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EFFECTS OF AGE ON HEMATOLOGY AND SERUM BIOCHEMISTRY OF SOKOTO GUDALI BREED OF CATTLE UNDER EXTENSIVE MANAGEMENT

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Abstract

The knowledge of age estimation, haematological and biochemical variables are tools used as medical decision through anamnesis, clinical diagnosis and animal health status. The effects of age on haematological and serum biochemical parameters of Sokoto Gudali breed of cattle under semi-intensive management in Ilorin, Nigeria. forty apparently healthy cattle were used for the study, 10 per age group. Ages of cattle were determined using already established records and compared with Sokoto Gudali local breeds available in Ilorin. Blood samples were aseptically collected for serum and further analyzed within 24h for selected blood chemistry. Data from haematological and biochemical studies were analysed for the effect of age on PCV, RBC count, Hb concentration, MCV, MCH, and MCHC, total protein, albumin, Ca^{2+} , P^4 , Mg^{2+} , HCO^- , and Cl^- using one way-ANOVA with $p < 0.05$ considered significant. The results showed that the age had effect on dentition vis-à-vis the ages of eruption for central (I_1), intermediate (I_2), lateral (I_3) and corner (I_4) incisors are 2yrs, 3yrs, 4yrs and 5yrs respectively. After 5yrs, incisors start wearing and age estimation becomes difficult, with less significant influence on the haematological parameters, total protein and albumin, while calcium, magnesium, bicarbonate and chloride significantly ($p < 0.05$) increased with age and phosphorus significantly ($p > 0.05$) decreased with age in these breed of cattle under extensive management. It was concluded that the results obtained may serve as reference values for Sokoto Gudali breed of cattle under extensive management within Ilorin and its metropolis for the farmers and the researchers.

Keywords: Age, Haematology, Biochemistry, Cattle, Ilorin

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Introduction

Ruminant animals largely constitute the farm animals reared by farmers in Nigeria (Ogbeh, 2016). This country has been reported to have cattle population of 13.9 million (Bourn, 2012) but recent National Agricultural

Sample Survey in 2011 indicated that Nigeria was endowed with an estimated 19.5 million cattle, 72.5 million goats, 41.3 million sheep, 7.1 million pigs and 28,000 camels. The country had 145 million chickens, 11.6 million ducks, 1.2 million turkeys and 974,

499 donkeys (NASS, 2011). This impressive statistics which had made Nigeria a leading livestock producer in Africa had not met the national demand of animal protein or contributed to the gross domestic product over the years (NASS, 2011). Cattle play significant roles in the nutrition and socio-economic life of Nigerians. Sometimes, they serve as savings, gifts as well as meat animals at weddings and other festivities. The most accurate method of estimating the age of an animal is through knowing its date of birth. In the absence of birth record, various anatomical features (such as teeth) are used to estimate age (De-Lahunta, 1986). The most convenient teeth for ageing are the incisors (front teeth), while the remaining teeth are hidden by the cheeks. There is a dearth of information on the use of rostral dentition in Nigeria. However, the available references on rostral dentition were obtained in other parts of the world and the breeds of animals used differ from those used in the present study. The various authors have reported that knowledge of age of animals forms an essential part of anamnesis and aid in clinical diagnosis (RAR, 2009, Ferguson, 1990, Harvey, 1985) and similarly in chemotherapy, the choice and dosage of some drugs are influenced by the age of the subject (Latshaw and Decker, 1987). Haematological and biochemical variables are widely used decision-making tools in veterinary medical practice. If performed properly, these variables can provide significant insights regarding diseases and therapeutic approaches (Thrall, 2004). During diagnostic procedure, it is very useful to compare the values obtained from ill animal with normal reference values of healthy animals. Reference values vary with species, age and environmental conditions. Therefore, specific reference intervals are needed for each age group of a species, in a particular environment, for appropriate interpretation of

results of haematological analyses (Meyer and Harvey, 2004). The management system in which animals are kept affect a wide range of haematological and serum biochemical parameters. The animals under intensive system were raised with commercial feeds or concentrate while their counterparts under extensive system raised with greens and forages with deficiency of macro and micro-nutrients which may elicit variation in haematological and serum biochemistry of livestock animals (Al-Zadjail *et al.*, 2004). Trace element like cobalt has been found to play a very significant role in the biosynthesis of vitamin B12 in livestock animals and its deficiency in animal feeds could lead to vitamin B12 deficiencies related diseases in animal under extensive system (Al-Zadjail *et al.*, 2004). Moreover, animals reared under extensive systems tend to have lower glucose levels compared to those reared under extensive systems due to physical exercises and ranging activities (Onasanya *et al.*, 2015). This study was designed to examine the relationship between age using dentition and haematological and serum biochemical parameters among Sokoto Gudali cattle breed under extensive management in Ilorin.

Materials and Methods

Study location and study animals

This study was carried out in 'Ipata' Abattoir, Emir Farm, Islamic Institute farm, Ilorin, Faculty of Agriculture and Faculty of Veterinary Medicine, Teaching and Research Farm, both at University of Ilorin, Ilorin, Kwara State, located at North-central geopolitical zone of Nigeria from December, 2015 to July, 2016. A total of 40 apparently healthy Sokoto Gudali breed of Cattle under extensive management, 10 per age group were sampled and relevant images taken from farms in Ilorin. The ages were grouped as follows: <2yrs (Plate1), 2yrs (Plate 2) , 3-4yrs, (Plate 3) and $\geq$ 5yrs (Plate 4), as

adopted from the knowledge of changes in eruption and wearing of the deciduous and permanent teeth from literature was used to compare with Sokoto Gudali cattle breeds sourced in Ilorin. The animals were allowed to graze on pasture which includes greens and forages from 10.00am in the morning till 5.00pm in the evening with available water from ponds.

Sample collection

Blood samples were aseptically collected between 7.00 am and 9.00 am via jugular venipuncture using disposable syringes and needles (Medline Inc.). Blood (3ml) was collected from each animal into disodium-EDTA (Vacutainer® EDTA Tubes, Thomas Sc.Inc.,) tubes and another 3ml collected into non coated vacutainer tubes (BD Vacutainer® Plus Plastic Serum Tubes, Thomas Sc.Inc.,) for serum analysis and transported in cold chain to the laboratory in Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin. Samples were analysed within 24hrs of collection. Red blood cells (RBC) count was determined by using haemocytometer (Schalm *et al.*, 1975), and Haemoglobin concentration was determined using Cyanomethaemoglobin (Kackmar, 1970). Packed cell volume was determined by using Microhaematocrit method as previously described by (Coles, 1986). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration were calculated according to (Mitruka and Rawnsley, 1977). Total protein, and Albumin were determined as described by (Doumas and Biggs, 1972), Phosphorus and Calcium, were determined by flame photometry method (Corning 400R: S/400/3888 Essex, UK) (Cheesbrough, 1991). Magnesium was determined as described by (Daisy *et al.*, 1947), Bicarbonate was determined using

titrametric method (Cheesbrough, 1991) and Serum chloride was determined by mercuric nitrate titrametric method of (Schales and Schales, 1941).

Statistical analysis

Statistical analysis was done using SPSS version 17 at 5% levels of errors and data were expressed as mean $\pm$ standard deviation. One way analysis of variance (ANOVA) was applied to compare significant differences within the age groups. Duncan multiple range test was used to examine the age group that differs from another.

Results and Discussions

Deciduous teeth were small sized, whitish and narrow necked. Permanent incisors were large sized, dirt-coloured and broad necked. Ages of eruption of permanent incisors are summarized in Table 1. The mean values of haematological parameters for cattle were shown in Table 2. It was found that the mean values of PCV ($29.00\% \pm 3.94$ - $30.20\% \pm 3.65$), RBC ($5.68 \times 10^{12}/L \pm 1.67$ - $4.68 \times 10^{12}/L \pm 0.56$), Hb (9.24 g/dL ± 1.18 - 9.60 g/dL ± 1.19), MCV (53.50 fL ± 12.16 - 63.30 fL ± 5.65), MCH (17.14 pg ± 3.81 - 20.51 pg ± 1.66) and MCHC (31.86 g/dL ± 1.28 - 32.93 g/dL ± 3.16) were not significantly affected by age ($p > 0.05$), among the different age groups of cattle. The mean values of total protein (6.40 g/dL ± 0.80 - 7.20 g/dL ± 0.75 and albumin (4.00 g/dL ± 0.5 - 4.50 g/dL ± 0.2) were not significantly affected by age while calcium (8.6 mg/dL ± 0.03 - 9.3 mg/dL ± 0.05), magnesium (2.0 mg/dL ± 0.01 - 3.4 mg/dL ± 0.03), bicarbonate (24.58 mEq/L ± 1.14 - 28.70 mEq/L ± 2.04), and chloride (101 mEq/L ± 0.02 - 105 mEq/L ± 0.03) significantly ($p < 0.05$) increased with age and phosphorus (5.6 mEq/L ± 0.1 - 3.2 mEq/L ± 0.2) significantly decreased ($p < 0.05$) with age (Table 3). It has been reported by (Fradson *et al.*, 2009) that tooth development in non-human mammals is

similar to human tooth development with the variations lie in the morphology, number, development timeline, and types of teeth as they aged. This had impact to some extent on haematological and biochemical parameters of various species of animals. In cattle, the ages of eruption of permanent incisors obtained agrees with Kahn, (2010). The features of the teeth after eruption can be attributed to age (whether early or late maturing) and geographical distribution. Also, it depends on the type of pasture or feed available. On long, soft pasture the teeth will grow long from lack of wear, but remain in good condition. On short feed, where close grazing is necessary, particularly if the soil is sandy or gravelly, the teeth will wear down. After the eight permanent incisors have appeared, progressive deterioration (wearing) starts the rate depending on the conditions, particularly the feed or pasture, under which the animal is managed. Estimation of age at this stage is very difficult. In the present study, age had no remarkable influence on the haematological parameters of cattle which may be due to chronic parasitic infection such as helminthosis or malnutrition or a combination of both as seen in animal under extensive management (Onansanya *et al.*, 2015, Oduye and Okunaiya, 1971). Although numerical increased in PCV, RBC and Hb concentration were observed up to 3-4 years, which is suggestive of erythropoiesis. According to Bedenicki *et al.*, (2014). The insignificant differences in PCV, RBC, haemoglobin concentration and MCV values of cattle from 2yrs upward. This agrees with (Mohri *et al.*, 2007, Meyer and Harvey, 2004), who reported that many values vary with age of the animal, with major changes occurring before puberty. In the present study, all cattle sampled had attained puberty, including those less than 2yrs. The mean values are consistent with reports of the following authors (Latimer, 2011, RAR, 2009, Jain, 1998) but

different (Olayemi *et al.*, 2007) which can be attributed to influence of nutrition, physiological condition, geographical location and seasonal variation (Kaneko *et al.*, 2008, Kaneko *et al.*, 1997, Schalm, 1984). The biochemical variables in the blood serum with other physiological variables are important for assessment of tissue and organ damage in different diseases and for assessment of development from welfare aspect (Klinkon and Jezek, 2012, Steinhardt and Thielscher, 200a,b). The high concentration of total protein and albumin in this study is influenced by nutrition and functioning of the liver (Klinkon and Jezek, 2012). The albumins are predominantly synthesized in the liver (Dubreuil *et al.*, 2005), so the amount depends on maturity and functioning of the liver (Steinhardt and Thielscher, 200a,b). The calcium homeostatic system depends on several factors such parathyroid hormone (PTH), vitamin D, phosphate, and magnesium metabolism. The increased of calcium and magnesium while the decreased of phosphorus with age is associated with parathyroid hormonal influence which maintain the steady-state level of serum calcium. A sudden fall in ionized calcium will quickly lead to a rise in PTH secretion and the result of which leads to a rapid release of calcium from bone. Thyroid hormone also acts to maintain the steady- state level of serum calcium by its action on the kidney. It increases the tubular reabsorption of calcium and magnesium and decreases the tubular reabsorption of phosphate, sodium, bicarbonate, potassium, and amino acids. PTH activates the adenylate cyclase system by binding with receptor sites in the renal cortex. It thus leads to an increase in cyclic adeno side monophosphate. It has been reported that the serum phosphorus level also plays a role in the maintenance of a steady-state

Concentration of serum calcium is such that a rise in serum phosphate usually leads to a fall in serum calcium. Some of this decrement may be caused by enhanced formation of CaHPO_4 complexes in the serum. A fall in the level of serum phosphate will conversely lead to an increase in the serum ionized and bone ECF calcium. The phosphorus significantly decreased with age. It has been reported that phosphorus is absorbed in form of inorganic phosphate (iP) in the fore stomach and in the front part of the small intestine and its excretion from faeces and urine. The serum concentration of iP increased in younger animal because of growth hormone increases the reabsorption of phosphate in the kidney (Rosol and Capen, 1997) while the decreased observed in the adult cattle may be associated with the fact that in the rumen it acts as working buffer for volatile fatty acids and substrate for microorganism and its deficiency lower microbe synthesis of proteins in the rumen (Underwood and Suttle, 2001). Chloride has been reported to be absorbed in small intestine and excreted by the kidney. It is the most important anion in the extracellular fluid and together with Na it is responsible for maintenance of osmotic pressure in the extracellular fluid (Klinkon and Jezek, 2012).

Moreover, Increased in the serum HCO_3^- is due to metabolic alkalosis as the animal increased in the age which may be due its excretion and reabsorption by the kidney as well as its regulation by the lung. In this study, it was observed that age had influence on some biochemical parameters. The calcium, magnesium, chloride, bicarbonate significantly increased, while phosphorus significantly decreased (Klinkon and Jezek, 2012).

Conclusion

The ages of eruption for central (I_1), intermediate (I_2), lateral (I_3) and corner (I_4) incisors can be estimated as 2yrs, 3yrs, 4yrs and 5yrs respectively. After 5yrs, incisors start wearing and estimation of age becomes difficult. The PCV, RBC, Hgb, MCV, MCH and MCHC are not significantly influenced by age. In serum chemistry, total protein and albumin had no influence with age but calcium, magnesium, bicarbonate and chloride significantly increased with age while phosphorus significantly decreased with age in the study area.

Conflicts of Interests:

There are no conflicts of Interests

Plate 1: Estimated <2years: All teeth are deciduous, with narrow neck, much smaller and whitish compared to permanent teeth

Plate 3. Estimated 3years: Central (I_1) and Intermediate (I_2) permanent incisors have erupted

Plate 2: Estimated 2years: Central permanent incisors (I_1) have erupted

Plate 4. Estimated 5years old: Full mouth, all permanent incisors (I_1 - I_4) have erupted

Table 1: Ages of eruption of permanent incisors in cattle

Age	Features of incisors
Less than 2yrs	All incisors were deciduous; whitish, small and narrow neck.
2yrs	Central permanent incisors (I_1) had erupted
3yrs	Intermediate permanent incisors (I_2) had erupted
4yrs	Lateral permanent incisors (I_3) had erupted
5yrs	Corner permanent incisors (I_4) had erupted
Above 5yrs	Permanent incisors start wearing.

Table 2: Comparison of haematological parameters (mean $\pm$ S.D) between age groups of cattle

Age (years)	n	PCV (%)	RBC ($\times 10^{12}/L$)	Hgb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
<2	10		5.68 $\pm$ 1.67	9.24 $\pm$ 1.18	53.50 $\pm$ 12.16	17.14 $\pm$ 3.81	31.86 $\pm$ 1.28
2	10	31.60 $\pm$ 4.30	5.40 $\pm$ 1.22	10.33 $\pm$ 1.60	59.6 $\pm$ 6.75	19.46 $\pm$ 2.61	32.63 $\pm$ 1.42
3-4	10	31.10 $\pm$ 3.28	5.94 $\pm$ 1.46	10.37 $\pm$ 0.92	54.7 $\pm$ 13.42	18.36 $\pm$ 4.42	33.29 $\pm$ 1.45
.	10	30.20 $\pm$ 3.65	4.68 $\pm$ 0.56	9.60 $\pm$ 1.19	63.20 $\pm$ 5.65	20.51 $\pm$ 1.66	32.93 $\pm$ 3.16

Table 3: Comparison of serum biochemical parameters (mean $\pm$ S.D) between age groups of cattle

Age (years)	n	Total protein (g/dL)	Albumin (g/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Magnesium mEq/L	Bicarbonate mEq/L	Chloride (mEq/L)
<2	10	6.40 $\pm$ 0.80	4.0 $\pm$ 0.5	8.6 $\pm$ 0.03	5.6 $\pm$ 0.1	2.0 $\pm$ 0.01	24.58 $\pm$ 1.14	101 $\pm$ 0.02
2	10	6.50 $\pm$ 0.70	4.2 $\pm$ 0.3	8.8 $\pm$ 0.05	5.2 $\pm$ 0.2	2.1 $\pm$ 0.02	25.40 $\pm$ 1.42	102 $\pm$ 0.04
3-4	10	7.15 $\pm$ 0.40	4.3 $\pm$ 0.3	9.1 $\pm$ 0.05	3.5 $\pm$ 0.1	3.0 $\pm$ 0.02	28.63 $\pm$ 4.80	103 $\pm$ 0.02
.	10	7.20 $\pm$ 0.75	4.5 $\pm$ 0.2	9.3 $\pm$ 0.05	3.2 $\pm$ 0.2	3.4 $\pm$ 0.03	28.70 $\pm$ 2.04	105 $\pm$ 0.03

References

- Al-Zadjali, A., Johnson, E. H., Srikanthakumar, A. 2004. Serum Vitamin B12 in Omani Goats. *Tropical Animal Health and Production* **36**, 473-482.
- Bedenicki M., Dalibor P., Ivica H., Berislav R., Marko S., Mario K., Damir Z., Drazen D., Ljiljana, B. 2014. **Haematological and biochemical parameters in the blood of an indigenous Croatian breed – Istrian cattle.** *Archive Tierzucht* **57(18)**, 1-7.
- Bourn, D. 2012. Highlights of the Nigerian livestock resources reports. Retrieved April 11, 2012, from <http://www.odi.org.uk/work/projects/pdn/papers/35d.pdf>
- Cheesbrough, M. 1991. Medical Laboratory Manual for Tropical Countries. 2nd Edn., University Press, Cambridge. pp. 508-511.
- Coles, E. H. 1986.** Veterinary Clinical Pathology 4th Edition. W.B. Saunders Co. Philadelphia.
- Daisy, G. S., Leola, M. W., Maxine, W. 1947. The determination of serum magnesium by the molybdivanadate method for phosphate. *Journal of Biological Chemistry* **169**, 39-47.
- De-Lahunta, A. 1986. *Applied Veterinary Anatomy*, 5th ed., W.B. Sanders Company, Philadelphia. pp. 5-15.
- Doumas, B. T., Biggs, H. C. 1972. Determination of serum protein and albumin. In: Standard Methods of Clinical chemistry (Copper, G. R. ed) Academic Press, New York. pp. 175-188
- Dubreuil, P., Arsenault, J., Bélanger, D. 2005. Biochemical Reference Ranges for Groups of Ewes of Different Age. *Veterinary Record* **156**, 636-638.
- Ferguson, M. 1990. The dentition throughout life. In: Elderton R. J. (ed). The Dentition and Dental Care, Vol. 3, Oxford Heinemann Medical Books, Oxford. pp. 1-18.
- Frandsen, D. R., Wilke, W. L., Fails, A. D. 2009. *Anatomy and Physiology of Farm Animals*. pp. 335-360.
- Harvey, C. E. 1985. Function and Formation of Oral Cavity. In: Harvey C.E. (ed). Veterinary Dentistry, W. B. Sanders Company, Philadelphia. pp. 5-22.
- Jain N. C. 1998. *Essentials of Veterinary Haematology*. 2nd ed. Lea and Febiger, Philadelphia. pp. 65-68.
- Kachmar, J. F. 1970. Determination of blood haematology by the cyanomethaemoglobin procedure. In: Teitz N.W. ed. Fundamentals of Clinical chemistry, W.B. Saunders Company, Philadelphia. pp. 268-269.
- Kahn, C. M. 2010. *The Merck Veterinary Manual*, 10th ed. Merck and Co. Inc., New Jersey. pp. 17-156.
- Kaneko, J. J., Harvey, J. W., Bruss, M. L. 1997. *Clinical Biochemistry of Domestic Animals*. 5th ed., Academic Press, San Diego. pp. 882-891.
- Kaneko, J. J., Harvey, J. W., Bruss, M. L. 2008. *Clinical Biochemistry of Domestic Animals*. 7th ed., Academic Press, San Diego. pp. 530-559.
- Klinkon, M., Jezek, J. 2012. Values of blood variables in Calves, *A Bird-Eye view of Veterinary Medicine* **16**, 301-320.
- Latimer, K. S., 2011. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed. John Wiley and Sons Inc., West Sussex. pp. 3-80.
- Latshaw, W. K., Decker B. C. 1987. Face, mouth and pharynx. In: Latshaw W. K. and Decker B. C. (eds) *Veterinary Developmental Anatomy—A Clinically Oriented Approach*, Toronto.
- Meyer, D. J. and Harvey, J. W. 2004. Veterinary Laboratory Medicine: Interpretation and Diagnosis. 3rd ed. Saunders, St. Louis. pp. 5.
- Mitruka, B. M., Rawnsley, H. M. 1977. Clinical Biochemistry and Haematological Reference Values in Normal Experimental Animals. Masson Publishing Inc., U.S.A.
- Mohri, M., Sharifi, K., Eidi, S. 2007. Hematology and Serum biochemistry of Holstein

dairy calves: age related changes and comparison with blood composition in adults. *Research in Veterinary Science* 83, 30-39.

NASS. 2011. National Agricultural Sample Survey on Animal Census, *AgroNigeria* ask@agronigeria.com

Oduye, O. O., Okunaiya, O. A. 1971. Haematological studies on the white Fulani and N'dama breeds of cattle. *Bulletin of Epizootiology of Disease in Africa* 19, 213-218.

Ogbeh, A. 2016. Nigeria releases census of goats, sheep, pigs, other livestock in country, *Premium Times* @ <http://www.premiumtimesng.com/.../204577>

Olayemi, F. O., Nwandu C. N., Aiyedun, O. 2007. Haematology of Sokoto Gudali Cattle as Influenced by Sex and Breed. *Journal of Animal and Veterinary Advances.*, 6(6), 816-818.

Onasanya, G. O., Oke, F. O., Sanni, T. M., Muhammad, A. I. 2015. Parameters Influencing Haematological, Serum and Bio-Chemical References in Livestock Animals under Different Management Systems, *Open Journal of Veterinary Medicine* 5, 181-189. Published Online August 2015 in Sci Res. <http://www.scirp.org/journal/ojvm>, <http://dx.doi.org/10.4236/ojvm.2015.58025>

Radostitis, O. M., Gay, C. C., Blood, D. C. 1994. *Veterinary Medicine: (A Text Book of Disease of Cattle, Sheep Goats, Pigs and Horses)* W.B. Sander. pp. 304.

R.A.R. 2009. *Reference values for Laboratory Animals: Normal Haematological values. Research Animal Resource (RAR), University of Minnesota. Retrieved on the 13th of January, 2015 from <http://www.ahc.umn.edu/rar/refvalues.html>.*

Rosol, T. J., Capen, C. C. 1997. Calcium regulating hormones and diseases of abnormal

mineral (calcium, phosphorus, magnesium) metabolism. In: *Clinical Biochemistry of Domestic Animals*, J.J. Kaneko, J.W. Harvey and M.L. Bruss, (Ed.), Academic Press, San Diego, California. pp. 619-702,

Schales, O., Schales, S. S. 1941. A Simple and Accurate Method for the Determination of Chloride in Biological fluids, *Journal of Biological Chemistry* 140, 879-884. <http://www.jbc.org/content/140/3/879.citation>

Schalm, O. W. 1984. *Manual of Bovine Hematology: Anemias/ Leukocytes/Testing. Veterinary Practice Publ. Co., Santa Barbara, CA, USA. pp. 31-57.*

Schalm, O. W., Jain, N. C., Carroll, E. J. 1975. *Veterinary Haematology* (3rd ed.). USA: Lea and Fabiger, Philadelphia. pp. 15-218.

Steinhardt, M., Thielscher, H. H. 2000a. Reaktionen von Milchrindkälbern auf ACTH Applikation und Flüssignahrungsaufnahme An verschiedenen Alterspunkten vor und während der Aufzucht am Tränkeautomaten Plasmacortisol, Speichelcortisol, hämatologische, metabolische Variablen und Herzschlagfrequenz. *Deutsche Tierärztliche Wochenschrift* 107(5), 180-187.

Steinhardt, M., Thielscher, H. H. 2000b. Physiologische Variable und Wachstumsleistung bei Saugkälbern der Mutterkuhhaltung in den ersten beiden Lebensmonaten. *Tierärztliche Umschau* 55(7), 380-389,

Thrall, M.A. 2004. *Veterinary Hematology and Clinical Chemistry*. Lippincott Williams and Wilkins, Baltimore. pp. 45-46.

Underwood, E. J., Suttle, N. F., 2001. *The Mineral Nutrition of the Livestock*. pp. 105-397, CABI Publishing, ISBN 0-85199-128-9, Wallingford, United Kingdom