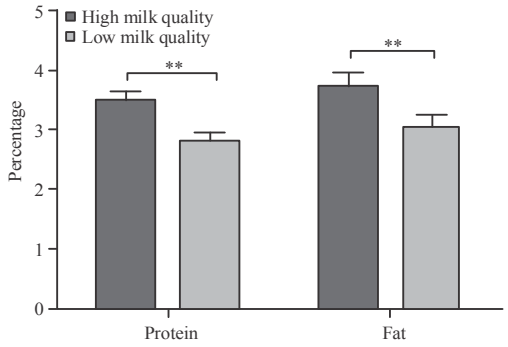

Fig. 1: The results of milk nutrition quality data classified (n = 10). **indicates differences were extremely significant (p<0.01)

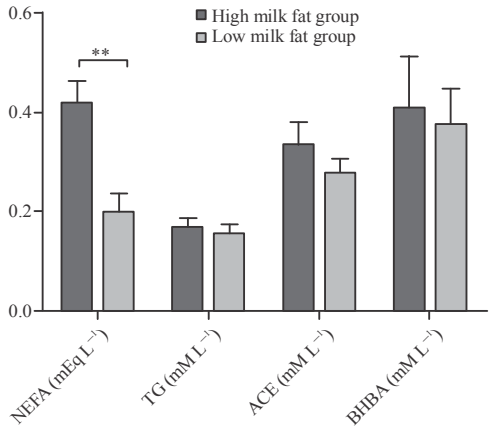

Fig. 2: The differences of precursors of milk fat concentration in serum between high milk fat group and low milk fat group (n = 10). **indicates differences were extremely significant (p<0.01)

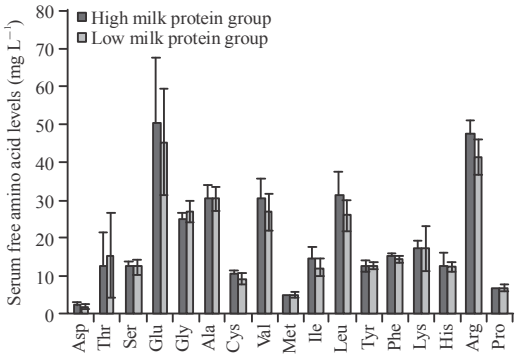

Fig. 3: The differences of free amino acid in serum between high milk protein group and low milk protein group (n = 10)

of high milk protein group was higher extremely significantly compared with low milk protein group (p<0.01). Serum aspartic acid, serine, glutamic acid, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine levels of high milk protein group were higher than low milk protein group but the differences among these parameters were not noticeable (p>0.05). Serum proline, methionine, glycine and threonine levels of high milk protein group were lower than low milk protein group but the differences among these parameters were not noticeable (p>0.05).

As the major nutrition component in milk and accounts for many of the physical properties, manufacturing characteristics and organoleptic qualities of milk and milk products, milk fat content is emphasized[7, 8]. In dairy cows, milk fatty acids arise from *de-novo* synthesis and the uptake of preformed long chain fatty acids[9]. During ruminal fermentation, the population of microorganisms ferments the carbohydrates (cellulose and hemicellulose) to produce Volatile Fatty Acids (VFA) which include ACE, propionic acid and butyric acid. Then the VFA are absorbed through the rumen wall into blood. Most of the acetate and all the propionate are transported to the liver but the majority of butyrate is converted in the rumen wall to BHBA. Most of the propionate is converted to glucose by the liver. Acetate and BHBA are used for the formation of the fatty acids that are attached to glycerol to form milk fat. Fatty acids having between 4 and 14 carbon atoms are synthesised *de-novo* in the mammary gland whereas those with 18 carbon atoms are of dietary origin and are absorbed from the blood stream. Palmitic acid (16 carbon atoms) is supplied approximately equally from the diet and *de-novo* synthesis[10]. Thus, the levels of NEFA, TG, ACE and BHBA were measured to study relationships between these parameters and milk fat content.

A clear relationship between changes in the rumen VFA pattern and the reduction in milk fat yield has been established for a range of diets. This led to suggestions that decreased rumen production of acetate will limit milk fat synthesis[11]. Some studies reported that plasma NEFA and BHBA concentrations can be used as a measure of adequate energy intake with increased concentrations associated with a negative energy balance[12, 13]. Increased concentrations of serum NEFA and BHBA had a detrimental effect on reproductive performance and milk production[13]. To the knowledge, the data provided herein are the first to study the relationships between NEFA and BHBA concentration in serum and milk fat content. In the present study, NEFA, BHBA and ACE were proved to be the main parameters which affect milk fat content. The

results showed that significant positive relationship between ACE and MF. It imply that decreased concentrations of serum ACE had a detrimental effect on fatty acid *de-novo* synthesis and milk fat content. Mammary uptake of NEFAs is directly related to their circulating concentration[14]. This study found that the positive relationship between NEFA and MF. Therefore, it indicates that increased concentrations of serum NEFA had a beneficial effect on milk fat content. The correlations between BHBA/MF and BHBA/ACE were negative relationships. It was showed that increased concentrations of serum BHBA had a detrimental effect on milk fat content. We propose that the increased concentrations of serum BHBA may decrease concentrations of serum ACE and decrease the rate of fatty acids *de-novo* synthesis. Based on this finding, we recommend that dairy farmers should use proper ration formulation to maintain optimization fiber level, maximize feed intake and maximize milk fat content.

Milk protein is an important component of milk and a source of nutrition for human consumption. Protein synthesis consumes amino acid and ATP and an adequate supply of both is essential to meet the demands of lactation[15]. Feed proteins are degraded by microorganisms in the rumen via amino acids into ammonia. The bacterial population uses ammonia in order to grow. A portion of the bacterial protein is broken down within the rumen but the majority flows to the abomasum attached to feed particles. The strong acids secreted by the abomasum stop all microbial activity and the digestive enzymes start breaking down the protein into amino acids. Approximately 60% of the amino acids absorbed through the small intestine is from bacterial protein and the remaining 40% is from ruminally undegraded dietary protein. Uptake and use of amino acid by the udder is a complex process involving changes in blood flow kinetics, plasma amino acid concentrations, amino acid transport activity by the udder and endocrine control[16]. Lysine and methionine co-limit milk protein synthesis in cows fed corn-based diets. Appuhamy proposed that methionine and lysine deficiencies themselves may represent lack of stimulation on milk protein synthesis or corn-based diets may be deficient in some other amino acid that limit milk protein synthesis, besides the substrate limiting effects of lysine and methionine[17]. Infusions containing isoleucine and other branched chain amino acids caused an increase in milk protein concentration, a decrease in milk production and no increase in total protein production[18]. Clark etc. observed that methionine and threonine were limiting for milk protein synthesis in bovine mammary cell culture. Studies in animal and cell culture models have revealed that amino acid play a role in regulating protein synthesis not only as substrates but also through direct signaling to the protein synthetic machinery[19]. Thus, dietary crude protein concentration or amino acid profile could affect milk protein percentage.

This study found that positive relationships between cysteine, phenylalanine, arginine, aspartic acid, leucine, isoleucine and milk protein content. Leucine and isoleucine could regulate mTOR signaling to stimulate milk protein synthesis[17]. Arginine could increase growth hormone and prolactin secretion of anterior pituitary cell. Phenylalanine, aspartic acid and leucine could increase growth hormone secretion of anterior pituitary cell[20]. Growth hormone administration in lactating cows increase the yield of milk proteins via GH receptors and signaling pathways such as JAK-STAT and mTOR[21]. Prolactin regulates amino acid uptake and increase the yield of milk proteins via., JAK-STAT pathway[22]. Thus, amino acid could affect milk protein synthesis via., regulation hormone secretion. The results of relationships between free amino acid concentration in serum and milk protein content suggested that optimization ration formulation for maximize milk protein content should increase the levels of cysteine, phenylalanine, arginine, aspartic acid, leucine and isoleucine, control the levels of proline, glycine and tyrosine.

CONCLUSION

In this study, the relationships between the concentration of milk fat precursors and milk protein precursors in the blood and fat and protein contents in cow's milk were analyzed. We found out thatNEFA, BHBA and ACE were the main parameters in that the relationships between precursors of milk fat concentration in serum and milk fat content. Arginine, histidine, methionine, tyrosine and serine were the main parameters in that the relationships between precursors of milk protein concentration in serum and milk protein content. Based on this finding, reasonable suggestions and countermeasures for maximize milk fat or protein content were provided. These results will provide scientific basis to further study the regulatory mechanism of fat and protein synthesis of cow's milk and improve the nutritional quality of cow's milk.

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