Veterinary Medicine and Science

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Equine

The Milk Compositions and Blood Parameters of Lactating Dezhou Donkeys Changes With Lactation Stages

Miaomiao Zhou | Zongjie Ma | Xinyi Du | Changfa Wang | Guiqin Liu

College of Agriculture and Biology, Liaocheng Research Institute of Donkey High-Efficiency Breeding and Ecological Feeding, Liaocheng University, Liaocheng, China

Correspondence: Miaomiao Zhou (zhoumm0329@163.com) | Guiqin Liu (liuguiqin@lcu.edu.cn)

Received: 28 September 2024 | **Revised:** 5 February 2025 | **Accepted:** 17 February 2025

Funding: This research was funded by the Key Technology Research and Development Program of Shandong (2021TZXD012), the Shandong Provincial Natural Science Foundation (ZR2023MC149), the National Key R & D Program of China (2022YFD1600103) and the Open Project of Liaocheng University Animal Husbandry Discipline (319312105-5).

Keywords: blood biochemical parameters | donkey | hormones | lactation stages | milk compositions

ABSTRACT

The aim of this study was to investigate the changes in milk compositions and blood parameters of Dezhou donkeys during the lactation. Milk and serum samples from six lactating Dezhou donkeys were collected at 1, 30, 60, 90, 120, 150 and 180 days of lactation. The results displayed that the milk yield during the lactation first increased and then gradually decreased, with the peak milk production period of 30–60 days after parturition. Compared with other lactation stages, milk fat, milk protein, milk solids and solids non-fat (SNF) were higher in early lactation ($p < 0.05$). Conversely, the milk of late lactation stage had a higher lactose content ($p < 0.05$); The mineral elements content in donkey milk decreased with the prolongation of lactation period ($p < 0.05$); The blood serum prolactin (PRL), insulin-like growth factor 1 (IGF-1), total protein (TP), albumin (ALB), glucose (GLU), total cholesterol (TG), triglyceride (TC), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), high density lipoprotein (HDL) and low density lipoprotein (LDL) were higher in early lactation stage ($p < 0.05$), similar to changes in milk compositions during lactation. The correlation analysis results showed that the milk yield was positively correlated with PRL, IGF-1 and ALP, and negatively correlated with ALB ($p < 0.05$); the milk composition, such as milk fat, protein, solids and SNF were positively correlated with blood GLU, LDH, TP, ALB, TC, TG, HDL and LDL ($p < 0.05$). The above results indicated that the lactation stage had significant effects on milk production, milk composition, blood biochemical indicators and hormone of lactating donkeys. This study investigated the changes of Dezhou donkeys' milk performance and blood parameters with lactation stages for the first time, which may contribute to the exploration of the nutritional value of donkey milk and provide theoretical support for the nutritional regulation and utilisation of donkey milk.

1 | Introduction

In recent years, donkey milk (DM) has received increasing attention from people. Donkey milk is a high-quality milk source with nutrients that are very close to human milk (Ning et al. 2023). It is low in fat and casein and rich in lactose, whey proteins, calcium (Ca), selenium (Se) and vitamin D_3. It also contains some bioactive molecules such as lysozyme, lactoferrin, lactalbumin and lactoperoxidase (Fantuz et al. 2020; Vincenzetti et al. 2021; Živkov Baloš et al. 2023). Donkey milk has many biological activities, such as antioxidant, immunoregulation and antibacterial (De Oliveira Moura Araújo et al. 2023; Parastouei et al. 2023; Zhou et al. 2023). It is an excellent nutrition for infants with milk protein allergy, convalescent patients and the elderly

(Papademas et al. 2021; Spada et al. 2021). In addition, donkey milk is also used for cosmetic and medical purposes, as well as in its innovative use as an inhibitor of late blowing in cheese and in farm management practices (Zhou et al. 2023; Cosentino et al. 2016; Adduci et al. 2019). However, due to low milk production, the commercialization of DM is still limited.

The lactation performance of donkeys is influenced by many factors such as breed, diet, season and lactation stage (Huang et al., 2024; Zhou et al., 2024). Research had shown that the average milk production of Jiangyue donkeys is 1.28 kg/d, while the average milk production of Martina Franca donkeys is 1.82 kg/d (Guo et al. 2007; Salimei et al. 2004). Zhang et al. (2019) compared the whey proteins of different breeds of donkeys and found that 19 types of whey proteins showed significant differences in high-yield milk samples (16 upregulated and 3 downregulated). Another study compared the differences in milk composition and metabolites between the Sanfen donkey and the Wutou donkey strains in Dezhou donkey, and found that the fat, total solids and solids non-fat (SNF) content of the Sanfen DM were higher than those of the Wutou donkey (Li et al. 2022). Diet is another important factor affecting the production performance of lactating donkeys. Supplementing concentrate to lactating donkeys is beneficial for improving the weight gain of foals (Salari et al. 2020). DM metabolome can be modulated by probiotic supplementation (Laus et al. 2023). The production season also has an impact on the milk production and quality of donkeys. Some study investigated the effects of seasons on the milk production and composition of Amiata donkeys, and found that in summer and autumn, milk production, casein and lactose content were higher; in spring, milk protein, Ca and ash content were higher; the concentration of vitamin D_3 in DM was higher in spring and summer (Martini et al. 2014; Martini et al. 2018).

The milk production of donkeys, as well as the milk components varies with the lactation stage. It was found that the peak milk production period for donkeys is 4–5 weeks after parturition (Raspa et al. 2019). The results of Martini et al. (2014) indicated that the protein content in DM gradually decreased in the first 6 months of lactation and the content of casein and lactose remained constant after 60 days of lactation. Li et al. (2020) found that the composition of DM fat varies with lactation, with 60 different lipids and 11 different free fatty acids between donkey colostrum and mature milk. Martemucci and D'Alessandro (2012) found that the content of saturated fatty acids in milk decreased while the milk unsaturated fatty acids content increased during the lactation. Research of Messias et al. (2021) found that lactation period can affect the content of α-linolenic acid (18:3 n-3), with the lowest content in milk samples of 30th day in milk (DIM) sample and the highest content in milk of 90th DIM.

The biochemical parameters and hormones of blood reflect the metabolic processes of animals and are indicators of their health and physiological conditions (Buryakov et al. 2022; Bruckmaier and Gross 2017). It was found that blood concentration of total protein (TP), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and total cholesterol (TC) of the dairy cows were effected by parity, stage of lactation and season of production (Cozzi et al. 2011). Endocrine changes also occur during lactation (Bruckmaier and Gross 2017; Lacasse et al. 2016). Thus, the blood biochemical parameters and hormones can be used to assess the metabolic status of lactating animals.

TABLE 1 | Composition and nutritional level of experimental concentrate (dry-matter basis).

Items	%
Feed ingredients	
Corn	31.75
Wheat bran	12.00
Wheat flour middling	21.00
Distillers dried grains with solubles	5.00
Wheat germ	18.00
Soybean meal	6.65
NaCl	0.63
$CaCO_3$	3.72
$CaHPO_4$	0.25
Premix[1]	1.00
Total	100
Nutrient level	
Dry matter	88.53
Crude protein	17.18
Ash	8.37
Crude fiber	4.27
Ether extract	4.59
Calcium	1.27
Phosphorus	0.52
Lysine	0.75

[1]Premix/kg: VA, 600 KIU; VD3, 125 KIU; VE, 3500 IU; Fe, 2 g; Cu, 800 mg; Zn, 6 g; Mn, 13 g; I, 95 mg; Se, 50 mg.

Donkey milk is progressively recommended as a substitute for infants, young children and the elderly. However, the DM yield and composition, such as protein, fat, mineral elements etc., are still unclear. Therefore, the dynamic changes in milk yield, milk chemical and mineral composition and their relationship with blood biochemical parameters and hormones during the lactation period (0-180 DIM) were investigated in this study.

2 | Materials and Methods

2.1 | Samples Collection and Preparation

Milk and serum samples were from 6 healthy multiparous lactating Dezhou jennies with similar ages (an average age of 5 years) and weights (an average bodyweight 284 ± 21 kg) at different DIM: 1 (A), 30 (B), 60 (C), 90 (D), 120 (E), 150 (F.) and 180 (G) after foaling. The Jennies were housed in a farm in Dong'E, Liaocheng City, China. The donkeys were raised in a semi-closed house. All donkeys drank freely and offered the same diet of beanstalk (ad libitum) supplemented with 2 kg concentrate per head per day. The composition and nutritional level of the concentrate were shown in Table 1.

The lactating donkeys were manual-milked twice a day (11 am and 3 pm). The foals were removed from the mother for 4 h before the milking. The milk yield of Jennies is recorded. The milk samples were collected during milking twice a day, frozen quickly in liquid nitrogen and stored at -80°C until analysis. The blood samples were collected from the jugular vein on an empty stomach. After stratification, the blood samples were centrifuged at 3500 rpm, 4°C for 15 min to separate the serum. Then, the serum was frozen quickly with liquid nitrogen and stored at -80°C.

2.2 | Milk Composition Determination

The milk components (fat, protein, lactose, urea, lactoferrin, solids and solids non-fat) were determined using the CombiScope FTIR300 (Delta Instruments B.V., Drachten, The Netherlands). There were three replicates for each sample.

2.3 | Determination of Blood Hormone

The concentration of insulin-like growth factor 1 (IGF-1), estradiol (E2), progesterone (Pr), prolactin (PRL) and thyroid stimulating hormone (TSH) were measured using enzyme linked immunosorbent assay (ELISA) (DR-200BS, Huaweidelang, Wuxi, China). The ELISA Kits for IGF-1, E2, P, PRL and TSH detection were purchased from Beijing Huaying Biotechnology Research Institute (Beijing, China).

2.4 | Measurement of Blood Biochemical Index

The concentration or international units (IU) of serum glucose (GLU), TP, TC, albumin (ALB), triglyceride (TG), urea, AST, high density lipoprotein (HDL), lactate dehydrogenase (LDH), low density lipoprotein (LDL) and ALP were measured using the automatic biochemical analyser (Mindray BS-420, Shenzhen, China) and the test kits were provided by BioSino Bio-Technology and Science Inc (Beijing, China).

2.5 | Determination of Milk Mineral Elements

Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500, Beijing, China) was used to detect Ca, phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), zinc (Zn) and Se in DM and blood. Firstly, the samples were pre-processed in the microwave digestion instrument (CEM, MARS6, North Carolina, United States of America).

Simply, 2 g of milk sample and 10 mL of nitric acid (65%, guaranteed reagent, Merck, Darmstadt, Germany) were put into the microwave digestion instrument for 1 h. Then the digestion was performed according to the digestion conditions (Table 2). After the digestion was completed, cooled and removed, the digestion tank was slowly opened to exhaust. The digestion tank was heated at 160° C for 30 min, added water to 50 mL and mixed well, then used for ICP-MS detection (Table 3). The purified water replaced DM as blank controls.

TABLE 2 | The conditions of microwave digestion.

Phases	Heating up time (min)	Holding time (min)	Temperature (°C)
1	5	5	120
2	5	10	150
3	5	20	190

2.6 | Statistical Analysis

The data were analysed in GraphPad Prism 6 software by using One-way repeated measure ANOVA method. The correlation analysis was performed using SPSS 27.0 software. The results were shown as mean ± SD. $P < 0.05$ was defined as significant difference.

3 | Results

3.1 | Effect of Lactation Stages on DM Yield and Compositions

The milk yield and composition of Dezhou donkey were displayed in Table 4. The milk yield first increased and then gradually decreased from 30 DIM to 180 DIM ($p < 0.01$). The content of milk fat, protein, solids and SNF was highest in colostrum, followed by milk of the 30th DIM ($p < 0.01$). However, as the duration of lactation period, the lactose and lactoferrin content significantly increased ($p < 0.01$). The lactose content in the middle and late lactation stage (90–180 DIM) was significantly higher than that in colostrum and early lactation stage (30 DIM) and the lactose content in colostrum was the lowest (1 DIM) ($p < 0.01$). Similarly, the lactoferrin content in colostrum was the lowest, then gradually increased and remained constant ($p < 0.01$). The lactation stage had no significantly effect on donkey milk urea.

The content of mineral elements (Ca, Na, K, Mg, P, Zn and Se) in DM of different lactation stages were shown in Table 5. Overall, compared to the milk, colostrum contained higher levels of Na, K, Mg, Zn and Se ($p < 0.01$). The content of Ca was gradually decreased with the prolongation of lactation period ($p < 0.05$). The P content in milk of early lactation (1–60 DIM) was significantly higher than that in mid to late lactation milk (90–180 DIM) ($p < 0.01$).

3.2 | Effect of Lactation Stage on Blood Hormones Level

There were significant changes in blood hormones during lactation (Figure 1). The blood PRL levels in the early and middle lactation stages were significantly higher than those in the late lactation stage (120–180 DIM), with the highest levels on 1st and 90th DIM (Figure 1A, $p < 0.01$). On the day of parturition, the blood IGF–1 level was highest and then decreased with the prolongation of lactation (Figure 1B, $p < 0.01$). The Pr level significantly decreased in 30th DIM and then increased to the level at parturition (Figure 1C, $p < 0.05$). Conversely, the concentration of E2 in the blood of the 30th DIM was highest (Figure 1D, $p < 0.01$).

TABLE 3 | The parameters of inductively coupled plasma mass spectrometry.

Items	Parameters	Items	Parameters
RF power (W)	1500	Atomizer	Concentric nebulizer
Plasma gas flow rate (L/min)	15	Sampling cone	Platinum cone
Carrier gas flow rate (L/min)	0.80	Sampling depth	8 mm
Auxiliary Gas Flow (L/min)	0.40	Acquisition mode	Spectrum
Helium flow rate (mL/min)	5	Detection method	Automatic
Temperature of atomization chamber (°C)	2	Measurement points per peak	3
Sample improvement rate (r/s)	0.3	Repetitions	3

TABLE 4 | Milk yield and composition of Dezhou donkeys at different lactation stages.

Items	Lactation stages (DIM) $n = 6$							p
	1	30	60	90	120	150	180	
Milk yield (g)		1075 ± 174^{a}	1137 ± 123^{a}	971 ± 172^{ab}	785 ± 136^{b}	604 ± 122^{bc}	575 ± 115^{c}	<0.0001
Milk compositions								
Fat (%)	0.92 ± 0.19^{a}	0.43 ± 0.10^{b}	0.20 ± 0.05^{c}	0.12 ± 0.06^{cd}	0.14 ± 0.03^{d}	0.09 ± 0.07^{d}	0.20 ± 0.18^{cd}	0.0001
Protein (%)	7.67 ± 2.61^{a}	1.84 ± 0.18^{b}	1.65 ± 0.08^{c}	1.51 ± 0.05^{d}	1.45 ± 0.09^{de}	1.41 ± 0.09^{e}	1.39 ± 0.10^{e}	0.0021
Lactose (%)	3.94 ± 0.61^{d}	6.39 ± 0.23^{c}	6.55 ± 0.09^{c}	6.72 ± 0.03^{b}	6.85 ± 0.08^{a}	6.74 ± 0.09^{ab}	6.69 ± 0.18^{ab}	<0.0001
Urea (mg/dL)	14.37 ± 0.57	14.62 ± 0.83	14.95 ± 1.11	15.20 ± 0.88	14.08 ± 0.88	14.13 ± 1.45	12.92 ± 1.14	0.0796
Lactoferrin (g/L)	0.085 ± 0.0003^{c}	0.091 ± 0.0019^{b}	0.094 ± 0.0016^{ab}	0.091 ± 0.0019^{ab}	0.094 ± 0.0013^{a}	0.094 ± 0.0037^{ab}	0.092 ± 0.0011^{ab}	0.0001
Solids (%)	14.13 ± 2.27^{a}	9.55 ± 0.29^{b}	9.15 ± 0.12^{c}	9.08 ± 0.15^{c}	9.13 ± 0.12^{c}	8.97 ± 0.16^{c}	8.99 ± 0.35^{c}	0.0023
Solids non-fat (%)	13.41 ± 2.54^{a}	9.13 ± 0.27^{b}	8.99 ± 0.14^{b}	9.03 ± 0.12^{b}	9.04 ± 0.09^{b}	8.92 ± 0.16^{b}	8.83 ± 0.26^{b}	0.0073

[a–d] Means within a row with different superscripts were significant ($p < 0.05$).

Throughout the lactation period, the blood concentration of TSH increased and then decreased, with the highest concentration occurring in the 90th DIM (Figure 1E, $p < 0.01$).

The correlation analysis between milk yield or milk composition and blood hormones were displayed in Table 6. The correlation analysis found that milk yield was positively correlated with PRL and IGF–1 ($p < 0.05$). The milk fat was positively correlated with IGF–1 ($p < 0.05$). And the urea in donkey milk was positively correlated with PRL ($p < 0.05$). Conversely, the lactoferrin was negatively correlated with IGF–1 ($p < 0.05$).

3.3 | Effect of Lactation Stage on Blood Biochemical Parameters

Except for AST and urea, all other blood biochemical parameters detected in this study were significantly affected by lactation stage (Figure 2, $p < 0.05$). From the 1st DIM to the 60th DIM, the GLU concentration gradually decreased and then remained stable (Figure 2A, $p < 0.01$). On the day of parturition, the concentrations of TP, ALB, TC, TG, HDL, LDL and LDH were significantly higher than those in other lactation periods and then significantly decreased and remained stable (30–180 DIM) (Figure 2, $p < 0.05$). The content of ALP in donkey blood remained stable in the early and middle lactation stages (1–120 DIM) and significantly decreased in the late stage (150–180 DIM) (Figure 2B, $p < 0.01$).

The correlation analysis between milk yield or milk composition and blood biochemical indicators were shown in Table 7. The results found that milk yield was positively correlated with ALP and negatively correlated with ALB ($p < 0.05$). Most of the milk composition, such as milk fat, protein, solids and SNF were positively correlated with blood GLU, LDH, TP, ALB, TC, TG, HDL and LDL ($p < 0.05$). On the contrary, milk lactose and lactoferrin were negatively correlated with the aforementioned blood indicators ($p < 0.05$). The milk urea was positively correlated with ALP ($p < 0.05$).

4 | Discussion

4.1 | Effects of Lactation Stage on Milk Yield of Donkey

In this study, the milk yield of Dezhou donkeys during lactation (0–180 DIM) was investigated. It varied with the lactation stages. The milk yield at 30 DIM, 60 DIM and 90 DIM was significantly higher than other groups, indicating that the peak milk

TABLE 5 | Mineral elements concentration in milk of Dezhou donkeys at different lactation stages.

Items	Lactation stages (DIM) $n = 6$							p
	1	30	60	90	120	150	180	
Ca (mg/kg)	935.69 ± 107.47^{a}	957.20 ± 279.24^{ab}	872.25 ± 45.14^{ab}	815.57 ± 52.75^{b}	771.34 ± 71.23^{bc}	752.17 ± 70.90^{bc}	592.59 ± 40.67^{d}	0.0383
Na (mg/kg)	260.33 ± 14.29^{a}	176.53 ± 29.72^{b}	137.92 ± 14.59^{c}	129.51 ± 14.21^{c}	135.56 ± 6.10^{c}	133.130 ± 26.58^{c}	120.34 ± 11.62^{c}	<0.0001
K (mg/kg)	1463.82 ± 72.23^{a}	879.06 ± 73.29^{b}	679.74 ± 59.73^{c}	638.43 ± 46.94^{cd}	625.86 ± 43.00^{cd}	638.34 ± 69.18^{cd}	592.66 ± 40.28^{d}	<0.0001
Mg (mg/kg)	414.09 ± 32.32^{a}	115.02 ± 14.66^{b}	73.75 ± 7.73^{c}	75.74 ± 4.72^{c}	69.85 ± 2.62^{c}	62.93 ± 3.07^{c}	63.39 ± 6.91^{c}	<0.0001
P (mg/kg)	714.26 ± 58.53^{a}	727.56 ± 143.46^{ab}	624.37 ± 22.29^{b}	541.34 ± 35.17^{c}	559.72 ± 33.34^{c}	543.93 ± 58.54^{c}	492.42 ± 28.41^{c}	0.0067
Zn (μg/kg)	3.49 ± 0.289^{a}	2.56 ± 0.37^{bc}	2.32 ± 0.22^{c}	2.54 ± 0.30^{bc}	2.91 ± 0.20^{b}	2.66 ± 0.24^{bc}	2.85 ± 0.24^{b}	0.0001
Se (μg/kg)	66.77 ± 14.78^{a}	20.70 ± 6.33^{b}	9.60 ± 4.27^{c}	9.89 ± 2.11^{c}	10.56 ± 1.22^{c}	12.17 ± 2.90^{c}	12.10 ± 3.89^{c}	<0.0001

a–d means within a row with different superscripts were significant ($p < 0.05$).

production period of Dezhou donkeys was about 30–90 DIM. The milk yield during the lactation decreased from 1.137 kg/d (60 DIM) to 0.575 kg/d (180 DIM), with an average of 0.858 kg/d. The results of Raspa et al. (2019) showed that the peak milk production period for donkeys was 4–5 weeks after parturition, which is different from the conclusion of this study. There is currently no clear conclusion regarding donkey milk yield. The study of Živkov Baloš et al. (2023) demonstrated that the average milk yield of female donkeys was 1.57 kg/d. Another study showed that the average milk yield of Ragusano donkeys gradually decreased from about 2 kg/day (0–160 DIM) to 0.13 kg/day (250–279 DIM), with an average of 1.64 kg/d (Malacarne et al. 2019). In addition, the results of Fantuz et al. (2020) displayed that the Italy DM yield per milking was 765.6 mL during 70–182 DIM. The average milk yield of Dezhou donkeys in this study is different from the above studies. This difference may be caused by differences in breed, parity, milking method, milk interval, diet and management.

4.2 | Effects of Lactation Stage on DM Chemical Composition

Donkey milk is characterised by high lactose, low dry matter, protein and extremely low fat, which is closer to human milk than dairy cows milk (Cimmino et al. 2022). Fantuz et al. (2020) found that the lactose, protein and fat content of Italy DM in the middle and late stages of lactation (70–182 DIM) were 6.59%, 1.33% and 0.41%, respectively. Similarly, the dry matter, lactose, protein and fat content of Ragusano DM were 8.19%, 6.07%, 1.34% and 0.16%, respectively (Malacarne et al. 2019). The milk of Indian small grey donkeys contained 6.30% lactose, 1.96% protein and 0.76% fat (Nayak et al. 2020). Study of Garhwal et al. (2023) showed that the India Halari DM between 147 DIM and 210 DIM has high lactose (5.75%), low protein (2.03%) and fat (0.86%). Our results displayed that the average content of solids, lactose, protein, fat and lactoferrin in Dezhou DM during the lactation period (30–180 DIM) was 9.15%, 6.66%, 1.54%, 0.20% and 0.093 g/L, respectively. The content of milk components in Dezhou DM is similar to that of above mentioned milk.

Lactation has a significant impact on milk composition. Malacarne et al. (2019) investigated the effect of lactation on DM chemical composition. They found that the dry matter and lactose content gradually decreased during lactation; the crude protein content decreased before 100–129th DIM and then remained constant; the milk fat content had a maximum value between 100th DIM and 219th DIM. Study of Martini et al. (2014) indicated that the protein content in DM gradually decreased in 180 DIM and the content of lactose remained constant after 60 DIM. The results of Andjelić et al. (2022) showed that milk fat and lactose levels were lower in early lactation, whereas milk protein was highly greater in early lactation. In this study, the content of milk fat, protein and solids was highest in colostrum, followed by milk of the 30th DIM and significantly higher than milk from other groups. Conversely, the lactose content in the middle and late lactation stage (90–180 DIM) was significantly higher than that in colostrum and early lactation stage (30 DIM), the lactose content in colostrum was the lowest (1 DIM). Overall, the changes of milk solid and protein content during lactation

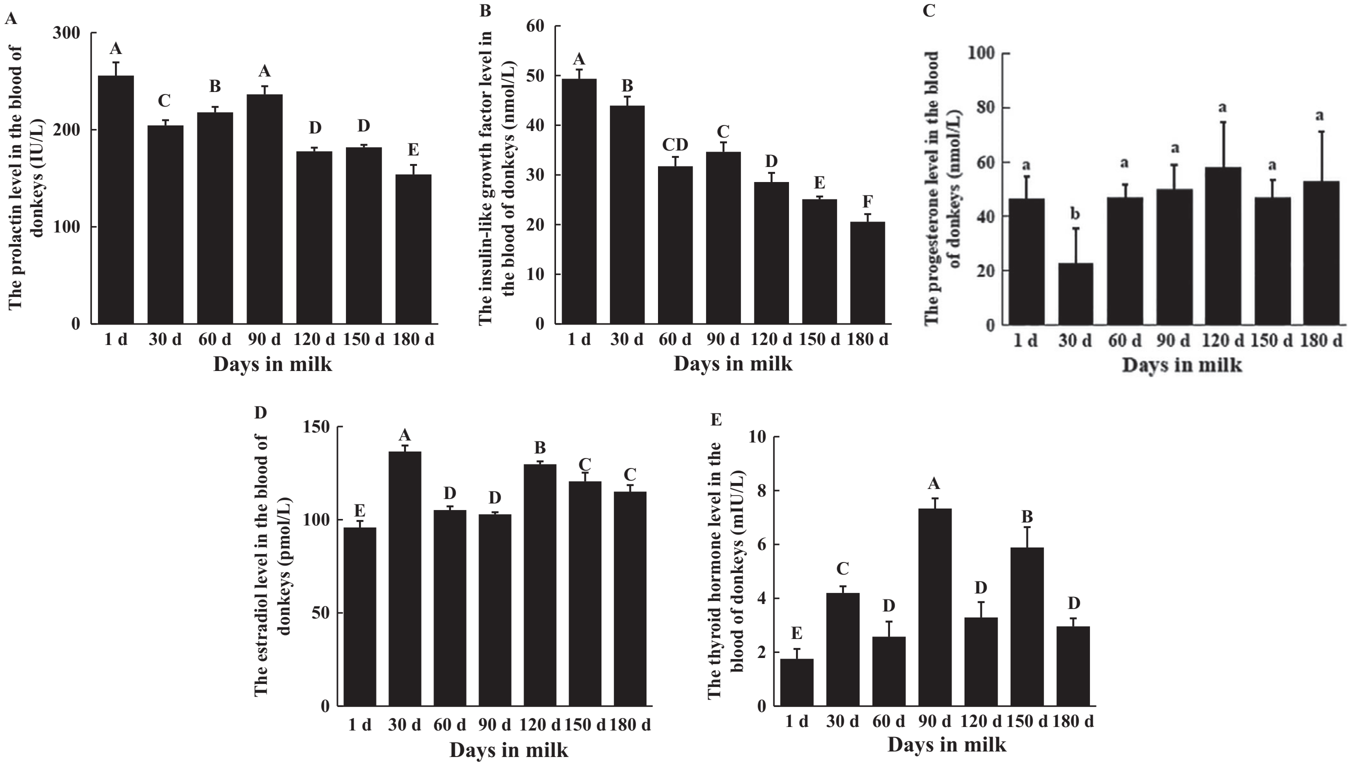

FIGURE 1 | Hormones level (mean ± SD) in the blood of Dezhou donkeys at different lactation stages. Different letters revealed a significant difference (a–b, $p < 0.05$; A–F, $p < 0.01$).

TABLE 6 | The correlation analysis between milk yield or milk composition and blood hormones.

Blood hormones	Milk yield	Milk composition						
		Fat	Protein	Lactose	Solids	Solids non-fat	Urea	Lactoferrin
Thyroid stimulating hormone	−0.05	−0.57	−0.51	0.52	−0.51	−0.49	0.37	0.32
Estradiol	−0.12	−0.36	−0.55	0.54	−0.52	−0.55	−0.24	0.50
Progesterone	−0.51	−0.32	−0.05	0.12	−0.08	−0.03	−0.31	0.25
Prolactin	0.82*	0.62	0.67	−0.67	0.66	0.67	0.76*	−0.70
Insulin-like growth factor 1	0.83*	0.83*	0.73	−0.75	0.75	0.72	0.54	−0.81*

Statistical probability for comparisons: *, $p < 0.05$.

were consistent with the above results; the changes in milk lactose and fat were different from the results of Malacarne et al. (2019) and Martini et al. (2014), but consistent with the results of Andjelić et al. (2022). The differences in milk composition mentioned above may be caused by factors such as breed, season, diet and data statistics.

4.3 | Effects of Lactation Stage on DM Mineral Composition

Donkey milk is also rich in minerals. Study of Nayak et al. (2020) showed that K (2009.67 mg/L), Na (910.55 mg/L), Ca (466.68 mg/L) and Mg (248.88 mg/L) were the most abundant mineral elements in Indian small grey DM and Zn (28.66 mg/L) and Fe (3.74 mg/L) showed lower amount. Result of Garhwal et al. (2023) revealed that contents of Ca, K, P, Na, Mg and Zn of Halari DM were 612.5, 473.9, 329.9, 138.4, 72.7 and 18.3 mg/L, respectively. The study of Malacarne et al. (2019) demonstrated that most abundant element in DM was K (1102.7 mg/kg), followed by Ca (543.6 mg/kg), Na (437.7 mg/kg), P (434.4 mg/kg), Fe (2.29 mg/kg) and Zn (2.24 mg/kg). Fantuz et al. (2020) evaluated the concentrations of macroelements in Italy DM and found that the concentrations of Ca, K, P, Na and Mg were 793.8, 708.5, 485.8, 143.6 and 78.0, respectively. The results of Fantuz et al. (2022) showed that the concentrations of Zn and Se in DM were 2.730 mg/L and 4.13 μg/L, respectively. In this study, the average contents of K, P, Na, Ca, Mg, Zn and Se in Dezhou DM were 675.68 mg/kg, 581.56 mg/kg, 138.83 mg/kg, 793.52 mg/kg, 76.78 mg/kg, 2.64 mg/kg and 12.50 μg/kg, respectively. In summary, although the mineral content varies numerically due to differences in breed and breeding environment, the macroelements in DM mainly include K, P, Na, Ca, Mg and the main microelements include Zn and Se.

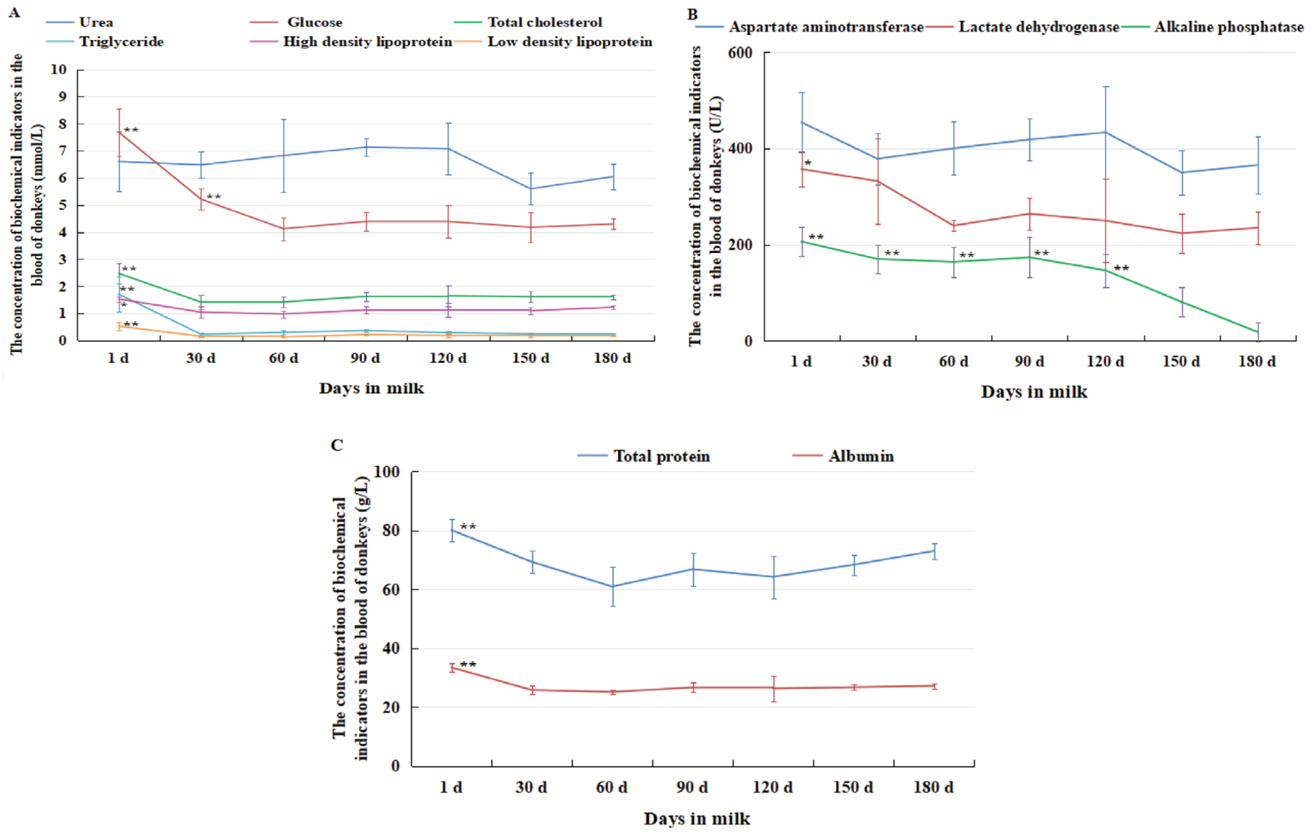

FIGURE 2 | The concentration of biochemical indicators (mean ± SD) in the blood of donkeys at different lactation stages. Statistical probability for comparisons: *, $p < 0.05$; **, $p < 0.01$.

TABLE 7 | The correlation analysis between milk yield or milk composition and blood biochemical indicators.

Blood biochemical indicators	Milk yield	Milk composition						
		Fat	Protein	Lactose	Solids	Solids non fat	Urea	Lactoferrin
Urea	0.65	0.06	0.08	−0.05	0.08	0.09	0.58	−0.10
Glucose	0.39	0.98**	0.97**	−0.98**	0.98**	0.97**	0.07	−0.96**
Aspartate aminotransferase	0.46	0.54	0.64	−0.59	0.64	0.65	0.37	−0.56
Lactate dehydrogenase	0.59	0.90**	0.76*	−0.79*	0.79*	0.76*	0.26	−0.87*
Alkaline phosphatase	0.88*	0.52	0.51	−0.51	0.52	0.52	0.82*	−0.51
Total protein	−0.61	0.78*	0.78*	−0.79*	0.78*	0.77*	−0.39	−0.85*
Albumin	−0.85*	0.86*	0.96**	−0.94**	0.95**	0.96**	−0.13	−0.90**
Total cholesterol	−0.74	0.81*	0.95**	−0.92**	0.94**	0.96**	−0.07	−0.86*
Triglyceride	0.40	0.91**	0.99**	−0.98**	0.99**	1.00**	0.09	−0.91**
High density lipoprotein	−0.79	0.79*	0.88**	−0.86*	0.88**	0.89**	−0.29	−0.85*
Low density lipoprotein	−0.54	0.87*	0.97**	−0.95**	0.96**	0.97**	0.01	−0.92**

Statistical probability for comparisons: *, $p < 0.05$; **, $p < 0.01$.

The mineral content in milk is significantly affected by the lactation stage. Malacarne et al. (2019) studied the effect of lactation on Ragusano DM mineral composition and found that the content of Ca, P and Mg changed significantly with lactation. Results of this study displayed that the mineral elements content in Dezhou DM decreased with the prolongation of lactation period. Compared to the mature milk, concentrations of Na, K, Mg, Zn, Se and Fe in colostrum were significantly higher.

4.4 | Relationship Between Milk Composition and Blood Hormones and Biochemical Parameters

Lactation is an important physiological period that affects the production performance of lactating donkeys. Understanding the biochemical processes and physiological status of lactating donkeys is beneficial for evaluating their nutritional needs (Bonelli et al. 2016). In our study, the blood PRL levels in early lactation (1–90 DIM) were higher than those in late lactation stage (120–180 DIM). The Pr level significantly decreased during 1–30 DIM. The concentration of E2 in the blood first increased and then decreased. The blood IGF–1 was also detected and its level decreased with the prolongation of lactation. It has been found that endocrine changes occur at calving: a reduction in blood Pr level and an increment in E2 and PRL (Bruckmaier and Gross 2017; Lacasse et al. 2016). Our conclusion is consistent with the above research. Estradiol can promote the development of mammary gland ducts and PRL initiates and maintains the lactation (Lacasse et al. 2016). IGF–1 is another extremely important regulator of mammary gland development and lactation. In this study, the correlation analysis found that milk yield was positively correlated with PRL and IGF–1.

Blood biochemical parameters can evaluate the metabolic status of dairy animals and provide reference for the nutrient and energy requirement; high blood GLU level can provide sufficient energy for parturition and early lactation (Afzal et al. 2022). The process of gluconeogenesis caused by endocrine changes after parturition can rapidly increase blood GLU level (Bell and Bauman 1997). In this study, the GLU content and LDH activity in donkey blood were highest on the day of parturition, then decreased and remained constant. The above results indicated that parturition and lactation initiation require a significant amount of energy. There were similar changes in blood lipids, such as TG and TC. The blood lipids also provide energy for dairy animals and promote the synthesis of milk fat (Buryakov et al. 2023). After parturition, blood protein level increases to meet the demand for production of immunoglobulin (Afzal et al. 2022). In this study, blood content of TP and ALB on 1 DIM were significantly higher than other lactation stages, consistent with the highest protein content in colostrum. The ALP activity was also detected. It is reported that the ALP activity can reflect the liver function of dairy cows and is also related to the deposition of Ca and P in animal bones (He et al. 2020). In this study, ALP activity in donkey blood was significantly higher in the early and middle lactation stages than in the late stage, indicating a decrease in liver function and an aggravation of bone Ca and P loss in the late lactation stage. In summary, the changes of the above blood biochemical indicators were similar to those in milk compositions during lactation. The correlation analysis found that the milk fat, protein, solids and SNF were positively correlated with blood GLU, LDH, TP, ALB, TC, TG, HDL and LDL.

5 | Conclusion

In conclusion, the Dezhou donkey milk is characterised by high lactose, low solids and protein and extremely low fat. The milk yield first increased and then gradually decreased, with the peak milk production period of 30–60 DIM. The milk fat, protein, solids and SNF gradually decreased during the lactation. The blood serum PRL, IGF-1, GLU, LDH, TP, ALB, TC, TG, HDL and LDL were higher in early lactation stage and gradually decreased, similar to the changes in milk compositions during lactation. The milk yield was positively correlated with PRL and IGF-1 and the milk fat, protein, solids and SNF were positively correlated with blood GLU, LDH, TP, ALB, TC, TG, HDL and LDL. This is the first study on the dynamic changes in milk, blood metabolic indicators and hormones during lactation of Dezhou donkey, which may contribute to the exploration of the nutritional value of donkey milk and provide theoretical support for the nutritional regulation of donkey milk and donkey breeding.

Author Contributions

Miaomiao Zhou: Conceptualisation, writing—original draft, funding acquisition; **Zongjie Ma**: Investigation, visualisation; **Xinyi Du**: Methodology, investigation, data curation; **Guiqin Liu and Changfa Wang**: Funding acquisition, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

This research was funded by the Key Technology Research and Development Program of Shandong (2021TZXD012), the Shandong Provincial Natural Science Foundation (ZR2023MC149), the National Key R & D Program of China (2022YFD1600103) and the Open Project of Liaocheng University Animal Husbandry Discipline (319312105-5).

Conflicts of Interest

The authors declare no conflicts of interest.

Institutional Review Board Statement

The study was approved by the Animal Care and Use Committee of Liaocheng University (Shandong, China) (2022111002).

Data Availability Statement

The original contributions presented in the study are included in the article and supplementary material, further inquiries can be directed to the corresponding author.

References

Adduci, F., H. S. Elshafie, C. Labella, et al. 2019. “Abatement of the Clostridial Load in the Teats of Lactating Cows With Lysozyme Derived From Donkey Milk.” *Journal of Dairy Science* 102: 6750–6755. https://doi.org/10.3168/jds.2019-16311.

Afzal, A., T. Hussain, A. Hameed, M. Shahzad, M. U. Mazhar, and G. Yang. 2022. “Dietary *Moringa Oleifera* Alters Periparturient Plasma and Milk Biochemical Indicators and Promotes Productive Performance in Goats.” *Frontiers in Veterinary Science* 8: 787719. https://doi.org/10.3389/fvets.2021.787719.

Andjelić, B., R. Djoković, M. Cincović, et al. 2022. “Relationships Between Milk and Blood Biochemical Parameters and Metabolic Status in Dairy Cows During Lactation.” *Metabolites* 12: 733. https://doi.org/10.3390/metabo12080733.

Bell, A. W., and D. E Bauman. 1997. “Adaptations of Glucose Metabolism During Pregnancy and Lactation.” *Journal of Mammary Gland Biology and Neoplasia* 2: 265–278. https://doi.org/10.1023/a:1026336505343.

Bonelli, F., A. Rota, M. Corazza, D. Serio, and M. Sgorbini. 2016. “Hematological and Biochemical Findings in Pregnant, Postfoaling, and Lactating Jennies.” *Theriogenology* 85: 1233–1238. https://doi.org/10.1016/j.theriogenology.2015.12.004.

Bruckmaier, R. M., and J. J. Gross. 2017. “Lactational Challenges in Transition Dairy Cows.” *Animal Production Science* 57: 1471.

Buryakov, N. P., L. V. Sycheva, V. I. Trukhachev, et al. 2023. “Role of Dietary Inclusion of Phytobiotics and Mineral Adsorbent Combination on Dairy Cows’ Milk Production, Nutrient Digestibility, Nitrogen Utilization, and Biochemical Parameters.” *Veterinary Science* 10, no. 3: 238. https://doi.org/10.3390/vetsci10030238.

Buryakov, N., D. Aleshin, M. Buryakova, et al. 2022. "Productive Performance and Blood Biochemical Parameters of Dairy Cows Fed Different Levels of High-Protein Concentrate." *Frontiers in Veterinary Science* 11, no. 9: 852240. https://doi.org/10.3389/fvets.2022.852240.

Cimmino, F., A. Catapano, I. Villano, et al. 2022. "Invited Review: Human, Cow, and Donkey Milk Comparison: Focus on Metabolic Effects." *Journal of Dairy Science* 106: 3072–3085. https://doi.org/10.3168/jds.2022-22465.

Cosentino, C., D. Faraone, R. Paolino, P. Freschi, and M. Musto. 2016. "Short Communication: Sensory Profile and Acceptability of a Cow Milk Cheese Manufactured by Adding Jenny Milk." *Journal of Dairy Science* 99: 228–233. https://doi.org/10.3168/jds.2015-10107.

Cozzi, G., L. Ravarotto, F. Gottardo, et al. 2011. "Short Communication: Reference Values for Blood Parameters in Holstein Dairy Cows: Effects of Parity, Stage of Lactation, and Season of Production." *Journal of Dairy Science* 94, no. 8: 3895–3901. https://doi.org/10.3168/jds.2010-3687.

De Oliveira Moura Araújo, E., E. C. Gavioli, V. A. D. Holanda, et al. 2023. "Repeated Donkey Milk Consumption Reduces Anxiety-Like Behaviors and Brain Oxidative Damage to Lipids in Mice." *Behavioural Brain Research* 449: 114477. https://doi.org/10.1016/j.bbr.2023.114477.

Fantuz, F., S. Ferraro, L. Todini, et al. 2020. "Distribution of Calcium, Phosphorus, Sulfur, Magnesium, Potassium, and Sodium in Major Fractions of Donkey Milk." *Journal of Dairy Science* 103: 8741–8749. https://doi.org/10.3168/jds.2020-18251.

Fantuz, F., S. Ferraro, L. Todini, et al. 2022. "Distribution of Selected Trace Elements in the Major Fractions of Donkey Milk." *Journal of Dairy Science* 105: 6422–6430. https://doi.org/10.3168/jds.2022-21901.

Garhwal, R., A. Bhardwaj, K. Sangwan, et al. 2023. "Milk From Halari Donkey Breed: Nutritional Analysis, Vitamins, Minerals, and Amino Acids Profiling." *Foods* 12, no. 4: 853. https://doi.org/10.3390/foods12040853.

Guo, H. Y., K. Pang, X. Y. Zhang, et al. 2007. "Composition, Physiochemical Properties, Nitrogen Fraction Distribution, and Amino Acid Profile of Donkey Milk." *Journal of Dairy Science* 90: 1635–1643. https://doi.org/10.3168/jds.2006-600.

He, Y., R. Zhong, L. Cheng, P. You, Y. Li, and X. Sun. 2020. "Effects of the Supplementation of Lysophospholipids Through Pelleted Total Mixed Rations on Blood BioChemical Parameters and Milk Production and Composition of Mid-Lactation Dairy Cows." *Animals (Basel)* 10, no. 2: 215. https://doi.org/10.3390/ani10020215.

Huang, F., X. Du, Z. Ma, et al. 2004. "Effects of Methionine on Milk Performance and Milk Constituents of Lactating Donkeys." *Animals* 24, no. 14: 3027. https://doi.org/10.3390/ani14203027.

Lacasse, P., S. Ollier, V. Lollivier, and M. Boutinaud. 2016. "New Insights Into the Importance of Prolactin in Dairy Ruminants." *Journal of Dairy Science* 99: 864–874. https://doi.org/10.3168/jds.2015-10035.

Laus, F., L. Laghi, M. Bazzano, et al. 2023. "Donkey Colostrum and Milk: How Dietary Probiotics Can Affect Metabolomic Profile, Alkaline Sphingomyelinase and Alkaline Phosphatase Activity." *Metabolites* 13, no. 5: 622. https://doi.org/10.3390/metabo13050622.

Li, M., W. Li, J. Wu, et al. 2020. "Quantitative Lipidomics Reveals Alterations in Donkey Milk Lipids According to Lactation." *Food Chemistry* 310: 125866. https://doi.org/10.1016/j.foodchem.2019.125866.

Li, M., Y. Liu, Q. Li, et al. 2020. "Comparative Exploration of Free Fatty Acids in Donkey Colostrum and Mature Milk Based on a Metabolomics Approach." *Journal of Dairy Science* 103: 6022–6031. https://doi.org/10.3168/jds.2019-17720.

Li, Y., Q. Ma, M. Zhou, et al. 2022. "A Metabolomics Comparison in Milk From Two Dezhou Donkey Strains." *European Food Research and Technology* 248: 1267–1275.

Malacarne, M., A. Criscione, P. Franceschi, et al. 2019. "New Insights Into Chemical and Mineral Composition of Donkey Milk Throughout Nine Months of Lactation." *Animals (Basel)* 9, no. 12: 1161. https://doi.org/10.3390/ani9121161.

Martemucci, G., and A. G D'Alessandro. 2012. "Fat Content, Energy Value and Fatty Acid Profile of Donkey Milk During Lactation and Implications for Human Nutrition." *Lipids in Health and Disease* 11: 113. https://doi.org/10.1186/1476-511X-11-113.

Martini, M., I. Altomonte, R. Licitra, and F. Salari. 2018. "Short Communication: Technological and Seasonal Variations of Vitamin D and Other Nutritional Components in Donkey Milk." *Journal of Dairy Science* 101: 8721–8725. https://doi.org/10.3168/jds.2018-14776.

Martini, M., I. Altomonte, F. Salari, and A. M Caroli. 2014. "Short Communication: Monitoring Nutritional Quality of Amiata Donkey Milk: Effects of Lactation and Productive Season." *Journal of Dairy Science* 97: 6819–6822. https://doi.org/10.3168/jds.2014-8544.

Messias, T., S. P. Alves, R. J. B. Bessa, M. S. Madruga, M. T. B. Pacheco, and R. Queiroga. 2021. "Fatty Acid Profile of Milk From Nordestina Donkey Breed Raised on Caatinga Pasture." *Journal of Dairy Research* 88, no. 2: 205–209. https://doi.org/10.1017/S0022029921000388.

Nayak, C. M., C. T. Ramachandra, U. Nidoni, S. Hiregoudar, J. Ram, and N. Naik. 2020. "Physico-Chemical Composition, Minerals, Vitamins, Amino Acids, Fatty Acid Profile and Sensory Evaluation of Donkey Milk From Indian Small Grey Breed." *Journal of Food Science and Technology* 57, no. 8: 2967–2974. https://doi.org/10.1007/s13197-020-04329-1.

Ning, J., M. Yang, W. Liu, X. Luo, and X. Yue. 2023. "Proteomics and Peptidomics as a Tool to Compare the Proteins and Endogenous Peptides in Human, Cow, and Donkey Milk." *Journal of Agricultural and Food Chemistry* 71, no. 44: 16435–16451. https://doi.org/10.1021/acs.jafc.3c04534.

Papademas, P., E. Kamilari, M. Aspri, et al. 2021. "Investigation of Donkey Milk Bacterial Diversity by 16S rDNA High-Throughput Sequencing on a Cyprus Donkey Farm." *Journal of Dairy Science* 104: 167–178. https://doi.org/10.3168/jds.2020-19242.

Parastouei, K., M. Jabbari, F. Javanmardi, et al. 2023. "Estimation of Bioactive Peptide Content of Milk From Different Species Using an in Silico Method." *Amino Acids* 55, no. 10: 1261–1278. https://doi.org/10.1007/s00726-022-03152-6.

Raspa, F., L. Cavallarin, A. K. McLean, D. Bergero, and E. Valle. 2019. "A Review of the Appropriate Nutrition Welfare Criteria of Dairy Donkeys: Nutritional Requirements, Farm Management Requirements and Animal-Based Indicators." *Animals (Basel)* 9: 315. https://doi.org/10.3390/ani9060315.

Salari, F., R. Licitra, I. Altomonte, and M. Martini. 2020. "Donkey Feeding During Maintenance, Pregnancy, and Lactation: Effects on Body Weight, Milk Production, and Foal Growth." *Journal of Equine Veterinary Science* 91: 103131. https://doi.org/10.1016/j.jevs.2020.103131.

Salimei, E., F. Fantuz, R. Coppola, B. Chiofalo, and G. Varisco. 2004. "Composition and Characteristics of Ass's Milk." *Animal Research* 53: 67–78.

Spada, V., P. Ferranti, L. Chianese, E. Salimei, F. Addeo, and G. Picariello. 2021. "Antibacterial Potential of Donkey's Milk Disclosed by Untargeted Proteomics." *Journal of Proteomics* 231: 104007. https://doi.org/10.1016/j.jprot.2020.104007.

Vincenzetti, S., G. Santini, V. Polzonetti, S. Pucciarelli, Y. Klimanova, and P. Polidori. 2021. "Vitamins in Human and Donkey Milk: Functional and Nutritional Role." *Nutrients* 13: 1509. https://doi.org/10.3390/nu13051509.

Zhang, X., H. Li, J. Yu, et al. 2019. "Label-free Based Comparative Proteomic Analysis of Whey Proteins Between Different Milk Yields of Dezhou Donkey." *Biochemical and Biophysical Research Communications* 508: 237e242. https://doi.org/10.1016/j.bbrc.2018.11.130.

Zhou, M., F. Huang, X. Du, G. Liu, and C. Wang. 2023. "Analysis of the Differentially Expressed Proteins in Donkey Milk in Different Lactation Stages." *Foods* 12, no. 24: 4466. https://doi.org/10.3390/foods12244466.

Zhou, M., F. Huang, X. Du, et al. 2024. "Fermented *Codonopsis Pilosula* Residue Improved Milk Performance of Lactating Donkeys by Enhancing Antioxidant Capacity and Regulating Metabolism." *Frontiers in Veterinary Science* 11: 1489480. https://doi.org/10.3389/fvets.2024.1489480.

Živkov Baloš, M., D. Ljubojević Pelić, S. Jakšić, and S. Lazić. 2023. "Donkey Milk: An Overview of Its Chemical Composition and Main Nutritional Properties or Human Health Benefit Properties." *Journal of Equine Veterinary Science* 121: 104225. https://doi.org/10.1016/j.jevs.2023.104225.