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# Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia

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ABSTRACT

The Shetland ponies, which originate from harshest environments in the world islands in the north of the Atlantic Ocean, are now most popular pony breed distributed in almost every corner of the world. Reference ranges of physiological, biochemical and haematological values, which are widely used in veterinary clinics, may differ according to season, age, sex, type of feeding, and environmental circumstances of the area. Reference ranges of haematological and biochemical values of Shetland ponies are rare. The present study was therefore undertaken to evaluate the impact of season, age and sex on some haematological and biochemical values and cortisol levels in healthy ponies in Saudi Arabia. The study was conducted between December 2016 to June 2017 on ponies in and around Al-Hasa, Saudi Arabia. Twenty-three clinically healthy ponies males and females of different ages were included for haematological and biochemical analysis in this study. For each animal, blood samples were collected in summer and winter. Four physiological, fourteen haematological, sixteen biochemical parameters as well as serum cortisol levels were analysed.

Heart rate, respiratory rate, pulse and rectal temperature were increased in summer in comparison to winter. Values of heart rate proved significant at $P < 0.05$. The results of blood haematology and biochemistry of the ponies revealed that there was no significant variation between summer and winter in most of the haematological and biochemical parameters, while there was a slight significant difference in leucocyte counts, monocytes, MCH, MPVK$^{+}$, platelets and AST activity. There were significant differences in serum cortisol concentration regarding season and age but not regarding gender.

## 1. Introduction

The Shetland pony (*Equus ferus caballus*) originates, as the name indicates, from the one of the harshest environments in the world islands in the north of the Atlantic Ocean [1]. It is one of the oldest, smallest and most popular pony breed distributed in almost every corner of the world [1,2]. Ponies have spread over the past decades also in the Arabic Gulf countries specially Shetland ponies. Reference ranges of physiological, biochemical and haematological values are widely used in veterinary clinics for disease prognosis, differential disease diagnosis, nutritional and therapeutic monitoring [3,4]. It is also well established that the standard values of haematological and biochemical parameters referred for a particular breed of animals may not hold good for other breeds as these have been reported to vary in different breeds [5,6].

For a given breed, haematological and biochemical values may differ according to the environmental circumstances of the area where the animal lives [7]. Knowledge about breed-specific blood values represents an important prerequisite for the application of haematological analyses in the evaluation of animal welfare in health and disease [8,9].

Factors that may affect haematological and serum biochemical parameters include gender, age, season and animal nutrition [10,11]. The measurement of serum cortisol as stress hormone is the most frequently used screening test to evaluate the effect of season and the adaptation of animals in the environmental circumstances of the area [12,13].

Reference ranges of haematological and biochemical values of Shetland ponies are rare [14]. The present study was therefore undertaken to evaluate the impact of season, age and sex on some clinical, haematological and biochemical values and cortisol levels in healthy ponies in Saudi Arabia.

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## 2. Material and methods

The study was conducted between December 2016 to June 2017 on ponies in and around Al-Hasa, Saudi Arabia. Twenty-three clinically healthy ponies (12 females, 11 males) were included in this study. Based on the animal's age, animals were divided into two groups, the first group including young animals (5 animals) aged between 2.5 and 3 years and the second group (18 animals) including animals aged above 3 years and under 17 years. Information about species, sex, age and management system of the studied animals were gathered from the owners. Ponies examination and blood sampling were done between 11:00 and 12:00 h in two phases: the first time was in late December (winter), when the weather temperature was 20 °C and the second time was in mid-June (summer), when weather temperature was 44 °C [15]. All procedures in this study were performed according to the guidelines of the Research Ethics Committee- by the King Faisal University-Saudi Arabia.

### 2.1. Clinical examination and blood sampling

Each pony was examined and clinical parameters (heart rate, respiratory rate, pulse and rectal temperature) were recorded. After clinical examination, a blood sample was taken from the jugular vein using a 21-gauge needle into vacuum blood tubes, (5 mL BD Vacutainer, United Kingdom). Tubes of Ethylene Diamino Tetraacetic Acid (EDTA) were used for blood haematology and the tubes without EDTA were used for blood biochemical examination.

### 2.2. Haematological and serum biochemical analysis

Blood samples were analysed within 45 min after sample collection for total Red Blood Cells (RBC) count, total and differential White Blood Cells (WBC) count, Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red cell Width Distribution (RWD) and Mean Platelet Volume (MPV) using CELL-DYN 3700 analyser. Blood samples collected without EDTA were centrifuged at 3000 U/min for 10 min to obtain serum. Concentrations of serum total protein (TP), albumin (Alb), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), total bilirubin (TBIL), urea (Urea), creatinine (Crea), glucose (Glu) were determined, and (CK) creatine kinase, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and the gama glutamine transferase (GGT) activities were measured using an automated blood chemistry analyzer Hitachi 705 (Hitachi, Japan) and the DIAS (Diagnostic Systems GmbH, Germany) reagents.

### 2.3. Serum cortisol analysis

Serum cortisol was measured using a commercial horse cortisol (COR) Elisa Kit (DEMEDITEC, Kiel, Germany). The detection rate of the assay was between 20 and 800 ng/mL. For ELISA plate, a logarithmic standard curve was made. A 4-parameter Logistics curve fit was used to calculate the concentration of serum cortisol in each sample.

### 2.4. Statistical analysis

Statistical analysis was performed using the descriptive statistical analysis of Graph Pad Prism 7. Comparison between summer and winter and the effect of age and gender was analysed using Student's *t*-test. Differences between groups were considered significant when $P < 0.05$. Normal distribution of values was evaluated by D'Agostino and Pearson omnibus normality tests.

**Table 1**
Effect of season on four physiological values of 23 ponies of different age and sex at Al-Hasa region-eastern province of Saudi Arabia.

| Items | Season | Mean ± SEM | Range | *P* value |
|---|---|---|---|---|
| Heart rate (beats/min) | Summer | 45.5 ± 2.5 | 30–54 | 0.063 |
| | Winter | 41.0 ± 1.4 | 28–44 | 0.02* |
| Respiratory rate (respiration/min) | Summer | 16.50 ± 1.41 | 10–18 | 0.052 |
| | Winter | 14.8 ± 0.85 | 9–16 | 0.12 |
| Pulse (puls/min) | Summer | 46.80 ± 2.75 | 36–52 | 0.32 |
| | Winter | 42.60 ± 1.85 | 34–49 | 0.08 |
| Rectal temperature (°C) | Summer | 37.45 ± 0.12 | 36.2–37.7 | 0.242 |
| | Winter | 36.9 ± 0.25 | 36.2–37.6 | 0.09 |

* $P < 0.05$.

**Table 2**
Effect of season on haematological parameters of 23 ponies of different age and sex at Al-Hasa region-eastern province of Saudi Arabia.

| Parameters | Season | Mean ± SEM | Range | *P* Value |
|---|---|---|---|---|
| Leucocyte counts ($10^9$/l) | Summer | 11.72 ± 1.83 | 7.5–16.1 | 0.04* |
| | Winter | 8.72 ± 0.44 | 6.5–10.2 | |
| Lymphocytes % | Summer | 42.73 ± 1.88 | 33.6–51.9 | 0.3 |
| | Winter | 41.12 ± 1.5 | 34.6–48.9 | |
| Monocytes % | Summer | 4.36 ± 0.27 | 3.3–5.4 | 0.008** |
| | Winter | 3.43 ± 0.12 | 2.7–4 | |
| Neutrophils % | Summer | 52.79 ± 2.0 | 43–63 | 0.23 |
| | Winter | 54.47 ± 1.3 | 61.8–47.8 | |
| Eosinophils % | Summer | 3.88 ± 0.61 | 2.1–7.8 | 0.17 |
| | Winter | 4.55 ± 0.55 | 2.5–7.8 | |
| Erythrocyte counts ($10^{12}$/l) | Summer | 7.59 ± 0.51 | 5.64–10.17 | 0.18 |
| | Winter | 8.12 ± 0.50 | 5.99–10.69 | |
| Haemoglobin (g/dL) | Summer | 7.183 ± 0.66 | 5.28–10.37 | 0.13 |
| | Winter | 8.149 ± 0.36 | 6.89–9.63 | |
| Haematocrit (g/dL) | Summer | 0.32 ± 0.03 | 0.24–0.48 | 0.12 |
| | Winter | 0.36 ± 0.01 | 0.3–0.43 | |
| MCV (fl) | Summer | 41.11 ± 1.7 | 36–52 | 0.07 |
| | Winter | 45 ± 1.8 | 35.2–53.5 | |
| MCH (pg) | Summer | 13.5 ± 2. 37 | 10.78–15.65 | 0.02* |
| | Winter | 9.1 ± 1.12 | 8.2–10.26 | |
| MCHC (g/dL) | Summer | 22.11 ± 0.09 | 21.8–22.33 | 0.09 |
| | Winter | 22.28 ± 0.20 | 21.3–23.92 | |
| RDW (%) | Summer | 17.97 ± 0.08 | 17.5–18.3 | 0.06 |
| | Winter | 25.75 ± 7.04 | 17.6–41.9 | |
| Platelets ($10^9$/l) | Summer | 244.1 ± 17.03 | 133–298 | 0.007** |
| | Winter | 194.1 ± 7.04 | 145–227 | |
| MPV (fl) | Summer | 5.69 ± 0.15 | 4.9–6.2 | 0.18 |
| | Winter | 5.52 ± 0.075 | 5.2–5.8 | |

* $P < 0.05$.
** $P < 0.01$.

## 3. Results

Results of clinical, haematological and biochemical analyses are presented in Tables 1–3 as Mean ± SEM (Standard Error of Mean), range and *P* values (PV). No significant effects of age and gender could be seen on the tested physiological, haematological and serum biochemical parameters. Although no significant variation was seen in most studied clinical parameters including heart rate, respiratory rate, pulse and rectal temperature, there was a significant increase in heart rate in summer (Table 1).

The results of blood haematology revealed that there was no significant variation between summer and winter in most of the haematological parameters, while there was significant difference in leucocyte