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Values of Blood Variables in Calves

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1. Introduction

Haematological and biochemical variables are most widely used medical decision making tool. Haematological and biochemical analyses of blood are very useful to get an insight in metabolic and health status of animal. During diagnostic procedure it is very useful to compare the values obtained from ill animal with normal values in healthy animals. Therefore specific reference intervals are needed for each animal species for appropriate interpretation of results of haematological and biochemical analyses. The reference values of different blood variables are well established for adult cattle, but for calves there is not a lot of data available. The values of different blood variables in calves and other young animals are changing with age. The values of haematological variables change after birth because of colostrums intake, short life time of erythrocyte and decrease of concentration of foetal haemoglobin. In growing calves the feeding and rearing system has an important influence on the values of different blood variables. Influence of feeding becomes more apparent after 5th week when consummation of dry food (hay, starter) increases

In our contribution we would like to present the results of our research about different blood variables (haematological, biochemical) in calves and their dependence from age (from birth till age of 6 months). Further we will discuss about factors which influence the values of different blood variables like; laboratory methods, feeding and rearing systems, health status, etc. We would like to explain how the knowledge about values of blood variables could be used for proper interpretation of results of laboratory analyses and for assessment of health or metabolic status in an animal.

2. Haematological variables in calves

The number of erythrocytes (E) in blood is lower in young animals and indicates more signs of regeneration (higher number of reticulocytes) as in adult animals (Kraft, 1999a). The life span of E in cattle lasts 160 days. Different authors studied values of haematological variables in calves and observed that the values are changing with age (Creatorex, 1954; Scheidegger, 1973; Hanschke & Schulz, 1982; Steinhardt & Thielscher, 2000a; Steinhardt & Thielscher, 2000b; Klinkon et al., 2000; Brun-Hansen et al., 2006; Moosavian et al., 2010; Mohri et al., 2010). Immediately after parturition the values of (PCV), haemoglobin (Hb) and

number of E are higher and they decreased in first days of life what could be associated with colostrum intake, which increase the plasma volume due to osmotic effect (Scheidegger, 1973; Harvey, 1997). To decreased values contribute also lower production of erythrocytes in first days after parturition and shorter life span of intrauterine produced E (Harvey, 1997). The findings of Kurz and Willet (1991) are in agreement with these establishments. They studied the dynamics of Hb and PCV values and number of E in calves from the 1st to the 6th day after calving and established continuous decrease of mentioned variables from birth till the 6th day when the Hb concentration was 9.3 g/dl, value of PCV was 27.2 % and number of E was $7.1 \times 10^{12}/L$. The different time of first colostrum intake did not influence the dynamics of these variables. Similar dynamics till 5th day after birth observed also Muri et al. (2005) in calves of Simmental and Holstein Friesian breed only they established slightly lower values of Hb (6.6 g/dl), PCV (24 %) and E ($7.8 \times 10^{12}/L$) as were established in other studies (Scheidegger, 1973; Kurz & Willet, 1991; Egli & Blum, 1998). Mohri et al. (2007) observed a decrease of PCV and Hb from birth to the age of 28 days later the values increased with age. Heidarpour Bami et al. (2008) found lower values of Hb and PCV to the age of 28 d in calves, which did not received iron and higher values in those, which received iron injections. In veal calves the highest values of some variables were established immediately after birth E ($9.35 \times 10^{12}/L$), Hb (12.86 g/dl), PCV (41 %), MCV (43.2 fL) in L ($13.99 \times 10^9/L$). The values decreased to the age of 48 hours and at the age of 3 weeks they observed higher values except MCV value which still decreased (Adams et al., 1992). Higher values of E, Hb and PCV are response of calf's organism to the hypoxia which could appear at the end of the pregnancy (Steinhardt et al., 1993). The decrease of haematological variables in the first weeks after calving was observed by majority of authors and is influenced also by decline of foetal erythrocytes which contain foetal Hb (HbF), they are replacing with E containing haemoglobin of adult animals HbA (Scheidegger 1973; Harvey, 1997) and have smaller volume. Smaller MCV is compensated with higher number of E to retain normal Hb amount which is nearly on the level of adult animals (Brun-Hansen et al. 2006).

In growing calves feeding and rearing system has an important influence on the values of haematological variables (Reece & Hotchkiss, 1987; Scheidegger, 1973). Influence of feeding regime becomes more apparent after the 5th week when consummation of dry food (hay, starter) increases. In this period the values of RBC, Hb and Ht increase. In veal calves fed predominantly with milk the values of mentioned variables decreased and calves become anaemic due to iron deficiency. In calves fed exclusively with milk replacer (feeding for white meat) the number of E decreased from birth to the age of 15 weeks when it was only $3.96 \pm 0.66 \times 10^{12}/L$. Similar dynamics was observed by Hb concentration which was 7.65 ± 1.28 g% at the age of 15 weeks and PCV value which decreased from 41.40 ± 5.86 % in the 1st week to 26.82 ± 5.03 % in the 15th week (Jazbec et al., 1973).

The number of leucocytes (WBC) in blood of calves is higher in comparison with adult animals and is more variable as values of other haematological variables. Different types of leucocytes have different life spans so their number can change rapidly and blood serves only as transport medium from the place of origin to the place of inflammation (Kraft, 1999a). The number of WBC usually increases at the presence of disease, especially in association with inflammatory processes and possibly at stress. The number of WBC

increased from birth to the age of 10 weeks, than it oscillated more or less to the age of 1 year and is slightly higher as in adult animals the most of values are between $6.5\text{--}11.5 \times 10^9/\text{L}$ (Greator, 1954). Terosky et al. (1997) observed the oscillation of WBC number in the period from birth to the age of 18 months; the number was between reference values for adult animals. In calves fed exclusively with milk replacer the number of WBC decreased after the 1st week ($10.42 \pm 2.29 \times 10^9/\text{L}$) and was $7.30 \pm 1.21 \times 10^9/\text{L}$ at the age of 21 weeks (Jazbec et al., 1973).

The number of platelets (PLT) in calves is around $400 \times 10^9/\text{L}$ in the first three days of life, then it increase rapidly to $900 \times 10^9/\text{L}$ at 10 days of age. Then after the PLT number increase slowly when it amount $1100 \times 10^9/\text{L}$, later it decrease slightly (Knowles et al. 2000). In Norwegian research performed on calves of Norwegian Red breed the increasing of PLT number to the age of 2 weeks was observed when it was $987 \times 10^9/\text{L}$. Then the PLT number decreased slowly to the age of 27-29 weeks, when it was $518 \times 10^9/\text{L}$ (Brun-Hansen et al., 2006). In Simmental calves the PLT number increased rapidly from the 1st to the 7th day of life, later it almost did not change to the age of 84 days (Egli & Blum, 1998). The observations of different studies indicate that PLT number increase rapidly in the first days of life, and for later the establishments of studies about PLT dynamics differ.

The results of various studies on age dependent dynamics of haematological variables are different what established also the other researchers (Jain, 1986; Knowles et al., 2000). The differences are influenced by the fact that blood samples were taken in different age periods, breeds, rearing systems, geographic regions, and different feeding system.

By analysing of results of different studies was established, that the most marked differences are between calves which are fed with milk or milk replacer and receive starter and hay and veal calves which receive predominantly or exclusively milk or milk replacer and no hay. The differences in values of haematological variables are partly influenced with the use various analysing methods. In older studies the blood cells were counted manually with Neubauer chambers, PCV values were estimated with centrifugation in microhaematocrit tubes, and Hb concentration was measured photometrical (Greator, 1954; Hanschke & Schulz, 1982). In later studies the blood cell numbers were counted with automated counters from different producers, PCV value was still estimated by microhaematocrit method (Scheidegger, 1973; Terosky, 1997; Steinhardt & Thielscher, 2000a; Steinhardt & Thielscher, 2000b; Reece & Hotchkiss, 1987). In most of recent researches, which were published in last years the haematological variables were measured with various automated counters (Egli & Blum, 1998; Muri et al., 2005; Knowles et al., 2000; Klinkon et al., 2000; Mohri et al., 2007, Heidarpour Bami et al., 2008).

3. Biochemical variables in calves

The knowledge about normal values of biochemical variables in blood serum and other physiological variables is important for assessment of damage of organs and tissues in different diseases and for assessment of development from the welfare aspect (Steinhardt & Thilescher, 2000c; Terosky et al., 1997). The values of biochemical variables in calf's serum differ from the values in adult animals. Different authors ascertained that there are deficient data available about physiological values of biochemical variables in calves and that results

of various studies differ (Hanschke & Schulz, 1982; Bouda & Jagoš, 1984; Steinhardt et al., 1993; Knowles et al., 2000 Mohri et al., 2007). The majority of data available is for the calves in the first days after birth, and for the calves of few weeks or months of age there is very few data available. The changing of values of biochemical variables in the first days of life is the consequence of adaptation on the extra uterine life and is importantly influenced by maturation of organs and intake of nutrients.

3.1 Aspartat Aminotransferase (AST)

The enzyme AST is present in different tissues and is a sensitive indicator of soft tissue damage. In heart and skeleton musculature as in liver there is high activity of AST. The AST is present in cytoplasm and in mitochondria so its activity is increased chiefly by cell necrosis in smaller amount also by damage of the cell membrane (Kraft & Dürr, 1999a). Measuring of AST activity in combination with CK is used for diagnostics of muscle damage (Kaneko, 1997). High activity of AST is also in liver and in the case of liver damage AST activity in serum increase.

After first colostrum intake the AST activity in serum increased from 23 U/L before intake, to 38 U/L at the age of 3 hours, what is most likely due to absorption from colostrum or because of activation of enzymes in calf intestine as consequence of colostrum intake (Kurz & Willet, 1991). However Hammon and Blum (1998) established that in calves which received only milk replacer instead of colostrum, activity of AST increased on the second day after birth so they are of the opinion that also other factors influence on the increased activity of some enzymes. The activity of AST decreased after the first week, and from 42nd to 84th day of life it increased slowly (Egli & Blum, 1998). Mohri et al. (2007) observed the increase of AST activity from the 14th to the 84th day of age.

3.2 Lactate Dehydrogenase (LDH)

Enzyme LDH catalyse reversible oxidation of pyruvate to lactate. The enzyme is present in numerous organs and tissues.

The activity of LDH in calves increased slowly in the first 24 hours of life, from 421 U/L immediately after birth to 759 U/L at the age of 24 hours and it is more likely this is a physiological event than it is due to absorption from colostrum (Kurz & Willet, 1991). The LDH activity increased slowly to the age of 56 days, later it remains on the same level to the age of 84 days (Egli & Blum, 1998).

3.3 Creatin Kinase (CK)

The highest activity of CK is in the skeleton and heart musculature. Measuring of the activity of CK in serum is first of all used for diagnostics of skeleton musculature damage. The activity of CK could be increased also after effort, long-lasting lying of the animal or convulsions. Miopathias as the consequence of vitamin E and Selenium deficiency which usually appear in veal animals (calves, lambs), sometimes also in adult animals, cause increased activity of CK (Smith et al., 1994).

The activity of CK in calves is high after birth later it decreased rapidly and almost did not change to the age of 60 days, at the age of 80 days it increased slightly (Knowles et al., 2000).

The mean activity of CK in calves of Simmental breed was $11.2 \pm 2 \mu\text{kat/L}$ ($671.8 \pm 119.9 \text{ U/L}$) at birth then it decreased to the age of 7 days and remained on this level to the age of 42 days, later it increased to the 84th day when it was $21.3 \pm 10.7 \mu\text{kat/L}$ ($1277.7 \pm 641.8 \text{ U/L}$) (Egli & Blum, 1998). Increased activity of CK after birth could be associated with the parturition and adaptation to the extra uterine life which represent for the newborn calf an effort on which it was not used in intrauterine life. The increasing of CK activity with age could be attributed to the growth of calves and gaining of muscle mass, partly also to the increased activity of calves, at this age they are in group pens where they have enough space for movement.

3.4 Alkaline Phosphatase (ALP)

For a long time the enzyme ALP is used in diagnostics as indicator of liver damage. The ALP is important also by diseases of skeleton. The enzyme was found in the intestine, liver, kidney and bones. In serum of young fast growing animals predominates isoenzyme from bones, in older animals which grow slower its activity decrease (Kaneko, 1997). Serum activity of ALP is higher in young animals than in adult ones and it decrease with age. After first colostrum intake serum activity of ALP increased from 235 U/L before intake to 364 U/L at the age of 3 hours, what is most likely due to absorption from colostrum and activation of enzymes in the calf's intestine because of colostrum intake (Kurz & Willet, 1991). The activity of ALP was high in calves after birth then it decreased and remained stable to the age of 60 days, later it decreased slightly more (Knowles et al., 2000). In calves to the age of 6 months the activity of ALP can reach 1800 U/L, in young cattle to the age of 3 years it decrease to 500 U/L (Kraft & Dürr, 1999a). In adult animals activity of ALP can increase at increased activity of osteoblasts. Activity of ALP is increased at acute and chronic liver diseases (especially cholestatic hepatopathias) and in diseases of bones (rachitis, periostitis)

3.5 Gamma Glutamyl Transferase (GGT)

The highest activity of GGT is in bile ducts epithelium and in kidney. The enzyme is located in membrane structures of the cells. The increased serum activity of GGT is usually associated with cholestasis and bile ducts damage. Very high activity of GGT is also in colostrum of cattle, sheep and goats. Hammon and Blum (1998) measured in colostrum of cows mean activity of GGT 22.432 U/L. After colostrum intake the enzyme is absorbed through intestinal wall, consequently the GGT activity is very increased in this period and can be used for indirect estimation of colostrum supply (Bostedt, 1983). The GGT activity in newborn calves was 10-31 U/L, after colostrum intake it increased to 370-5000 U/L, then it slowly decreased to the age of 20 days when it stabilised (Braun et al., 1982). In calves which received only milk or milk replacer instead of colostrum, the GGT activity did not increase (Boediker, 1991). In the first week of life GGT activity was high later it decreased rapidly (Knowles et al., 2000; Egli & Blum, 1998). The GGT activity below 100 U/L at the age of 2 days indicates insufficient colostrum supply or disturbed absorption (Klee, 1985). Tyler et al. (1999b) claimed that activity of GGT above 50 U/L in the calves serum indicate sufficient colostrum supply, Perino et al. (1993) stated 200 U/L for the boundary value.

By comparison of various data from the literature in some cases considerable differences in the activity of enzymes were established. There are also considerable differences between

reference values of enzyme activity claimed by different authors. Enzymes are very sensitive indicators of cell damage, their activity change by tiny alternations, so it is understandable that enzyme activity could rather differ between single animals. Consequently there are different results between studies. Reasons for these differences are partly differences between breeds and breeding conditions where the results were obtained. Great influences on the measurements have also analytical procedures and temperatures at which the activity of enzymes was measured. In majority of literature the mentioned analytical procedures are deficiently described, so it is very difficult to compare the data, because it is not clear at which temperature the activity of enzyme was measured. Some sources (Egli & Blum, 1998) claimed twice as high activity of LDH, as was measured by us. The reason is most likely in measuring procedure because the activity of LDH is measured with reaction of transformation pyruvate to lactate which can expire in both directions. With experiences in our laboratory was established that if the LDH activity is measured with reaction from lactate to pyruvate almost for a half lower values are obtained, than when LDH activity is measured with reaction from pyruvate to lactate.

3.6 Total serum bilirubin

Bilirubin is formatted by enzymatic decay of hem at disintegration of mature erythrocytes. These processes are taking course in spleen, liver and bone marrow. In blood plasma the unconjugated (indirect) bilirubin is bound to the albumin and is carried to the liver. With help of special protein called ligandin, bilirubin pass into hepatocytes. In the liver of newborns there is very low concentration of ligandin, so their ability to excrete the bilirubin is decreased. They can have for 50-100% higher serum concentrations as the adult animals (Jazbec, 1990; Tennant, 1997). At the end phase conjugated (direct) bilirubin pass into bile and than in the intestine. Increased concentration of total serum bilirubin in cattle is usually associated with cholestasis, fatty liver or haemolytic anaemia (Tennant, 1997), but can also be in connection with decreased appetite (Kraft & Dürr, 1999a).

The total serum bilirubin concentration in one day old calves was higher (9.58 $\mu\text{mol/L}$) than in adult animals. Later it decreased slowly and at 31-60 days of age it was 4.45 $\mu\text{mol/L}$ (Hanschke & Schulz, 1982; Egli & Blum, 1998). Kurz and Willett (1991) observed increase of total serum bilirubin concentration in the first 12 hours of life for approximately five times and until 6th day the concentration decreased approximately to the value at birth. In the research of Mohri et al. (2007) bilirubin concentration decreased from 1st to the 14th day of age and later it remain stable. Higher concentration of total serum bilirubin after birth is associated with destruction of foetal haemoglobine and slower excretion of bilirubin because of lower concentration of transport protein ligandin. Ligandin enables passing of indirect bilirubin into hepatocytes where it is transformed to direct bilirubine which can be excreted (Tennant, 1997). After the second week of age the concentration of total serum bilirubin is inside reference values for adult animals. What can be associated with improvement of glomerular filtration and liver function with age.

3.7 Serum iron

Iron is present in the milk in very low concentration, but it is absorbed very efficiently from the gut (Underwood & Suttle, 2001). Efficiency of iron absorption depends from the needs of

the organism and is controlled by iron amount in the intestine mucosa. The majority of iron is absorbed in the duodenum. The absorption is more efficient in animals with iron deficiency (Underwood & Suttle, 2001). The iron is very important for synthesis of haemoglobin. Approximately 60-75% of iron in the body is bound to haemoglobin (Kraft, 1999a). Haemoglobin is composed from four molecules of hem and each of them contains one atom of iron. The main occupation of iron is transportation of oxygen. The rest of iron in the body is bound to transferrin and ferritin, small amount also to myoglobin. Iron is important for normal functioning of numerous enzymes. Iron deficiency in calves cause decreasing of haemoglobin and myoglobin concentration (Underwood & Suttle, 2001). In milk the iron is in relatively small quantity for all that in this period intensive synthesis of haemoglobin is expiring in calves (Kraft, 1999a). Long lasting iron deficiency influence on lost of appetite, growth retardation, whitening of mucous membranes due to progressive hypochromic anaemia (Underwood & Suttle, 2001). In fast growing suckling calves mild anaemia can appear, but usually it does not come to iron deficiency in ruminants, because there is enough iron in forage.

The calves were born with iron concentration around $27.7 \pm 9.6 \mu\text{mol/L}$, then it decreased on $18.0 \pm 3.0 \mu\text{mol/L}$ at the age of 4 days. After the 2nd week of life iron concentration started to increase and at the age of 6 weeks it reached similar level as at birth ($28.0 \pm 7.8 \mu\text{mol/L}$) (Bostedt et al., 1990). Knowles et al. (2000) established gradually increasing of iron concentration from 30th to 80th day of age, but at the age of 80 days the concentration was still on the lower limit of reference values for adult cattle. Other studies observed gradual increase of iron concentration to the age of 60 days (Steinhardt & Thielscher, 2000a; Steinhardt & Thielscher, 2000b).

Iron dynamics with age is influenced by feeding regime of calves. By suckler calves the iron concentration was low in the first weeks (around $9 \mu\text{mol/L}$) later it increased to the age of 14 weeks (approximately $36 \mu\text{mol/L}$). The calves which received milk replacer, have already at the age of 1-2 weeks, higher iron concentration as suckler calves. In calves which received starter and hay in addition to milk replacer the iron concentration increased to the 3rd week of age later it slightly oscillated but it did not decrease to the age of 13 weeks. In calves fed exclusively with milk replacer the iron concentration decreased after 2nd week of age and at the age of 14 weeks it was only $4 \mu\text{mol/L}$ (Reece & Hotchkiss, 1987; Scheidegger, 1973). In the calves fed only with milk replacer the iron concentration decreased from birth to the 10th week when it was $5.19 \pm 4.29 \mu\text{mol/L}$, later it increased slightly, but the lowest concentration was established at the age of 22 weeks when it was only $1.79 \pm 1.43 \mu\text{mol/L}$ (Jazbec et al., 1975).

3.8 Urea

The concentration of urea in blood depends from nutrition, diagnostically is important also at diseases of kidneys (Kraft & Dürr, 1999b; Jazbec, 1990). Increased concentration of urea in calves' serum indicates increased catabolism of proteins and appears at long lasting diarrhoeas (Jazbec, 1990).

The colostrum intake did not influence the urea concentration (Steinhardt et al., 1993). In calves the urea concentration slightly decreased from birth to the age of 60 days when it was 2.7 mmol/L (Steinhardt & Thielscher, 2000d). Knowles et al. (2000) observed increasing of

urea concentration from 40th to 80th day of age. Hanschke and Schulz (1982) established higher values of urea in the age 31-60 days in calves in subtropical climate, where the concentration of urea was 5.14 mmol/L. In calves with diarrhoea at the age 4-15 days twice as high mean urea concentration (7.98 mmol/L) in plasma was established as in healthy calves of the same age (3.89 mmol/L) (Maach et al., 1992). The authors claimed that measuring of urea concentration is very helpful for assessment of dehydration and disturbances of acid-base balance in calves with diarrhoea. Hugi et al. (1997) ascertained association between protein amount in forage and serum urea concentration in calves at the age 8 to 15 weeks.

3.9 Creatinin

Creatinin is synthesised at endogen metabolism in muscles. Creatinin is excreted with urine; its concentration in serum does not depend from the nutrition. Diagnostically it is important for the assessment of functioning of the glomerular system in the kidneys, but its concentration increase only at serious damage (Kraft & Dürr, 1999b). In calves after birth very high serum concentration of creatinin was established ($256 \pm 106 \mu\text{mol/L}$), the value normalised to the 4th day of age ($108 \pm 28 \mu\text{mol/L}$) (Klee, 1985). Similar was established by Maach et al. (1991), and after 15th day of age they observed increase of creatinin concentration to the age of 60 days when it was $146.7 \pm 23.9 \mu\text{mol/L}$. In the research of Mohri et al. (2007) the concentration of creatinin in Holstein calves decreased from 1st to the 70th day of age.

3.10 Total Serum Protein (TSP)

The absorption of proteins which are degraded into amino acids takes place in small intestine. The main source of proteins in ruminants is microbe synthesis in the rumen. In liver the majority of body's own proteins are synthesised. Albumins (Alb) are proteins in blood plasma which are synthesised in the liver. Other part of plasma proteins are globulins which are produced by the immune system. Albumins carry 75% of osmotic activity in plasma and they serve as transport proteins in metabolic processes. Globulins can be divided into three fractions with help of electrophoresis. In α fraction there are proteins of acute phase of inflammation (haptoglobin, serum amyloid A). In β fraction are parts of complement (C_3 and C_4), transferrin, C reactive protein and partly also some immunoglobulin (IgA, IgM). Gamma fraction consists of immunoglobulin (Kaneko, 1997). Measuring of concentration of TSP and quantity of albumin and globulin is very important in diagnostics of numerous diseases and disturbances in functioning of organism.

The concentration of TSP and proportion between albumin and globulin is changing with age. Usually the calves have lower concentration of TSP (50-70 g/L) as adult animals (60-80 g/L) (Kraft & Dürr, 1999c). Pregnancy, lactation, nutrition and inflammation can influence on TSP concentration (Kaneko, 1997). In calves after birth the TSP concentration is almost for a half lower than in cows (45.8 g/L), after colostrum intake the concentration increases (54.5 g/L), but is still lower than in adult animals. Numerous studies found correlation between TSP and concentration of immunoglobulin in calves' serum. Measuring of TSP concentration in the 1st week of age can be used as indirect indicator of colostrum supply (Tyler et al., 1998; Tyler et al., 1996a; Selim et al. 1995).

The concentration of albumin decreased after colostrum intake (27.5 g/L), and was approximately on the lower limit of reference values for adult cattle (Steinhardt et al., 1993; Kurz & Willet, 1991). In calves, which received colostrum, higher concentration of TSP was established, as in calves which received only milk replacer, what is associated with immunoglobulin absorption in the first ones (Muri et al., 2005). The concentration of TSP and albumin is influenced with nutrition of calves and functioning of the liver. The albumins are predominantly synthesised in the liver, so their amount depends on maturity and functional ability of the liver (Steinhardt & Thielscher, 2000c). Hypoalbuminemia in calves could be the consequence of liver damage or protein catabolism at long lasting diarrhoeas (Jazbec, 1990). In calves at the age from 8 to 15 weeks statistically significant differences in albumin concentration were established when they received meals with different amount of proteins (Hugi et al. 1997). Hammon et al. (2002) compared the calves which received limited or unlimited amounts of milk and established significantly lower albumin concentration in calves, to the age of 28 days, which were fed with unlimited amount of milk. Lower concentration of albumin in healthy animals could be the consequence of insufficient supply with amino acids (Whitaker, 1997). In calves from the 5th to the 40th day of age the concentration of TSP decreased slightly, after the 60th day it increased again and was 55.7 g/L. The concentration of albumin gradually increased from the 5th to the 60th respectively the 80th day of age (Steinhardt & Thielscher, 2000d; Knowles et al., 2000). In sucker calves gradual increase of TSP and albumin concentration was established from birth to the age of two months. The values of both variables were higher than in calves which were fed with limited amounts of milk (Steinhardt & Thielscher, 2000b).

3.11 Inorganic Phosphate (iP)

The absorption of iP is taking place in the forestomachs and in the front part of the small intestine. The iP is excreted with faeces and urine in cows in lactation also with milk. Phosphorus is important for normal growth and mineralization of bones. In the skeleton of adult animals is stored approximately 80% of all phosphorus in the body, which could be mobilised at need. The rest 20% of phosphorus is in soft tissues and body fluids where it collaborates in numerous important processes. It is the ingredient of deoxy- and ribonucleic acids, as phospholipids it is the part of cell membranes, as phosphate is important by regulation of osmotic and acid-base balance in the organism. Phosphorus has an important role in metabolism of energy, where it collaborates by transport of energy and fatty acids, synthesis of amino acids and proteins and working of Na/K pump (Underwood & Suttle, 2001). The serum concentration of iP is higher in young animals because the growth hormone increases the reabsorption of phosphate in the kidney (Rosol & Capen, 1997). In the rumen the phosphate is working as buffer for volatile fatty acids and as substrate for microorganisms. In ruminants with phosphorus deficiency, lower microbe synthesis of proteins in rumen is established. Phosphorus is important for the control of appetite and efficiently use of nutrients (Underwood & Suttle, 2001). By decrease of iP concentration in serum, increase the activity of ALP. Phosphorus deficiency in young animals causes the lost of appetite and retarded growth. In rachitic calves and lambs the concentration of iP is decreased for 30-50 % (Jazbec, 1990). Normal plasma concentration of iP in calves should be 1.3-1.9 mmol/L and 1.0-1.5 mmol/L in adult cattle (Underwood & Suttle, 2001). Kraft (1999b) claimed slightly higher values in calves serum namely; at the age until 2 months 2.6-3.5 mmol/L, from 2 to 6 months it should be 2.5-3.1 mmol/L and from 12 to 18 months the

concentrations are on the level of adult animals (1.6-2.3 mmol/L). The colostrum intake did not influence the iP concentration in calves' serum (Steinhardt et al., 1993). Kurz and Willett (1991) established decrease of iP concentration in first 24 hours of age. In sucker calves the increase of iP concentration in the first 14 days was established later it remained stable. The values were all the time higher as in adult animals (Egli & Blum, 1998). At the age of 60 days the concentration of iP in calves' serum was 2.6 mmol/L (Steinhardt & Thielscher, 2000d).

3.12 Calcium (Ca)

The majority of calcium (99 %) in organism is stored in bones and teeth. Calcium is important for activation of numerous enzymes and hormones. The calcium collaborates in processes of blood coagulation, nerves stimulation and muscles contraction. In blood serum approximately 55% of Ca is in ionised form and this is biologically active. The part of ionised Ca depends from blood pH, by decrease of pH the part of ionised Ca increase. A part of Ca in serum is bound to the albumins (40%) and smaller part (5%) to organic acids (Jazbec, 1990; Kraft, 1999b). The most of Ca is absorbed in small intestine.

In newborn calves the mean serum concentration of Ca was 3.35 ± 0.27 mmol/L. Six hours after birth the Ca level decreased to 2.41 ± 0.18 mmol/L and in the next days and weeks almost did not change (Bostedt & Schramel, 1982). Kurz and Willett (1991) observed decrease of Ca concentration in the first 24 hours of life. In the first two months of life the concentration of Ca was around 2.7 mmol/L and did not change a lot. After the 3rd month the Ca concentration started to decrease and at the age of 6 months it was 2.53 ± 0.10 mmol/L (Bouda & Jagoš, 1984). Similar dynamics was established in other study in calves which were fed with milk replacer, only they have slightly higher Ca concentration at the age of 5 days (3.02 ± 0.2 mmol/L) and from the age of 15 days to 2 months it was 2.8 ± 0.1 mmol/L (Steinhardt & Thielscher, 2000d). In sucker calves of Simmental breed the decrease of Ca concentration from birth to the age of 28 days was established (2.6 mmol/L), later the concentration almost did not change to the age of 84 days (Egli & Blum, 1998). Mohri et al. (2007) established a decrease of Ca concentration in the first two weeks of life, later the Ca concentration slowly increased.

3.13 Potassium (K)

In ruminants the potassium is absorbed from rumen and small intestine and is excreted over the kidney and with faeces. The K is main cation in milk (36 mmol/L). High concentrations of K in organism have citotoxic effect. Potassium is the main intracellular ion, its concentration in the cells is 25 to 30 times higher than in plasma (Ward, 1966), 96-98 % of total amount of K is in the cells (Wirth, 1999). Potassium is important for making electrical potential for transport of nervous impulses and for maintenance of muscle tonus. Hypokalemia increase the membrane potential and cause hyper polarisation block, what influence on lower muscle tonus and paralysis (Carlson, 1997). Potassium is important for regulation of acid-base balance in the body. The forage of ruminants usually contains enough potassium; more often comes to the surplus than to potassium deficiency in the meal. Tucker et al. (1991) and Weil et al. (1988) established association between potassium amount in the meal and concentration of K in calves' serum, and between mean daily weight gains. The mean K concentration in plasma of 8 weeks old calves was from 5.13 mmol/L, at 0.35% K in the meal, to 5.60 mmol/L at 0.53 % K in the dry matter of the meal. The authors claimed that the measuring of K concentration in serum and control of daily weight gain is a good indicator of supply with this element in

calves. In calves with diarrhoea comes to hyperkalemia which is connected with grade of acidosis (Maach et al., 1992).

The colostrum intake influence on increase of K concentration in calves' serum, what is most likely, the consequence of higher amount of this mineral in the colostrum (Steinhardt et al., 1993). In the age from 1 week to 2-3 months the K concentration in calves' serum almost did not change and was around 5 mmol/L (Maach et al. 1991). Bouda and Jagoš (1984) measured slightly higher values, around 5.4 ± 0.4 mmol/L, at the age of 6 month K concentration slightly decreased to 4.7 ± 0.4 mmol/L. Reece (1980) Established a decrease of K concentration from the 1st week of age when it was 7.2 mmol/L to the age of 15 weeks when it fell to 4.4 mmol/L.

3.14 Sodium (Na)

The sodium is the most important cation in extracellular fluid, where it is responsible for maintenance of osmotic pressure. Together with chlorine (Cl) collaborates in metabolism of water and regulation of acid-base balance in the organism (Jazbec, 1990). In ruminants a big part of Na which comes in to digestive tract originates from saliva (rotation in the organism). The rumen could contain up to 50% of whole amount of Na which is available for the organism. From the body is excreted with the urine, faeces and milk (Underwood & Suttle, 2001). At diarrhoeas the calves lose higher amounts of Na. Maach et al. (1992) established significantly lower concentration of Na in serum of calves with acute diarrhoea (131.2 ± 7.2 mmol/L) in comparison to healthy calves of same age (140.0 ± 9.9 mmol/L). In newborn calves after colostrum intake the Na concentration increased what was attributed to absorption from the colostrum (Steinhardt et al., 1993). But Maach et al. (1991) established higher concentration of Na before colostrum intake when it was 145.7 ± 3.7 mmol/L as then after (137.8 ± 6.8 mmol/L), later it almost did not change. The concentration of Na did not change a lot in the first three months, it was about 145 mmol/L, and at the age of 6 months it was slightly lower, about 136.6 ± 5.1 mmol/L (Bouda & Jagoš, 1984). Reece (1980) established higher concentration of Na in serum of calves which received milk replacer in comparison to the calves which were fed with milk.

3.15 Chlorine (Cl)

Chlorine is the most important anion in the extracellular fluid, inside the cells there is only 12% of total amount in organism. Together with Na it is responsible for maintenance of osmotic pressure in extracellular fluid. Chlorine is absorbed in small intestine, and is excreted mainly through the kidney (Wirth, 1999). The calves with acute diarrhoea, in which the volume of faeces can increase for 40 times, with the fluid lose also electrolytes. In such calves significantly lower concentration of Cl was established (95.6 ± 6.9 mmol/L) in comparison to healthy calves (103.3 ± 6.9 mmol/L) (Maach et al., 1992).

In calves immediately after birth higher concentration of Cl was established (107.3 ± 12.3 mmol/L), then it decreased to the 7th day of age to 95.9 ± 6.6 mmol/L, later it increased slightly to 102.3 ± 6.2 mmol/L at the age of 2 months (Maach et al., 1991). In suckler calves the concentration of Cl increased with age from 98.0 mmol/L in the 1st week of age to 102.4 mmol/L at the age of 14 weeks (Reece, 1984). In calves which were fed with limited amounts of milk the increase of Cl concentration was established to the age of 5 weeks, later it decreased slightly and oscillated between 97.7 and 99.3 mmol/L (Reece, 1980).

4. Results of our study

The aim of the study was to define the physiological pattern of haematological and biochemical variables for different age groups of dairy calves, which should help in interpretation of laboratory results.

The study was performed on 65 Holstein-Friesian calves (31 females, 34 males) from two dairy farms. After calving, the calves received 1-1.5 l of colostrum from their mother *via* nipple bottle. The colostrum and milk were supplied three times a day for the first 4 day; later they received milk two times a day. After 10 day of age, they had free access to commercial starter and hay. They were weaned at the age of 16 weeks.

Blood samples were taken from the jugular vein into evacuated tubes containing K₃EDTA (Venoject, Terumo, Belgium) for haematology and in tubes without additives for biochemical analysis. They were taken once weekly till the age of 6 weeks, and then at the age of 8, 12, 16, 20, and 24 weeks. During the research period, health status of calves was monitored regularly. When the calf was found to be sick at sampling time, the sample was excluded from the study. Some calves were lost from the study before the end because they were sold.

Age (weeks)	n	RBC × 10 ¹² /L	Hb g/L	MCV fL	PCV L/L	WBC × 10 ⁹ /L	PLT × 10 ⁹ /L
1	61	7.63 ± 1.49 8,12,16,20,24	104.1 ± 22.1 20	42.8 ± 3.5 2,3,4,5,6,8,12, 16,20,24	0.3 ± 0.07 6	9.6 ± 3,4 16	451 ± 212 2,3,4,5,6,8,12, 16
2	64	8.35 ± 1.70 12,16,20,24	108.8 ± 23.7 5,6,8	40.5 ± 2.6 1,4,5,6,8,12,16,20,24	0.34 ± 0.07 5,6,8	10.9 ± 4.1	723 ± 218 1,16,20,24
3	58	8.22 ± 1.55 12,16,20,24	103.7 ± 22.7 16,20	38.9 ± 2. 1,5,6,8,12,16, 20,24	0.32 ± 0.07	9.9 ± 3.2	730 ± 221 1,16,20,24
4	62	8.11 ± 1.54 12,16,20,24	100.1 ± 22.5 16,20,24	37.7 ± 2.9 1,2,6,8,12,16, 20,24	0.31 ± 0.07	10.1 ± 3.1	746 ± 201 1,16,20,24
5	63	7.98 ± 1.47 12,16,20,24	94.9 ± 20.4 2,12,16,20,24	36.4 ± 2.7 ^{1,2,3}	0.29 ± 0.07 2,12,16,20,24	10.9 ± 3.1	745 ± 194 1,16,20,24
6	63	8.05 ± 1.33 12,16,20,24	94.5 ± 18.3 2,12,16,20,24	35.7 ± 2.5 1,2,3,4	0.29 ± 0.06 1,2,12,16,20,24	9.7 ± 3.1 16	682 ± 206 1,24
8	65	8.53 ± 1.01 12,16,20,24	98.3 ± 12.9 2,12,16,20,24	35.1 ± 2.5 1,2,3,4	0.30 ± 0.05 2,16,20	9.8 ± 3.3 16	681 ± 252 1,24
12	61	9.33 ± 0.89 1,2,3,4,5,6,8	109.7 ± 9.0 5,6,8	35.2 ± 2.7 1,2,3,4	0.33 ± 0.04 5,6	10.8 ± 2.6	659 ± 198 1,24
16	62	9.72 ± 0.76 1,2,3,4,5,6,8	114.6 ± 8.6 3,4,5,6,8	35.0 ± 2,0 1,2,3,4	0.34 ± 0.03 5,6,8	11.8 ± 3.1 1,6,8	585 ± 201 1,2,3,4,5
20	56	9.85 ± 0.81 1,2,3,4,5,6,8	116.2 ± 10.1 1,3,4,5,6,8	34.8 ± 2.2 1,2,3,4	0.34 ± 0.03 5,6,8	11.4 ± 3.0	573 ± 168 2,3,4,5
24	38	9.59 ± 1.11 1,2,3,4,5,6,8	113.8 ± 12.3 4,5,6,8	35.3 ± 2,1 1,2,3,4	0.34 ± 4.0 5,6	11.5 ± 4.1	481 ± 213 2,3,4,5,6,8,12

The age group differs significantly (P<0.05) from age groups in superscript

Table 1. Haematological variables in calves (Ježek, 2007)

Age (weeks)	n	AST U/L	LDH U/L	CK U/L	ALP U/L	GGT U/L
1	64	38.7 ± 16.1 3,12,16,20,24	483.2 ± 108.8 8,12,16,20,24	168.8 ± 211 2	262.2 ± 185 2,3,4,5,12,16,20,24	329.8 ± 358.1 2,3,4,5,6,8,12,16,20,24
2	64	32.7 ± 11.1 8,12,16,20,24	451.3 ± 105.2 5,6,8,12,16,20,24	99.6 ± 69.7 1	143.1 ± 61.3 1,6,8	79.8 ± 72.1 1
3	59	31.5 ± 6.2 1,8,12,16,20,24	459.8 ± 88.9 5,6,8,12,16,20,24	121.9 ± 48.4 24	132.8 ± 57.0 1,5,6,8,12	45.1 ± 34.5 1
4	62	33.3 ± 11.0 8,12,16,20,24	503.1 ± 160.9 12,16,20,24	106.3 ± 71.8 16,20,24	157.8 ± 79.7 1,8	30.5 ± 18.3 1
5	63	33.9 ± 12.1 12,16,20,24	532.9 ± 125.9 2,3,16,20,24	113.3 ± 69.9 16,24	189.5 ± 93.4 1,3	24.6 ± 11.2 1
6	63	35.7 ± 7.4 12,16,20,24	544.9 ± 115.3 2,3,12,16,20,24	106.9 ± 46.9 16,20,24	208.1 ± 104.3 2,3	20.2 ± 7.7 1
8	64	40.3 ± 10.2 2,3,4,12,16,20,24	575.8 ± 122.4 1,2,3,16,20,24	140.4 ± 70.5	216.2 ± 97.7 2,3,4,20	16.1 ± 3.5 1
12	61	47.6 ± 9.2 1,2,3,4,5,6,8	633.6 ± 100.9 1,2,3,4,6,20,24	154.5 ± 103.1	196.9 ± 67.9 1,4	13.4 ± 3.0 1
16	61	48.4 ± 8.1 1,2,3,4,5,6,8	671.1 ± 102.7 1,2,3,4,5,6,8	181.9 ± 94.2 2,4,5,6	175.6 ± 65.2 1	13.0 ± 2.6 1
20	56	50.2 ± 10.3 1,2,3,4,5,6,8	722.6,3 1,2,3,4,5,6,8,12	166.5 ± 96.3 2,4,6	158.9 ± 55.4 1,8	13.9 ± 3.4 1
24	39	53.6 ± 10.1 1,2,3,4,5,6,8	770.7 ± 125.5 1,2,3,4,5,6,8,12	177.0 ± 115.5 2,3,4,5,6	162.4 ± 58.5 1	14.2 ± 4.9 1

The age group differs significantly (P<0.05) from age groups in superscript

Table 2. Activity of enzymes in calves (Ježek, 2007)

Age (weeks)	n	Bilirubin μmol/L	Fe μmol/L	Urea mmol/L	Creatinin μmol/L	TSP g/L	Alb g/L
1	64	16.08 ± 13.16 2,3,4,5,6,8,12,16,20,24	16.91 ± 10.95 8,12,16,20,24	3.64 ± 1.33 2,3	113.76 ± 36.20 3,4,5,6,8,12,16,20,24	52.09 ± 7.09 8,12,16,20,24	26.35 ± 2.10 2,3,4,5,6,8,12,16,20,24
2	64	8.23 ± 3.41 1,12,16,20,24	14.52 ± 7.16 5,8,12,16,20,24	5.90 ± 2.01 1,4,5,6,8,12,16,20,24	116.53 ± 19.41 3,4,5,6,8,12,16,20,24	52.92 ± 5.48 8,12,16,20,24	28.94 ± 2.79 1,4,5,6,8,12,16,20,24
3	59	6.99 ± 1.94 1	16.61 ± 7.88 8,12,16,20,24	5.12 ± 3.42 1,4,5,6,8,12	100.79 ± 21.66 1,2,5,6,8,12,16,20,24	52.71 ± 4.85 8,12,16,20,24	30.24 ± 2.48 1,16,20,24
4	62	6.96 ± 1.52 1	20.97 ± 12.16 12,16,20,24	4.01 ± 1.21 2,3	93.55 ± 16.66 1,2,8,12,16,20,24	52.41 ± 3.91 8,12,16,20,24	30.99 ± 2.53 1,2,20,24
5	63	6.88 ± 2.11 1	22.84 ± 13.47 2,12,16,20,24	3.85 ± 1.34 2,3	89.35 ± 16.67 1,2,3,12,16,20	53.77 ± 4.47 8,12,16,20,24	31.67 ± 2.48 1,2
6	63	6.79 ± 3.05 1	20.93 ± 11.81 12,16,20,24	3.79 ± 1.26 2,3	87.89 ± 15.67 1,2,3,12,16,20	54.95 ± 5.21 12,16,20,24	31.79 ± 2.91 1,2

Age (weeks)	n	Bilirubin $\mu\text{mol/L}$	Fe $\mu\text{mol/L}$	Urea mmol/L	Creatinin $\mu\text{mol/L}$	TSP g/L	Alb g/L
8	64	5.82 ± 1.72 1	25.67 ± 12.53 1,2,3,16,20	3.90 ± 1.19 2,3	82.34 ± 15.10 1,2,3,4,16	56.94 ± 5.19 1,2,3,4,5,12,16,20,24	31.76 ± 2.68 1,2
12	61	5.19 ± 1.32 1,2	30.43 ± 10.48 1,2,3,4,5,6	4.07 ± 1.21 2,3	75.31 ± 10.60 1,2,3,4,5,6	61.09 ± 4.98 1,2,3,4,5,6,8,16,20,24	31.71 ± 2.82 1,2
16	61	4.82 ± 0.98 1,2	34.08 ± 13.49 1,2,3,4,5,6,8	4.49 ± 1.29 2	69.92 ± 8.79 1,2,3,4,5,6,8	64.69 ± 5.17 1,2,3,4,5,6,8,12	32.52 ± 2.40 1,2,3
20	56	4.42 ± 1.15 1,2	32.90 ± 9.27 1,2,3,4,5,6,8	4.52 ± 1.36 2	75.96 ± 11.06 1,2,3,4,5,6	67.55 ± 4.37 1,2,3,4,5,6,8,12	33.21 ± 2.91 1,2,3,4
24	39	4.47 ± 1.52 1,2	30.12 ± 9.88 1,2,3,4,5,6	4.48 ± 1.04 2	78.38 ± 9.25 1,2,3,4	66.92 ± 5.03 1,2,3,4,5,6,8,12	32.89 ± 2.84 1,2,3,4

The age group differs significantly ($P<0.05$) from age groups in superscript

Table 3. Biochemical variables in calves (Ježek, 2007)

Age (weeks)	n	iP mmol/L	Ca mmol/L	K mmol/L	Na mmol/L	Cl mmol/L
1	64	2.94 ± 0.48 2	2.97 ± 0.30 2,3,4,5,6,8,12,16,20,24	5.72 ± 0.60 2,3,4	144.9 ± 4.1 2,20,24	100.0 ± 2.6 5,6,8,12,16,20,24
2	64	3.27 ± 0.42 1,4,6,8,12	2.0 ± 0.23 1	6.49 ± 0.69 1,4,5,6,8,12,16,20,24	142.6 ± 3.9 1,4,5,6,8,12,16,20,24	98.6 ± 3.1 3,4,5,6,8,12,16, 20,24
3	59	3.11 ± 0.42	2.63 ± 0.23 1	6.17 ± 0.65 1,12,16,20,24	143.8 ± 4.4 16,20,24	100.5 ± 2.9 2,6,8,12,16,20,24
4	62	3.02 ± 0.42 2	2.66 ± 0.25 1	6.08 ± 0.53 1,2,16,24	144.6 ± 3.2 2,16,20,24	101.3 ± 2.6 2,20
5	63	3.04 ± 0.49	2.66 ± 0.23 1	6.02 ± 0.53 2,16,24	144.9 ± 3.0 2,16,20,24	101.8 ± 2.6 1,2
6	63	2.94 ± 0.45 2	2.60 ± 0.19 1,16,20	5.95 ± 0.48 2,16,24	144.6 ± 2.9 2,16,20,24	102.3 ± 2.7 1,2,3
8	64	2.88 ± 0.33 2	2.65 ± 0.20 1	5.85 ± 0.37 2	145.1 ± 2.3 2,20,24	102.7 ± 2.5 1,2,3
12	61	3.01 ± 0.35 2	2.73 ± 0.18 1	5.79 ± 0.54 2,3	145.2 ± 2.5 2,20,24	102.3 ± 2.6 1,2,3
16	61	3.06 ± 0.36	2.74 ± 0.20 1,6	5.61 ± 0.57 2,3,4,5,6	146.8 ± 2.5 2,3,4,5,6	102.2 ± 2.1 1,2,3
20	56	3.07 ± 0.34	2.76 ± 0.20 1,6	5.76 ± 0.48 2,3	148.4 ± 2.4 1,2,3,4,5,6,8,12	102.9 ± 1.9 1,2,3,4
24	39	2.99 ± 0.33	2.67 ± 0.21 1	5.52 ± 0.57 2,3,4,5,6	148.6 ± 2.8 1,2,3,4,5,6,8,12	102.3 ± 1.7 1,2,3

The age group differs significantly ($P<0.05$) from age groups in superscript

Table 4. Mineral concentration in calves' serum (Ježek, 2007)

The data were processed with the statistical software SPSS (Ver 15.0). For investigated haematological and biochemical variables the descriptive statistics were calculated regarding to the age. Bonferroni test was used to investigate significant difference between age groups.

The age of calves influenced the investigated haematological and biochemical variables what should be considered by interpretation of laboratory results.

5. Conclusion

The age of calves influence the haematological and biochemical variables what should be considered by interpretation of laboratory results.

The results of various studies on age dependent dynamics of haematological and biochemical variables in calves are different. The differences are influenced by the fact that blood samples were taken in different age periods, breeds, rearing systems, geographic regions, and were analysed with different methods.

For proper interpretation of laboratory results it is the best to use the reference values from the laboratory which performed the analyses of blood.

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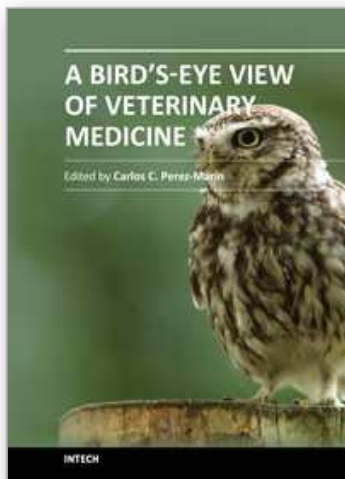
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Veterinary medicine is advancing at a very rapid pace, particularly given the breadth of the discipline. This book examines new developments covering a wide range of issues from health and welfare in livestock, pets, and wild animals to public health supervision and biomedical research. As well as containing reviews offering fresh insight into specific issues, this book includes a selection of scientific articles which help to chart the advance of this science. The book is divided into several sections. The opening chapters cover the veterinary profession and veterinary science in general, while later chapters look at specific aspects of applied veterinary medicine in pets and in livestock. Finally, research papers are grouped by specialisms with a view to exploring progress in areas such as organ transplantation, therapeutic use of natural substances, and the use of new diagnostic techniques for disease control. This book was produced during World Veterinary Year 2011, which marked the 250th anniversary of the veterinary profession. It provides a fittingly concise and enjoyable overview of the whole science of veterinary medicine.

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