International Journal of Veterinary Science and Medicine 6 (2018) 61–64

HOSTED BY

Contents lists available at ScienceDirect

International Journal of Veterinary Science and Medicine

journal homepage: www.elsevier.com/locate/ijvsm

Full Length Article

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

Turke Shawaf[a,*], Jamal Hussen[b], Mohammed Al-Zoubi[a], Hassein Hamaash[a], Khalid Al-Busadah[c]

[a] Department of Clinical Studies, College of Veterinary Studies, King Faisal University, Al Hasa Box 400, Saudi Arabia
[b] Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, 400 Al-Hasa, 31982, Saudi Arabia
[c] Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, King Faisal University, Al-Hasa, Saudi Arabia

ARTICLE INFO

Keywords:
Chemistry
Cortisol
Elisa
Haematology
Pony
Season

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.

Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.
* Corresponding author at: College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, P.O. Box: 400, Saudi Arabia.
E-mail address: tshawaf@kfu.edu.sa (T. Shawaf).

https://doi.org/10.1016/j.ijvsm.2018.03.007
Received 26 February 2018; Received in revised form 19 March 2018; Accepted 19 March 2018
Available online 24 March 2018

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

Table 3
Effect of season on serum biochemical values of 23 ponies of different age and sex at Al-Hasa region-eastern province of Saudi Arabia.

Parameters	Season	Mean ± SEM	Range	*P* Value
ALP (IU/L)	Summer	246.4 ± 17.94	212–384	0.24
	Winter	221.4 ± 19.21	121–289	
AST (IU/l)	Summer	249.3 ± 17.94	173–345	0.01*
	Winter	358.1 ± 36.8	216–547	
GGT (IU/l)	Summer	15.11 ± 2.63	8–32	0.15
	Winter	24.78 ± 6.78	10–65	
TP (g/dL)	Summer	7.1 ± 0.34	5.2–8.8	0.48
	Winter	7.32 ± 0.44	6.5–10.9	
ALB (g/dL)	Summer	3.47 ± 0.28	2.1–4.7	0.26
	Winter	3.79 ± 0.17	3.3–4.9	
Glob (g/dL)	Summer	3.72 ± 0.25	2.2–4.9	0.107
	Winter	3.62 ± 0.31	3–6	
BUN (mg/dL)	Summer	11.37 ± 0.79	9–15.5	0.26
	Winter	12.63 ± 2.73	6.8–32	
CK (IU/l)	Summer	209.9 ± 22.8	106–322	0.08
	Winter	249.7 ± 19.6	155–328	
CA (mmol/l)	Summer	11.69 ± 0.23	10.3–12.7	0.31
	Winter	11.56 ± 0.21	10.7–12.5	
Na+ (mmol/l)	Summer	131.8 ± 1.86	126 – 141	0.28
	Winter	133.4 ± 2.8	177 – 148	
Cl (mmol/l)	Summer	117.6 ± 1.6	109 – 123	0.42
	Winter	117 ± 1.13	112–121	
K+ (mmol/l)	Summer	5 ± 0.1041	4.5–5.6	0.05
	Winter	4.48 ± 0.25	3.4 – 6	
TBIL (μmol/l)	Summer	1.03 ± 0.11	0.6–1.5	0.39
	Winter	0.98 ± 0.28	0.65–1.35	
CRE (mg/dL)	Summer	1.09 ± 0.075	0.7–1.4	0.23
	Winter	0.98 ± 0.65	0.8 – 1.1	
GLU (mg/dL)	Summer	94 ± 4.56	77–111	0.21
	Winter	103.2 ± 7.7	78 – 154	
Mg (mmol/l)	Summer	2.14 ± 0.15	2.1–2.7	0.37
	Winter	2.09 ± 0.15	1.5–2.8	

* $P < 0.05$.

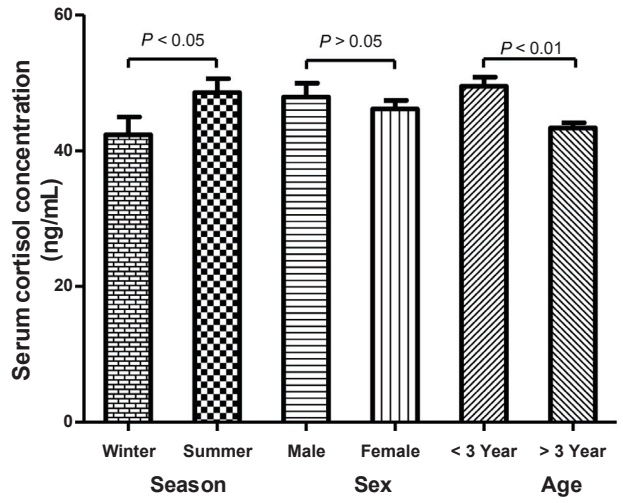

Fig. 1. Impact of season, age and sex on serum cortisol level in 23 Shetland Ponies in Al-Hasa region- eastern Province of Saudi Arabia.

counts, monocyte counts, MCH and platelets counts (Table 2). Comparative analysis of serum biochemicals parameters in winter and summer revealed no significant effect of season on most studied parameters, while there was significant difference in AST ($P = 0.01$, Table 3). For serum cortisol concentration, significant differences were shown under age and seasonal effects, while no significant variation could be seen between different genders (Fig. 1).

4. Discussion

Reference ranges of physiological, biochemical and haematological values may differ according to many factors like breed, age, sex, type of feeding, seasonal and environmental circumstances [3–7]. As a stress hormone, serum cortisol has been widely used to evaluate seasonal and environmental adaptation of the animal [12,13].

Weather temperature changes registered in Saudi Arabia between summer and winter may have an impact on haematological and serum biochemical parameters and cortisol levels in ponies [16].

Our results showed that all mean physiological parameters (heart rate, respiratory rate, pulse and rectal temperature) were tendentially increased in summer in comparison to winter season (Table 1). The results of increased heart rate in summer in the present study is in agreement with Brinkmann et al. [17,18], although the differences between summer and winter heart rate values were less obvious in our study than that reported by Brinkmann et al. (Mean heart rate: summer (58/beats/m), winter (35 beats/m)) [19]. In agreement with Scheibe et al. [20], increased heart rate in summer may indicate the adaptation ability of Shetland pony to hot temperatures [20] for body temperature regulation. Brinkmann et al. reported that the decreased heart rate in winter season could be explained by the feed restriction in ponies during winter [18].

In comparison to results obtained from studies conducted in other geographical areas, our study revealed higher WBC in Shetland ponies ($8.72 \pm 0.44\,10^9$/l) than that reported in Bosnian ponies ($6.2 \pm 2.39\,10^9$/l) [8]. On the other hand, RBC ($8.12 \pm 0.50\,10^{12}$/l), HGB (8.15 ± 0.36 g/dL), MCH (9.1 ± 1.12 pg), MCHC (22.28 ± 0.2 g/dL) were lower than that reported in Bosnian ponies: RBC ($9.11.12 \pm 1.34\,10^{12}$/l), HGB (15.9 ± 2.4 g/dL), MCH (17.58 ± 3.1 pg), MCHC (38.2 ± 6.47 g/dL) [8]. However, with the exception of platelets, Shetland ponies have lower values of WBC, Lymphocyte, RBC and HGB in comparison to horses [21]. In addition, comparison of our results with values of haematological parameters of Hassawi donkeys that live in the same geographical area [4] showed that Shetland ponies have lower values in most haematological parameters like WBC, RBC, Lymphocyte, HBG, MCV, MCH, MCHC and MPV. Such differences observed between our results and results from previous studies may be due to differences in group size, geographical locations and assay methodology.

For the biochemical parameters, previous study found higher values of ALP, GGT, ALB, Ca, Cl, K and GLU but lower values of TP and CREA in comparison with results reported in Shetland pony [22]. According to the present results, most biochemical parameters in Shetland ponies were lower than that reported for Arabian horses except TP, which was more in Shetland ponies [21]. However, when comparing the present results with values reported in Hassawi donkey from the same area, it found higher values of GLOB, BUN, CK, TBIL and GLU and lower GGT and ALB in Shetland ponies than that reported in Hassawi donkey [4]. Differences in biochemical parameters values among previous studies could be explained by many factors like life condition, muscle mass, type of feeding and type of exercise.[23].

Our study found higher concentrations of serum cortisol levels in summer than in winter, which is in agreement with previous studies [13,21,24]. In a recent study [13], it has been suggested that elevation of serum cortisol in summer season might result from direct effects of increased temperature and daylight on endocrine functions. Previous studies have yielded conflicting results on the impact of season on cortisol concentrations in healthy animals, with some studies showing no effect of season on cortisol concentration [24,25], others demonstrating increased cortisol secretion in the spring [26]. Differences between studies may be influenced by the differences of geographic

location, time of sample collection, and assay methodology [27]. It is therefore important to consider using these area-specific values for the interpretation of laboratory diagnostic findings.

In contrast to other studies, where no impact of age on serum cortisol concentration was reported [24], significant higher values of serum cortisol were recorded in young ponies than in old ponies (Fig. 1). The decreased hormone secretion in old ponies could be due to loss of complex variability to physiological stressors associated with aging [28]. Similar to results reported on horses [27], we found no effect of sex on cortisol concentration in Shetland ponies.

5. Conclusions

This is the first study on haematological and serum biochemical parameters in healthy ponies in Saudi Arabia. Although higher animal numbers are needed to confirm reference values for blood parameters, our results would be useful for interpretation of laboratory data of ponies in Saudi Arabia. However further studies with higher animal numbers are needed to confirm these results.

Competing interests

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors would like to thank the Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University (Al-Hasa, Saudi Arabia) for their kind cooperation in conducting this study. Many thanks for Prof. Sabri Al-Bahar for his support in ELISA.

References

[1] Russell V. Shetland ponies. London, U.K: Whittet Books Ltd; 1996.
[2] Khadka R. Global horse population with respect to breeds and risk status. Second cycle, A2E. Uppsala: Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics; 2010.
[3] Al-Bulushi S, Shawaf T, Al-Hasani A. Some hematological and biochemical parameters of different goat breeds in Sultanate of Oman “A preliminary study”. Vet World 2017;10:461–6.
[4] Shawaf T. Some haematological and serum biochemical parameters in apparently clinically healthy Hassawi donkey. Alex J Vet Sci 2017;53:125–30.
[5] Burlikowska K, Boguslawska-Tryk M, Szymeczko R, Piotrowska A. Haematological and biochemical blood parameters in horses used for sport and recreation. J Cent Eur Agric 2015;16:370–82.
[6] Cruz AM, Maninchedda UE, Burger D, Wanda S, Vidondo B. Repeatability of gait pattern variables measured by use of extremity-mounted inertial measurement units in nonlame horses during trotting. Am J Vet Res 2017;78:1011–8.
[7] Zinkl JG, Mae D, Guzman Merida P, Farver TB, Humble JA. Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (*Equus asinus*). Am J Vet Res 1990;51:408–13.
[8] Dekic R, Ivanc D, Cetkovic D, Dolicanin Z, Obradovic S. Hematology of Bosnian pony. Bulg J Agric Sci 2014;20:1237–44.
[9] Lacerda L, Campos R, Sperb M, Soares E, Barbosa P, Godinho E, et al. Hematological and biochemical parameters in three high performance horse breeds from Southern Brazil. Arch Vet Sci 2006;11:40–4.
[10] Plotka ED, Eagle TC, Gaulke SJ, Tester JR, Sniff DB. Hematological and blood chemical characteristics of feral horses from three management areas. J Wildl Dis 1988;24:231–9.
[11] Raymond SL, Smith TK, Swamy HV. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J Anim Sci 2003;81:2123–30.
[12] Nilssen KJ, Bye K, Sundsfjord JA, Blix AS. Seasonal changes in T3, FT4, and cortisol in free-ranging Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Gen Comp Endocrinol 1985;59:210–3.
[13] Tangyuenyong S, Sato F, Nambo Y, Murase H, Endo Y, Tanaka T, et al. Comparison of physical body growth and metabolic and reproductive endocrine functions between north and south climates of Japan in trained Thoroughbred yearling horses. J Equi Sci 2017;28:77–86.
[14] Snow DH. Haematological, biochemical and physiological changes in horses and ponies during the cross country stage of driving trial competitions. Vet Rec 1990;126:233–9.
[15] Protection TGAoMaE. Weather Status In Ihsaa Currently. Saudi Arabia, 2017.
[16] Mizukami H, Suzuki T, Nambo Y, Ishimaru M, Naito H, Korosue K, et al. Comparison of growth and endocrine changes in Thoroughbred colts and fillies reared under different climate conditions. J Equi Sci 2015;56:49–56.
[17] Brinkmann L, Gerken M, Riek A. Seasonal changes of total body water and water intake in Shetland ponies measured by an isotope dilution technique. J Anim Sci 2013;91:3750–8.
[18] Brinkmann L, Riek A, Gerken M. Long-term adaptation capacity of ponies: effect of season and feed restriction on blood and physiological parameters. Anim J 2017;12:1–10.
[19] Brinkmann L, Gerken M, Riek A. Adaptation strategies to seasonal changes in environmental conditions of a domesticated horse breed, the Shetland pony (*Equus ferus caballus*). J Exp Biol 2012;215:1061–8.
[20] Scheibe KM, Berger A, Langbein J, Streich WJ, Eichhorn K. Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. Biol Rhythm Res 1999;30:216–23.
[21] Mikniene Z, Maslauskas K, Kerzienė S, Kučinskienė J, Kučinskas A. The effect of age and gender on blood haematological and serum biochemical parameters in žemaitukai horses. Vet Med Zoot 2014;65:37–43.
[22] Rico AG, Braun JP, Benard P, Bardies J, Thouvenot JP, Periquet B, et al. Blood serum biochemistry of ponies. Ann Rech Vet 1978;9:393–9.
[23] Cywinska A, Czopowicz M, Witkowski L, Gorecka R, Degorski A, Guzera M, et al. Reference intervals for selected hematological and biochemical variables in Hucul horses. Pol J Vet Sci 2015;18:439–45.
[24] Borer-Weir KE, Menzies-Gow NJ, Bailey SR, Harris PA, Elliott J. Seasonal and annual influence on insulin and cortisol results from overnight dexamethasone suppression tests in normal ponies and ponies predisposed to laminitis. J Equi Vet 2013;45:688–93.
[25] Place NJ, McGowan CM, Lamb SV, Schanbacher BJ, McGowan T, Walsh DM. Seasonal variation in serum concentrations of selected metabolic hormones in horses. J Vet Int Med 2010;24:650–4.
[26] Cordero M, Brorsen BW, McFarlane D. Circadian and circannual rhythms of cortisol, ACTH, and alpha-melanocyte-stimulating hormone in healthy horses. Dom Anim Endocrinol 2012;43:317–24.
[27] Hart KA, Wochele DM, Norton NA, McFarlane D, Wooldridge AA, Frank N. Effect of age, season, body condition, and endocrine status on serum free cortisol fraction and insulin concentration in horses. J Vet Int Med 2016;30:653–63.
[28] Vaillancourt DE, Newell KM. Changing complexity in human behavior and physiology through aging and disease. Neurobiol Aging 2002;23:1–11.