

Shahbazkia, Homayon Reza; Aminlari, Mahmoud; Tavasoli, Atoosa; Mohamadnia, Ahmad Reza; Cravador, Alfredo. Associations among milk production traits and glycosylated haemoglobin in dairy cattle: importance of lactose synthesis potential. *Veterinary Research Communications*, 34, 1, 1-9, 2010.

Associations among milk production traits and glycosylated haemoglobin in dairy cattle; importance of lactose synthesis potential

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Abstract Glucose is the major precursor of lactose synthesis in the mammary gland. Lactose the major carbohydrate and osmolyte of milk, controls milk volume and its concentration. Glycosylated haemoglobin (HbG) is a retrospective measure of mean blood glucose level and it is largely unaffected by recent physiological conditions and environmental events. The purposes of this study were to determine the correlations between lactose traits and other milk production traits in dairy cattle and to investigate whether HbG level can be correlated with milk and lactose production traits. Here, HbG percentage, milk and lactose production traits including milk yield, lactose, protein, SNF, total solid and fat percentages and yields were measured in 485 second calved Iranian Holstein cattle. Statistically significant negative correlations were established between HbG and milk yield ($r=-0.88$), lactose yield ($r=-0.83$), SNF yield ($r=-0.81$), protein yield ($r=-0.79$) and total solid yield ($r=-0.74$). Positive correlations were established between lactose yield and milk ($r=0.96$), protein ($r=0.81$), SNF ($r=0.92$) and total solid ($r=0.79$) yields. The negative correlation between HbG and milk and total lactose production is

probably related to the higher glucose demands in the lactating mammary gland of more productive cows. The positive correlation between lactose yield and milk, protein, SNF and total solid yield indicates that the level of lactose synthesis influences milk production traits in ways other than merely via its osmolytic action.

Keywords Glycosylated haemoglobin · Milk production traits · Lactose synthesis · Dairy cattle

Introduction

Lactose, the major carbohydrate of milk, controls milk volume by maintaining its osmolarity. Therefore, the rate of lactose synthesis in the epithelial cells of the mammary gland serves as a major factor influencing milk volume (Cant et al. 2002; Neville et al. 1983; Zhao and Keating 2007). Glucose is the main precursor of lactose synthesis in mammary gland epithelial cells (Neville et al. 1983). However; a lactating mammary gland cannot synthesize glucose from other precursors due to the absence of glucose-6-phosphatase (Threadgold and Kuhn 1979) and so is dependent on the blood supply for its glucose requirement. Mammary glucose uptake, which is performed by both active and passive transport processes (Zhao et al. 1999, 2005), is independent of the concentration of glucose and insulin in the arterial blood during lactation. It has also been suggested that the blood glucose concentration affects the rate of synthesis of milk lactose, hence milk volume by osmotic association (Kronfeld 1982). A negative correlation has been demonstrated between milk production and plasma glucose concentration (Jenny et al. 1974) and genetically superior cows which have high milk yields maintain a relatively low plasma glucose level (Snijders et al. 2001). The general consensus at present is that lactose acts primarily as an osmolyte in milk, so that the effect of increasing lactose synthesis is to draw more water into the milk. Thus, the higher the synthesis of lactose, the greater the volume of milk produced. The effect of this process is to leave the total amount of other milk constituents such as proteins and solids unchanged. Therefore, although milk yield is increased, concentration of its constituents is decreased. A negative correlation has been also reported between milk yield and milk composition in sheep (Flamant and Morand-Fehr 1982; Barillet et al. 1986; Casu and Sanna 1990). This relationship applies not only to the more productive breeds when compared with the less productive breeds (Flamant and Morand-Fehr 1982; Casu and Sanna 1990), but also within a flock, to those animals that produce more milk (Barillet et al. 1986), and even to an animal producing at different levels throughout its lactation period (Casoli et al. 1989; Pulina 1990). This relationship has been generally attributed to the fact that milk volume is determined by lactose secretion and in highly productive dairy animals the synthesis of fat and protein (and therefore total solids) does not keep up with that of lactose when high rates of milk secretion are achieved (Bencini and Pulina 1997; Holmes and Wilson 1984).

The level of glycosylated haemoglobin (HbG) has been used in humans and some domestic animals as a stable indicator of glucose status (Gabbay et al. 1977; Jovanovic and Peterson 1981; Shahbazkia and Nazifi 2008a,b). The HbG level represents a measure of mean blood glucose level over the previous several weeks and is largely unaffected by recent stress events, or the nutritional or physiological condition experienced by the animal (Latimer et al. 2003). As such, it offers a better indicator of glucose status than other tests. Although HbG has been measured in a range of both wild and domestic animals (Al-Ali et

al. 1990; Alayash and Wilson 1987; Alayash et al. 1988; Ardia 2006; Elliott et al. 1997; Jenks et al. 1991; Lose and Marca 2001; Richter 1986; Shahbazkia and Nazifi 2005; Shahbazkia and Nazifi 2008a,b), the relationship of HbG to milk production traits in dairy cattle has not been explored to date.

The aims of this study were to determine the correlations between lactose traits and the other milk production traits in dairy cattle to study the importance of lactose synthesis potential and to investigate whether HbG level can be correlated with milk and lactose production traits.

Materials and methods

Animal selection, milk production records and milk analysis

A sample of 485 second-calved Holsteins cows was randomly selected from a herd of 4500 cows on a major Iranian dairy farm. All selected cattle were raised under the same nutritional, environmental and management conditions. The animals were kept in free-stall housing, and the calving season was from February to March. Parturient cows in the transition period (3–4 weeks before parturition) were housed in a separated dry lot. All diets were based on alfalfa, corn silage, and a combination of concentrates including corn, soya meal, and bone meal. Health, fertility and production records were maintained on all animals. The mean productive values of first calved cattle of the farm were as follows: milk yield 10195.9 ± 1809.7 l; fat yield 220.1 ± 79.6 kg; protein yield 300.2 ± 52.2 kg; lactose yield 460.95 ± 83.6 kg; Total solid yield 1188.7 ± 229.5 kg; fat% 2.19 ± 0.76 ; protein% 2.96 ± 0.24 ; lactose% 4.52 ± 0.23 ; total solid% 11.72 ± 1.09 .

Official milking records taken between 2006 and 2007, covering 485 lactations were used to supply the monthly production of each cow until the end of its lactation period, and these were converted to an equivalent 305 day milk production. After parturition and the initiation of the lactation period, a milk sample was obtained from each animal every month until the end of its lactation period. The first sample was obtained at least 15 days after parturition to exclude any bias related to the presence of colostrum. As the animals were milked three times daily, errors related to the influence of milking time on milk composition were avoided by bulking a 10 ml sample from each of the three milking sessions.

Milk composition (milk fat, protein, lactose, urea, SNF (solid not fat) and TS (total solid)) was determined by a Milko Scan 4000 (Foss, USA) automatic milk analyser. The total production of each milk constituent was calculated by extrapolating its mean relative content over the 305 day milk production period.

Blood sampling and HbG determination

Peripheral whole blood samples (5 ml) were collected at the peak of the lactations from the jugular vein into a vacuum plastic tube containing citrate as an anticoagulant. The samples were immediately placed on ice water and transferred to the laboratory. Sample processing last less than 1 h until analysis was performed without interruption. HbG was measured by the same cation exchange chromatography method used in previous investigations (Shahbazkia and Nazifi 2005; Shahbazkia and Nazifi 2008a,b). To validate the method, human HbG level was determined and this delivered results within the expected range (Burtis and Ashwood 1999).

The samples were centrifuged at 2,000×g for 15 min. Erythrocytes were incubated for 30 min at 37°C in a volume of normal saline (0.9% w/v NaCl in distilled water) equal to that of the removed plasma to dissolve any labile glucose adducts (pre-glycosylated Hbs), and then the sample was re-centrifuged at 2,000×g for 10 min. Two volumes of cold water were added to lyse the erythrocytes, and the cell debris was removed by centrifugation (15 min at 2,000×g). The haemolysate was removed and processed according to Peterson et al. (1998). For the determination of HbG, a volume of prepared haemolysate was mixed with an equal volume of phosphate buffer (10 mM, pH 6.2), of which 100 µl was loaded onto a chromatography column (1×5 cm) packed with CM-Sephadex cation exchange resin. The mobile phase was a 10–95 mM linear gradient of phosphate buffer (pH6.2). The Hb concentration in the chromatography fractions and haemolysate was determined using light absorbance at 415 nm. For the in vitro glycosylation of Hb, haemolysate was incubated in 10 mmol D-glucose solution for 96 h at room temperature. The HbG level of the in vitro glycosylated sample was determined as described above. As HbG is less positively charged than Hb, it elutes earlier. To confirm that the faster running peak was indeed HbG, in vitro prepared glycosylated haemolysates were added to haemolysate samples, and the resulting mixture subjected to chromatography. Thus, weak cation exchange chromatography using a linear gradient of ionic strength can be used to determine HbG level in cattle blood haemolysate. For comparison, the HbG level of ten healthy humans was determined using the method described above.

Statistical analysis

Statistical analysis was performed by SPSS 9.05 software. The Pearson product-moment correlation coefficients were considered as indicators of the strength and direction of linear relationships between different variables.

Results

This is the first study relating HbG to milk production traits in cattle. As expected, two peaks corresponding to HbG and Hb were observed in the chromatogram of each haemolysate sample (Fig. 1). The mean HbG content as a proportion of total Hb across all the cows was $3.58 \pm 0.47\%$ compared to $5.45 \pm 0.43\%$ in human sample. In vitro glycosylation of haemolysate raised the level of HbG to $6.2 \pm 0.91\%$. In these samples the first peak of the chromatogram were spiked (Fig. 2) indicating that the first peak is related to HbG. Negative correlations were established between HbG and milk yield ($r=0.88$), lactose yield ($r=0.83$), protein yield ($r=0.79$), total solid yield ($r=0.74$) and SNF yield ($r=0.81$) (Table 1). No significant correlation was obtained between HbG and the lactose percentage ($r=0.007$) or with any of the other milk constituents.

Strong positive correlations of lactose yield were established with milk, protein, SNF and total solid yields. Correlation with fat yield was also positive but not as strong (Table 1). Although very weak negative correlations were found between milk volume and protein, lactose, total solid, SNF and fat percentages, correlations between milk volume and protein, lactose, total solid and SNF yields were strongly positive indicating that with increasing milk volume, protein and total solids production also increased (Table 1).

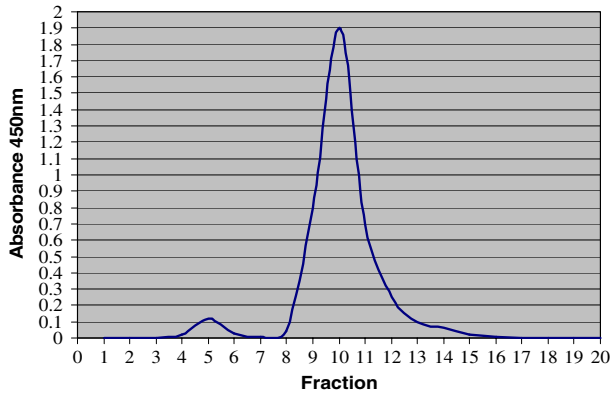


Fig. 1 Chromatogram of a dairy cow haemolysates

Discussion

The mean Holstein cattle HbG appears different in this study from that ($1.52 \pm 0.79\%$ and $1.86 \pm 0.78\%$) reported by Ganaba et al. (1993); lower than that of human (3–6%: Burtis and Ashwood 1999), camel (5.5%: Alayash and Wilson 1987) and goat (4.0%: Alayash et al. 1988); similar to that of sheep (3.2%: Alayash et al. 1988), dog (3.3%: Elliott et al. 1997) and horse (3.2%: Shahbazkia and Nazifi 2005); and higher than that of dog (1.4%: Loste and Marca 2001), ostrich (1.2%: Shahbazkia and Nazifi 2008b) and kestrel (1.25%: Ardia 2006). As HbG percentage is dependent on long term blood glucose concentration, erythrocyte life span (Burtis and Ashwood 1999), the permeability of the erythrocytes to blood glucose (Higgins et al. 1982) and the potential of Hb to bind with glucose, the interspecies variations in HbG may be related to one or a combination of all these factors. In addition, differences in the methods used to determine HbG in different studies may also be a potential source of variations in HbG values.

The negative correlation between HbG and milk production is consistent with the negative correlation established between blood glucose level and milk production (Jenny et al. 1974; Snijders et al. 2001). As HbG concentration appears to be a good indicator of long

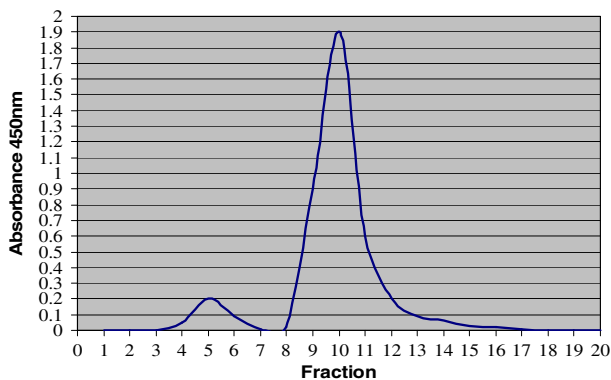


Fig. 2 Chromatogram of in vitro glycosylated haemolysate sample shown in Fig. 1, The first peak was spiked; indicating that the first peak is related to the HbG

Table 1 Correlations between different milk production traits and glycosylated haemoglobin in Iranian Holsteins. Pearson product-moment correlation coefficients have been shown

Traits	Milk Volume	Fat yield	Protein yield	Lactose yield	Total solid yield	SNF yield	Fat %	Protein %	Lactose %	Total solid %	SNF %	HBG%
Milk Volume	-	+0.067	+0.794	+0.958	+0.711	+0.851	-0.053	-0.0861	-0.001	-0.0289	-0.0330	-0.882
Fat yield	+0.067	-	+0.017	+0.102	+0.022	+0.065	+0.745	-0.101	+0.062	-0.032	0.000	-0.098
Protein yield	+0.794	+0.017	-	+0.806	+0.768	+0.857	-0.102	+0.019	0.000	0.000	0.000	-0.792
Lactose yield	+0.958	+0.102	+0.806	-	+0.788	+0.922	-0.022	-0.085	+0.063	-0.005	-0.001	-0.834
Total solid yield	+0.711	+0.022	+0.768	+0.788	-	+0.837	-0.076	-0.005	+0.018	+0.102	+0.006	-0.737
SNF yield	+0.851	+0.065	+0.857	+0.922	+0.837	-	-0.044	-0.020	+0.019	0.000	+0.014	-0.812
Fat %	-0.053	+0.745	-0.102	-0.022	-0.076	-0.044	-	-0.034	+0.075	-0.007	+0.008	+0.004
Protein %	-0.0861	-0.101	+0.019	-0.085	-0.005	-0.020	-0.034	-	-0.002	+0.173	+0.211	-0.007
Lactose %	-0.001	+0.062	0.000	+0.063	+0.018	+0.019	+0.075	-0.002	-	+0.110	+0.269	-0.002
Total solid %	-0.0289	-0.032	0.000	-0.005	+0.102	0.000	-0.007	+0.173	+0.110	-	+0.304	+0.006
SNF %	-0.0330	0.000	0.000	-0.001	+0.006	+0.014	+0.008	+0.211	+0.269	+0.304	-	+0.011
HBG%	-0.882	-0.098	-0.792	-0.834	-0.737	-0.812	+0.004	-0.007	-0.002	+0.006	+0.011	-

term mean blood glucose level, the negative correlation between HbG percentage and milk production is no doubt related to lower blood glucose concentration in more productive cows. We suggest that cows having a higher potential for lactose synthesis maintain a lower HbG content. This hypothesis was tested by searching for a correlation between HbG and lactose percentages, but no such correlation existed. However, the percentage of milk lactose does not necessarily correlate with the potential for lactose synthesis in the mammary gland. For example, one cow with a 305 day milk yield of 12 litres, with a mean lactose percentage of 4% will produce approximately twice as much lactose as a second cow with a 305 day milk yield of 6 litres with the same lactose percentage. Milk lactose percentage was also uncorrelated with total lactose production ($r=0.063$). Thus the total 305 day lactose production was taken as an indicator of potential lactose synthesis and this was strongly correlated with HbG. Lactose synthesis and milk yield show a linear positive correlation with glucose uptake in the mammary gland of goats and cows (Kronfeld 1982; Nielsen and Jakobsen 1993; Hurtaud et al. 2000; Kim et al. 2001; Nielsen et al. 2001; Cant et al. 2002; Huhtanen et al. 2002). Therefore higher lactose synthesis potential is accompanied by greater glucose uptake by the lactating mammary gland; however, this demand may be insufficiently satisfied by the rate of liver gluconeogenic activity leading to a fall in blood glucose concentration followed by a fall in HbG.

Strong correlations between lactose yield and protein yield and milk volume (0.92 and 0.98 respectively) have been also reported in cattle by Miglior et al. (2007). Our results are in agreement with this report and add more data on milk concentration and total solid content and yield.

Because lactose maintains the osmolarity of the milk, its rate of synthesis is the major factor influencing milk volume (Neville et al. 1983; Cant et al. 2002). This mechanism can explain the strong positive correlation between lactose yield and milk production. Very weak correlation between lactose percentage and milk production could be related to the osmotic regulator role of lactose. Higher or lower lactose production leaves the lactose percent of the milk almost unchanged but it can strongly affect milk volume.

In genetically modified mice, it has been reported that deficiency of lactose synthase complex in the mammary gland induced by diminution of α -lactalbumin or β 1-4-galactosyltransferase (two parts of the lactose synthase complex) can lead to decreases in milk volume caused by decreased biosynthesis of lactose (Vilotte 2002). Although the strong correlation between lactose yield and milk volume in the studied cattle can be explained by the mechanism mentioned above, the effect of this process would be to leave the total amount of other constituents such as protein and solids unchanged and therefore, milk concentration is decreased as it has been reported in mice (Vilotte 2002) and in dairy animals (Bencini and Pulina 1997; Holmes and Wilson 1984).

However, we have shown in the present study that there are no strong negative correlations between lactose yield and milk concentration (total solid percentage), between lactose yield and the percentages of other constituents of milk and between milk volume and total solid content of milk (Table 1). This suggests that higher lactose production is accompanied by higher milk volume production, higher protein and other solids (except fat) secretion into the milk to leave protein and total solid content and milk concentration almost unchanged.

Considering correlations between percentages and yields of the different constituents of milk, the strongest correlation was found between fat percent and its yield indicating that fat percentage of milk and not milk volume is the most important factor influencing fat yield. In the case of protein and total solid yields, the correlations between percentages and yields were very weak but correlations between yields and milk volumes were very strong,

indicating that milk volume, which is determined by the level of lactose synthesis, is the most important factor affecting protein and total solid yields.

Our results suggest that lactose or mechanisms involved in its synthesis and secretion may also affect the synthesis and/or secretion of the other milk constituents.

Conclusion

The observed negative correlations between HbG and milk, lactose, SNF, protein and total solid yields, suggest that higher milk production accompanies lower mean blood glucose. Positive correlations were established between lactose yield and milk, protein, SNF and total solid yields. We concluded that the mechanism of lactose secretion and its controlling effects on milk volume, the lactose synthase enzyme and related genes can be considered as important factors that must be taken in account in milk production and animal breeding strategies. Considering these findings, we are presently studying the β 1-4-galactosyltransferase I gene, which encodes the catalytic part of lactose synthase and its possible associations with different milk production traits.

Acknowledgements We acknowledge the Ghiam Dairy Co. and its management team for facilitating this study and for providing technical support for sampling, milk and herd history analysis. This research was jointly supported by grant number 87-GR-VT-11, Shiraz University Research Council and by FCT (Fundação para a Ciência e Tecnologia) and Shahrekord University.

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