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HAEMATOLOGICAL AND BIOCHEMICAL REFERENCE VALUES OF VARIOUS AGE AND SEX IN ANATOLIAN WATER BUFFALOES (*Bubalus bubalus*)

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Farklı yaş ve cinsiyetteki Anadolu mandalarında (*Bubalus bubalus*) hematolojik ve biyokimyasal değerler

ÖZET

Bu çalışma Anadolu Mandası'nın yaş ve cinsiyet ile ilişkili olarak değişen kan değerlerinin saptanması amacıyla yapılmıştır. Hematolojik hesaplamalar ve serum biyokimyasal analizler Afyon ilinde bulunan klinik olarak sağlıklı 160 Anadolu mandasında yapılmıştır. Eritrosit, total ve diferansiyel lökosit sayımları, hematokrit, hemoglobin düzeyleri hesaplandı. Serum biyokimyasal analizlerinde; total bilirubin, üre, kreatinin, glikoz, total protein, trigliserid ve total kolesterol konsantrasyonlarının yanı sıra alanin aminotransferaz, aspartat aminotransferaz, gamma-glutamiltransferaz, kreatin kinaz, laktik dehidrogenaz ve sorbitol dehidrogenaz aktiviteleri ölçüldü.

KEY WORDS: Anadolu mandası, kan, biyokimya, hematoloji

SUMMARY

The objective of this study was to investigate to determine the age and sex-related changes in blood of Anatolian Water Buffaloes. Haematological estimations and serum biochemical analyses were made on collected 160 samples from clinically hea1thy Anatolian Water Buffa1oes (Bubalus bubalus) in Afyon Province. The red blood cell counts, packed cell volume, haemoglobin concentration, total and differencial white blood cell counts were estimated. In the serum biochemica1 ana1yses, total bilirubin, urea, creatinine, glucose, total protein, trigyliceride and total cholesterol concentrations were measured, as were the alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, creatine kinase, lactic dehydrogenase and sorbitol dehidrogenase activities.

ANAHTAR KELİMELER: Anatolian water buffalo, blood, biochemistry, hematology

GİRİŞ

Buffalo breeding was characterized by economic advantages with relatively low costs. Although buffalo products (meat and milk and their production) are high in protein, low in fat, and the taste is appreciated by consumers (Ognjanovic et al. 1970, Hoang 1978, Dinen 1986, Pradhan et al 1991, Sing and Sing 1994), in Turkey, main problem is marketing; in the country buffalo meat is only used in meat products like sausage. People do not accustom to eat buffalo meat in their meals. Therefore, at the end of the fattening period, buffaloes can be marketed to different sausage factories in Afyon Province. The situation prevents competition as regards to meat price, although the price of concentrate mixture is getting higher.

When compared with other domestic livestocks, the water buffalo generally is a healthy animal, but there are numerous health problems in buffaloes reared in captivity and include infectious diseases (viral, bacterial, micotic and parasitic) and non-infectious syndromes associated with husbandry, incubation, diet and metabolism (Hoffman et al 1984, Hung 1985, Dwivedi et al 1997). As a result, veterinarians are frequently requested to examine these animals, and determination of a serum biochemical profıle is occasionally part of this

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examination. For many tests, there is no single reference range that applies to everyone because the tests performed may be affected by the age and sex of the patient, as well as many other considerations. Both age and sex are known to affect the normal levels of many important and commonly measured blood constituents (Hoang 1978, Bush et al 1982, Canfield et al 1984)

For a clinico-pathological test reported to be meaningful, there must be a standard of reference values. The purpose of the study reported was to describe normal values for selected clinic-pathologic variables in different age and gender of healthy Anatolian Water Buffalo residing in Afyonkarahisar province in Turkey.

MATERIALS and METHODS

Totally 160 Anatolian water buffaloes different age and sex from 15 ranches in Afyon province were used in the study. The animals were used for this study categorised by neonate < 1 month), nursing (1 to 6 months), juvenile (6 to 18 months) and adult (> 18 months) males and females at different physiological states (pregnant, non-pregnant and lactating). In the cold seasons, buffaloes were fed with alfalfa hay, straw, cotton seeds; sugarbeet pulp, dried breed. In summer wheat bran, straw and barely were added to grazing. The animals were clinically health.

Blood was obtained from the jugular vein in vacuated glass tubes (Vacutainer tubes, Becton, Dickonson & Co, Rutherford, NJ). Samples for hematologic analyses were collected in 5 ml EDTA containing tubes. Blood collected for serum biochemical analysis was allawed to clot in serum separation tubes and was centrifuged for 10 minutes at 600 g. Enzymes were analyzed within 48 hours from its withdrawal and the rest was storaged at -20 ° C until analyzes. Most samples were collected in 2003 September and October. Blood were also collected from pregnant and lactating buffaloes in February.

For each age group, except for neonate group (n=40), 20 buffalo were sampled for hematologic analysis. Packed cell volume (PCV) was determined in microhematocrit tubes arter centrifugation for 5 minutes. Total red blood cell (RBC) and white blood cell (WBC) counts were determined manually with Thoma lam and lamel using Hayem's solution (for RBC count) and Turkey's solution (for WBC count). Blood smears were made using EDTA anticoagulated blood and stained with Giemsa stain for differential counts. Hemoglobin was determined using Sahli method. Haematological values were determined by rutine methods (Schalm et al 1975).

Serum biochemical analyses were including creatinine, total protein, albumin, urea nitrogen, total bilirubin, cholesterol, triglyceride, glucose, aspartate transaminase (AST) and alanine aminotransaminase (ALT), lactate dehydrogenase (LDH), creatinin kinase (CK), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). Biochemical assays, except for sorbitol dehydrogenase activities (SDH), were performed in Roche/Hitachi 917 Clinical Chemistry Analyzer by using commerically available diagnostic kits supplied from RoscheDiagnostics (D-68298, Mannheim, Germany). SDH activities were determined on a recording spectrophotometer (Shimadzu VV-I 200 series PIN 206-62409) using standard reagents (Sigma Chemical Co), and activity of this enzyme was not obtained for the frozen samples, beceause assays for SDH, are meaningless unless fresh samples are used.

The haematological and biochemical values were evaluated independently by using of a general linear model ANOVA (SAS/STAT, Version 11.0) with age and sex groups as the main effect. Differences between for each groups were compared, using the Duncan multiple test, with $p<0.01$ considered significant. Suggested reference values represent 95^{th} percentile estimates.

RESULTS

The effect of age and sex on mean hematological and biochemical values for clinically normal Anatolian water buffaloes were determined (Table 1). Furthermore, the levels of blood analyses in Anatolian water buffaloes classified according to their physiological states are shown in Table 2.

DISCUSSION

Although buffaloes breeding largely diffused in different regions and it is economically important in the world, their hematological and biochemical analyses are not used extensively in water buffalo medicine due to the lack of reference intervals for various buffaloes species; these analyses have been investigated only by few authors particularly in Asia and Italy (Said et al 1987, Horadagoda et al 2002).

A previous report (Ferrer et al 2000) of hematological values for 200 buffalo calves indicated that PCV, erytrocyte count and Hb to be significantly increased with age. That finding generally agrees with our results, although they did not select a juvenile group. Results of this study agree with previous reports that leukocyte count was gradually decreased with age. The difference between lympocyte and neutrophil absolute count narrowed increasing age, but the lymphocyte was always the predominant leucocyte (Pospisil et al 1985, Jain 1994). In the present study, absolute lymphocyte count (68.91±3.47 %) was higher than the absolute neutrophyl count (36.24±1.46 %) in young buffaloes. However, there was significant and marked decreases in the absolute number of lymphoctes between young (68.91±3,47 %) and adult (56.27±2.24 %) bulls.

Table 1. Hematologic and serum biochemiacal values in Anatolian water buffaloes

Values	Neonatal <1 mo	Nursing 1 to 6 mo	Juvenile 6 to 18 mo	All young	Adult male	Adult female	All adults	All buffaloes
Haematology	40	20	20	80	20	20	40	120
Erythrocyte count x 10^6/µl	5.34±1.23^{c}	5.86±1.03^{bc}	6.43±1.11^{a}	5.74±1.20^{bc}	6.74±1.35^{a}	6.58±0.93^{a}	6.64±1.19^{a}	6.44±1.28^{a}
Hemoglobin (g/dl)	10.88±2.04^{c}	11.46±1.62^{bc}	12.39±1.28^{a}	11.08±1.65^{b}	12.58±1.17^{a}	12.88±1.01^{a}	12.48±1.12^{a}	11.88±1.69^{b}
Packed cell volume (%)	28±3^{c}	33±2^{bc}	35±2^{a}	33±1^{b}	34±2^{ab}	34±1^{ab}	34±1^{ab}	33±6^{b}
Leukocytes x 10^3/µl	10.642±3.99^{a}	10.432±2.301^{a}	8.362±2.444^{c}	9.568±3.991^{b}	8.642±2.720^{c}	8.823±1.590^{c}	8.645±2.771^{c}	9.656±3.225^{b}
Neutrophils (%)	34.97±1.928^{c}	35.18±1.543^{c}	37.77±1.568^{b}	36.24±1.468^{b}	38.56±1.456^{a}	38.42±1.113^{a}	38.47±1.347	37.17±1.224^{b}
Lymphocytes (%)	71.71±3.54^{a}	70.41±2.68^{a}	67.60±3.12^{b}	68.91±3.47^{b}	56.42±2.32^{c}	56.11±2.24^{c}	56.27±2.24^{c}	57.91±2.84^{c}
Monocytes (%)	652±480	656±361	663±360	658±371	680±580	681±552	681±286	671±380
Eosinophils (%)	348±416	358±232	398±113	381±350	357±377	359±330	358±152	368±234
Basophils (%)	39±14	39±16	40±24	39±20	39±44	39±43	39±44	39±35
Proteins								
Total proteins (g/dl)	6.2±1.10^{c}	6.4±1.23^{b}	7.4±1.12^{a}	6.7±1.43^{b}	7.8±1.33^{a}	7.6±1.28^{a}	7.7±1.25^{a}	7.4±1.32^{a}
Albumin (g/dl)	3.80±0.15^{a}	3.45±0.45^{a}	3.10±0.26^{a}	3.60±0.27^{a}	2.76±0.40^{b}	2.47±0.21^{b}	2.56±0.33^{b}	3.10±0.12^{a}
Metabolites								
Glucose (mmol/L)	2.68±0.44^{b}	2.72±0.57^{b}	3.22±0.33^{a}	3.02±0.31^{a}	3.47±0.87^{a}	3.44±0.76^{a}	3.45±0.34^{a}	3.22±0.87^{a}
Cholesterol (mmol/L)	65±13^{b}	66±18^{b}	68±10^{b}	66±72^{b}	70±18^{a}	70±03^{a}	70±14^{a}	68±43^{b}
Creatinine (µmol/L)	120.20±21.25^{c}	123.28±45.20^{c}	126.17±15.85^{c}	124.18±30.12^{c}	130.14±37.23^{b}	134.12±12.23^{a}	132.47±22.20^{b}	128.14±32.28^{b}
Urea (mmol/l)	3.20±1.20^{c}	3.62±1.04^{c}	3.80±1.12^{c}	3.56±1.15^{c}	4.08±1.27^{b}	5.12±1.32^{a}	4.58±1.18^{b}	4.42±1.26^{b}
Total bilirubine (µmol/1)	4.3±4.2	4.1±4.3	4.7±4.0	4.4±4.2	4.9±4.4	4.0±4.0	4.5±4.3	4.4±4.3
Direct bilirubine (µmol/1)	2.05±1.7	2.00±1.3	2.06±1.2	2.03±1.4	2.05±1.6	2.01±1.4	2.03±1.5	2.04±1.3
Trigliceride	22.7±11.0^{b}	22.8±10.8^{b}	23.6±10.3^{b}	22.9±11.0^{b}	27.7±12.4^{a}	28.2±11.0^{a}	27.9±10.5^{a}	24.9±10.3^{b}
Enzymes (IU/L)								
Alanine aminotransferase	35.2±11.0^{c}	35.7±10.8^{c}	38.2±10.6^{b}	36.9±11.6^{c}	39.2±10.4^{a}	39.0±10.2^{a}	39.1±10.3^{a}	37.6±11.0^{b}
Asparate aminotransferase	105±36^{c}	110±20^{c}	118±28^{b}	113±22^{c}	125±42^{a}	125±16^{a}	118±33^{b}	115±24^{bc}
Alkaline phosphatase	148.6±64.3a	132.4±54.1b	118.1±44.3c	137.5±74.6b	116.1±34.6c	117.2±44.1c	116.5±84.4c	126.3±84.2c
Gamma glutamyltransferase	28.8±16.1a	20.1±10.2b	16.4±12.2c	24.7±13.6ab	16.8±10.3c	17.1±9.1c	16.8±9.8c	18.4±11.2
Creatine kinase	86.45±38.4	70.45±25.5	64.75±32.4	76.45±25.8	68.70±25.3	67.24±15.8	67.76±24.2	70.15±23.2
Lactic dehydrogenase	1342±332.43c	1532±230.23bc	1768±440.24a	1586±363.28bc	1754±432.26a	1768±414.22a	1758±422.18a	1672±313.44b
Sorbitol dehidrogenase	25.40±8.12c	26.32±7.19bc	27.45±8.02bc	26.37±7.79bc	31.68±7.72a	31.32±7.86a	31.51±7.74a	28.86±7.75b

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Table 2. Haematologic and serum biochemiacal values in Anatolian water buffaloes different physiologic state

Values	Pregnant	Non-pregnant	Lactating
Haematology	20	20	20
Erythrocyte count x 10^6/µl	6.84±1.23	6.58±0.93	6.93±1.20
Hemoglobin (g/dl)	13.23±1.42	12.88±1.01	13.08±1.12
Packed cell volume (%)	35±1[b]	34±1[c]	37±2[a]
Leukocytes x 10^3/µl	10.512±3.23	8.823±1.590	10.642±3.91
Neutrophils (%)	40.47±1.643	38.42±1.113	39.17±1.128
Lymphocytes (%)	60.71±3.54	56.11±2.24	58.48±3.26
Monocytes (%)	682±580	681±552	678±860
Eosinophils (%)	388±350	389±330	376±343
Basophils (%)	39±28	39±43	39±15
Proteins			
Total proteins (g/dl)	6.9±1.15	7.6±1.28	7.0±1.28
Albumin (g/dl)	2.3±0.45	2.4±0.21	2.6±0.43
Metabolites			
Glucose (mmol/L)	2.72±0.86[b]	3.44±0.76[a]	2.22±0.87[b]
Cholesterol (mmol/L)	84±13[b]	70±03[c]	91±13[a]
Creatinine (µmol/L)	138.27±27.25	131.12±22.23	135.27±27.25
Urea (mmol/l)	5.02±1.62	4.82±1.32	4.96±1.60
Total bilirubine (µmol/1)	4.8±4.2	4.0±4.0	4.9±4.4
Direct bilirubine (µmol/1)	2.15±1.4	2.01±1.4	2.05±1.7
Trigliceride	35.7±11.2[b]	28.2±11.0[c]	39.8±11.5[a]
Enzymes			
Alanine aminotransferase (IU/L)	42.2±11.8	38.0±10.2	40.2±10.4
Asparate aminotransferase (IU/L)	135±28	115±26	126±24
Alkaline phosphatase (IU/L)	108.5±34.2	117.2±44.1	110.5±62.3
Gamma glutamyltransferase (IU/L)	18.8±16.3	17.1±9.1	18.8±16.3
Creatine kinase (IU/L)	66.75±35.5	67.24±15.8	66.75±35.5
Lactic dehydrogenase (IU/L)	1748±210.12	1768±414.22	1747±382.26
Sorbitol dehidrogenase (IU/L)	33.47±7.45	31.32±7.86	35.14±6.81

[a,b,c] Within each hematologic or serum biochemical variable, a different superscript (a,b,c) indicates the age and sex groups differ (p= 0.01).
Data are presented as mean ± SD

Levels of eosinophil of animals in juvenile groups were higher according to levels of the other groups in this study. Persistent eosinophilia could suggest a disease process involving degranulation of mast cells (Jain 1994, Monke et al 1998). The other study (Dorner 1986) reported a gradually increasing eosinophil count in healthy calves, with relative eosinophil count up to 15 % between 4 and 8 months of age. Therefore, is not clear whether the high values in eosinophil count reflects some recognized antigenic stimulation or simply wide normal limits.

Determination of reference ranges for a variety of serum biochemical variables was an important component of this study. Our biochemical results suggest the following considerations.

In this study, very small and insignificant variations between male and female Anatolian water buffaloes were seen in the levels of the various blood analyses. Significant differences were associated with age and we identified for several variables. Among the measured enzymes ALP showed a significant difference, being much higher in neonatal animals (<1 month) (Ottol et al 2000), values tend to fall progressively with age in both sexes. Reference values for ALP that include yearling and adult bulls are not usefull because of the age-related difference, which is correlated with the high tissue activity of ALP in the bones of young (Dorner 1986, Horadagoda et al 2002). Higher values of ALP were found in the first 40 days of life due to the more intense bone remodeling (Campanile et al 1991), and leakage of the enzyme from the growing bones and intestines into the blood (Zicarelli et al 1982).

The growth and therefore, the progressive and ever more complete functionality of the organs, leads to greater activity of these enzymes at tissue level and consequently to their increase at serum level. Hepatocellular injury may be evaluated by measuring AST, because it has high activity in hepatocytes. However, skeletal muscle tissue also has high AST activity: Differentiation may be made by measuring CK, a muscle-specific enzyme (Zicarelli et al 1982, Canfield et al 1984, Pospisil et al 1985). CK and LDH are indicators of muscle damage, both skeletal and cardiac, and neoplasia. Second tend to decrease progressively with age in both sexes (Campanile et al 1993). In the present study, an increase on the 30 th day of life is followed by progressive reduction; this progress could represent the resulting increase of muscular tissue and functional gymnastics. The cytoplasmatic enzyme, GGT, is the first to increase even in conditions of slight hepatic sufferance. It is

hightly correlated with SDH enzyme, those which more specifically stabilize hepatic functionality, even if difficult to analyze. The progressively decreasing GGT values in our study, would be linked to the return within physiological limits of two constants which rise noticeably during the colostral phase, in as far as absorbed with the colostrum (Tardati et al 1982, Campanile et al 1993, Lombardi et al 1996).

Evaluation of blood protein variables provides an indication of the health of animal. Nevertheless, total protein was significantly affected by feeding system (Houpt 1970, Di Lella 1995), because the animals on pasture received more protein than heifers fed maize silage; (Pradhan et al 11991) and think total protein could be modified with diets in buffaloes, may be for the increased protein breakdown required for gluconeogenesis (Zicarelli et al 1986), even if found no influence of the lactation stage and of meal in this species (Campanile et al 1993). The progressively decreasing albumin values with age were determined in this study (Table 1). According to Meyer et al (Myer and Enrich 1992), hypoalbuminemi results secondary to the hyperglobulinemia, because increased osmotic presure of the blood signals the liver to reduce albumin production.

In the present study, mean creatinin concentration tend to increase progressively with age in sexes. According to Monke (17), higher creatinin value in adult animals was correlated with protein metabolism associated with the ir large muscle mass. Blood urea is present in peripheral blood and has been shown to be an indirect indicator of feed protein composition in farm animals (Houpt 1970, Di Lella et al 1995). Significant differences were not observed in values of bilirubin according to ages or gender in the study.

The normal values for glucose in this study were found to increase with age. Compared with cattle (Ottol et al 2000), higher glucose concentration was found in this study is not obvious, a transient in serum glucose concentration due to excitement associated with simply and subsequent epinephrine (Cesar et al 1997) can not be ruled out if this were so, Anatolian water buffaloes seem to be more prone to this phenomon than other domesticated ruminants. The concentration of glucose in this study seems likely that Anatolian water buffalo simply have higher baseline glucose concentrations, and the buffalo adapts itself better than bovine to the relatively good digestibilty of low energy diets.

The physiological status of the buffaloes significantly affected the serum levels of some blood constituents. Highest cholesterol and triglyceride levels with higher hematocrit were seen in the lactating buffaloes and glucose level was highest ($P<0.01$) in the non-pregnant and non-lactating buffaloes (Table 2). Glucose level was significantly affected by puberty onset also as puberty probably involves a more intensive energy metabolism. All these differences could be related to the differences in the animals metabolism, needs for milk production and metabolic changes related to the development of the fetus (Campanile et al 1991). Rising glycemia, on the other hand, is obtained only during the anabolic phase of lactation when energy intake is equal to, or superior to the energy release.

Cholesterol is not affected by feeding system it shows increasing trend after puberty. It is well know, in fact, that the NEF A which are released at early lactation following intense fat mobilization, are used hepatically for the syntesis of the triglycerides only if the balance between energy absorbed in the diet and that emitted due to production is not especially deficit (Zicarelli et al 1982, Zicarelli et al 1986).

The research provides that the most comprehensive investigation haematological and biochemical varibles of Anatolian water buffaloes in World to date.

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