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Physiological Constraints to Milk Production in High Yielding Dairy Cows

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A B S T R A C T

Milk yields and their relationships with blood and milk metabolic parameters were assessed in 59 high-yielding Holstein cows over the course of their lactation to identify metabolic constraints to daily milk production. Over an 11 month lactation period the cows, which were milked thrice daily and fed a total mix ration, had a mean daily milk yield of 35.5 l. Blood parameters monitored were haematocrit (PCV), erythrocytes (RBC), leucocytes (WCC), hemoglobin, neutrophils, lymphocytes, monocytes, eosinophils, urea, protein, creatinine, triglycerides, cholesterol, magnesium (Mg), phosphorus (P), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), aspartates aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and glutamic gammatransferase (GGT). Stepwise regression indicated that blood haemoglobin concentration was most closely and positively correlated with milk yield, indicating that oxygen-carrying capacity was potentially a limit to milk production. Secondly, milk Na was negatively correlated with milk yield, and milk protein yield was negatively correlated with milk Mg, Ca and Na, demonstrating lack of homeostatic control of these elements in milk. Principal component analysis identified a primary metabonomic axis of hemoglobin and RBC concentrations at one end and blood K, Na and milk lactose at the other, which appeared related to milk production. A second axis was apparent of milk divalent cations at one end and monovalent cations at the other. It is concluded that constraints to milk production in high yielding cows may exist due to limited oxygen-carrying capacity of the blood, as well as monovalent cations.

Introduction

The bovine mammary gland yields considerably more milk and milk constituents than are required by a calf. As a result of the deficit of nutrient intake dairy cows are under metabolic stress for the early part of their lactation, and there has been considerable research in the evaluation of metabolic markers for such nutritional stress (Ollier *et al.,* 2016). However, although the use of metabolic profiles is well established in humans, and methods have been widely researched and developed in dairy cows they have not entered widespread use in the dairy industry **(**Djoković *et al.,* 2017**).** Their value is predicated on a relationship between metabolic constituents of blood or milk and the health and productivity of the dairy cows **(**Strzałkowska and Jóźwik 2021**).** In addition, the research on metabolic profiles has been almost universally conducted with low yielding dairy cows (Kumar and Pachhauri, 2000), whereas it is the high yielding cows that are increasingly used in intensive systems of production which are more likely to be challenged physiologically **(**Hasan *et al.,* 2021**).** A new science of metabonomics (the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification, Nicholson, 2006, Sun *et al.,* 2015) is emerging that may offer diagnostic capabilities to veterinarians using the metabolic profiles of high yielding cows (Donat *et al.,* 2016).

The bovine mammary gland is capable of extracting nutrients from large volumes of blood, with more than 500 l processed to produce l l of milk **(**Delamaire and Guinard-Flament 2006). However, intake restrictions in early lactation and excessive nutrient demands render adipose tissue loss inevitable **(**Contreras *et al.,* 2016**).** This places the cow at high risk of metabolic disorders, and it is therefore beneficial to understand the metabolism of high yielding Holstein dairy cows in relation to the correlations between milk yield, blood (Nozad *et al.,* 2013) and milk components (Nozad *et al.,* 2012). The risk of metabolic challenges is particularly high in the newly developing intensive dairies, such as in Azerbaijan in Iran, where the genetics of the dairy cows have been radically changed from native breeds (Hashemi *et al.,* 2009) to Holstein Friesian cows (Ramin *et al.,* 2005) over the last 40 years, mainly by importing pure breeds from Canada and Germany. In such situations, adequate feed quality and quantity are not always as well assured as in systems that have been operating in Western countries for several decades. The metabolic profile test can assist such developing dairy industries by better quantifying dairy metabolic parameters (Bashtani *et al.,* 2009; Hossein-Zadeh *et al.,* 2011) to prevent disease and improve milk yield, using key indices in blood, milk and feed (Sakhaee *et al.,* 2011, Mokhber *et al.,* 2011). The aims of this study were to investigate the relationships between milk yield and a variety of key milk and blood metabolic indicators to identify metabolic constraints to daily milk production in a newly developed intensive milk production system in Azerbaijan.

Materials and Methods

Animals

A commercial dairy herd of 1500 Holstein cows in Azerbaijan (mean daily temperature range 13-34^{o}C, altitude 1361 m, mean annual humidity 58% and total rainfall 288 mm) was used as the basis for the study of metabonomic parameters related to milk production. One hundred and forty-seven cows were considered to be high yielding (Dobson *et al.,* 2007) and in good health and condition, which was determined by the absence of clinical

disease symptoms and from blood analyses. From these 78 cows were randomly selected for the study, aged 5 to 6 years old and in their third or fourth parity, which entered the study over a three-month period. Fifty-nine of the selected cows remained throughout the study (due to selling, missing data, mastitis and emergency culling) within the main herd, which was split into three groups of high (up to 4 months), medium (5^{th} to 8^{th} month) and low yielding cows (9^{th} to 11^{th} month of lactation). Annual welfare management included vaccination, deworming, hoof trimming, CMT tests and sanitary control.

Feed and feed Analysis

Cows were fed three times per day, after milking, two different total mixed rations in October to February (TMR1) and March to September (TMR2) *ad libitum* (Table 1).

These were prepared in and fed from 1 ton feeders in feed bunks (EzeFeed, Australia) with free access for all cows in the group. Mean DM intakes were 26.2 and 24.7 kg/d, respectively. Feed subsamples of mean wet matter mass at least 50 g were taken monthly from February to December 2011 for chemical composition analysis. Crude protein content was measured by the Kjeldahl method and GE content by calculation of the carbohydrate (CHO), dry matter (DM), CP, ash, ether extract (EE) and crude fiber (CF) according to the following formula:

CHO%=100 - (Moisture % + CP % + Ash % + EE % + CF %)

GE (Cal/100 gm diet) = CP % x 5.65 + EE % x 9.40 + CHO % x 4.15

Mean DM content of the diet was 582 g/kg and mean concentrations in DM of CF, ash, CP, and EE were 20.4%, 5.5%, 34%, and 2.39%, respectively, over the 11-month period (Nozad *et al.*, 2013). Ca and P concentrations were measured at 1 and 0.5% of feed DM, respectively.

Blood sampling and analysis

Blood samples were taken monthly from February to December 2011. Five ml of blood were extracted from each cow after morning milking starts from 8 am by jugular venipuncture monthly into a test tube without EDTA. Blood samples were separated by centrifuge at 3000 *g* for 5 min and stored at -20ºC before analysis.

Two ml was mixed with EDTA and used for 9 hematological tests and the remaining 3 ml was used after the separation of serum for 16 biochemical, mineral, and enzymatic tests.

PCV was determined by centrifuging blood in a microhaematocrit tube at 1500 g. RBC and WCC were determined after dilution of the blood using a haemocytometer with Neubaurer grid, and hemoglobin (mg/dl) was measured by the cyanomethaemoglobin method. Leucocytes were differentiated into neutrophils, lymphocytes, monocytes and eosinophils by counting 200 leucocytes in each microscopic evaluation and converting % into an absolute count.

Blood sera were also used to determine concentrations of urea, protein, creatinine, triglycerides, cholesterol, Mg, P, Cl, AST, ALT, ALP, and GGT using an auto analyzer (RA-1000, USA) and the appropriate commercial kits (Pars Azmoun, Iran).

Serum Na and K were measured by flame photometer using appropriate standards (Jenway, Clinical PFP7, UK). The concentration of β-hydroxybutyrate was determined by spectrophotometry (Ultra-violet method), using a Runbut kit (Randox Laboratories, Crumlin, UK).

Milk sampling and analysis

Milk yield was measured daily and mean values presented that were weighted for the number of cows still lactating. Milk samples were taken monthly over the 11-month period, from February to December 2011. Cows were milked three times daily in a Westphalia parlor (GEA-Farm Technologies, Germany), and 10 ml daily milk samples from each teat were taken from a morning milking of each cow monthly and refrigerated to 4^{o}C until analysis. Milk serum was obtained by centrifugation at 3000 *g* for 5 minutes. Milk casein was separated by 0.1 N HCl in pH 3.6 (Nozad *et al.*, 2013). Milk serum was used to determine the protein and macro-mineral concentrations. Ca, P and Mg concentrations were measured using Ca, P and Mg kits (Pars Azmoon Co., Tehran, Iran) in an auto-analyzer (RA-1000, Pharmacia Co., LKB, Novaspec, USA). Milk Na and K concentrations were assessed by flame photometer (Jenway PFP-7, Essex, UK) using a standard Na^{+} and K^{+} test (Ziest Chimi Diagnostics, Tehran, Iran). Milk lactose was assessed by Polarimeter (Bellingham and Stanley, UK). Milk urea was measured first by precipitation of total protein, separation of the milk serum and then was run as blood urea. Milk protein was determined in milk serum by using spectrophotometry commercial kits.

Statistical analysis

$SPSS_{13}$ and Minitab statistical programs were used for all analyses. ANOVA was used to determine the significance of monthly variation in mean concentrations of all parameters, with month as a factor in the model and cow as a random factor. Residuals were tested for normal distribution with results confirmed by Mood's median test where necessary. A forwards-backwards stepwise regression was conducted with the cows with entire datasets of milk yield and milk protein yield on 16 predictors with alpha values to enter and remove variables set at 0.10.

Results and Discussion

Mean daily milk yield was 35.5 l/d, and after an initial increase in the second month to a mean of 44.8 l/d, it declined to 27.4 l/d at a rate of 0.068 l/d (0.16%/d) (Table 2, Fig1). The range in milk yields is presented in Table 2. The mean±SE monthly concentrations in blood of PCV, hemoglobin, RBC and WBC were 31%±0.05, 10.6±0.02 mg/dl, 5.3±0.005 x 10^{6} cells/mm^{3} and 8919±33.2 cells/mm^{3}, respectively. Mean urea, protein, glucose, creatinine, cholesterol, triglycerides and β-hydroxybutyrate concentrations were 33.1±0.18, 9.7±0.06, 44.7±0.16, 0.83±0.004, 148.1±1.54, 116.8±2.7 mg/dl and 0.61±0.004 mmol/l. Mean Ca, P, Mg, Na, K and Cl concentrations were 9.42±0.03, 5.36±0.09, 2.97±0.02 mg/dl, 137.2±0.52, 4.9±0.02 and 103.2±0.13 mmol/l and AST, ALT, ALP and GGT concentrations were 109.1±0.67, 116.7±0.10, 31.4±1.45, 19.2±0.08 mg/dl, respectively.

All parameters differed significantly between months of lactation ($P<0.05$). Blood triglycerides increased rapidly after the first month, remained high for 5 months and then low for the remainder of the lactation period (Fig 2). Blood protein was low for the first 5 months, and then increased for the remainder of the lactation period (Fig 3). Blood urea N increased erratically for the first 7 months and then decreased (Fig 4). Blood P increased between months one and two, and then declined until month 6, after which it was stable (Fig 5). Blood Mg and Ca both increased erratically throughout the lactation (Fig 6 and 7). Blood alkaline phosphatase increased between months one and two, and then declined (Fig 8). Blood hemoglobin declined erratically over the course of the lactation (Fig 9).

A stepwise regression of milk yield for the cows with complete datasets for the 17 blood and milk parameters produced two significant correlations: first, a positive correlation with hemoglobin ($P < 0.001$) and second, a negative correlation with milk Na ($P = 0.007$) (Table 3). There was a trend for a positive correlation with milk P concentration ($P = 0.06$).

The regression equation for milk yield and hemoglobin was:

Milk yield (kg/d) = 3.4 + 0.083 hemoglobin, s = 6.79, ($P< 0.001$)

The stepwise regression of milk protein yield with 17 blood and milk parameters produced six steps introducing significant variables: first the inclusion of milk Mg, then milk Ca, then blood Na, all with negative coefficients, then milk P and WBC, both with positive coefficients (Table 4).

Principle component analysis (Table 5) produced two initial components for which the loading plot described the main clusters of responses (Fig 1). A cluster of variables in the top right-hand corner, with high responses in both components, included milk K, Na, P, protein and urea, together with neutrophil concentrations in blood. At the opposite corner is milk Ca, Mg, and blood lymphocytes. Milk yield and blood Cl were centrally located, therefore unrelated to these two components. In the other diagonal, RBC, hemoglobin and PCV were opposite blood K, Na and milk lactose.

In this study, the mean daily milk yield was 35.5 l/d, and in peak lactation 44.8 l/d, with an individual maximum monthly yield of 67 l/d, indicating a milk yield comparable with top dairy cow production in the world (Sun *et al.*, 2015, Strzałkowska and Jóźwik 2021). By the definition of Dobson *et al.*, (2007) for Holstein cows, these cows were high yielding. The key variable to relate to milk yield was hemoglobin concentration in blood, which showed an erratic decline over the lactation and positive correlation with milk yield. Other authors have indicated a reduction in hemoglobin with increasing milk yield (Kumar and Pachauri, 2000), but this was probably confounded by season, milk yield level and other factors. They used crossbred cows, including heifers, many of which were low yielding and their study was conducted at an altitude of 1700 m in the central Himalayas (Kumar and Pachauri 2000). As milk yields have increased over the last 50 years, hemoglobin concentrations in dairy cows have declined (George *et al.*, 2010), again suggesting an antagonistic relationship. Another study observed a reduction in hemoglobin concentration over the early part of lactation in dairy cows. The antagonistic relationship between hemoglobin status and milk yield in our study may differ from previous ones because our high yielding cows had considerably greater metabolic needs. Sustaining such high milk yields would have had a high metabolic requirement for feed digestion, nutrient processing and milk production (Djoković *et al.*, 2017, Hong 2019). All this requires large amounts of energy, and it is therefore evident that oxygen requirement is one of the main drivers of hemoglobin status (Abd Ellah 2016). In support of this, alpine cows increase hemoglobin concentration to cope with high altitude (Berry *et al.*, 2001), and ewes increase hemoglobin concentration in response to walking stress (Seijan *et al.*, 2012). Oxygen requirements for metabolism in the dairy cow are considerable and are increased by high milk yields (Sordillo 2005). Because of this, it is not surprising that hypoxia is one factor reducing productivity at high altitudes, at least before adaptation (Leiber *et al.*, 2004). In the early lactation cow, nutrient demands exceed the provision from intake, and mobilization of

body tissues is essential to sustain high production. The visceral hypertrophy in response to these demands, in particular the splanchnic tissues and increased hepatic artery flow, facilitate increased oxygen supply to the liver (Reynolds *et al.,* 2003). By 12 weeks' postpartum oxygen consumption by the splanchnic tissues has increased by more than blood flow, which may be due to increased hemoglobin concentrations in high yielding cows.

Many other factors affect hemoglobin status and these must be verified; for example, the hemoglobin status of dairy cow's cattle can also be reduced if Fe or Cu status is low (Yang *et al.,* 2007). Hemoglobin status is also increased by heat stress (Timisoar *et al.,* 2018), and hot temperatures can increase hemoglobin in blood in Holstein-Friesian cows, probably due to enhanced respiration rates (Sammad *et al.,* 2020). The temperature experienced in this experiment ranged from 13 to 34ºC, but hot temperatures did not coincide with high hemoglobin status of the cows, which was highest in the early part of lactation in March.

It is possible that any positive correlation between milk yield and hemoglobin is not causal. Khaled *et al.,* (1999) observed that hemoglobin was negatively correlated with milk fat content in dairy goats, which could produce a positive correlation with milk yield. Near infrared spectroscopy offers the potential for non-invasive measurements of hemoglobin that can distinguish between oxyhemoglobin and deoxyhemaglobin (Oya *et al.,* 2003), and this could be used to examine oxygen carrying capacity of hemoglobin in future studies.

There was evidence of antagonistic relationships between milk lactose, one of the primary milk osmolytes, together with associated monovalent cations in blood, and the RBC, hemoglobin, PCV complex, although the latter is confounded with other constituents. Milk lactose concentration declines in late lactation, especially if cows are underfed, and demonstrates an antagonistic relationship with milk yield (Phillips, 2010). Na and K often change antagonistically in the mammary gland, the former increasing in response to damage to the epithelial tissue during mastitis, which allows the Na to diffuse into the mammary gland, however, our loading plot indicated a close relationship between variation in Na and K in blood and milk lactose. These are related to the osmotic pressure of blood and have been observed previously (Bijl *et al.,* 2013).

The second axis to emerge from the PCA was one apparently related in part to the valence of milk cations. Also active at this end of the axis was the neutrophil concentration, which is the central reaction to invasion of the gland by bacteria. This indicates that responses to subclinical or clinical mastitis were driving this end of the axis. Lymphocytes are usually depleted in mastitic milk, at the same time as neutrophils increase in number (Hussain *et al.,* 2012), and their proliferation is dependent on Ca, which is often deficient at this time (Kimura *et al.,* 2006). Divalent cations, however, are neither osmotically active nor homeostatically controlled in milk, yet they are potentially deficient in their support for the lymphocyte response to bacterial infection of the mammary gland. Ca and Mg absorption are inextricably linked in ruminants (Chiy and Phillips, 1993), and it is therefore plausible that Mg would play an equivalent role to Ca in supporting lymphocyte proliferation.

Milk protein yield indicated a negative regression with milk Mg, Ca and Na concentration, and milk yield similarly had a negative correlation with milk Na concentration (Salim and Salman 2020).

Table.1 Feed ingredients, diet composition and DM intake (% unless otherwise specified) for October to February (TMR1) and March to September (TMR2) diets in dairy cows

Ingredients	TMR 1 October to February	TMR2 March to September
Alfalfa hay (chopped)	20.8	20.5
Corn Silage	16.31	14.7
Dry Apple Pulp	3.45	5.1
Wet cane molasses	2.13	1.58
Meat Meal	1.83	1.72
Whole barley grains	9.77	15.86
Cottonseed meal	2.65	2.68
Cottonseed lint	6.33	6.43
Corn Stover	16.51	11.07
Wheat straw (chopped)	1.81	1.88
Calcium carbonate ($CaCO_3$)	0.70	0.70
Salt (NaCl)	0.31	0.28
Sodium bicarbonate ($NaHCO_3$)	0.94	0.93
Fishmeal	1.07	1.07
Tallow	1.56	1.54
Soya meal	7.31	7.41
Vitamins	1.95	1.91
Canola meal	3.53	3.66
Wheat bran	1.04	1.08
[1]Composition		
Net energy (MJ/kg)	6.78	6.78
Crude protein	17.1	17.4
NDF	29.1	29.9
ADF	19.0	19.2
Ca[†]	1.0	1.0
P[†]	0.5	0.5
DM intake, kg/d	26.6	24.7

[†]Mean measured values, [1]=Assessed based on standard units with current laboratory methods (Nozad et al., 2013).

Table.2 Weighted least square means, SEM and range of milk yields in Holstein dairy cows over the 11 months of lactation in high, moderate and low milk yielding group

Months of lactation	Mean ± SE (l/d)	Range (l/d)
High milk yielding group		
1	50.6 ± 0.59	46.0-65.0
2	43.2 ± 0.17	41.0-45.6
3	42.8 ± 0.32	41.6-45.6
4	40.8 ± 0.50	40.6-41.0
Moderate milk yielding group		
5	39.2 ± 0.11	38.0-40.4
6	36.7 ± 0.11	35.0-38.0
7	33.6 ± 0.13	32.0-35.0
8	30.1 ± 0.14	28.0-32.0
Low milk yielding group		
9	25.5 ± 0.20	23.2-28.0
10	19.4 ± 0.32	16.6-23.0
11	11.4 ± 0.58	3.5-16.5
Mean	35.5 ± 0.06	3.5-65.0

Table.3 Regression coefficients, T and P values for a stepwise regression of mean milk yields of 59 cows on 17 blood and milk parameters

Step	1	2	3
haemoglobin	3.4	6.1	5.3
T-Value	40.1	4.4	3.8
P-Value	0.000	0.000	0.000
Milk Na		-1.36	-2.69
T-value		-1.98	-2.78
P-value		0.05	0.007
Milk P			2.4
T-Value			1.91
P-Value			0.06

Table.4 Regression coefficients, T and P values for a stepwise regression of mean milk protein yields of 59 cows on 17 serum and milk parameters

Step	1	2	3	4	5	6
Constant	58.6	123.2	194.8	166.6	164.1	155.5
Milk Mg	-14.4	-12.0	-9.0	-5.7		
T-value	-3.11	-2.65	-1.99	-1.22		
P-value	0.003	0.01	0.05	0.23		
Milk Ca		-3.9	-4.6	-4.1	-4.4	-5.0
T-value		-2.51	-3.01	-2.73	-2.98	-3.34
P-value		0.01	0.004	0.009	0.004	0.002
Blood Na			-0.49	-0.57	-0.65	-0.66
T-value			-2.43	-2.86	-3.40	-3.54
P-value			0.02	0.006	0.001	0.001
Milk P				1.37	1.63	1.51
T-value				2.15	2.69	2.54
P-value				0.04	0.009	0.01
WBC						0.0025
T-value						1.81
P-value						0.076

Table.5 Principle component analysis of 18 serum and milk parameters in Holstein dairy cows (n=59)

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Blood						
Na	-0.093	0.115	-0.497	0.125	-0.267	0.349
K	-0.198	0.189	-0.412	0.028	-0.325	-0.046
Cl	-0.083	-0.138	-0.348	0.148	0.286	-0.287
PCV	0.306	-0.437	-0.100	0.006	-0.067	0.074
Haemoglobin	0.278	-0.441	-0.091	-0.004	-0.114	0.051
RBC	0.265	-0.428	-0.085	-0.101	-0.044	0.050
WBC	0.057	-0.104	-0.248	0.204	0.020	-0.423
Neutrophil	0.284	0.208	0.167	0.401	-0.283	-0.106
Lymphocyte	-0.232	-0.177	-0.165	-0.532	0.211	0.150
Milk						
Urea	0.173	0.254	-0.182	-0.262	0.257	-0.090
Lactose	-0.098	0.155	0.110	-0.420	-0.367	-0.127
Protein	0.347	0.160	0.279	-0.017	0.162	-0.003
Ca	-0.214	-0.017	-0.080	0.225	0.327	-0.357
P	0.332	0.260	-0.258	-0.116	0.008	-0.003
Mg	-0.226	-0.081	-0.048	0.375	0.115	0.454
Na	0.333	0.184	-0.205	0.043	0.047	0.267
K	0.296	0.184	-0.272	-0.094	0.185	-0.099
Yield	-0.027	-0.147	-0.075	-0.071	-0.461	-0.363
Eigenvalue	4.0	2.8	2.0	1.7	1.5	1.3
Proportion	0.22	0.16	0.11	0.09	0.08	0.07
S	4.77	4.56	4.37	4.23	4.25	4.17
R-Sq	14.5	23.1	30.6	36.1	34.3	38.1

Fig.1 Mean milk yield of dairy cows (n=59) over 11 months of lactation

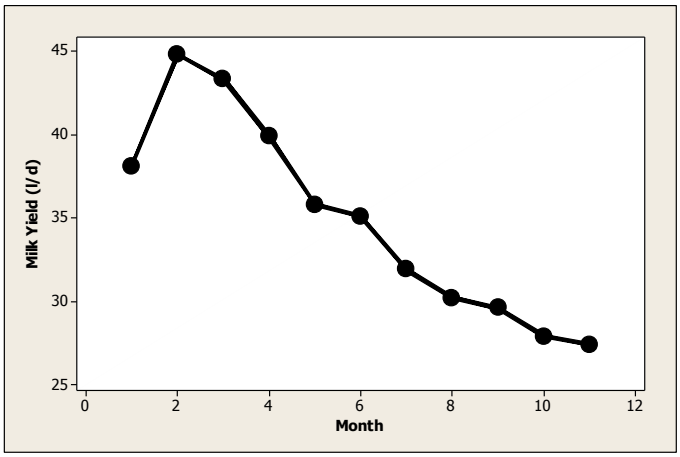


Fig.2 Mean serum triglyceride concentrations of dairy cows (n=59) over 11 months of lactation

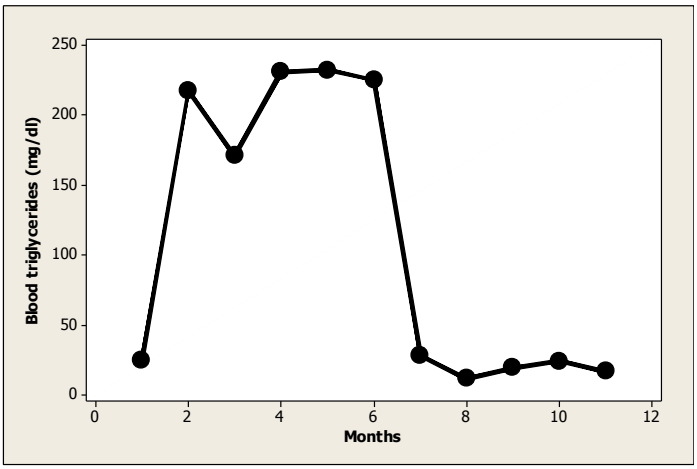


Fig.3 Mean serum protein concentrations of dairy cows (n=59) over 11 months of lactation

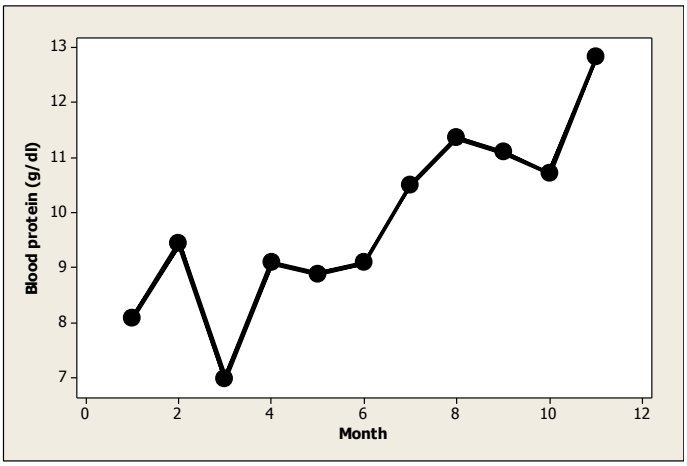


Fig.4 Mean serum urea nitrogen concentrations of dairy cows (n=59) over 11 months of lactation

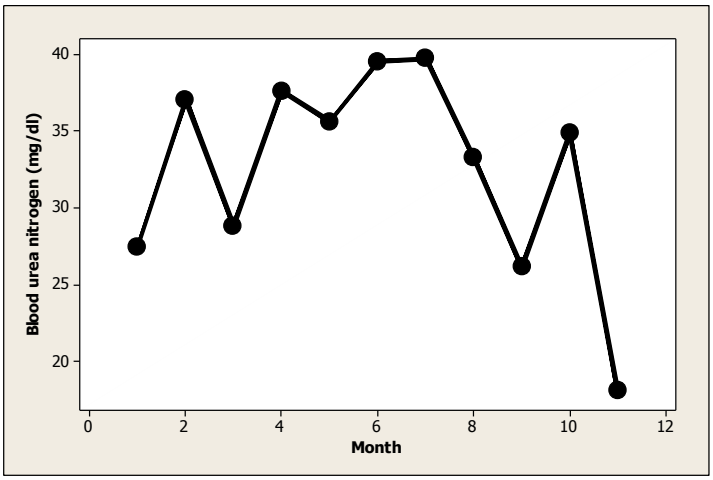


Fig.5 Mean serum P concentrations of dairy cows (n=59) over 11 months of lactation

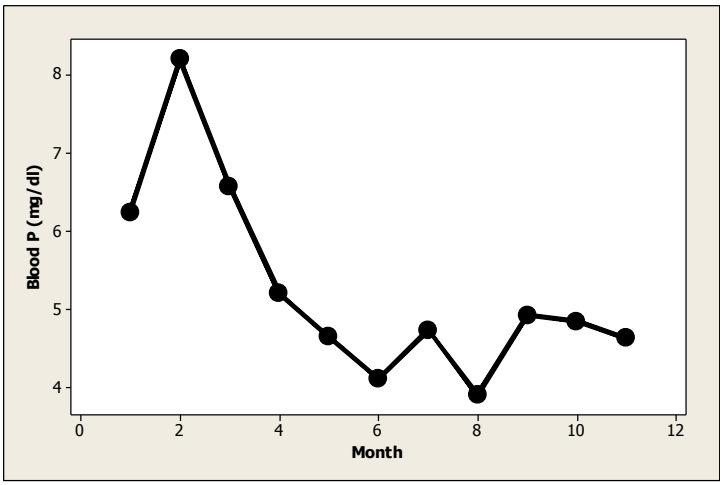


Fig.6 Mean serum Mg concentrations of dairy cows (n=59) over 11 months of lactation

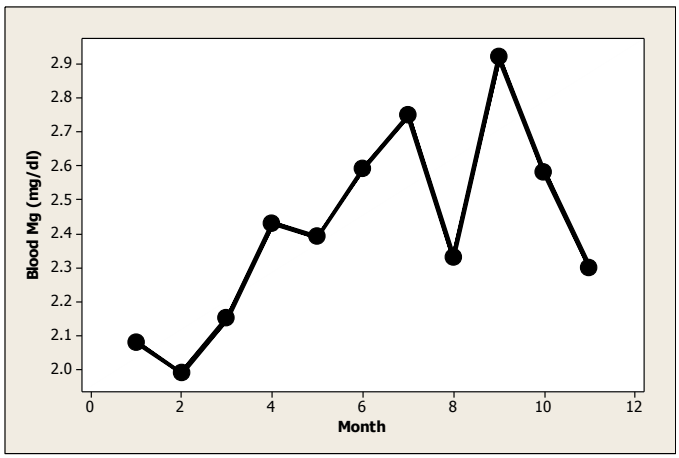


Fig.7 Mean milk Ca concentrations of dairy cows (n=59) over 11 months of lactation

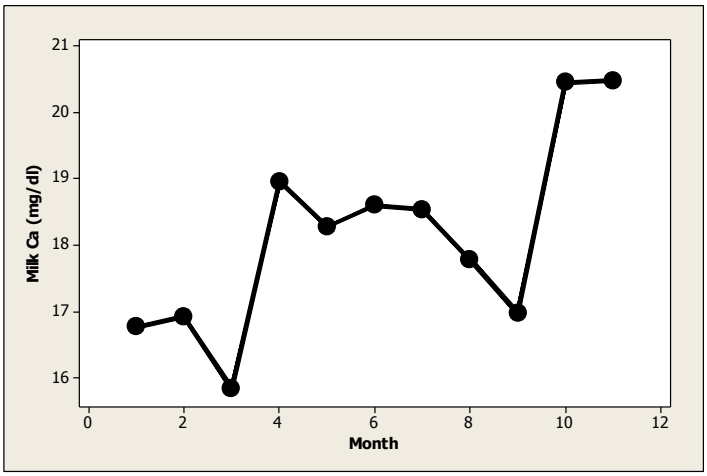


Fig.8 Mean serum alkaline phosphatase of dairy cows (n=59) over 11 months of lactation

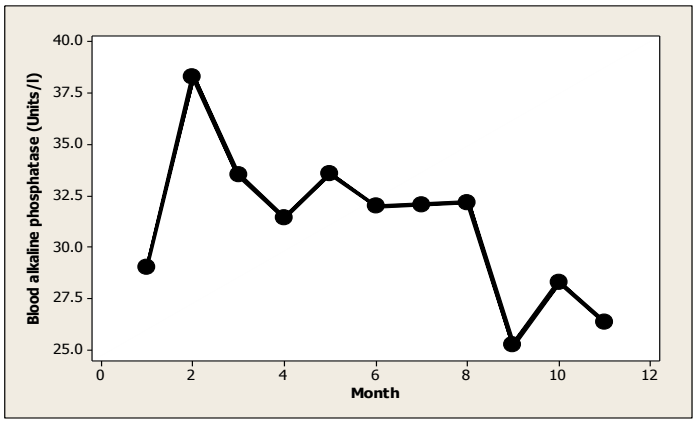


Fig.9 Mean serum haemoglobin concentrations of dairy cows (n=59) over 11 months of lactation

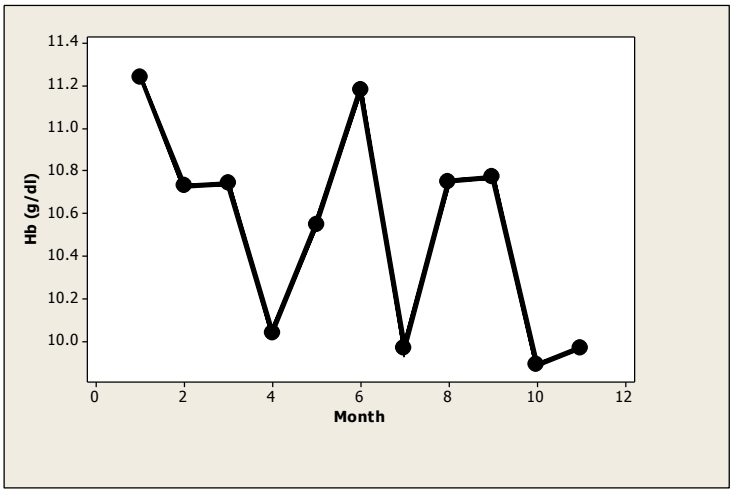
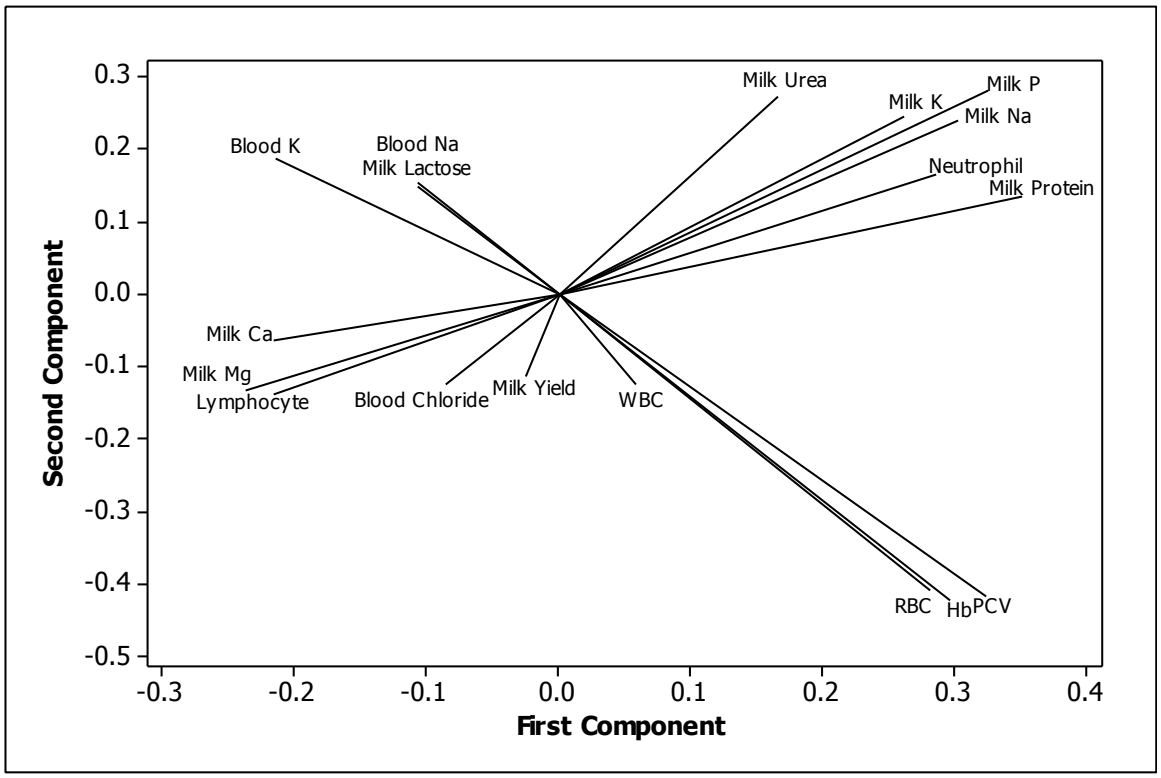


Fig.10 Loading plot of principal component analysis of the relationships between 18 serum and milk parameters



This is probably due to the reduction in the concentration of elements that are not homeostatically regulated as milk yield increases, rather than the protein concentration, because 70% of Ca and 30% of Mg are dispersed in colloidal calcium phosphate nanoclusters bound to caseins, with the remainder as free ions in milk serum (Bijl *et al.*, 2013). Therefore, Ca and protein concentrations in milk are positively correlated, and Mg is also correlated with Ca (Bijl *et al.*, 2013, Mordak *et al.*, 2021). Metabolic profiles can help to identify constraints to milk production in high yielding industrial dairies. Our study found that a key metabolite in the conditions under which our high yielding cows were kept is hemoglobin, suggesting that hypoxia may limit nutrient utilization. This is the first time that hemoglobin has been detected as a possible constraint to milk production. Two major axes of metabolites were identified, one related to milk yield, with hemoglobin and RBC at one extreme and blood monovalent cations and milk lactose, all potentially involved in regulating milk yield. The second axis focused on responses to invasive bacteria, with neutrophils and the milk monovalent cations principally involved in mastitis responses at one end and the milk divalent cations and lymphocytes at the other.

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