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Changes in some blood parameters associated with the age of lambs*)

ZVONKO ANTUNOVIĆ, ĐURO SENČIĆ, MATIJA DOMAĆINOVIĆ, MARCELA ŠPERANDA, ZVONIMIR STEINER

Department of Animal Science, Faculty of Agriculture in Osijek, University of J. J. Strossmayer, Trg sv. Trojstva 3, 31000 Osijek, Croatia

Antunović Z., Senčić Đ., Domaćinović M., Šperanda M., Steiner Z.

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Summary

The influence of age on the concentration of changes of some blood parameters has been researched within the blood serum of 120 lambs of the Merinolandrace breed over the weaning period (10th and 40th day) and beyond (80th, 120th, 160th and 300th day). The lambs were in equal proportions as to sex and health and were all in good physical condition. The lambs were at the pasture together with sheep, and they had been fed with fodders and quality meadow hay. With the increase of age of the lambs the concentrations of calcium, inorganic phosphorus, magnesium, glucose and the activity of the enzyme AP, CK and GGT in the blood serum decreased and the concentrations of iron, urea, total proteins and cholesterol increased. The concentration of blood parameters changed with the increase of age of the lambs, which should be taken into consideration in a nutritive, metabolic and health status evaluation of the sheep.

Keywords: lamb, blood

With the aim of detecting the feeding errors at the right time, while their consequences are not visible on the animals, there are certain blood analysis to be made (2, 3, 15). The blood analysis are important in establishing feeding status and metabolic profile of the highly productive animals wheather their increase or the production is all about. The weaning lambs and those in increase are very sensitive to the feeding errors, especially if the sheep milk is low quality. Ewes milk yield is an important component for lamb growth (26). Also, the weaning lambs are still adaptable to the new environment conditions (6). Therefore, it is necessary to establish certain blood parameters, in the aim of explaining the substance change in the lamb organism (7). To evaluate the provision of lambs with energy and proteins in blood, there is an urea, glucose and cholesterol detection and to evaluate the provision with mineral substances, there is a calcium concentration detection, inorganic phosphorus, natrium and potassium detection. The aim of this research has to establish certain blood parameters concentration flow in lambs over their growth for establishing their metabolic profile.

Material and methods

Biological investigations have been made on a sheep farm „Jasenje" (Slavonian, Croatia) on 120 lambs Merinolandrace breed. The influence of age to the concentration changes of some blood parameters has been researched within the blood serum of lambs over the weening period (10th and 40th day) and after that (80th, 120th, 160th and 300th day). The lambs were in an equal sex proportion (50% male : 50% female), healthy and in good physical condition. The lambs were, together with sheep, at the pasture (*Lolium perenne*, *Lolium italicum*, *Phleum phleoides*, *Trifolium repens* and *Dactylis glomerata*) all the time during the investigation and at the return to the barn they have been fed with fodders with: 18.0% crude proteins, 4.5% crude fibers, 5.2% ashes, 3.18% fat and 60.55 NET and quality meadow hay.

The blood was collected from the jugular vein (10 ml) into the sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium). After that, the serum was separated by centrifugation (10 min.) at 3000 revolutions/min. and placed into the Boehringen Mannheim/Hitachi 911 auto-analyser (Boehringer Mannheim GmbH, Mannheim, Germany). Within the blood serum there have been found the concentrations of the mineral indicators (calcium, inorganic phosphorus, potassium, sodium, magnesium, chloride and iron, concentrations of the biochemical indicators (urea, glucose, total proteins, albumin, cholesterol and bilirubin total) and enzyme activity (ALT-alanine amino-

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transferase, AST-aspartate aminotransferase, alpha--amylase, AP-alkaline phosphatase, CK-creatine kinase, GGT-γ-glutamyl transferase, cholinesterase and LDH--lactate dehydrogenase).

Statistical analysis of data was performed by computer program Statistica. The results were statistically evaluated using Duncan's multiple range test.

Results and discussion

The change of mineral concentration in the blood serum of the lambs depending on their age is prominent in the tab. 1.

The age of lambs had a statistically significant influence on the differences in the calcium, inorganic phosphorus, magnesium and iron concentrations in the blood serum. Calcium, inorganic phosphorus and magnesium concentrations were much higher in the younger lambs in comparison to the older lambs. Baker et al. (4) have stated that changes in minerals needs over times are most obvious in young growing animals. The decrease of calcium could be explained with a decreased capacity for resorption of calcium with increased age (24). With the rise of age kids (from 8 days to 6 moths) the Ca concentration dropped in the blood plasma (22). The inorganic phosphorus concentration dropped in the blood of older lambs and could be the cause of a lessened capacity to assimilate phosphorus from diet as the age of animals rises (10). With the rise of age the P concentration dropped in the blood of ruminants (20). Kaneko et al. (18) quotes that the serum phosphate may be higher in younger animals because the growth hormone increases renal phosphate resorption. Similar observations for calcium and inorganic phosphorus concentration have been obtained by Bickhardt et al. (9). High magnesium concentration in the blood serum of suckling lambs is caused by its high content in milk of dams, which exceeds over six times its serum concentrations (17). Baranowski et al. (7) have stated that higher concentrations of magnesium in blood of young lambs are connected with a body mass increase, intensifying the metabolism and activating the main cell structures (ribosoms and mitochondries). Shiga et al. (28) have in the weening lambs detected the fall of calcium and magnesium concentrations with the increase of their age. With the increase of lambs' age, the natrium concentration levels dropped in the blood serum, but not statistically significant. The concentrations of potassium and chloride in the blood serum were very changeable and did not depend on the age, although, the younger lambs had some more of these mineral concentrations in comparison to the older lambs. Statistically high increase of the iron concentrations has been found in the blood serum of the older lambs in comparison to those that were younger. Transient iron deficiency occurs in the young of most species in association with rapid growth and an all-milk diet (18). In the first days after birth there was noticed a low level of iron in the blood serum because colostrum as a milk contains small amounts of iron and does not cover the needs of a young animal (7). The reason for that could be a high ability of iron bondage by some proteins from the colostrum (firstly transferrin and apoferritine) in the first days of life (14).

From tab. 2 it may be clearly seen the increase of the urea with the increase of age. There have been detected highly significant differences in the urea concentrations in the blood serum of the lambs depending on age. Also, with the increased age, we have detected a statistically significant fall of glucose and albumin concentration and not have detected significant fall in bilirubin level. It is a fact that the blood glucose, cholesterol and total protein analyses were used as an indicator for the ongoing growth processes (19). Feeding has a significant influence to the urea concentrations in the serum of animals (1). With the advance of age in kids (from 8 days to 6 moths) the plasma urea concentration increased (21). Glucose as an index for energy status has its limitations as peripheral glucose concentration is homeostatically controlled and quickly responding to adrenal corticol hyperactivity (23). The glusoce decrease up to 1 year of age in the ruminants is associated with the rumen development and with metabolic activities. The concentrations of the total proteins and cholesterol in the blood serum of the lambs have been increased with the advanced age, except in the first evaluation (10^{th} day) for total proteins, first and second evaluation (10^{th} and 40^{th} day) for cholesterol concentration. As the age rises, the concentration of total protein in the blood of sheep increases (9, 13). Namely, with increasing age, the blood proteins seems to increase and it is shown in a lessend decrease of albumin concentration and a progressive increase in the globulin fraction (18). High total protein content after birth can be a result of serum immunoglobulin content growth and this demonstrates good alimentary canal absorption and has some effect on later clinical state (8). The cholesterol concentration is influenced by the degree of stress (27). Therefore, higher cholesterol with age might be expected from stress (particularly by weaning lambs). High cholesterol concentration after birth can be a result of higher fat assimilation in younger lambs. Similar observations for cholesterol concentration in serum lambs have been obtained by Ramos et al. (25). With the rise of age kids (from 8 days to 6 moths) the bilirubin concentration drops in the blood plasma (21). Similar observations for urea, bilirubin and glucose concentrations have been obtained by Bickhardt et al. (9). It is possible that the increase in these blood parameters (total proteins, cholesterol) in lambs after birth is due to the increase of the sympathetic activity, anabolic metabolism function (5), lipolytic potential (11) and growth hormones for influencing the energy support needed for lean tissue development and maintenance of good health in this early stage of its life (13).

Tab. 1. Mineral concentration (mmol/l) in the blood serum of the lambs depending on their age (n = 20; $\bar{x} \pm s$)

Indicators	Age											
	10 day		40 day		80 day		120 day		160 day		300 day	
Ca	2.75^{A}	± 0.19	$2.72^{b,A}$	± 0.16	$2.67^{b,A}$	± 0.16	$2.62^{c,A,B}$	± 0.11	$2.54^{B,C}$	± 0.13	2.43^{D}	± 0.17
P	3.40^{A}	± 0.19	3.06^{B}	± 0.36	$3.00^{B,C}$	± 0.45	$2.90^{b,C,D}$	± 0.40	$2.73^{C,D}$	± 0.21	2.65^{D}	± 0.32
Na	154.05	± 5.03	153.90	± 2.36	152.70	± 2.45	152.75	± 3.20	150.70	± 5.79	150.65	± 5.19
K	5.58	± 0.55	5.51	± 0.38	5.63	± 0.56	5.30	± 0.87	5.41	± 0.35	5.32	± 0.85
Mg	1.21^{A}	± 0.17	1.10^{B}	± 0.14	$1.07^{B,C}$	± 0.17	$0.98^{B,C,D}$	± 0.09	$0.95^{C,D}$	± 0.08	$0.91^{c,D}$	± 0.11
Cl	103.85	± 5.84	103.25	± 5.12	104.45	± 2.89	103.50	± 2.24	102.65	± 3.86	102.80	± 4.99
Fe (µmol/l)	$21.91^{e,D}$	± 3.84	25.16^{D}	± 2.01	$25.50^{C,D}$	± 4.68	$29.15^{B,C}$	± 5.33	32.50^{B}	± 4.92	39.20^{A}	± 6.85

Explanations: a, b, c, d, e, A, B, C, D – means with different superscript letters differ significantly: small letters $p < 0.05$, capitals at $p < 0.01$

Tab. 2. Biochemical concentration in the blood serum of the lambs depending on their age (n = 20; $\bar{x} \pm s$)

Indicators	Age											
	10 day		40 day		80 day		120 day		160 day		300 day	
Urea (mmol/l)	$4.11^{d,C}$	± 1.44	4.94^{C}	± 0.91	6.53^{B}	± 0.98	$6.92^{a,A,B}$	± 1.13	$7.34^{A,B}$	± 1.34	7.51^{A}	± 0.84
Glucose (mmol/l)	4.30^{A}	± 0.53	$3.92^{A,B}$	± 0.84	3.75^{B}	± 0.45	3.65^{B}	± 0.48	2.92^{C}	± 0.28	2.86^{C}	± 0.46
Total proteins (g/l)	$66.71^{c,A,B}$	± 3.74	$63.25^{d,B}$	± 4.12	$64.54^{c,d,B}$	± 4.90	$66.91^{c,B}$	± 3.03	$69.46^{b,A}$	± 5.70	70.55^{A}	± 7.92
Albumins (g/l)	30.09^{A}	± 2.30	29.98^{A}	± 1.52	$28.88^{A,B}$	± 5.11	$26.10^{B,B}$	± 3.24	$26.27^{B,C}$	± 2.90	$25.66^{b,C}$	± 3.71
Bilirubin total (µmol/l)	5.80	± 1.97	5.25	± 1.59	4.93	± 0.69	4.40	± 0.72	4.05	± 1.79	3.85	± 1.06
Cholesterol (mmol/l)	2.65^{A}	± 0.82	2.39^{A}	± 0.83	$1.35^{A,B}$	± 0.24	$1.42^{B,C}$	± 0.19	1.51^{B}	± 0.26	1.65^{B}	± 0.24

Explanations: a, b, c, d, e, A, B, C, D, E – means with different superscript letters differ significantly: small letters $p < 0.05$, capitals at $p < 0.01$

Tab. 3. The activity of enzymes (U/l) in the blood serum of the lambs depending on their age (n = 20; $\bar{x} \pm s$)

Indicators	Age											
	10 day		40 day		80 day		120 day		160 day		300 day	
ALT	22.80	± 10.78	21.90	± 8.39	22.50	± 10.85	24.15	± 10.17	24.90	± 6.12	29.45	± 9.09
AST	208.10	± 145.82	204.25	± 100.30	195.90	± 96.99	169.60	± 79.92	148.65	± 77.80	149.70	± 88.44
AP	394.15^{A}	± 89.06	$360.65^{A,B}$	± 73.55	$332.90^{A,B,C}$	± 90.33	$294.95^{d,B,C}$	± 50.58	$271.80^{C,D}$	± 48.35	$219.00^{e,D}$	± 76.71
Alpha amylase	20.80	± 10.71	20.30	± 10.90	15.35	± 7.30	19.75	± 11.11	16.40	± 8.88	16.00	± 10.27
Cholinesterase	150.12	± 15.87	149.14	± 14.89	144.71	± 16.28	146.29	± 17.15	142.87	± 12.84	144.54	± 9.89
CK	464.45^{A}	± 136.47	342.65^{B}	± 135.86	$295.15^{B,C}$	± 119.59	$250.90^{d,C}$	± 68.90	$225.75^{d,C}$	± 37.46	$128.40^{e,D}$	± 31.52
GGT	74.50^{A}	± 11.59	$68.25^{A,B}$	± 6.58	$62.95^{B,C}$	± 7.54	60.70^{C}	± 9.73	50.30^{D}	± 5.28	$44.25^{e,D}$	± 6.02
LDH	856.45	± 372.64	807.80	± 376.15	787.75	± 328.35	791.35	± 297.74	685.05	± 205.67	648.60	± 280.51

Explanations: a, b, c, d, e, A, B, C, D – means with different superscript letters differ significantly: small letters $p < 0.05$, capitals at $p < 0.01$

The activity of enzymes in the blood serum of the lambs is shown in the tab. 3.

It is prominent that the age of lambs significantly influenced to the activity of the majority of enzymes researched. Statistically higher enzyme activity (AP, CK, GGT), regarding the age, has been detected in the younger comparing to the older lambs. In young growing animals bone AP is the predominant form of serum AP which decreases as maturation progresses and the epishysis closes (18). Increased CK activity in the blood serum of younger lambs could be an indicator that CK is a specific marker of muscle damage (12). Similar observations for the activity of AP and GGT have been obtained by Bickhardt et al. (9) and for the activity of GGT by Ramos et al. (25). Enzyme LDH, alpha amylase and AST activity were also higher in younger in comparison to the older lambs but the differences were not statistically significant. Concerning the age of sheep, there have been detected differences in the enzyme AST activities (18). Baranowski (6) has detected in the 14-day old lambs much lower activity of AST than in 70-days old lambs. Enzyme ALT acti-

vity was, however, higher in the blood serum of the older lambs than in the younger but without statistically significance. The increase of ALT enzyme with age is probably a consequence of the body mass increase and intensifying the metabolic activity. Similar observations for the activity ALT have been obtained by Ramos et al. (25). Cholinesterase is a strategy for biomonitoring environmental background levels of organophosphate contamination (16), although in these researches we did not find any significant variations concerning age and therefore, we can not talk about the environmental pollution.

In conclusion, the age of lambs significantly influences the concentration of some researched indicators in blood (calcium, inoranic phosphorus, magnesium, iron, urea, glucose, total proteins, albumins, cholesterol, AP, CK and GGT). We have not detected statistically significant influence on the concentrations of natrium, potassium, chloride, total bilirubin, ALT, AST, alpha amylase, cholinesterase and LDH in the blood serum of the lambs. With the increase of age of the lambs the concentrations of calcium, inorganic phosphorus, magnesium, glucose and the activity of the enzyme AP, CK and GGT in the blood serum have been decreasing and the concentrations of iron, urea, total proteins and cholesterol have been increasing.

The concentration of blood parameters changes with the increase of age of the lambs, which should be taken into consideration in a nutritive, metabolic and health status evaluation of the sheep.

References

1. *Antunović Z., Bukvić G., Steiner Z., Antunović M., Rastija D.*: Dynamic of rotation pastures quality and influence on some biochemical indicators in sheep blood. Krmiva. 2001, 43, 301-308.
2. *Antunović Z., Senčić Đ., Šperanda M., Liker B.*: Influence of the season and the reproductive status of ewes on the blood parameters. Small Rumin. Res. 2002, 145, 39-44.
3. *Antunović Z., Šperanda M., Steiner Z.*: The influence of age and the reproductive status to the blood indicators of the ewes. Arch. Tierzucht Dummerstorf. 2004, 47, 265-273.
4. *Baker D. S., Ahola J. K., Burns P. D., Engle T. E.*: Trace mineral metabolism in ruminants: impact on production, reproduction and the environment. Nutritional Biotechnology in the Feed and Food Industries (Ed. Lyons T. P. and Jaques K. A.) Proc. Alltech's 19th Annual Symp. Nottingham, UK 2003, May 11-14, pp. 275-288.
5. *Ball A. J., Thompson J. M., Hinch G. N., Fennessy P. E., Blakley A. R.*: Feed requirements for maintenance of mature rams and ewes from lines selected for differences in body composition. Proc. New Zealand Soc. Animal Prod. 1995, 55, 133-136.
6. *Baranowski P.*: Niektóre hematologiczne i biochemiczne wskaźniki krwi jagniąt w okresie pourodzeniowym. Magazyn Wet. 1995, 4, 521-522.
7. *Baranowski P., Baranow-Baranowski S., Klata W.*: Wybrane hematologiczne i biochemiczne wskaźniki krwi 14-dniowych jagniąt różnych ras. Magazyn Wet. 1997, 6, 128-132.
8. *Baranowski P., Baranow-Baranowski S., Klata W.*: Some haematological and biochemical serum and bone tissu indices of lambs derived from ewes fed on vitamin-and mineral-vitamin supplements during pregnancy. Bull. Vet. Inst. Pulawy. 2000, 44, 204-214.
9. *Bickhardt K., Dudziak D., Ganter M., Henze P.*: Dependence of haematological and chemical blood parameters on the age of healthy lambs-definition of reference values in sheep. Dt. Tierärztl. Wschr. 1999, 106, 445-451.
10. *Blood D. C., Radostits O. M.*: Medicina Veterinaria. 7th ed. Interamericana. McGraw-Hill, Madrid. 1993, pp. 1293-1294.
11. *Bocquier F., Ferlay A., Chilliard Y.*: Energy Metabolism of Farm Animals. Proc. of the 14th Symp. Energy Metabolism. New Castle, Co. Down, Norther Ireland 1997, 14-20, pp. 167-170.
12. *Braun J. P., Lefebvre H. P., Hamliri A., Kessabi M., Toutain P. L.*: Creatine-Kinase in sheep-a review. Rev. Med. Vet. 1993, 111, 659-664.
13. *El-Barody M. A. A., Abdalla E. B., El-Hakeam Abd. A. A.*: The changes in some blood metabolites associated with the physiological responses in sheep. Liv. Prod. Sci. 2002, 75, 45-50.
14. *Gürter H., Schutzler H.*: Zum Verhalten des Eisen und Kuplerstatus bei Kalbern nach Geburt. Mh. Vet. Med. 1971, 26, 255-264.
15. *Herdt T. H., Rumbeiha W., Braselton W. E.*: The use of blood analyses to evaluate mineral status in livestock. Vet. Clin. North Am. Food Anim. Pract. 2000, 16, 423-444.
16. *Halbrook R. S., Shugart L. R., Watson A. P., Munro N. B., Linnabary R. D.*: Characterizing biological variability in livestock blood cholinesterase activity for biomonitoring organophosphate nerve agent exposure. J. Am. Vet. Med. Assoc. 1992, 201, 714-725.
17. *Jelinek P., Gajdušek S., Illek J.*: Relationship between selected indicators of milk and blood in sheep. Small. Rum. Res. 1996, 20, 53-57.
18. *Kaneko J. J., Harvey J. W., Bruss M. L.*: Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, London, Boston, New York, Sydney, Tokio, Toronto 1997, p. 932.
19. *Kumar G. A. R., Rottan P. J. S.*: Plasma thyroidal and adrenocoritcal hormones durin different development stages in buffalo heifers. Indian J. Anim. Sci. 1992, 62, 747-754.
20. *Little W., Kay R. M., Manston R., Rowlands G. J., Stark A. J.*: The effects of age, live weight gain and feed intake on the blood composition of young calves. J. Agric. Sci. Camb. 1977, 89, 291-296.
21. *Löhle K. U.*: Untersuchungen über den Gehalt an Harnstoff, Gesamtbilirubin, Gesamteweiß und Kreatinin im Blutplasma Afrikanischer Zwergziegen. Mh. Vet.-Med. 1992, 47, 487-493.
22. *Löhle K. U.*: Die Blutbestandteile Natrium, Kalium, Kalzium, Chlorid und Phosphat bei Afrikanischen Zwergziegen im Vergleich zu Literaturergebnissen, bezogen auf andere Ziegenrassen und Schafe. Mh. Vet.-Med. 1994, 49, 85-91.
23. *O'doherty J. V., Crosby T. F.*: Blood metabolite concentrations in late pregnant ewes as indicators of nutritional status. Anim. Sci. 1998, 66, 675-683.
24. *Ramberg C. F., Mayer Jr. G. P., Kronfeld D. S., Potts J. T. Jr.*: Dietary calcium, calcium kinetics and plasma parathyroid hormone concentration in cows. J. Nutr. 1976, 106, 671-679.
25. *Ramos J. J., Verde M. T., Marca M. C., Fernandez A.*: Clinical chemical values and variations in Rasa Argonesa ewes and lambs. Small Rumin. Res. 1994, 13, 133-139.
26. *Rhind S. M., Bass J., Doney J. M.*: Pattern of milk production of East Friesland and Scottish Blackface ewes and associated blood metabolite and hormone profiles. Anim. Prod. 1992, 54, 265-273.
27. *Shaffer L., Roussel J. D., Koonce K. L.*: Effects of Age, Temperature-Season, and Breed on Blood Characteristics of Dairy Cattle. J. Dairy Sci. 1981, 64, 62-70.
28. *Shiga A., Nagamine S., Tsuchiya Y.*: Effect of Weaning on Calcium, Phosphorus and Magnesium Metabolisms in Suckling Ewes and Their Lambs. Anim. Sci. Technol. (Jpn.) 1995, 66, 862-868.
29. *Zadnik T., Klinkon M.*: Dynamics of some blood parameters in dairy cattle at early spring pasture-managment, nutrition and health control. Krmiva 1999, 41, 71-79.

Author's address: PhD. Zvonko Antunović, Faculty of Agriculture, University of J. J. Strossmayer, Trg sv. Trojstva 3, 31000 Osijek, Croatia; e-mail: zantun@pfos.hr

GUDMUNDSDOTTIR K. B., SVANSSON V., AALBAEK B., GUNNARSSON E., SIGURDARSON S.: *Listeria monocytogenes* u koni z Islandii. (*Listeria monocytogenes* in horses in Iceland). Vet. Rec. 155, 456- 459, 2004 (15)

Poddano analizie w pulsacyjnym polu elektrycznym i rybotypowaniu 20 izolatów *Listeria monocytogenes*. Pięć izolatów pochodziło z potwierdzonych przypadków listeriozy koni, które zakończyły się padnięciem zwierząt, 15 izolatów pochodziło z 4 ognisk chorób koni, gdzie podejrzewano listeriozę. Zarazek wyosobniono z kału 12 koni podejrzanych o listeriozę i z 3 próbek kiszonki stosowanej w żywieniu koni. Duża liczba *L. monocytogenes* występowała w kale koni z ostrymi objawami choroby. W obrębie 20 izolatów wyróżniono 6 genotypów. Każdy incydent choroby wywoływał jeden genotyp. Serotyp 1/2a spowodował 3 ogniska choroby w 1991, 1993 i 1997 r. W jednym ognisku choroby ten sam genotyp wyizolowano z wątroby i śledziony konia z listeriozą oraz z kiszonki.

G.