Comparative analysis of haematological, biochemical and nutritional parameters of Dezhou donkey with healthy and weak foals (Equus asinus)

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ABSTRACT
The study was conducted to compare the haematological, biochemical and nutritional parameters between healthy foals (HF) and weak foals (WF, malformation, lameness and drooping ears), and between the corresponding female donkeys to provide a reference for the assessment of healthy status. A total of 34 donkeys with HF (DHF) and 34 with WF (DWF) were selected. Compared to HF, WF had higher leukocyte, neutrophil, platelets (PLT) and the percentage of neutrophil, and their corresponding female donkeys showed the similar tendency; WF had lower birth weight and body measurements. The concentrations of serum urea, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low-density lipoprotein cholesterol, β-hydroxybutyric acid (BHBA) and non-esterified fatty acid (NEFA) were increased in WF compared with HF, whereas serum glucose (GLU) was decreased. The concentrations of serum urea, total protein, AST, alkaline phosphatase (ALP), BHBA, NEFA, calcium and phosphorus were increased in DWF compared with DHF, whereas serum albumin (ALB), GLU, kalium, chromium, iron, manganese, lead and zinc were decreased. The hair concentrations of minerals had the same tendency as well, but calcium concentration of hair was opposite. There were significant correlations of kalium, copper, iron and zinc contents between serum and hair. In plasma, the contents of threonine, glutamic acid, alanine, arginine in DWF were greater than DHF, whereas valine content was lower. In conclusion, there were significant differences in some haematological, biochemical and nutritional parameters, which suggested that the malnutrition of the corresponding female donkeys was probably responsible for malformation, lameness and drooping ears of foals.

HIGHLIGHTS
- Weak foals (WF) had higher leukocyte and neutrophil count, platelets (PLT) and the percentage of neutrophil than healthy foals (HF), and their corresponding female donkeys showed the similar tendency.
- WF had higher concentrations than HF in serum urea, total protein, AST, alkaline phosphatase (ALP), β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), but had lower concentration in serum glucose (GLU), and their corresponding female donkeys showed the similar tendency.
- Donkeys with WF had higher concentrations of threonine, glutamic acid, alanine, arginine in plasma than donkeys with HF, but lower concentrations of valine in plasma and some mineral elements in serum and hair.

Introduction
Donkeys (Equus asinus) are counted as close companions to humans and used as working animals mainly in transportation of goods and agricultural produce for a long time (Kimura et al. 2011). Today donkeys are still play an essential role of transport for people living in many mountainous and poor regions of the world (Smith and Pearson 2005). But donkeys are increasingly considered as economic animals. Donkey meat possesses high nutritional value, and donkey-hide is the raw materials for making colla Corii Asini (donkey glue) which is a traditional Chinese medicine (Kumeta et al. 2014). In addition, donkey milk is much more similar to human milk than cow’s milk (Martini...
et al. 2018) and it is highly tolerated by infants with protein allergies (Salimei and Fantuz 2012). However, donkeys have a long reproductive cycle because of the gestation period with averaging 12 months (Tosi et al. 2013), and the special physiological structure without milk cistern of the mammary glands result in the lower milk performance in donkeys compared with dairy cows (D’Alessandro and Martemucc 2012). At present, there is scarce study on the nutrient requirement of donkeys and the feeding and management of donkeys are offending. The phenomena of malformation, lameness, drooping ears and limb atony often occur in newborn foals (Lempe et al. 2012; Carli 2006; Smith 2010), which has profound and negative implications on the foal growth development in many donkey farms. We made an investigation on the incidence of weak newborn foals with malformation, lameness and drooping ears, and found the incidence exceeded 10%, and these foals, in most cases, would be eliminated, which affected the reproduction of donkeys and decreased economic benefit of donkey feeding. The probable reason is related to malnutrition of female donkeys during pregnant period (Janardhan et al. 2020). Therefore, evaluating the nutritional status of female donkeys reasonably carry major implications for safeguarding the health of female donkeys and foals and improving the reproductive performance, milk performance of donkeys and growth performance of foals. But little data are available.

Biochemical profile and haematological parameters are useful means to evaluate health, nutritional and physiological status of equine (Bonelli et al. 2016; Zakari et al. 2015). Many studies have shown a marked change for biochemical and haematological parameters in horses or donkeys while suffering from malnutrition or developing a disease (Oikawa and Yamaoka 2003; Dixon et al. 1983; Muñoz et al. 2010; Gupta et al. 1999). The leukocyte (WBC), platelet (PLT) count and alkaline phosphatase (ALP) contents were increased, and glucose (GLU) content were deceased in horse with endotoxemia (Oikawa and Yamaoka 2003). Dixon et al. (1983) found the WBC count, aspartate aminotransferase (AST) and blood urea content were elevated in donkey foal with steatitis and myonecrosis compared with values for healthy donkey. In a study on horses with malnutrition, increased neutrophils (NEU) count and decreased serum triglycerides (TGs) concentration has been reported (Muñoz et al. 2010). Gupta et al. (1999) indicated feed deprivation resulted in the increase of serum urea and decrease of GLU in equids. Geor (2000) stated that energy deficits are probably greater in foals with more severe illnesses as a result of increased metabolic demands. The donkey with liver disease needs a low protein diet to decrease intestinal ammonia production (Burden and Bell 2019), and needs to be offered soluble carbohydrates that are easily utilised by the donkey and will decrease the demand for hepatic gluconeogenesis (MacLeod and Shellim 2008). Little information is available on biochemical and haematological parameters of healthy and weak donkey foals with malformation, lameness and drooping ears at present, especially the information on minerals and amino acids (AA) concentration in blood is much less.

There are 50.6 million donkeys in the world, and China has a large donkey population with 5.14% of them (from FAO’s corporate database, 2019). Dezhou donkey is one of the five biggest native donkey breeds in north of China. Therefore, the purpose of this study was to compare the haematological, biochemical, nutritional parameters between healthy foals and weak foals (WF), and between the corresponding female donkeys to provide a reference for the assessment of healthy status.

Materials and methods

The experiment was conducted in the experimental farm of Inner Mongolia Agricultural University (Hohhot, China). All procedures were approved by the Technical Committee for Laboratory Animal Sciences of the Standardisation Administration of China (SAC/TC281), and performed under the national standard Guidelines for Ethical Review of Animal Welfare (GB/T 35892-2018).

Animals

Thirty-four female Dezhou donkeys (average age, 7.06 ± 0.86; parity, 3.97 ± 0.90; gestational time, 368.60 ± 7.92) with healthy foals (HF) and thirty-four female Dezhou donkeys (average age, 6.85 ± 0.78; parity, 3.76 ± 0.78; gestational time, 366.80 ± 10.45) with WF from the same farm located in Hohhot (Inner Mongolia, China) were selected to collect blood and hair samples, respectively. The foals in abnormal symptoms: malformation, lameness and drooping ears were considered as WF, and the corresponding female donkeys were also assigned to the same group and marked as DWF (donkeys with WF). The foals in good spirit without abnormal symptoms above were assigned to HF group, and the corresponding female donkeys were also assigned to the same group and marked as donkeys with HF (DHF). The foals from HF
and WF group had the same genetic background of paternity. There were eighteen male foals and sixteen female foals in WF group, and HF group had the same sex ratio. All female donkeys were lived together in same paddock and transferred to delivery pen one week before birthing, by the special care. The female donkeys were offered the same diet in the whole gestation period, and the dietary composition and nutrient level was shown in Table 1. The concentrate, silage and alfalfa were mixed to feed donkeys twice daily at 0700 and 1400 h, respectively. Millet straw was offered ad libitum. The female donkeys were vaccinated anthrax vaccine, tetanus toxoid and combined braxy, struck and enterotoxaemia vaccine, and expelled parasite by injecting ivermectin according to obstetric history prior to this delivery, and all the donkeys were offered the same diet in the whole gestation period, and the dietary composition and nutrient level was shown in Table 1. The concentrate, silage and alfalfa were mixed to feed donkeys twice daily at 0700 and 1400 h, respectively. Millet straw was added three times at 1000, 1500 and 2000 h. Water was provided ad libitum. The female donkeys were vaccinated anthrax vaccine, tetanus toxoid and combined braxy, struck and enterotoxaemia vaccine, and expelled parasite by injecting ivermectin according to normal procedure. All the female donkeys with no bad obstetric history prior to this delivery, and all the donkey foals were delivered naturally. The donkey farm is located in Horinger (a county in Hohhot city) which is located at 111.8° east longitude and 40.4° north latitude. The study took place from April to May, and there was a suitable environment with a mild climate during the experimental period, and the mean temperature was about 16 °C.

### Sample collection

All samples including serum, plasma and hair were collected within postpartum 24 h. The jugular blood was collected in EDTA-containing vacutainer tubes for haematological determination and plasma separation, and in vacutainer tubes without anticoagulant for serum extraction after parturition. Plasma and serum were harvested by centrifugation at 2500×g for 10 min after standing 1 h at room temperature. Immediately after subpackage, the samples were stored at −20 °C prior to analysis. Hair samples were collected from withers using a stainless steel scissors in a quantity of not less than 1 g (Miroshniko et al. 2015).

### Chemical analyses

#### Analysis of haematological parameters

All haematologic parameters were analysed in an automated blood cell analyser (ADVIA 2120, Siemens Healthcare Diagnostic, Germany). The haematological parameters: white blood cell (WBC), and WBC differential count for NEU, lymphocytes (LYMPH), monocytes (MONO) as percentage as absolute count (n− cell 109/L); and red blood cell (RBC) count, haemoglobin concentration (HGB), haematocrit value (HCT), PLT count and neutrophil–lymphocyte ratio (NLR).

#### Analysis of biochemical parameters

All the biochemical profiles were carried on by using a biochemical autoanalyzer (HITACHI 7150, Hitachi Limited, Tokyo, Japan) with dedicated reagent kits according to standard methods. The biochemistry panel included the following parameters: AST, alanine aminotransferase (ALT), ALP, total serum protein (TP), albumin (ALB), TG, creatinine (CREA), total cholesterol (TC), urea, GLU, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), β-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA).

### Measurement of minerals

The collected hair samples were steeped in acetone for 10–15 min and then rinsed thrice in ultrapure water. After that, the samples were dried to constant mass at 65 °C and chopped for minerals analysis. Appropriate 0.05 g of hair and 1 mL serum samples were digested with 10 mL of mixture of nitric acid and perchloric acid (HNO3: HCLO4 = 2:1) in small beakers, which were allowed to react slowly at room temperature to prevent excessive foaming. Then, the digestion was heated to 200 °C on a hot plate and continued until the mixture was water-clear and less than 1 mL of solution remained. Each digested solution was quantitatively filtered into graduated test tube with rinsing wall of the breaker thrice by ultrapure water, and the liquid was diluted to 4 mL for trace elements.

### Table 1. The diet composition and nutrient level (air-dry basis, %).

| Composition            | Content (%) | Nutritional level |
|------------------------|-------------|-------------------|
| Millet straw           | 71.81       | DE (MJ/kg)b 9.60  |
| Alfalfa                | 1.99        | CP 9.50           |
| Corn silage            | 2.09        | EE 2.75           |
| Corn                   | 10.89       | NDF 51.97         |
| Soybean meal           | 7.17        | ADF 30.11         |
| Corn gluten meal       | 0.40        | Ca 1.06           |
| Corn germ meal         | 0.40        | P 0.29            |
| Distillers dried grains| 1.99        | –                 |
| Bran                   | 0.80        | –                 |
| Extruded full-fat soybean | 0.60   | –                 |
| CaCO3                  | 0.33        | –                 |
| CaHPO4                 | 0.66        | –                 |
| NaCl                   | 0.37        | –                 |
| Premixa                 | 0.50        | –                 |
| Total                   | 100.00      | –                 |

*Provided per kg of premix: VA 300,000 IU, VD 62,500 IU, VE 1500 IU, Fe 2.0 g, Cu 1.6 g, Zn 6.0 g, Mn 6.0 g, I 72 mg, Se 30 mg, Co 50 mg.

Digestible energy is calculated based on the ingredients of the diet and their digestible energy content, not based on the actual dry matter intake. DE, digestible energy; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; Ca, calcium; P, phosphate.
After dilution 400 times, the solution was used to analyse major elements.

Then, the minerals of serum and hair samples were performed according to standard procedures, using an inductively coupled plasma emission spectrometer (ICAP 6300Duo, Thermo Scientific, Waltham, MA). The determining elements included calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), cadmium (Cd), Cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), Manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se) and zinc (Zn).

### Measurement of amino acids

An aliquot of 1 mL of plasma samples adding 1 mL 8% sulphosalicylic acid was centrifuged at 10,000×g for 10 min after stable over night at 4 °C, and the supernatant was filtered into autosampler vials through hydrophobic membrane with the pore diameter of 0.22 μm. Then, the plasma amino acid profile was determined by automatic amino-acid analyser (Hitachi, Tokyo, Japan) with specified reagent kits according to standard procedures. The determining AA included aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), cysteine (Cys), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg) and proline (Pro).

### Statistical analysis

Statistical analyses were performed by using SAS software version 9.3 (SAS Institute Inc., Cary, NC). All the data were analysed by one-way analysis of variance (ANOVA) procedure. Variability in the data was expressed as the standard error of means. Differences were considered significant if \( p \leq 0.05 \). The Duncan’s Multiple Range Test was employed to gauge differences between the treatments. A parametric (Pearson) correlation for minerals in serum and hair were analysed using standard statistical methods. Correlations with \( p \leq 0.05 \) were considered as significant.

### Results

#### Birth weight and body measurement of foals

Compared with HF, birth weight \((p < 0.001)\), withers height \((p < 0.01)\), body length \((p < 0.01)\), chest circumference \((p < 0.05)\) and cannon circumference \((p < 0.01)\) of WF were significantly decreased (Table 2).

### Haematological parameters

The DWF and WF had a higher WBC, NEU, PLT, NLR and percentage of NEU than DHF (Table 3) and HF (Table 4), respectively \((p < 0.05)\). Other haematological parameters of DHF and DWF had no significant differences \((p > 0.05)\), with the same results on HF and WF.

### Serum biochemical parameters

Concentrations of TP \((p < 0.05)\), AST \((p < 0.01)\), ALP \((p < 0.01)\), UREA \((p < 0.01)\), BHBA \((p < 0.05)\) and NEFA \((p < 0.01)\) in serum of DWF were higher than those of DHF (Table 5). Concentrations of ALB \((p < 0.05)\) and GLU \((p < 0.05)\) in serum and ALB-total protein ratio \((p < 0.01)\) of DWF were lower than those of DHF \((p < 0.05)\). Concentrations of UREA \((p < 0.01)\), TP \((p < 0.05)\), ALT \((p < 0.01)\), AST \((p < 0.05)\), TC \((p < 0.05)\), LDL-C \((p < 0.01)\), BHBA \((p < 0.01)\) and NEFA \((p < 0.01)\) in serum of WF were higher than those of HF (Table 6). Serum GLU concentration and ALB-total protein ratio of WF was lower than that of HF \((p < 0.01)\).

### Minerals of serum and hair

Table 7 presents the results of serum mineral concentration in donkeys of DHF and DWF groups. DHF had
Table 4. The haematological parameters of foals in HF and WF.

| Items       | DHF | WF | SEM | p Value |
|-------------|-----|----|-----|---------|
| WBC (× 10^9/L) | 5.64^b | 6.78^a | 0.257 | 0.038 |
| LYMPH (× 10^9/L) | 1.37 | 1.56 | 0.055 | 0.179 |
| MONO (× 10^9/L) | 0.26 | 0.30 | 0.022 | 0.510 |
| NEU (× 10^9/L) | 3.19^b | 4.94^a | 0.219 | 0.004 |
| PLT (× 10^9/L) | 2.36^b | 3.27^a | 0.202 | 0.005 |
| LYMPH (%) | 26.75 | 22.89 | 1.480 | 0.302 |
| MONO (%) | 4.66 | 4.00 | 0.171 | 0.319 |
| NEU (%) | 63.50^b | 73.11^a | 1.103 | 0.010 |
| RBC (× 10^12)/L | 8.26 | 8.48 | 0.849 | 0.772 |
| HGB (g/L) | 147.80 | 141.10 | 4.479 | 0.545 |
| HCT (%) | 47.04 | 45.37 | 1.528 | 0.657 |
| NEU (%) | 63.50^b | 73.11^a | 1.103 | 0.010 |

*Means within a row with different letters differ significantly (p < 0.05).

HF: healthy foals; WF: weak foals; WBC: white blood cell; NEU: neutrophils; LYMPH: lymphocytes; MONO: monocytes; RBC: red blood cell count; HGB: haemoglobin concentration; HCT: haematocrit value; PLT: platelet count; NEU/LYMPH: SEM: standard error of the mean

Table 5. The serum biochemical parameters of donkeys in DHF and DWF.

| Items       | DHF | DWF | SEM | p Value |
|-------------|-----|-----|-----|---------|
| ALT (U/L) | 194.08 | 258.76 | 7.980 | 0.002 |
| AST (U/L) | 18.12 | 17.25 | 0.720 | 0.461 |
| TP (g/L) | 47.04 | 45.37 | 1.528 | 0.657 |
| ALB (g/L) | 32.94^a | 30.82^b | 0.691 | 0.038 |
| GLU (mmol/L) | 5.25^a | 4.28^b | 0.171 | 0.020 |
| TG (mmol/L) | 0.62 | 0.64 | 0.039 | 0.683 |
| UREA (mmol/L) | 14.88 | 15.43 | 0.418 | 0.445 |
| CRE (mmol/L) | 77.86 | 75.99 | 2.580 | 0.001 |
| ALA (mmol/L) | 8.81 | 5.21 | 0.569 | 0.001 |
| K (mg/L) | 93.32 | 72.61 | 7.215 | 0.001 |
| Ca (mg/L) | 133.71 | 9.80 | 1.37/10^9 | 0.001 |
| Mg (mg/L) | 72.61 | 81.15 | 3.922 | 0.132 |
| Fe (mg/L) | 25.64 | 4.66 | 0.248 | 0.009 |
| Mn (mg/L) | 37.11 | 1.37 | 0.602 | 0.001 |
| Cd (mg/L) | 72.61 | 81.15 | 3.922 | 0.132 |
| Zn (mg/L) | 13.01 | 1.37 | 0.602 | 0.001 |
| Cu (mg/L) | 0.15^b | 0.18^a | 0.005 | 0.005 |
| Pb (mg/L) | 0.26^b | 0.59^a | 0.019 | 0.001 |

*Means within a row with different letters differ significantly (p < 0.05).

HF: healthy foals; WF: weak foals; ALT: aspartate aminotransferase; AST: alanine aminotransferase; TP: total serum protein; ALB: albumin; TC: cholesterol; TG: triglyceride; CRE: creatinine; UREA: urea; GLU: glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BHBA: β-hydroxybutyric acid; NEFA: non-esterified fatty acids; SEM: standard error of the mean

Table 6. The serum biochemical parameters of foals in HF and WF.

| Items       | HF | WF | SEM | p Value |
|-------------|----|----|-----|---------|
| ALT (U/L) | 20.88^b | 25.64^a | 0.808 | 0.001 |
| AST (U/L) | 186.33^b | 251.66^a | 17.229 | 0.013 |
| ALP (U/L) | 300.66 | 371.60 | 36.282 | 0.173 |
| TP (g/L) | 51.18^b | 57.45^a | 1.602 | 0.024 |
| ALB (g/L) | 29.42 | 26.13 | 1.167 | 0.082 |
| ALB/TP | 0.58^a | 0.46^b | 0.020 | 0.002 |
| GLU (mmol/L) | 5.65^a | 4.66^b | 0.248 | 0.009 |
| TG (mmol/L) | 0.50 | 0.49 | 0.046 | 0.892 |
| UREA (mmol/L) | 3.57^b | 4.82^a | 0.179 | 0.001 |
| CRE (mmol/L) | 72.61 | 81.15 | 3.922 | 0.132 |
| TC (mmol/L) | 2.76^b | 3.03^a | 0.088 | 0.035 |
| LDL-C (mmol/L) | 1.43 | 1.40 | 0.076 | 0.777 |
| HDL-C (mmol/L) | 2.31 | 2.64 | 0.098 | 0.001 |
| NEFA (mmol/L) | 0.15^b | 0.18^a | 0.005 | 0.005 |
| NEFA (mmol/L) | 0.26^b | 0.59^a | 0.019 | 0.001 |

*Means within a row with different letters differ significantly (p < 0.05).

HF: healthy foals; WF: weak foals; ALT: aspartate aminotransferase; AST: alanine aminotransferase; TP: total serum protein; ALB: albumin; TC: cholesterol; TG: triglyceride; CRE: creatinine; UREA: urea; GLU: glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BHBA: β-hydroxybutyric acid; NEFA: non-esterified fatty acids; SEM: standard error of the mean

Plasma AA measurement

Table 10 shows the values of plasma AA concentrations in donkeys of DHF and DWF. The plasma concentrations of Thr (p < 0.01), Glu (p < 0.01), Ala (p < 0.05), Arg (p < 0.01), LAA (p < 0.05) and FAA (p < 0.01) in DWF were significantly increased compared with DHF, but Val (p < 0.01) and BCAA (p < 0.05) were decreased. There was no difference (p > 0.05) among the rest of AA.

Discussion

The newborn foals with malformation, lameness and drooping ears were more vulnerable in comparison with HF, and were limited in growth and production, which resulted in obsolescence of WF. Little research has been designed to investigate the blood parameters on foals with malformation, lameness and drooping ears.
BHBA (González et al. 2011). The increased NEFA concentration caused reduction of antioxidant and immunologic function, as well as the level of anti-inflammatory factors (Wang et al. 2020). In previous studies, the elevated concentrations of NEFA were shown to impair various immune and anti-inflammatory functions in dairy cows (Contreras and Sordillo 2011; Ospina et al. 2010), and similar metabolic responses may occur in WF. Adewuyi et al. (2005) indicated that increased concentrations of BHBA and NEFA, along with decreased concentrations of GLU in blood plasma also showed metabolic status of animals was impaired. In addition, in the condition of negative energy balance horses can turn to protein catabolism and increase significantly the gluconeogenic pathway from AA, with a consequent increase in ammonium production, converted to UREA for elimination (Henneke et al. 1983). In this study, the higher serum BHBA, NEFA and UREA concentrations and lower GLU concentration showed energy deficits were probably greater in WF and DWF compared with HF and DHF. LDL-C and TC participate in fat metabolism, and the increased concentration indicates the enhancement of lipolysis. These indicators, in this study, were significantly elevated in WF and their corresponding female donkeys, verifying the fact of energy deficit and fat mobilisation in WF and DWF. This may be influenced in part by the diet with low energy level, or alternatively, by feed intake. Ferris et al. (2001) reported feeding dairy cows with low energy diet significantly increased serum NEFA, BHBA and UREA concentrations, and resulted in negative energy balance. These results suggested that there is probably a deficiency

Table 8. The contents of hair minerals of donkeys in DHF and DWF.

| Items          | DHF (mg/kg) | DWF (mg/kg) | SEM   | p Value |
|----------------|-------------|-------------|-------|---------|
| Ca             | 3.95        | 3.59        | 0.108 | 0.057   |
| K              | 1.47<sup>a</sup> | 0.93<sup>b</sup> | 0.067 | <0.001  |
| Mg             | 1.37        | 1.25        | 0.047 | 0.135   |
| P              | 0.49        | 0.48        | 0.010 | 0.351   |
| Fe (mg/kg)     | 133.14<sup>a</sup> | 81.40<sup>b</sup> | 7.330 | 0.002   |
| Cu (mg/kg)     | 6.84        | 6.42        | 0.161 | 0.118   |
| Mn (mg/kg)     | 7.68<sup>a</sup> | 6.01<sup>b</sup> | 0.438 | 0.003   |
| Zn (mg/kg)     | 133.62<sup>a</sup> | 122.09<sup>b</sup> | 2.437 | 0.015   |
| Se (mg/kg)     | 0.53        | 0.52        | 0.024 | 0.777   |
| Mo (mg/kg)     | 0.29        | 0.29        | 0.011 | 0.859   |
| Co (ug/kg)     | 0.07        | 0.08        | 0.004 | 0.073   |
| Cr (mg/kg)     | 3.02<sup>b</sup> | 2.59<sup>b</sup> | 0.007 | 0.001   |
| Cd (mg/kg)     | 0.05        | 0.04        | 0.004 | 0.234   |
| Pb (mg/kg)     | 2.31        | 2.37        | 0.114 | 0.802   |

<sup>a,b</sup>Means within a row with different letters differ significantly (p < 0.05).

Table 9. Correlation analysis of mineral contents between serum and hair of female donkeys.

| Items  | Value | p Value |
|--------|-------|---------|
| Ca     | –0.0636 | 0.713   |
| K      | 0.5204  | 0.002   |
| Mg     | –0.0315 | 0.930   |
| P      | –0.0915 | 0.585   |
| Cd     | –0.3405 | 0.097   |
| Co     | –0.0047 | 0.979   |
| Cr     | 0.1587  | 0.402   |
| Cu     | 0.3890  | 0.019   |
| Fe     | 0.5981  | 0.001   |
| Mn     | 0.2460  | 0.175   |
| Mo     | 0.0396  | 0.842   |
| Pb     | –0.0825 | 0.677   |
| Se     | 0.0211  | 0.907   |
| Zn     | 0.5985  | <0.001  |

<sup>a,b</sup>Means within a row with different letters differ significantly (p < 0.05).

Table 10. The contents of plasma amino acids between donkeys in DHF and DWF, μmol/L.

| Items          | DHF | DWF | SEM | p Value |
|----------------|-----|-----|-----|---------|
| Asp            | 140.47 | 130.27 | 6.414 | 0.224  |
| Thr            | 90.39<sup>b</sup> | 110.06<sup>a</sup> | 3.222 | 0.002  |
| Ser            | 90.36  | 90.82  | 5.568 | 0.555  |
| Glu            | 90.45<sup>b</sup> | 100.82<sup>a</sup> | 3.686 | 0.004  |
| Gly            | 160.70 | 160.39 | 3.792 | 0.604  |
| Ala            | 90.50<sup>b</sup> | 110.23<sup>a</sup> | 5.140 | 0.036  |
| Cys            | 3.52   | 3.32   | 0.218 | 0.350  |
| Val            | 70.76<sup>a</sup> | 40.90<sup>b</sup> | 3.747 | <0.001 |
| Met            | 10.22  | 10.26  | 0.556 | 0.604  |
| Ile            | 20.49  | 20.43  | 1.761 | 0.845  |
| Leu            | 50.34  | 50.48  | 2.982 | 0.773  |
| Tyr            | 30.15  | 30.79  | 2.000 | 0.053  |
| Phe            | 30.48  | 30.44  | 1.258 | 0.851  |
| Lys            | 40.42  | 50.00  | 2.343 | 0.133  |
| His            | 40.01  | 40.02  | 2.499 | 0.990  |
| Arg            | 20.57<sup>b</sup> | 30.77<sup>a</sup> | 2.361 | 0.006  |
| Pro            | 86.99  | 87.09  | 1.589 | 0.966  |
| TAA            | 1110.40 | 1146.31 | 25.635 | 0.365  |
| EAA            | 400.83 | 400.73 | 13.252 | 0.962  |
| NEAA           | 702.13 | 741.29 | 15.689 | 0.109  |
| LAA            | 50.36<sup>b</sup> | 60.22<sup>a</sup> | 2.678 | 0.041  |
| BCAA           | 150.51<sup>a</sup> | 120.96<sup>b</sup> | 6.408 | 0.015  |
| FAA            | 170.25<sup>a</sup> | 200.15<sup>a</sup> | 6.013 | 0.004  |

<sup>a,b</sup>Means within a row with different letters differ significantly (p < 0.05).

Ca: calcium; P: phosphorus; Mg: magnesium; K: potassium; Cd: cadmium; Co: cobalt; Cr: chromium; Cu: copper; Fe: iron; Mn: manganese; Mo: molybdenum; Pb: lead; Se: selenium; Zn: zinc SEM: standard error of the mean.
of dietary energy in this study. However, few research on energy requirement in pregnant donkeys has been reported, and it need to be looked at further.

Maternal nutritional condition can influence offspring body weight by means of metabolic imprinting (Passos et al. 2000). Studies on sow (Campos et al. 2012) and rats (Passos et al. 2000) confirmed that energy deficit and malnutrition during gestation resulted in lower birth weight. Consistent with these studies, the birth weight and body measurement of foals in WF group were decreased, which was probably caused by malnutrition of female donkeys in DWF group. Serum CREA was often used to estimate renal function, and high CREA concentration indicated glomerular filtration rate was decreased (Go et al. 2018). In this study, the WF had higher serum CREA concentration (numerical), to some extent, which showed renal function of WF was impaired. However, there is limited literature to study the relation between neonatal serum CREA and gestational health in donkeys, and it deserves further investigation.

Augmented fat and protein catabolism aggravated the burden of liver, which had a negative effect on liver function. In a study on dairy cows in negative energy balance, an extremely high rate of fat mobilisation can cause TG accumulation in the liver, which may impair liver function (van den Top et al. 1995). Several studies (Nyblom et al. 2004; Farnsworth et al. 2012) and rats (Passos et al. 2000) confirmed that energy deficit and malnutrition during gestation resulted in lower birth weight. Consistent with these studies, the birth weight and body measurement of foals in WF group were decreased, which was probably caused by malnutrition of female donkeys in DWF group. Serum CREA was often used to estimate renal function, and high CREA concentration indicated glomerular filtration rate was decreased (Go et al. 2018). In this study, the WF had higher serum CREA concentration (numerical), to some extent, which showed renal function of WF was impaired. However, there is limited literature to study the relation between neonatal serum CREA and gestational health in donkeys, and it deserves further investigation.

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marker, and high NLR indicated the enhanced systemic inflammatory response (Yildirim et al. 2015). Meanwhile, the increase of WBC demonstrated the synergistic relationship between malnutrition and infections (Nathan 1990). A previous study on children indicated that children with malnutrition had higher mean value of WBC and PLT counts (Arya et al. 2017). In this study, it was found that significant increased WBC, NEU, PLT counts and NLR among WF and DWF as compared to HF and DHF, and the results were similar to studies above, suggesting malnutrition may resulted in the increase of WBC, NEU counts and NLR. Therefore, we speculated the newborn foals were influenced by the blood from matrix, and their abnormality including malformation, lameness and drooping ears may be related to changes of haematological parameters caused by malnutrition.

In the body, the minerals, especially the trace elements, assist in fluid balance, bone development and play a role in maintaining a healthy nervous system (Hostetler et al. 2003). Meanwhile, their role is often intensified or abated by nutritional disorder (Han et al. 2016). Lee et al. (1986) believed that a negative energy balance accelerated calcium catabolism, and restriction of energy intake to 60% definitely produced a lower femur ash or calcium content in rats. In a study on beef steers indicated high dietary energy level contributed to Zn absorption (Carmichael-Wyatt et al. 2020). In our study, the serum Ca and P in DWF were greater than DHF, and the serum K, Fe, Mn and Zn were lower than DHF. The increase of Ca concentration could have been caused by the augmented calcium catabolism due to the malnutrition in DWF. In addition, mineral malabsorption can result in multiple diseases. For example, the imbalance of Ca and P, and lack of Mn contributed to nutritional lameness of chicken (Chasity and Murugesan 2016), and the lameness was decreased by supplementing organic trace minerals (Mn and Zn), which also reduced lameness during lactation of gilts (Fabà et al. 2018). Mn was required by the animals for normal skeletal development and reproduction (Hostetler et al. 2003). In this study, Mn, Fe and Zn concentrations in serum of DWF were lower than those of DHF, which provided the interpretation for lameness in donkeys. But it is regrettable that we lack the evidence to demonstrate the standpoint without enough serum sample from newborn foals. Furthermore, Guyot et al. (2009) found the mean value of Zn higher in healthy herds as compared to the sick ones with digestive troubles. There is substantial evidence that Zn supplementation may well reduce the impact of the renal disease, chronic gastrointestinal disorders and acrodermatitis by preventing the dismantling of the immune system (Fraker et al. 2000), indicating the health status of donkey is closely bound up to Zn concentration. Also, Hanna et al. (1997) indicated Zn deficiency can have a negative influence on foetal development, and increase the odds of malformation occurring.

Combs (1987) found hair mineral analyses combined with other indicators of mineral status to provide a more precise assessment of mineral status in livestock. Dastgheib et al. (2014) indicated there was a significant correlation for concentrations of Zn, Cu and Fe between serum and hair. A study on cows showed a significant correlation for concentrations of Zn and Fe between serum and hair (Patra et al. 2006), and the serum Fe level had significant correlation with hair Fe level (Biricik et al. 2005). This study showed that the serum concentrations of K, Cu, Fe and Zn had significant correlation with those in hair, which were similar with the studies above. Meanwhile, we observed the variation tendencies of Fe, Mn and Zn elements in hair were consistent with those in serum of donkeys, suggesting the important minerals involved in malformation and lameness were all reflected in hair and serum. Hair mineral analyses contributed to assessment of mineral status in donkeys. In this study, we found that the hair of female donkeys with WF was slipped and lack of burnish. Biricik et al. (2005) also showed that the dermatomycosis inducing moult was diminished by increased serum and hair Zn concentrations in horses, suggesting weak donkeys moulted due to lacking of Zn probably. Few literatures were reported on hair minerals of donkey, but we can seek out relevant evidence from researches on human. Several reports indicated that some alopecia areata patients showed Zn and some other trace element deficiencies and some alopecia areata cases were cured with oral zinc sulphate (Sharquie et al. 2014). One explanation is that trace elements are requisite cofactors for numerous enzymes and play an important role in functional activities within the hair follicle. Further, Zn inhibits hair follicle regression and accelerates hair follicle recovery.

To sum up, there are significant differences in some haematological, biochemical and nutritional parameters such as WBC count, NEU count, AST, ALT, ALP, BHBA, NEFA, serum K, Fe, Mn, and Zn between healthy and weak donkey foals, also between their corresponding female donkeys, so these measurements can be used to evaluate health status of donkeys and foals, and may provide us an indication for improving nutritional status of female donkeys. In this study, all female donkeys were fed the same diet, but some situations of increased inflammatory response and fat
mobilisation arose in the female donkeys from DWF, and decreased birth weight and body measurements, malformation, lameness and drooping ears developed in foals from WF. The outcomes suggested the current diet might result in malnutrition of pregnant donkeys. It is necessary to adjust nutritional level in the current diet, but few data are available on nutrient requirement of pregnant donkeys and it needs further research. In addition, it is hardly to create a reference interval for HF and WF and their corresponding female donkeys because of the statistical limitations of the study due to the small sample size.

At present, no widely approved interpretation exists for malformation, lameness and drooping ears of the newborn foals. Our study may be able to provide some explanations of the diseases by the changed haematological, biochemical parameters and nutritive indexes. The results above have their limitations, and the exact evidences still need further researches.

**Conclusions**

In conclusion, there were significant differences in some haematological, biochemical and nutritional parameters such as WBC count, NEU count, AST, ALT, ALP, BHBA, NEFA, serum K, Fe, Mn and Zn between HF and WF, and between their corresponding female donkeys. The differences in these parameters suggested that the undernutrition of their corresponding female donkeys was probably responsible for malformation, lameness and drooping ears of foals.

**Ethic approval**

The experiment was conducted in the experimental farm of Inner Mongolia Agricultural University (Hohhot, China). All procedures were approved by the Technical Committee for Laboratory Animal Sciences of the Standardisation Administration of China (SAC/TC281), and performed under the national standard Guidelines for Ethical Review of Laboratory Animal Sciences of the Standardisation Administration of China (GB/T 35892-2018).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Data availability statement**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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