Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Hematologic, Metabolite and Hormone Responses to Weaning-Induced Stress in Foals of Different Breeds

Anas Sarwar Qureshi*, Fazeela Yaqoob[1] and Heinrich Enbergs[2]

Department of Anatomy, University of Agriculture, Faisalabad; [1]Department of Zoology, Government College University, Lahore, Pakistan; [2]Institute of Anatomy, Physiology and Hygiene of Domestic Animals, Bonn University, Bonn, Germany
*Corresponding author: anas-sarwar@hotmail.com

ARTICLE HISTORY

Received: August 10, 2012
Revised: November 24, 2012
Accepted: July 21, 2013

Key words:
Foals
Haematology
Hormones
Metabolites
Weaning

ABSTRACT

Eighty-four foals of different breeds (Thoroughbred, Standardbred, German Warmblood), aged 5-6 months, were used in a study to evaluate the effects of weaning, sex, breed on haematological, metabolic and hormonal parameters. The values of leukocytes, neutrophils, red blood cells, haemoglobin, packed cell volume, platelets, activity of γ-GT and concentrations of cholesterol, cortisol and thyroxin rose on day 1 after weaning, while the percentages of eosinophils, lymphocytes and serum concentration of triglycerides decreased one day following weaning. These parameters returned to pre-weaning values on day 14 post-weaning except serum cholesterol, which fell to a significantly lower level on day 14 post-weaning than pre-weaning value. Serum alkaline phosphatase declined markedly on day 14 post-weaning. The most pronounced rise was observed for cortisol (51%) and thyroxin (40%) values. Females presented higher mean corpuscular haemoglobin concentration and cortisol concentration than males ($P<0.05$). German Warmblood foals presented a less-altered picture following weaning as compared with Thoroughbreds and Standardbreds. It is concluded that foals respond immediately to weaning, which may lead to suppression of the activities of bone cells i.e., osteoblasts. In addition, serum concentrations of cortisol and thyroxin influence one another in weanling foals. The Thoroughbred foals appear to be the most sensitive to weaning.

To Cite This Article: Qureshi AS, F Yaqoob and H Enbergs, 2013. Hematologic, metabolite and hormone responses to weaning-induced stress in foals of different breeds. Pak Vet J, 33(4): 500-504.

INTRODUCTION

Recently our understanding of interaction between neuroendocrine pathway and immune related inflammatory reactions has expanded immensely (Suradhat, 2005). Physiological, molecular and cellular studies proved that nervous, immune and endocrine systems are not individual entities rather they interact with each other for proper maintenance of body functions including stress responses (ThyagaRajan and Priyanka, 2012). The central nervous system (CNS) responds to stress by signaling target cells of the immune system through autonomic and neuroendocrine pathways (Lorton *et al.,* 2006).

Weaning is a stressful event in a foal's life that normally occurs during rapidly growing phase of animal with the requirement of significant nutritional input. In various species such as rabbits (Bonanno *et al.,* 2004) and lambs (Bimczok *et al.,* 2005) abrupt weaning has led to weight loss, which lasts for a considerable time period. But complexity of weaning can trace back to the fact that it results in a number of physiological challenges that may have prolonged effects on foal welfare (Moons *et al.,* 2005). Research has proved that well-chosen weaning time for foal is 4.5 and 6.0 months (Warren *et al.,* 1998). Weaning elicits an immediate and somewhat short-lived acute stress response in the calf. The effects serve to enhance, rather than suppress, the immune response by means of a heightened inflammatory response and cellular mobilization (O'Loughlin *et al.,* 2011). Many studies further prove that secretion of many neuroendocrine hormones is affected by the stress, especially glucocorti- coids, which lead to immune system suppression (Li *et al.,* 2012).

The present study was carried out to investigate the effects of weaning on haematologic, metabolite and hormone parameters in foals from 4 days pre-weaning to 14 days after weaning. Besides weaning, the effects of sex, breed and farm were also evaluated in weanlings on selected blood profile.

MATERIALS AND METHODS

Foals: Eighty-four foals (46 females; 38 males), aged 5 - 6 months, from three breeds (Thoroughbred, Standardbred and German Warmblood) were used in this study. The foals belonged to five different stud farms located in Nordrhein Westfalen state of Germany. All foals available on these farms were included in the study with the consent of farm owners. The management and nutritional conditions were intensive and comparable to a large extent. All candidates of weaning were found clinically healthy throughout study. All foals were weaned abruptly and were unable to see their dams.

Sampling and analyses: Five blood samples were collected from each foal as per schedule: Day -4: 4 days before weaning; Day –2: 2 days before weaning; Day -1: one day before weaning; Day 1: one day after weaning; Day 14: 14 days after weaning. Blood samples were collected between 7^{00} and 9^{00} in the morning. A 10 ml blood was collected by the jugular venipuncture in a heparinized monovette tube, 2 ml in Fluoride-EDTA labelled tube and twice in 4.9 ml serum gel tubes. The blood was allowed to clot and centrifuged at 3000 rpm for 10 minutes for serum separation. The serum samples were frozen at -20°C until analysed. All haematological and biochemical tests except hormone profiles were performed at the same day.

Other than differential leukocyte counts (Table 1) all haematological parameters were measured with automated haematology system using a Technicon H1™. Differential leukocytes counts were performed manually (blinded and in randomized order). Serum activities of alkaline phosphatase (AP) and gamma - glutamyltransferase (γ-GT) and serum concentrations of cholesterol and triglycerides were determined with a spectrophotometer using test kits. Serum levels of cortisol and thyroxin (T4) were measured with enzyme immunoassay using test kits.

Statistical analysis: Mean + SD values and ranges of various parameters were computed. Each parameter studied was tested for normal distribution by the Scharpio-Wilk and Kolmogrove – Siminov tests. The effects of possible risk factors (weaning, sex, breed and farm) were tested on all parameters studied. A general linear model (GLM) multivariate repeated-measures test was used for Gaussian parameters. Different functions were attempted to transform non-Gaussian parameters (neutrophils, eosinophils, red blood cells, monocytes, MCHC, γ-GT and cholesterol) but no function was sufficient. The non-Gaussian parameters were therefore tested by Wilcoxon's test. Difference at $P<0.05$ was accepted as significant. The 'r' values between serum concentration of cortisol and all other parameters under study were worked out with help of Spearman's correlation coefficient.

RESULTS

Mean±SD and ranges of all parameters studied in total contingent of foals are presented in Table 1. Percentiles of all parameters in the total contingent of foals are presented in Table 2. Because of values of all parameters did not show important pre-weaning variation for any foal, only the values for D-1 are shown. P values for the effects of the risk factors are in Table 3. Correlation coefficients between serum cortisol concentration and all other parameters are presented in Table 4. Most parameters that were altered on day +1 returned pre-weaning values on day +14 except serum cholesterol. Serum cholesterol rose on D+1 after weaning and thereafter fell to a significantly lower level than preweaning values. Alkaline phosphatase was not changed on D+1 but markedly declined on day +14. The most-pronounced rises were found by serum cortisol (51%) and thyroxin (40%) concentrations following weaning. Sex affected MCHC and serum cortisol values significantly ($P<0.05$): females had higher MCHC and cortisol values than males.

DISCUSSION

The values of all haematological, metabolic and hormone parameters recorded in this study did not show significant variations during pre-weaning period. These values were largely comparable with physiological ranges for corresponding age groups, as reported in literature (Grondin and Dewitt, 2010). There are reports of relatively higher numbers of platelets in 5 and 6 months old foals, while at the same age there are changes in salivary cortisol, heart rate and behavioural markers of stress in foals (Moones *et al.,* 2005).

The findings of increased neutrophil/lymphocyte ratio (N:L) with concomitant leukocytosis, neutrocytosis, lymphopenia and eosinopenia following weaning represented a typical 'stress picture' in foals especially when both neutrophil ($r = +0.29$) and lymphocyte ($r = -0.28$) counts are correlated with serum cortisol levels. The hypothalamic - adrenohypophyseal - axis and the immune system have manifold interactions. A central feature of stress is release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which regulates cortisol secretion by the adrenal glands. The pituitary-adrenal response is stimulated typically by emotional perturbations such as uncertainty or social dislocation (Dantzer, 2004). Stress-induced elevation of ACTH has a direct effect on the immune system and reduces differentiation and proliferation of T- and B lymphocytes (Aoki *et al.,* 1990). Abrupt weaning can lead to increased plasma cortisol and noradrenaline concentrations that were complemented by attenuation of in vitro interferon-gamma production to novel mitogen and antigen complexes up to 7 d after weaning (Hickey *et al*., 2003). A contradict view given by (Magistrelli *et al.,* 2012) is difference observed in cortisol level that may not be due to stress but because of metabolic changes develop after diet change.

Increase in the number of neutrophils might be explained as result of demargination of cells from bone marrow caused by cortisol (Pyne, 1994). Significant increases in the numbers of leucocytes and neutrophils with a decrease in lymphocytes have also been observed in response to stress in pigs (Dalin *et al.,* 1993). The profound increase in neutrophil number and N: L ratio

Table 1: Mean±SD and ranges of all hematologic, metabolite and hormone parameters in all foals throughout study period

Analyte (unit)	Pre-weaning Period		Post-weaning Period		
	Day-4	Day-2	Day-1	Day1	Day14
Bands (%)	0.49±1.14 (0-7)	0.62±1.80 (0-14)	0.57±1.61 (0-12)	0.42±1.19 (0-9)	0.23±0.82 (0-6)
Basophils (%)	0.49±0.53 (0-2)	0.64±0.59 (0-2)	0.62±0.62 (0-2)	0.63±0.68 (0-2)	0.60±0.52 (0-2)
Eosinophils (%)	1.17±0.97 (0-6)	1.35±1.31 (0-7)	1.21±1.05 (0-5)	0.82±0.87* (0-4)	1.08±0.87 (0-4)
Hematocrit (%)	35.81±3.17 (29.1-42.9)	36.011±2.98 (28.9-43.6)	36.77±2.92 (29.8-43.2)	37.69±3.76* (31.9-47.9)	37.72±5.29 (27.8-48.9)
Hemoglobin (mmol/l)	7.81±0.70 (6.39-9.12)	7.89±0.68 (6.27-9.87)	8.12±0.83 (6.46-10.55)	8.12±0.83* (6.95-10.92)	8.20±1.04 (6.58-10.43)
Leucocytes (k/µl)	11.10±2.54 (5.65-24.25)	10.93±2.36 (5.25-22.66)	11.41±2.27 (7.31-22.88)	12.41±2.84* (7.57-25.65)	11.52±2.00 7.00±17.92
Lymphocytes (%)	45.93±7.40 (24-68)	46.81±8.80 (21-73)	45.56±6.83 (27-64)	38.31±6.68* (24-52)	44.99±6.62 (28-58)
MCV (fl)	36,50±2.17 (31.4-40.4)	36.59-1.98 (32.2-40.5)	36.61±2.07 (31.8-40.6)	36.35±2.52 (29.5-40.7)	36.54±2.35 (32.0-40.4)
MCH (pg)	12.88±0.66 (11.6-13.9)	12.94±0.65 (11.3-14.2)	12.93±064 (11.6-14.2)	12.97±0.64 (11.6-14.1)	12.86±0.70 (11.3-14.0)
MCHC (mmol/l)	21.95±0.81 (20.23-23.96)	22.00±0.68 (20.67-23.71)	21.98±0.81 (20.55-24.08)	22.19±1.27 (20.42-26.38)	21.92±1.07 (20.17-24.27)
Neutrophils (%)	47.04±7.85 (30-67)	46.03±8.51 (27-74)	46.85±6.78 (34-64)	54.51±7.04* (41-71)	48.16±6.46 (36-66)
Neutrophils/ lymphocyte ratio	109:100	106:100	107:100	150:100	112:100
Platelets (k/µl)	182.5±41.1 (61-323)	174.9±43.7 (64-287)	175.0±42.1 (36-272)	190.0±40.0* (78-290)	187.8±43.7 (87-323)
RBC (m/µl)	9.82±1.06 (7.69-12.42)	9.84±0.91 (7.56-12.36)	10.06±1.02 (8.08-12.71)	10.34±1.73* (8.39-13.92)	10.4±1.73 (7.39-14.90)
AP (U/l)	523±147 (315-1241)	543±143 (351-1204)	541±146 (329-1168)	531±149 (258-1274)	445±80* (314-793)
Cholesterol (mmol/l)	2.49±0.45 (0.041-0.100)	2.53±0.40 (0.043-0.092)	2.57±0.57 (0.049-0.11)	2.71±0.57* (0.048-0.108)	2.27±0.56* (0.034-0.102)
γ-GT (U/l)	9.41±4.57 (2.0-22.0)	9.05±5.15 (1.0-26.0)	9.70±5.59 (1.0-29.0)	11.38±5.62* (1.0-34.0)	10.72±5.95 (1.0-32.0)
Triglycerides (mmol/l)	0.298±0.10 (0.088-0.528)	0.300±0.10 (0.066-0.539)	0.279±0.09 (0.099-0.52)	0.225±0.10* (0.088-0.605)	0.262±0.11 (0.099-0.682)
Cortisol (ng/ml)	22.01±7.98 (7.0-41.0)	22.77±8.55 (6.4-41.3)	21.86±8.39 (7.0-50.5)	33.20±8.40* (5.5-60.0)	22.77±8.05 (0.0-53.20)
Thyroxin (ng/ml)	24.94±10.86 (6.4-56.9)	23.88±11.22 (5.0-55.0)	22.61±11.15 (5.60-56.9)	31.72±12.88* (11.5-72.30)	24.31±7.55 (11.0-53.6)

Asterisk indicates significantly (P<0.05) different values from previous observation. Values in parenthesis indicate range values.

Table 2: Percentiles of all parameters studied in 84 foals of various breeds under study

Analytes	Day around weaning								
	D -1			D +1			D +14		
	5th	50th	75th	5th	50th	75th	5th	50th	75th
Hematologic									
Bands (%)	0	0	0.5	0	0	0.5	0	0	0
Basophils (%)	0	0	0	1	0	1	1	1	1
Eosinophils (%)	0	1	1	0	1	1	1	1	1
Hematocrit (%)	34	36	38	34	38	40	34	38	42
Hemoglobin (mmol/l)	12	13	14	12	13	14	12	13	14
Leukocytes (k/ml)	10	12	12	11	12	14	10	11	12
Lymphocytes (%)	42	46	52	34	38	44	40	45	50
MCV (fl)	32	37	38	35	36	38	35	37	38
MCH (pg)	13	13	14	13	13	13	12	13	13
MCHC (mmol/l)	35	36	37	35	36	37	34	35	36
Monocytes (%)	2	5	5.	3	5	5	3	5	6
Neutrphils (%)	42	45	50	49	54	59	43	48	53
Neutrophil:lymphocyte	83	98	120	111	141	174	88	103	130
Platelets (k/µl)	156	177	206	169	186	212	164	190	210
RBC (m/µl)	9	10	10	9	10	11	9	10	12
Metabolic									
AP (U/l)	435	517	601	433	513	599	391	440	498
Cholesterol (mmol/l)	15	21	26	27	33	40	18	22	26
GGT (U/l)	7	9	12	8	10	13	7	10	12
Triglycerides (mmol/l)	20	24	30	14	20	26	19	24	31
Hormonal									
Cortisol (ng/ml)	15	21	26	27	33	40	18	22	26
Thyroxin (ng/ml)	15	19	29	222	30	39	19	23	28

together with reduced lymphocyte number in calves post-weaning suggests that the first may be more subtle to weaning stress. Within 24 hours, there was considerable increase in neutrophil numbers that was returned to baseline by day 11 (O'Loughlin *et al.*, 2011). Blanco *et al.* (2009) and (Lynch *et al.*, 2010) reported increased neutrophil percentage following weaning. Likewise, eosinophil elevation was observed in horses in stress period after strenuous exercise (Adamson and Slocombe., 1995).

In the present study, Thoroughbred solely contributed elevations in red blood cell parameters following weaning. These results are in agreement with (Qureshi *et al.*, 2002) who reported significantly increased RBC, Hb and PCV in response to mental stress in human beings. It is assumed that Thoroughbreds are probably more sensitive to corticosteroid induced haemoconcentration and activation of erythropoiesis than other breeds. Platelets elevated significantly (P<0.5) on day 1 following weaning. These results correspond with (Qureshi *et al.*, 2002) who reported an appreciable increase with difference (7270.27 ± 928.82) in platelets in response to mental stress in human beings. O'Loughlin *et al*. (2011) also observed increased platelets numbers while working with post weaning calves.

Table 3: P values for risk factors from repeated-measures general linear models of parameters considered possible indicators of stress in 84 weaned foals under study.

Outcome	Risk Factors			
	Breed	Sex	Weaning	Farm
Hematologic:				
Bands	0.01	0.16	0.20	0.00
Basophils	0.00	0.47	0.42	0.00
Eosinophils	0.00	0.73	0.00	0.00
Hematocrit	0.00	0.60	0.00	0.00
Hemoglobin	0.00	0.34	0.00	0.00
Leucocytes	0.19	0.24	0.00	0.09
Lymphocytes	0.06	0.41	0.00	0.13
MCV	0.00	0.192	0.71	0.00
MCH	0.00	0.644	0.19	0.00
MCHC	0.01	0.01	0.80	0.00
Monocytes	0.00	0.88	0.22	0.00
Neutrphils	0.09	0.38	0.00	0.23
Platelets	0.06	0.88	0.067	0.02
RBC	0.00	0.17	0.00	0.00
Metabolic:				
AP	0.10	0.30	0.00	0.19
Cholesterol	0.00	0.27	0.00**	0.00**
GGT	0.04	0.89	0.00	0.13
Triglycerides	0.36	0.74	0.00	0.41
Hormonal:				
Cortisol	0.69	0.01	0.00	0.40
Thyroxin	0.00	0.55	0.00	0.00

Table 4: Spearman's correlations between serum cortisol concentration and all other parameters in 84 foals under study

	Days around weaning		
Parameters	D-1	D+1	D+14
Hematologic:			
Bands	0.02	0.11	0.17
Basophils	0.12	0.95	0.78
Eosinophils	0.49	0.20	0.01
Hematocrit	0.67	0.08	0.40
Hemoglobin	0.73	0.03	0.26
Leucocytes	0.35	0.21	0.21
Lymphocytes	0.27	0.19	0.05
MCV	0.16	0.72	.028
MCH	0.88	0.72	0.05
MCHC	0.49	0.33	0.71
Monocytes	0.00	0.16	0.77
Neutrphils	0.79	0.05	0.08
Platelets	0.50	0.82	0.91
RBC	0.98	0.02	0.94
Metabolic:			
AP	0.61	0.74	0.41
Cholesterol	0.02	0.89	0.25
γ-GT	0.94	0.39	0.54
Triglycerides	0.38	0.08	0.40
Hormonal:			
Thyroxin	0.25	0.51	0.05

Spearman's correlations (r_{sp}) in bold indicates 2-tailed P<0.05.

In newborn and very young horses normally two different AP isoenzyme fractions appear in serum: liver and bone AP (Thorèn-Tolling, 1988). Many studies suggest that approximately 60% of total AP is derived from bones in foals up to one year old (Lepage *et al*., 1993; Price *et al.,* 1995). Glucocorticoides have strong suppressive effect on bone (Kim *et al*., 2006) likewise a marked decline (15%) in AP in the present study could be attributed to the suppression of the activity of bone cells (osteoblasts). In foals early weaning could have strong negative effects on bone mineral content (BMC) if growth parameters are taken into consideration (Reichmann *et al.,* 2004). Further research should be performed in order to establish the effect of weaning on bone metabolism in foals with the help of a more specific biochemical marker.

In all species and particularly in horses, γ-GT is regarded to be as a general indicator of hepatobiliary implications rather than a marker of cholestasis and there are indications that in some cases elevations might be associated with bile duct proliferation. However, a consistent but moderate elevation (9.70 to 11.38) in serum activity of γ-GT might be due to hepatobiliary involvement and/or an abrupt change in the postweaning diet.

Serum cholesterol levels markedly increased one day after weaning, but thereafter, declined significantly below preweaning values, whereas serum triglycerides exhibited a moderate decline on day 1 postweaning. Although the modulatory effect of dietary fatty acid composition and/or cholesterol intake on plasma lipid profiles and serum cholesterol levels, as well as cholesterol synthesis has been well documented in humans and animal species at weaning (Cavender *et al.,* 1995; Hugi and Blum 1997), a change in the post weaning diet as the source of these traits in the present study can only be speculated because we did not quantitate serum HDL-, LDL- and very low density lipoprotein (VLDL)-cholesterol concentrations.

In cattle, pigs and alpacas, cortisol is usually regarded as an indicator of weaning. Serum concentrations of cortisol illustrated an acute upswing following weaning, as reported previously (Mohamed, 2006). However, on day 14 post weaning, these values returned to preweaning level. (Mohamed, 2006) reported elevated levels of cortisol in camels during weaning, which was maintained until day 3 after weaning, again conform to basal level by day 5. Since the activities of the sympathoadrenal and pituitary–adrenal system are commonly considered as an indicator of state of stress (Mravec, 2011). Thus, elevations in cortisol in foals might be considered as a physiological reaction of body to weaning induced stress.

Like glucocorticoids, the synthesis and secretion of thyroid hormones are also controlled by the hypothalamo-hypophyseal-system. Serum thyroxin concentrations also increased following weaning and returned to preweaning level until day 14 postweaning in the present study. Carroll *et al.,* (1998) initially noticed no difference in T4 level but after diet change there was appreciable change in T4 level. Dvorak and Neumannova (1986) reported increased serum concentration of T4 among piglets in response of weaning induced stimulated adrenocortical activity. They suggested that both specific stressor effects and circulating corticosteroids are responsible for the changes of T3 and T4 concentrations in blood sera of stressed animals. Similarly induction of 4, 72 and 168 hours stress in rainbow trout (*Onchorynchus mykiss*) produced and acute increase in cortisol and thyroxin level (Bleau *et al.,* 1996). Similarly, induction of 4 hours stress produced an acute increase in cortisol and thyroxin concentrations in white rats (Lischmanov *et al.*, 1987).

REFERENCES

Adamson EJ and RF Slocombe, 1995. Flow cytometric studies of equine phagocytes following strenuous exercise. Equine Vet J, 27: 37-42.

Aoki N, Y Ohno and M Imamura, 1990. Physiological interactions between the immune and endocrine systems: are cytokines hormones? Med Sci Res, 18: 195-201.

Biondi M, and L Zannino, 1997. Psychological stress, neuroimmunomodulation and susceptibilty to infectious diseases in animal and man: a review. Psychther Psychosom, 66: 3-26.

Bimczok D, FW Röhl and M Ganter, 2005. Evaluation of lamb performance and costs in motherless rearing of GermanGrey Heath sheep under field conditions using automatic feeder systems. Small Rumin Res, 60: 255-265.

Blanco M, I Casasús and J Palacio, 2009. Effect of age at weaning on the physiological stress response and temperament of two beef cattle breeds. Anim, 3: 108-117.

Bleau H, C Daniel, G Chevalier, H van Tra and A Hontela, 1996. Effects of acute exposure to mercury chloride and methylmercury on plasma cortisol, T3, T4, glucose and liver glycogen in rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol, 34: 221-235.

Bonanno A, F Mazza, A Di Grigoli and M Alabiso, 2004. Effects of a split 48-h doe-litter separation on the productivity of free-nursing rabbit does and their litters. Livestock Prod Sci, 89: 287-295.

Carroll JA, TL Veum and RL Matteri, 1998. Endocrine Responses to Weaning and Changes in Post-Weaning Diet in the Young Pig. Domestic Anim. Endocrinol., 15: 183-194.

Cavender CP, SD Turley and JM Dietschy, 1995. Steroid metabolism in fetal, newborn, and suckled lambs and their response to cholesterol after weaning. Am J Physiol, 269: 331-340.

Dalin AM, U Magnusson, J Haeggendal and L Nyberg, 1993. The effects of transport stress on plasma levels of catecholamines, cortisol corticosteroid-binding globulin, blood cell count, and lymphocyte proliferation in pigs. Acta Vet Scand, 34: 59-68.

Dantzer R, 2004. Innate immunity at the forefront of psychoneuroimmunology. Brain Behav Immun, 18: 1 -6.

Dvorak M and M Neumannova, 1986. Effects of stimulated adrenocortical activity on the concentration of thyroxin and triiodothyronine in blood serum of piglets. Clin Endocrinol, 88: 339-345.

Grondin TM and SF Dewitt, 2010. Normal hematology of the horse and donkey. In: Schalm´s Veterinary Hematology. (Weiss DJ, Wardrop KJ eds): Wiley Blackwell Inc, Ames IA, pp: 821-828.

Hickey MC, M Drennan and B Earley, 2003. The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute phase proteins, and in vitro interferon-gamma production. J Anim Sci, 81: 2847-55.

Hugi D and JW Blum, 1997. Changes of blood metabolites and hormones in breeding calves associated with weaning. J Vet Med A, 44: 99-108.

Kim HJ, H Zhao, H Kitaura, S Bhattacharyya, JA Brewer, LJ Muglia, FP Ross and SL Teitelbaum, 2006. Glucocorticoids suppress bone formation via the osteoclast. J Clin Invest, 116: 2152-2160.

Lepage OM, S Laverty, M Marcoux and D Ghislain, 1993. Serum osteocalcin concentration in horses treated with triamcinolone acetonide. Am J Vet Res, 54: 1209 -1212.

Li Y F, RR He, B Tsoi, XD Li and WX Li, 2012. Anti-stress effects of carnosine on restraint-evoked immunocompromise in mice through spleen lymphocyte number maintenance. PLoS ONE 7: e33190.

Lischmanov I, Z Trifonova, AN Tsibin, LV Moslova and LA Damentève, 1987. Plasma ß-endorphin and stress hormones in stress and adaptation. Bull Eksp Biol Med, 103: 422-424.

Lorton D, CL Lubahn, C Estus, BA Millar, JL Carter, CA Wood and DL Bellinger, 2006. Bidirectional communication between the brain and the immune system: implications for physiological sleep and disorders with disrupted sleep. Neuroimmunomodulation, 13: 357-374.

Lynch EM, B Earley, M McGee and S Doyle, 2010. Effect of abrupt weaning at using on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. BMC Vet Res, 6: 39.

Magistrelli D, AA Aufy, L Pinotti and F Rosi. 2012. Analysis of weaning-induced stress in Saanen goat kids. J Anim Physiol Anim Nutr (Berl), 95: 40-6.

Mohamed HE, 2006. Factors affecting cortisol status in camels (*Camelus dromedarius*). J Anim Vet Adv, 5: 307-309.

Moons CPH, K Laughlin and AJ Zanella, 2005. Appl Anim Behav Sci, 91: 321-335.

Mravec B, 2011. Role of catecholamine-induced activation of vagal afferent pathways in regulation of sympathoadrenal system activity: negative feedback loop of stress response. Endocrinol Regul, 45: 37-41.

O'Loughlin A, M McGee, SM Waters, S Doyle and B Earley, 2011. Examination of the bovine leukocyte environment using immunogenetic biomarkers to assess immunocompetence following exposure to weaning stress. BMC Vet Res, 7: 45.

Price JS, B Jackson, R Eastell, AE Goodship, A Blumsohn, I Wright, S Stoneham, LE Lanyon and RGG Russel, 1995. Age related changes in biochemical markers of bone metabolism. Equine Vet J, 27: 201-207.

Pyne DB, 1994. Regulation of neutrophil function during exercise (Review). Sports Med, 17: 245-258.

Qureshi F, J Alam, MA Khan and G Sheraz, 2002. Effect of examination stress on blood cell parameters of students in a Pakistani medical college. J Ayub Med Col Abbottabad, 14: 20-22.

Reichmann P, A Moure and HR Gamba, 2004. Bone mineral content of the third metacarpal bone in quarter horse foals from birth to one year of age. J Equine Vet Sci, 24: 391-396.

Suradhat S, 2005. Relationship between the immune system and stress reactivity in pig: visualizing the immune-neuroendocrine framework in action. Thai J Vet Med, 36: 9-18.

Thorèn-Tolling K, 1988. Serum alkaline phosphatase isoenzymes in the horse-variation with age, training and in different pathological conditions. J Vet Med, 35: 13-23.

ThyagaRajan S and HP Priyanka, 2012. Bidirectional communication between the neuroendocrine system and the immune system: relevance to health and diseases. Ann of Neur, 19: 40-46.

Warren LK, LM Lawrence, AL Parker, T Barnes and AS Griffin, 1998. The effect of weaning age on foal growth and radiographic bone density. J Equine Vet Sci, 18: 355.