—FULL PAPER—

Relationship between lactational performance and metabolic parameters of Mongolian native grazing mares

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Information regarding the lactational performance of mares in relation to metabolic parameters can help practitioners to manipulate animal rearing management for sustainable mare milk production. The aim of this study was to characterize the lactational performance of Mongolian native mares grazing on natural pastureland by revealing the seasonal effects on metabolic parameters. In this study, 8 multiparous mares were used. Milk yield and composition and serum metabolic parameters, such as alanine aminotransferase, aspartate aminotransferase (AST), glucose (GLU), triacylglycerol, total cholesterol (TCH), non-esterified fatty acid (NEFA), albumin, urea, total protein, cortisol (Cort), and insulin, were determined at 30, 60, 90, 120, 150, 180, 210, 240, and 270 days of lactation. During the lactation period, milk yield peaked at around the 90th day and declined sharply in the following period. While the milk fat and protein contents decreased gradually from the early stages of lactation to the late stages, the lactose content was highest at mid-lactation and stayed constant until the end of the lactation period. Meanwhile, changes were observed between the stages of lactation, and the differences in metabolic parameters were significant ($P<0.05$), except for AST and GLU. The strongest correlation was found with NEFA ($P<0.01$), followed by the Cort ($P<0.05$) concentration, with both parameters showing negative correlation, and strong positive correlation was detected between the milk yield and TCH ($P<0.05$) concentration.

Key words: *lactation, metabolic parameter, milk composition, milk yield*

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Horse husbandry represents one of the most promising livestock sectors in Mongolia and involves not only activities in which horses are used for work, tourism, and racing but also activities in which they are used for food products, both meat and milk. Because of the small volume of the mammary gland [9], milk harvesting can be carried out several times per day, making the dairy equine enterprise different from that with conventional dairy species in terms of both the management of dams and foals and milking practice [22]. In Central Asia, mares are milked 4–5 times per day, with milking beginning at least 2 hr after foal separation from the mother [4]. The farming system is a major cause of variability in equine milk production [4, 20]. In Mongolia, fermented mare's milk, airag, has traditionally been produced at the household level, and its production is severely limited by seasonality due to the nature of pastoral livestock husbandry, which relies entirely on natural pastureland. Thus, milk from mares is basically only obtainable in

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warmer seasons (from June to October). Nowadays, airag is produced in an industrialised manner and is offered to consumers in urbanised communities year-round. For future interventions to get better production in terms of both quantity and quality, it is essential to understand the relationship of mare lactational performance with metabolic responses, with consideration of seasonal effects. Milk production is affected by many factors, including the farming system, nutrition and feeding, strategy and type of milking (manual or mechanical), individual milkability, stage of lactation, and size and body condition of the animals, in addition to genetics [26]. Lactation involves the mobilization and export of substantial quantities of nutrients in milk [25], which mainly affects the metabolism and induces stress, resulting in the variation of haemato-biochemical parameters [19] and biochemical constituents of blood through adjustments to maintain homeostasis [28]. The metabolic profile is a useful tool to determine if homeostatic mechanisms are capable of maintaining blood parameters under different feeding and housing conditions [17], and the determination of metabolic parameters can identify energy metabolism disturbances. The emerging niche market of equine milk raises questions on appropriate management strategies of dam and foals, as mainly related to animal nutrition as well as environmental issues, besides food security and animal welfare [22]. Barely any information in this regard is available in the Mongolian context and even in industrialized countries where the use of mare milk is emerging. Therefore, the aim of this study was to characterize lactational performance of mares kept on natural pastureland by revealing the seasonal effects on metabolic parameters.

Materials and Methods

Research site

The research was carried out 110 km east of Ulaanbaatar, Mongolia, in Bayandelger soum, Tuv province, next to Baganuur district (47°47.50 N, 108°20.40 E, 1,360 m above sea level). The area has been described as having a continental semi-arid climate and to be covered by steppe vegetation, and the annual mean air temperature and precipitation for 2004 to 2019 was −1.8°C and 256.8 mm, respectively [24].

Mares and rearing management

Eight mares, aged 7–12 years, from two separate harems of a herdsman living at the research site were used in this study. During the milking season, the herdsman milked around 30 mares, and those mares were divided into four harems, each headed by a stallion. Each harem was composed of milking mares and non-milking horses, such as fillies, colts, yearlings, and castrated male horses. In total, there were over a hundred horses, and the pasture was shared by other ruminant livestock species, including cattle, sheep, and goats belonging to the herdsman. Places where nomadic people were taking care of horses and milking horses in summer are shown in Fig. 1. The winter camp of the herdsman was around 15 km away from the summer camp. Horses were reared in the traditional manner of keeping them on natural pasture without the provision of any feed during the study, except for watering with well water when there was no snow or rain. The plant species where the mares were reared are shown in Table 1, and they were previously described by Nakano *et al*. [24]. The pasture conditions and air temperatures around the sampling area are described in a modified version of the schematic diagram (Fig. 1) presented in the previous study of Bat-Oyun *et al*. [3], due to the use of similar traditional Mongolian pastures.

Sample collection

Milk yield and composition and serum metabolic parameters were determined at about 30, 60, 90, 120, 150, 180, 210, 240, and 270 days postpartum, which corresponded to June, July, August, September, October, November, December, January, and February, respectively. First, five samples were collected during routine milking by the herdsman at his summer camp. The rest of the samples were collected at the herdsman's winter camp after gathering the horses from the pasture in the early morning on the day of sample collection. During sample collection (milking), foals were caught and tied up to prevent suckling. Milk was collected by a traditional method. Briefly, at each milking, the mare's foal was allowed to suckle the first stream of milk until ejection occurred, and then milk was collected by hand-milking, trying to empty the mammary gland as much as possible, with the foal in close proximity. Milking was repeated 5 times a day at two-hour intervals. Between each milking, mares were free to graze nearby. Samples for compositional analysis were obtained from daily pooled milk, cooled immediately, and kept cool until laboratory tests were performed. Serum was collected individually into vacutainer collection tubes from the jugular vein before the first milking was performed on each day of sample collection. Sample collection was terminated in February because of a significant decrease in the milk yield to below100 m*l* per milking.

Determination of the yield and composition of the milk

Immediately after milking, the milk yield was measured volumetrically on an individual basis, and the mean milk yield was calculated from the measurements taken for all mares on each day of sample collection or the measurements taken for all mares on each day of sampling. Milk

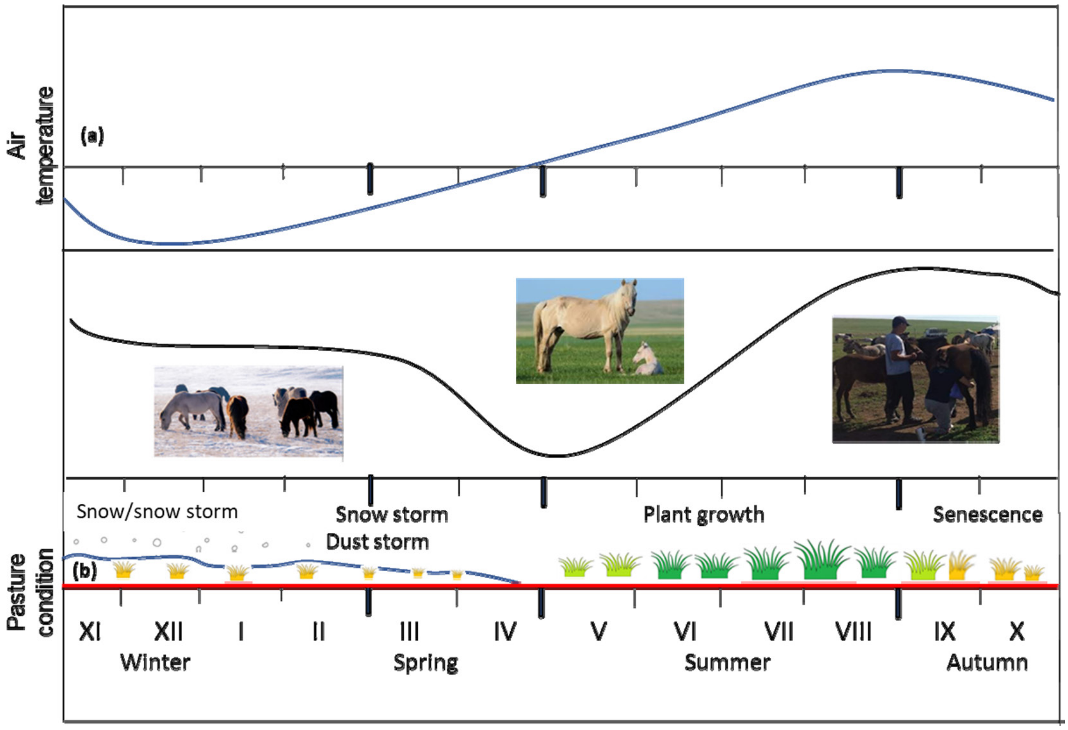

Fig. 1. Schematic diagram of the pasture conditions and air temperature of the sampling area.

Table 1. Plant species where the mares were reared (described by Nakano *et al.* [24])

Palatability	Life duration	Abbreviation	Plant species found at the study sites
Palatable	Annual	PAF	*Dontostemon integrifolius*, *Draba integrifolia*, *Heteropappus hispidus*, *Salsola collina*, *S. ruthenica*
Palatable	Annual	PAG	*Setaria viridis*
Palatable	Perennial	PPF	*Allium anisopodium*, *A. bidentatum*, *A. odorum*, *Arenaria capillaris*, *Asparagus dahuricus*, *Aster alpinus*, *Carex duriuscula*, *C. korshinskyi*, *Carum carvi*, *Gallium verum*, *Leontopodium ochroleucum*, *Medicago ruthenica*, *Plantago depressa*, *Potentilla acaulis*, *P. bifurca*, *Ptilotrichum canescens*, *Pulsatilla ambigua*, *Sibbaldianthe adpressa*, *Taraxacum dissectum*
Palatable	Perennial	PPG	*Agropyron cristatum*, *Agrostis trinii*, *Cleistogenes squarrosa*, *Elymus chinensis*, *Poa attenuata*, *Stipa krylovii*
Palatable	Perennial	PPL	*Astragalus laguroides*, *A. scaberrimus*, *Caragana microphylla*, *C. pygmaea*, *C. stenophylla*
Palatable	Perennial	PPS	*Artemisia frigida*
Unpalatable	Annual	UAF	*Bassia dasyphylla*, *Chenopodium acuminatum*, *C. album*, *C. aristatum*
Unpalatable	Annual	UAS	*Artemisia palustris*, *A. sieversiana*
Unpalatable	Perennial	UPF	*Convolvulus ammanii*, *Ephedra sinica*, *Haplophyllum dahuricum*, *Limonium flexuosum*, *Linaria acutiloba*, *Myosotis suaveolens*, *Potentilla tanacetifolia*, *Serratula centauroides*, *Stellera chamaejasme*
Unpalatable	Perennial	UPL	*Astragalus galactites*
Unpalatable	Perennial	UPS	*Artemisia adamsii*, *A. dracunculus*, *A. mongolica*

protein, fat, and lactose were measured using an infrared milk analyzer (MilkoScan Minor, Foss, Hillerød, Denmark).

Determination of the serum metabolic parameters

Blood samples were centrifuged to separate serum, and the sera were stored at −20°C until they were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), triacylglycerol (TAG), total cholesterol (TCH), albumin (ALB), urea (Ure), and total protein (TP) using an automatic biochemical analyzer

(Alizé, Lisabio, France). The concentrations of cortisol (Cort), insulin (Ins), and non-esterified fatty acid (NEFA) were determined by enzyme-linked immunosorbent assays (EIA-1887, DRG Instruments GmbH, Marburg, Germany; EIA-2935, DRG Instruments GmbH, Marburg, Germany; and Ab65341, Abcam, Cambridge, U.K., respectively). Samples were assayed in duplicate according to the instructions of the assay kits. Based on the standards provided with the kits, the intra- and inter-assay coefficients of variation were 6.0% and 8.7%, 5.4% and 9.5%, and 7.3% and 11.5% for NEFA, Cort, and Ins, respectively.

Statistical analysis

IBM SPSS Statistics for Windows version 20 (IBM Corp., Armonk, NY, U.S.A.) was used for statistical analyses. Repeated measures ANOVA, followed by the Fisher's LSD test, was used to evaluate changes in variables. The level of significance was set at $P<0.05$. Results are presented as the mean and standard deviation (SD). Pearson's correlation analysis was used to evaluate the relationship between milk yield metabolic parameters.

This research study was approved by the Ethical Committee on the Use of Animals in Research of the Mongolian University of Life Sciences.

Results

Changes in milk yield and milk composition

The changes in milk yield and composition during lactation are shown in Fig. 2. The peak of the milk yield occurred at about 90 days of lactation, and the milk yield declined along with lactation stage.

While the milk protein and fat contents decreased gradually from the early stages of lactation to the late lactation stages (Fig. 2B and 2C), the lactose content was highest at mid-lactation and remained constant until lactation ended (Fig. 2D). All these changes were statistically significant ($P<0.05$).

In total, eleven serum metabolic parameters including two hormones, Ins and Cort, were measured at different lactation stages along with milk yield. Table 2 shows the changes in these parameters. The results of statistical analysis indicated that the changes in the parameters were significant ($P<0.05$), except for AST and GLU.

To determine the association between milk yield and metabolic parameters, Pearson's correlation analysis was conducted. The strongest correlation was found with NEFA ($P<0.01$), followed by the Cort ($P<0.05$) concentration, with

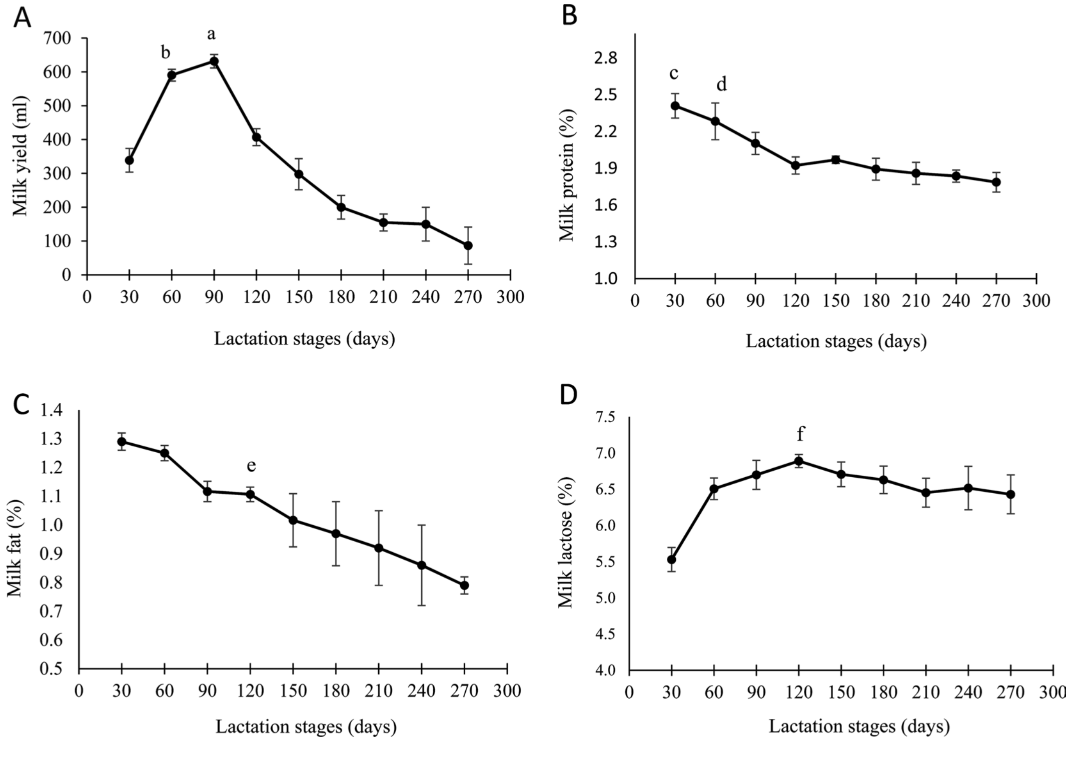

Fig. 2. Changes in milk yield and composition. A, yield; B, protein; C, fat; D, lactose. $^{a}P<0.05$ for 90 vs. 30, 120, 150, 180, 210, and 240 days. $^{b}P<0.05$ for 60 vs. 30 and 180 days. $^{c}P<0.05$ for 30 vs. 150, 180, 210, and 240 days. $^{d}P<0.05$ for 60 vs. 210 and 240 days. $^{e}P<0.05$ for 120 vs. 270 days. $^{f}P<0.05$ for 120 vs. 30 days.

both parameters showing negative correlation, and strong positive correlation was detected between the milk yield and TCH ($P<0.05$) concentration (Table 3).

Furthermore, when plotted graphically (Fig. 3A), the elevation in the NEFA concentration and decrease in the milk yield crossed at approximately the 165th day of lactation.

Table 2. Concentrations of serum metabolic parameters and milk yield during lactation

Metabolic parameters		Lactation stages, month									
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	*P*-value
ALT, IU/*l*	Mean	6.5^{b}	9.6^{b}	12.4^{a}	5.9	11.7^{b}	9.1^{a}	11.6	20.8	23.6	<0.05
	SD	2.0	0.7	3.5	2.7	0.7	4.3	6.9	9.6	11.4	
AST, IU/*l*	Mean	152.0	179.1	203.3	248.1	233.7	226.3	226.0	190.9	201.6	ns
	SD	47.5	3.8	88.4	19.5	9.1	40.4	42.0	3.5	38.8	
GLU, mg/d*l*	Mean	80.7	74.1	75.0	71.7	68.3	75.4	75.3	69.5	71.9	ns
	SD	17.6	3.9	21.2	9.7	3.3	6.5	6.5	3.3	18.7	
TAG, mmol/*l*	Mean	19.1	20.1	20.7^{a}	39.5	21.7^{b}	17.6^{a,b}	16.7^{a}	30.8^{a}	34.5	<0.05
	SD	6.3	0.5	2.4	23.3	1.5	0.9	1.5	4.5	12.8	
TCH, mmol/*l*	Mean	44.5	47.8^{b}	87.6	63.9^{a}	42.8^{b}	38.2	32.3^{a}	41.5	46.4	<0.05
	SD	5.5	3.3	33.9	14.8	6.5	1.2	1.6	3.8	6.1	
NEFA, mmol/*l*	Mean	106.4$^{a\text{-}d}$	33.6$^{a\text{-}d}$	42.0$^{a\text{-}d}$	33.6$^{a\text{-}d}$	182.0$^{a\text{-}c}$	264.0	263.3	333.3^{b}	446.7^{a}	<0.05
	SD	19.4	8.4	8.4	14.6	32.4	188.9	127.4	20.8	30.6	
Alb, g/*l*	Mean	19.1$^{a\text{-}c}$	28.2^{a,b,d}	43.0^{b}	25.1^{a}	28.6^{b,c}	21.4^{a,b}	24.4^{a,b,d}	44.0^{a}	31.7	<0.05
	SD	4.3	0.3	2.7	12.7	0.9	4.6	0.7	4.6	6.0	
Ure, mmol/*l*	Mean	69.0	95.2^{b}	92.7^{c}	128.4	101.1^{a}	88.8^{d}	61.2^{b,c}	63.6$^{a\text{-}c}$	62.4$^{a\text{-}c}$	<0.05
	SD	18.2	4.5	4.5	52.7	4.8	9.2	13.6	4.5	6.3	
TP, g/*l*	Mean	35.6	55.6$^{a\text{-}c}$	72.9	74.3	78.2^{a}	76.1^{b}	68.9	72.8	68.1^{a,c}	<0.05
	SD	22.0	5.1	8.5	8.9	1.5	2.3	11.6	17.4	4.1	
Cort, μg/m*l*	Mean	400.8^{a}	215.7$^{a\text{-}c,e\text{-}g}$	199.1$^{a\text{-}g}$	268.2$^{a\text{-}c,e}$	256.6$^{a\text{-}c,f}$	265.3$^{a\text{-}c}$	353.5^{a,c}	354.3^{a,b}	329.0^{d}	<0.05
	SD	4.5	5.2	7.4	4.9	5.4	29.6	21.8	7.1	32.7	
Ins, μIU	Mean	5.7^{a}	5.3	6.4	10.5	6.3	3.8^{a,b}	4.0^{a,b}	3.8^{a}	4.0^{a}	<0.05
	SD	0.4	1.0	1.1	4.3	1.3	0.0	0.0	0.1	0.1	

Alb: $^{a}P<0.05$ for Jan vs. Jun, Jul, Sep, Oct, Nov, and Dec. $^{b}P<0.05$ for Aug vs. Jun, Jul, Oct, Nov, and Dec. $^{c}P<0.05$ for Oct vs. Jun and Dec. $^{d}P<0.05$ for Jul vs. Dec. **Alt:** $^{a}P<0.05$ for Aug vs. Nov. $^{b}P<0.05$ for Oct vs. June and July. **Urea:** $^{a}P<0.05$ for Oct vs. Jan and Feb. $^{b}P<0.05$ for Jul vs. Dec, Jan, and Feb. $^{c}P<0.05$ for Aug vs. Dec, Jun, and Feb. **TCH:** $^{a}P<0.05$ for Sep vs. Dec. $^{b}P<0.05$ for Jul vs. Oct. **TAG:** $^{a}P<0.05$ for Jan vs. Aug, Nov, and Dec. $^{b}P<0.05$ for Oct vs. Nov. **TP:** $^{a}P<0.05$ for Oct vs. Jul and Feb. $^{b}P<0.05$ for Nov vs. Jul. $^{c}P<0.05$ for Feb vs. Jul. **NEFA:** $^{a}P<0.05$ for Feb vs. Jun, Jul, Aug, Sep, and Oct. $^{b}P<0.05$ for Jan vs. Jun, Jul, Aug, Sep, and Oct. $^{c}P<0.05$ for Oct vs. Jun, Jul, Aug, and Sep. $^{d}P<0.05$ for Jun vs. Jul, Aug, and Sep. **Cort:** $^{a}P<0.05$ for Jun vs. Jul, Aug, Sep, Oct, Nov, Dec, and Jan. $^{b}P<0.05$ for Jan vs. Jul, Aug, Sep, Oct, and Nov. $^{c}P<0.05$ for Dec vs. Jul, Aug, Sep, Oct, and Nov. $^{d}P<0.05$ for Feb vs. Jul, and Aug. $^{e}P<0.05$ for Sep vs. Jul and Aug. $^{f}P<0.05$ for Oct vs. Jul and Aug. $^{g}P<0.05$ for Jul vs. Aug. **Ins:** $^{a}P<0.05$ for Jun vs. Nov, Dec, Jan, and Feb. $^{b}P<0.05$ for Dec vs. Nov.

Table 3. Pearson Correlation between milk yield and serum metabolic parameters

	Alb	Alt	Ure	Cho	Tag	TP	NEFA	Cort	Ins	Yield
Alb	1									
Alt	0.638	1								
Ure	−0.135	−0.596	1							
TCH	0.484	−0.131	0.489	1						
TAG	0.272	0.359	0.277	0.231	1					
TP	0.405	0.295	0.314	0.135	0.276	1				
NEFA	0.139	0.800**	−0.695*	−0.566	0.182	0.267	1			
Cort	−0.263	0.220	−0.687*	−0.591	0.070	−0.457	0.510	1		
Ins	−0.127	−0.561	0.852**	0.564	0.469	0.074	−0.698*	−0.338	1	
Yield	0.155	−0.524	0.584	**0.730***	−0.188	−0.196	**−0.905****	**−0.699***	0.517	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

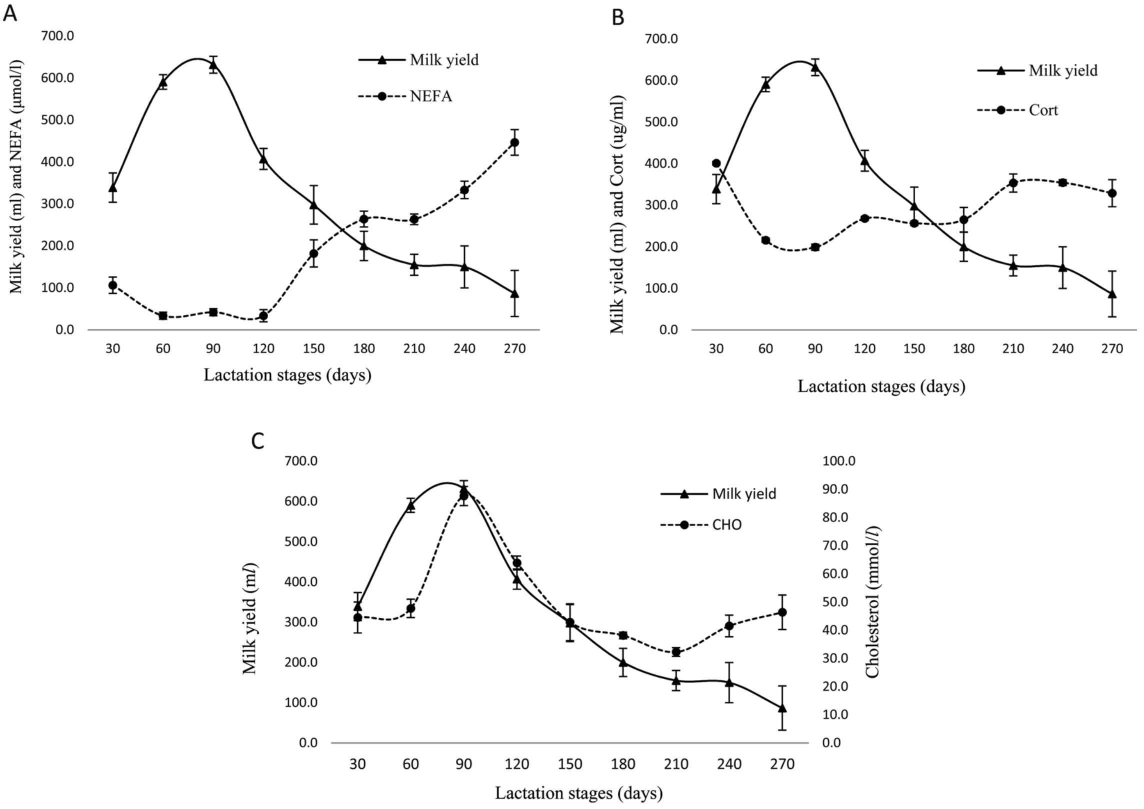

Fig. 3. Correlation between milk yield and non-esterified fatty acid (NEFA) (A), milk yield and cortisol (B), and milk yield and cholesterol (C).

Discussion

The patterns of changes in the fat, protein, and lactose contents of milk during lactation in the present study were generally consistent with those obtained from Quarter horse mares [12], Italian heavy draft mares [7], Lusitano mares [27], and Martina Franca jennies [8]. However, in the current study, the peak of the milk yield in the Mongolian native grazing mares was observed slightly later in the lactation period (at about 90 days) than in Quarter horse mares [12], Italian heavy draft mares [7], Lusitano mares [27], and Martina Franca jennies [8], among which the peaks occurred at about 30, 69, 31, and 48 days postpartum, respectively. The later peak of the milk yield may be characteristic of the abundance and preference of grassland forage from natural pastures.

Post-foaling energetic demands continue with milk production to meet foal nutritional needs, while mares also require energy to recover in the postpartum period [16]. After foaling, the mare's biochemical profile is altered [28], with adjustments occurring during gestation and lactation, even as homeostatic mechanisms ensure that individual components remain constant [15]. Therefore, the majority of these changes were partly characterized by the mares' responses to seasonality and the demands of reproductive physiology.

For the assessment of energy balance, NEFA is one of the most important tools when considering an animal's health, productive, and reproductive performance [10]. Serum NEFA is assessed as an indicator for the mobilization of lipids from the adipose tissues, thus reflecting the status of energetic balance in the body, and the serum NEFA concentration has been found to be elevated during times of insufficient feed supply [29, 30]. In this study, the lower serum NEFA concentrations and greater amounts of milk recorded from the 60 to 120 days of lactation period (Fig. 3A), which covers July to September, correspond to the blossomy season in Mongolia in terms of feed availability and physical condition for pasture-based livestock animals. Studies on dairy cows revealed that reduced concentrations of GLU and Ins in dairy cows led to elevated TAG hydrolysis and increased availability of NEFA for metabolism [5], but these processes have not been fully elucidated in horses [16]. The NEFA concentration is used as an indicator of lipid degradation in adipose tissue [1, 2] and reflects the status of the energy balance of the body [16]. The high levels of

NEFA in the present study during the last half of lactation could be a consequence of mobilization of body fat due to the restriction of feeding with natural grasses, and they were consistent with the above studies.

The point at which the elevation in NEFA crossed the decrease in milk yield may be a threshold indicating when lactating mares enter a negative energy balance state for lactational performance. The similar patterns of change in the serum NEFA and Cort concentrations (Fig. 3B) support the physiological background for this. Cort influences the intermediary metabolism of carbohydrates, proteins, and fats and accelerates gluconeogenesis and lipolysis via the release of glycerol and NEFA as an antagonist of Ins [11, 13]. High levels of Cort have been found in sheep in autumn and winter and might be associated with a decreased environmental temperature [29]. In the present study, a decrease in serum Cort was associated with an increase in milk yield or an increase in serum Cort was associated with a decrease in milk yield, and the two parameters crossed each other on the 160th day of lactation when plotted on a graph. Synthesis and secretion of Cort is accelerated by various stressors, like detrimental ambient temperatures, and feeding and handling of animals [6, 14, 21, 23, 31]. The serum Cort levels gradually increased from mid-lactation to the end of lactation in the present study, and the authors suggest that this could be attributed to the Mongolian mares with pastoral herding experiencing energy loss or a negative energy balance this during period.

Serum TCH is a part of the lipoprotein complex, and it is closely related to milk production [2]. Kida reported that serum TCH showed an obvious positive relationship with milk production in dairy cows [18]. In this study, TCH also showed a strong positive correlation with milk yield (Fig. 3C).

With barely any information available on this topic, this work attempted to characterize the lactational performance of Mongolian native grazing mares in relation to metabolic parameters. Further study is necessary to develop an assessment tool that can help obtain greater amounts of milk by increasing both the quantity obtained from milking and the length of the milking period through management of the nutrition and rearing of mares.

Acknowledgments

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