

Concentrations of NEFA, β -HBA, triglycerides, and certain blood metabolites in healthy colored Angora goats during the peripartum period

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Abstract: The aim of this study was to determine the changes in serum nonesterified fatty acid (NEFA), serum β -hydroxybutyric acid (β -HBA), triglycerides, Ca, Na, and other metabolites (bilirubin, glutamate dehydrogenase (GLDH)) in the blood of grazing, healthy goats at the time of parturition. Blood samples were taken weekly from the jugular vein of 11 goats, starting at week 2 antepartum (ap) until week 9 postpartum (pp). NEFA and β -HBA concentrations increased from week 2 ap to 2 weeks pp. The increase in NEFA level was not significant; however, the β -HBA levels were higher ($P < 0.05$) 2 weeks pp compared to the levels at 2 weeks ap. Triglycerides were recorded at maximum levels ($P < 0.05$) 2 weeks ap, with the lowest concentrations at 3 weeks pp. Bilirubin levels consistently increased up to 7 weeks pp, followed by a decrease. However, these changes were not significant. Similarly, GLDH activities increased until week 8 pp. A significant difference ($P < 0.05$) was recorded between the 1st week and 8th week pp. Ca and Na levels were lower during the 1st week pp and increased at 3 weeks pp. The results show that there are characteristic alterations of some metabolic blood parameters in goats around the time of parturition, which may be related to physiological changes.

Key words: Goats, grazing, NEFA, β -HBA, pre- and postpartum

1. Introduction

Goats are a very important source of animal protein for people in many countries, contributing to the food supply both in the form of milk and meat (1). Moreover, goat skin also possesses excellent qualities for the leather industry (2). Thus, productivity in goats is vital in many countries. Colored Angora goats are raised on small family farms in the southeastern part of Turkey for their colorful mohair. These animals are mainly kept on highland pastures during spring and summer to reduce feeding costs.

Pregnancy and lactation are metabolically stressful stages and certain hematological changes have been studied in cattle (3,4,5), sheep (6), and goats (7) raised on specific diets. However, there is a lack of information on animals producing low quantities of milk maintained on natural pastures. Periods requiring high nutrient supply, such as the pre- and postpartum periods in high-producing goats, which may be coupled with under- and/or unbalanced

nutrition, may involve the mobilization of body fat and protein, leading to a fatty liver and clinical ketosis (known as pregnancy toxemia) and eventually resulting in reduction in animal productivity and in some instances even death (8). Similar metabolic changes may take place even in low-producing goats if animals are underfed and/or fed with unbalanced nutrition. Blood serum concentrations of free fatty acids, triglycerides, cholesterol, urea, and creatinine have been reported to be higher in sheep and goats with pregnancy ketosis and multiple pregnancies compared to barren sheep and goats without pregnancy ketosis (9,10). As Ca intake may decrease due to a decrease in feed intake during ketosis, animals experiencing ketosis may also have hypocalcemia. In fact, Bickhardt et al. (9) noted that levels of serum Ca decreased significantly in sheep and goats with hypocalcemia and pregnancy ketosis. Reduced feed intake in grazing animals is the presumed cause for the hypocalcemia.

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It is highly likely that feed intake among grazing animals is low in early spring when dry matter content of pasture grasses is very low. Meeting the nutrient requirements for those animals with a low feed intake is challenging and can lead to metabolic problems. Thus, to reduce problems such as subclinical and clinical ketosis, nutrient intake needs to be enhanced. Therefore, it is important to identify periods when feed intake is low, so that animals can be nutritionally supplemented to meet their body requirements during these times. Measuring blood metabolite concentrations can help to identify these periods.

The objective of this study was to determine the changes in serum nonesterified fatty acid (NEFA), serum β -hydroxybutyric acid (β -HBA), triglyceride, calcium (Ca), sodium (Na), and blood metabolites (bilirubin, glutamate dehydrogenase (GLDH)) concentrations in grazing goats during the pre- and postpartum periods.

2. Materials and methods

The test animals included 11 colored Angora goats from the Eastern Anatolia region of Turkey, ranging in age from 3 to 6 years and weighing between 34 and 45 kg, all in late gestation according to the mating protocols. Animals were sampled from 2 weeks before the anticipated kidding date to 9 weeks after parturition. Goats were in their first to fourth gestation, with an average of 1.2 lambs per goat. Goats producing less than 1 kg/day of milk were considered low-producing goats and were used in the study. Goats were grazed on natural pastures without supplements. Immediately after parturition, does were supplemented with ground sainfoin hay at night. Energy content of natural pasture and sainfoin were approximately 63% and 56% total digestible nutrients. All does had free access to vitamin-mineral blocks and clean water. Vitamin-mineral blocks contained 1,500,000 IU vitamin A, 300,000 IU vitamin D3, 450 mg vitamin E, 9000 mg niacin, 12,000 mg P, 18,750 mg Ca, 15,000 mg Fe, 6000 mg Zn, 1500 mg Mn, 1500 mg Cu, 36,000 mg Mg, 300 mg I, and 300 mg Co.

Blood samples were taken from the jugular vein using tubes for withdrawal of serum at week 2 prepartum and on weeks 1, 2, 3, 4, 5, 6, 7, 8, and 9 postpartum. Blood samples were stored at room temperature and then centrifuged to obtain serum. Sera samples were stored at -20 °C until assayed. The serum NEFA levels were determined using the enzymatic color test with NEFA C Nr994 75409D kits (WAKO). β -HBA was assessed by the enzymatic UV test, using the Ranbut D-3 Hydroxybutyrate Kit (Randox). Serum triglyceride levels were determined by the photometric glycerin-3-phosphate oxidase-phenol amino phenazone (GOP-PAP) method, using Triglyceride GPO-PAP, Flüssig (Labor + Technik Eberhard Lehmann). Serum bilirubin was analyzed according to the Jendrassik and Grof method, using the BILIRUBIN-Test (Labor + Technik Eberhard Lehmann). Serum GLDH levels were

determined using the enzymatic ultraviolet test (UV), using GLDH Opt. DGKC (Labor + Technik Eberhard Lehmann). Serum Ca and Na levels were assessed using an ion-selective electrode of the ABL SYSTEM 615 (Radiometer). All data are expressed as the mean \pm standard error of the mean ($X \pm$ SEM) and were evaluated using one-way ANOVA with the statistical software SAS (11). Means were separated using Duncan's t-test (6). $P < 0.05$ was considered significant.

3. Results

Serum NEFA levels are illustrated in Figure 1 and the Table. The serum NEFA levels increased to 2 weeks postpartum, after which they steadily decreased; however, these changes were not statistically significant ($P > 0.05$). Similarly, β -HBA levels also increased until 2 weeks postpartum and then decreased, as shown in Figure 2 and the Table. Serum β -HBA levels were highest at 2 weeks postpartum ($P < 0.05$). The changes in serum triglyceride levels were opposite those of serum NEFA and β -HBA levels, as shown in Figure 3 and the Table: serum triglyceride levels were high during preparturition but significantly decreased after parturition as compared to the initial levels ($P < 0.05$).

Serum bilirubin and GLDH levels followed a similar pattern. Both these parameters were high during the 8th week postpartum and then decreased again. However, the changes in serum bilirubin levels were not significant, while the changes in the serum GLDH levels were significant ($P < 0.05$) as shown in the Table.

Serum Ca and Na levels were also significantly affected by parturition ($P < 0.05$). Serum Ca increased 3 weeks postpartum and then remained stable throughout the trial, whereas serum Na level increased 3 weeks postpartum and then started to decrease by week 5 postpartum (Table).

4. Discussion

It has been reported that feed intake is insufficient to meet the energy requirements of high-yielding dairy cows in the early phase of lactation, especially during the first 3–5 weeks and up to 2 or 3 months postpartum (12,13). Thus, such animals typically run into a state of negative energy balance (14). High-yielding dairy cows are reported to mobilize their long-chain fatty acids from adipose tissues in order to maintain high milk yield (15,16). Thus, dairy cows can only achieve maximal milk yield with intense lipid mobilization, but if mobilization is excessive or if normal homeostatic controls break down, metabolic disorders may occur in the dairy cows (17). It appears that similar mechanisms may take place in low-producing goat during periods of high nutrient demand, such as the pre- and postpartum periods, especially when coupled with under- and/or unbalanced feeding (8).

Table. Serum NEFA, β -HBA, triglyceride, GLDH, bilirubin, Ca, and Na concentrations in pre- and postpartum goats ($X \pm SEM$).

Sampling week	NEFA, mmol/L	β -HBA, mmol/L	Triglyceride, mmol/L	GLDH, U/L	Bilirubin, μ mol/L	Ca, mmol/L	Na, mmol/L
-2	506.24 \pm 91 ^a	0.227 \pm 0.033 ^c	0.293 \pm 0.042 ^a	4.86 \pm 1.34 ^{ab}	5.00 \pm 0.62 ^a	2.15 \pm 0.04 ^b	144.3 \pm 5.4 ^{bc}
1	530.9 \pm 67 ^a	0.305 \pm 0.051 ^{bc}	0.173 \pm 0.061 ^{ab}	4.09 \pm 1.22 ^b	5.13 \pm 1.56 ^a	2.11 \pm 0.05 ^b	145.2 \pm 4.9 ^{bc}
2	549.5 \pm 90 ^a	0.395 \pm 0.064 ^a	0.183 \pm 0.063 ^{ab}	4.80 \pm 1.15 ^{ab}	4.91 \pm 1.03 ^a	2.11 \pm 0.12 ^b	147.8 \pm 4.6 ^{ab}
3	467.9 \pm 97 ^a	0.295 \pm 0.055 ^{abc}	0.139 \pm 0.023 ^b	4.73 \pm 1.21 ^{ab}	4.97 \pm 0.75 ^a	2.24 \pm 0.06 ^a	149.9 \pm 5.9 ^a
4	411.6 \pm 109 ^a	0.305 \pm 0.075 ^{abc}	0.155 \pm 0.041 ^b	5.00 \pm 1.26 ^{ab}	5.31 \pm 1.36 ^a	2.24 \pm 0.07 ^a	149.5 \pm 3.9 ^a
5	392.5 \pm 116 ^a	0.312 \pm 0.065 ^{abc}	0.149 \pm 0.025 ^b	5.00 \pm 1.17 ^{ab}	5.55 \pm 1.12 ^a	2.19 \pm 0.05 ^b	147.6 \pm 5.2 ^{ab}
6	398.7 \pm 93 ^a	0.262 \pm 0.062 ^{bc}	0.165 \pm 0.042 ^{ab}	4.64 \pm 1.10 ^{ab}	5.43 \pm 1.14 ^a	2.16 \pm 0.06 ^b	146.1 \pm 4.8 ^{abc}
7	367.3 \pm 132 ^a	0.308 \pm 0.099 ^{abc}	0.178 \pm 0.021 ^{ab}	4.79 \pm 1.26 ^{ab}	5.78 \pm 1.13 ^a	2.20 \pm 0.07 ^b	144.9 \pm 6.2 ^{bc}
8	371.5 \pm 152 ^a	0.288 \pm 0.052 ^{bc}	0.162 \pm 0.013 ^b	5.59 \pm 1.18 ^a	5.66 \pm 0.72 ^a	2.19 \pm 0.06 ^{ab}	146.1 \pm 6.3 ^{abc}
9	348.2 \pm 117 ^a	0.298 \pm 0.062 ^{abc}	0.176 \pm 0.018 ^{ab}	4.92 \pm 1.32 ^{ab}	4.80 \pm 1.16 ^a	2.21 \pm 0.06 ^{ab}	143.4 \pm 5.4 ^c
SEM	100	0.031	0.039	0.52	0.43	0.03	1.30

$X \pm SEM$: Mean value \pm standard error of the mean.

^{abc}: different letters indicate significant differences ($P < 0.05$).

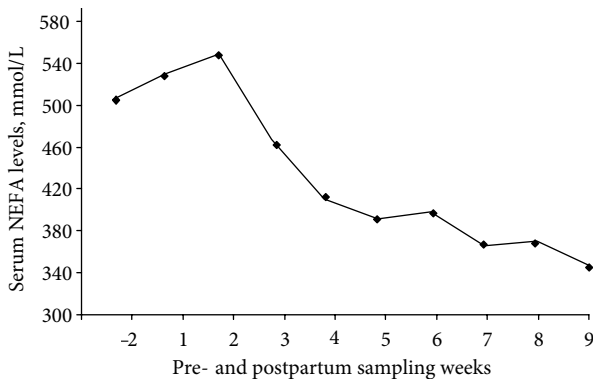


Figure 1. Mean serum NEFA (mmol/L) concentration of goats in pre- and postpartum periods. There was no significant difference among sampling weeks, $P > 0.05$ and SEM = 100.

During the postpartum period, plasma NEFA concentration reflects the rate of lipolysis (18) or lipomobilization; that is, NEFA levels model the balance between lipolysis and the reesterification of the fatty acids (19). Hence, evaluation of plasma NEFA concentrations during the periparturient period should provide insight into the time course of fatty liver development (20). In the present study, serum NEFA concentrations consistently increased 2 weeks prepartum until 2 weeks postpartum and then steadily decreased. The difference in serum NEFA concentration between weeks 2 and 9 postpartum was approximately 1.58-fold. Similarly, Blum

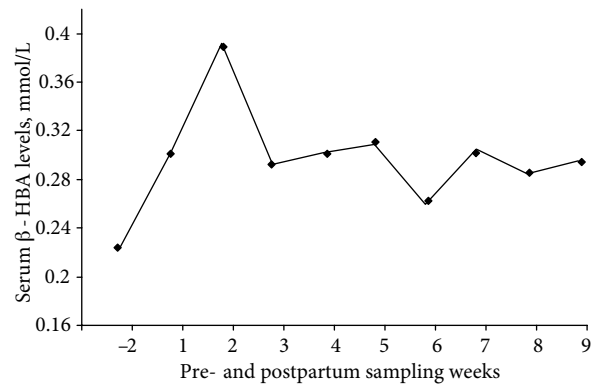


Figure 2. Mean serum β -HBA (mmol/L) concentration of goats in pre- and postpartum periods. There were significant differences among sampling weeks, $P < 0.05$ and SEM = 0.031.

et al. (12), and Kunz et al. (21) previously showed that plasma concentrations of NEFA typically increase after parturition and peak 2 weeks postpartum, reflecting the mobilization of body fat.

When excessive fat mobilization associated with significant formation of acetyl coenzyme A takes place, the tricarboxylic acid cycle cannot completely metabolize fatty acids, resulting in the conversion of acetyl coenzyme A to acetoacetate. Acetoacetate is then reduced to β -HBA by β -HBA dehydrogenase or spontaneously decarboxylized to acetone (14). However, β -HBA, synthesized from fatty acids during energy deficiency, composes the main part of the ketone bodies (19). If the concentrations of

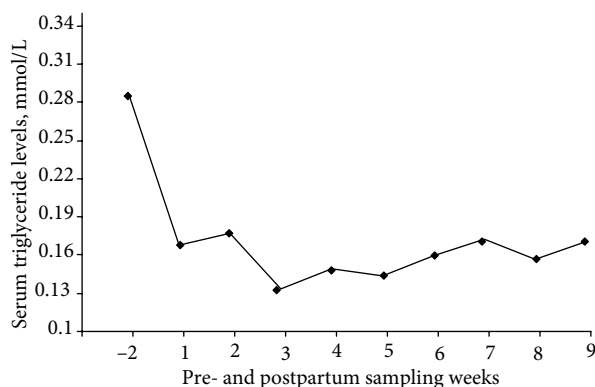


Figure 3. Serum triglyceride concentration (mmol/L) of goats in pre- and postpartum periods. There were significant differences among sampling weeks, $P < 0.05$ and SEM = 0.039.

the ketone bodies in body fluids exceed a certain level, the adaptability of metabolism is exceeded and whole-body homeostasis cannot be maintained (13,14). β -HBA concentration increased after parturition and peaked at week 2 postpartum, following the increase of NEFA; this suggests that NEFA provides the substrate for β -HBA synthesis. This increase in β -HBA concentration reveals incomplete oxidation of NEFA in the tricarboxylic acid cycle during negative energy balance (21). In addition to the increases in plasma NEFA levels due to feed deprivation (22), imposed experimental stress increased plasma NEFA concentration from 199.5 to 752.5 mEq/L in goats (23). In the present study, the observed increases in NEFA concentrations may be due to both feed deprivation and stress. Similarly, Pirmohammadi et al. (24) reported that the plasma NEFA concentrations are useful indicators to monitor the energy status of goats in the last month of gestation.

Serum triglyceride levels decreased until 3 weeks postpartum and then increased slightly. Serum triglyceride levels at weeks 3, 4, 5, and 8 postpartum were significantly lower than prepartum levels ($P < 0.05$). These results indicate that gluconeogenesis was taking place; goats were becoming ketotic as pregnancy advanced and lactation commenced. This result is consistent with results previously reported by Pancarci et al. (25) in ewes.

In the present study, both serum bilirubin and GLDH tended to increase at week 8 postpartum compared to the levels at birth, indicating a form of liver damage. Previously, a strong positive correlation among aspartate aminotransferase, blood bilirubin, and the state of ketosis was reported in dairy cows; plasma acetoacetate

concentration also has a significant effect on the concentrations of GLDH and sorbitol dehydrogenase (26,27).

It is well known that GLDH is a mitochondrial enzyme and also a sensitive and specific marker of liver disease in all animals. Normal GLDH levels in healthy small ruminants have generally been reported to be <10 U/L (28). The increase in GLDH level thus primarily reflects leakage from damaged or necrotic hepatocytes. In the present study, GLDH level did not surpass this critical threshold but significantly increased from 4.091 to 5.591 ($P < 0.05$).

Serum Ca levels in the goats also changed during the pre- and postpartum periods. Serum Ca levels were lowest during the first 2 weeks postpartum, then significantly increased after a further 2 weeks ($P < 0.05$) and then decreased slightly. This fluctuation can be explained by postpartum Ca mobilization. Azab and Abdel-Maksoud (7) also observed that plasma Ca levels in Baladi does markedly decreased during late pregnancy, reached minimum levels at parturition, and then continued to decrease for 3 weeks postpartum, ultimately returning to the level before pregnancy. Yameogo et al. (27) also reported lower average blood calcium concentrations in cows with clinical or subclinical ketosis. This was likely the result of mineral deficiencies that typically result from ketosis due to disturbances in feed consumption, as reported by other researchers (27,29). Furthermore, it has been speculated that ketosis impairs Ca metabolism. Thebault (30) reported that fat accumulation in the liver of ketogenic cows impairs the hydroxylation or conversion of vitamin D, resulting in decreased intestinal absorption of Ca (27).

It is well known that serum anion-cation levels are also affected by ketosis. Serum Na levels were low before and at parturition but started to increase after parturition. Serum Na levels were highest during weeks 3 and 4 postpartum ($P < 0.05$). However, by week 5, levels returned to those observed at parturition. Similarly, Azad and Abdel-Maksoud (7) noted that plasma Na decreased before parturition. In their study, there was a significant decrease 1 week before parturition and on the day of parturition, and then plasma Na increased 3 and 4 weeks postpartum.

The results of this study indicate characteristic changes in some metabolic blood parameters in healthy colored mohair goats around the time of parturition, resembling ketosis as seen in dairy cows. Thus, further detailed studies need to evaluate changes in blood metabolites in relation with changes in body weight.

References

1. Sousa NM, Garbayo JM, Figueiredo JR, Sulon J, Gonçalves PD, Beckers JE. Pregnancy-associated glycoprotein and progesterone profiles during pregnancy and postpartum in native goats from the north-east of Brazil. *Small Ruminant Res* 1999; 32: 137–147.
2. Oliveira AAP, Lima VPMS. Aspectos econômico da caprinocultura tropical brasileira. In: Leite ER, editor. Proc. I Semana da Caprinocultura e da Ovinocultura Tropical Brasileira. Sobral, Brazil; 1994. pp. 7–46 (in Portuguese).
3. Ghergariu S, Rowlands GJ, Danielescu AL, Pop N, Moldova A. A comparative study of metabolic profiles obtained in dairy herds in Romania. *Brit Vet J* 1984; 140: 600–608.
4. Holtenius K, Agenäs S, Delavaud C, Chilliard Y. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *J Dairy Sci* 2003; 86: 883–891.
5. Zom RL, van Baal J, Goselink RM, Bakker JA, de Veth MJ, van Vuuren AM. Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. *J Dairy Sci* 2011; 94: 4016–4027.
6. Steel RG, Torrie JH. Principles and Procedures of Statistics. 2nd ed. New York, NY, USA: McDonald Book Co.; 1980.
7. Azab ME, Abdel-Maksoud HA. Changes in some hematological and biochemical parameters during prepartum and postpartum periods in female Baladi goats. *Small Ruminant Res* 1999; 34: 77–85.
8. Kaçar C, Zonturlu AK, Karapehlivan M, Arı UÇ, Ögün M, Çitil M. The effects of L-carnitine administration on energy metabolism in pregnant Halep (Damascus) goats. *Turk J Vet Anim Sci* 2010; 34: 163–171.
9. Bickhardt K, Henze P, Ganter M. Clinical findings and differential diagnosis in ketosis and hypocalcemia in sheep. *Deut Tierärztl Woch* 1998; 105: 413–419 (in German with abstract in English).
10. Kolb E, Kaskom S. Patho-biochemical aspects of pregnancy ketosis in sheep and goats. *Tieraerztl Umschau* 2004; 59: 374–380 (in German with abstract in English).
11. SAS Institute Inc. SAS/STAT Software: Changes and Enhancements, Release 6.12. Cary, NC, USA: SAS Institute; 2005.
12. Blum JW, Kunz PL, Leuenberger H, Gautschi K, Keller M. Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. *Anim Prod* 1983; 36: 93–104.
13. Aeberhard KI, Bruckmaier RM, Blum JW. Metabolic, enzymatic and endocrine status in high-yielding dairy cows - part 2. *J Vet Med A* 2001; 48: 111–127.
14. Baird GD. Primary ketosis in the high-producing dairy cow: clinical and subclinical disorders, treatment, prevention, and outlook. *J Dairy Sci* 1982; 65: 1–10.
15. Brumby PE, Anderson M, Tuckley B, Storry JE, Hibbit KG. Lipid metabolism in the cow during starvation induced ketosis. *Biochem J* 1975; 146: 609–615.
16. Bell AW. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. *Prog Lipid Res* 1980; 18: 117–164.
17. Goff JP, Horst RL. Physiological changes at parturition and their relationship to metabolic disorders. *J Dairy Sci* 1997; 80: 1260–1268.
18. Pullen DL, Palmquist DL, Emery RS. Effect of days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. *J Dairy Sci* 1989; 72: 49–58.
19. Chilliard Y, Bocquier F, Doreau M. Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. *Reprod Nutr Dev* 1998; 38: 131–152.
20. Reist M, Koller A, Busato A, Kupfer U, Blum JW. First ovulation and ketone body status in the early postpartum period of dairy cows. *Theriogenology* 2000; 54: 685–701.
21. Kunz PL, Blum JW, Hart IC, Bickel H, Landis J. Effects of different energy intakes before and after calving on food intake, performance and blood hormones and metabolites in dairy cows. *Anim Prod* 1985; 40: 219–231.
22. Kouakou B, Gazal OS, Terrill TH, Kannan G, Gelaye S, Amoah EA. Effects of plane of nutrition on blood metabolites and hormone concentration in goats (abstract). *J Anim Sci* 1999; 77 (Suppl. 1): 267.
23. Kannan G, Kouakou B, Terrill TH, Gelaye S. Endocrine, blood metabolite, and meat quality changes in goats as influenced by short-term, preslaughter stress. *J Anim Sci* 2003; 81: 1499–1507.
24. Pirmohammadi R, Anassori E, Zakeri Z, Tahmouzi M. Effects of garlic supplementation on energy status of pre-partum Mahabadi goats. *Vet Res Forum* 2014; 5: 207–212.
25. Pancarcı ŞM, Kaçar Ş, Ögün M, Güngör Ö, Gürbulak K, Oral H, Karapehlivan M, Çitil M. Effect of L-carnitine administration on energy metabolism during periparturient period in ewes. *Kafkas Univ Vet Fak* 2007; 13: 149–154.
26. Ropstad E, Halse K, Refsdal AO. Variations in parameters of liver function and plasma progesterone related to underfeeding and ketosis in a dairy herd. *Acta Vet Scand* 1989; 30: 185–197.
27. Yameogo N, Ouedraogo GA, Kanyandekwe C, Sawadogo GJ. Relationship between ketosis and dairy cows' blood metabolites in intensive production farms of the periurban area of Dakar. *Trop Anim Health Pro* 2008; 40: 483–490.
28. Singh SP. Practical Manual in Biochemistry. 5th ed. New Delhi, India: CBS; 2004.
29. Curtis CR, Erb HN, Sniffen CJ, Smith RD, Powers PA, Smith MC, White ME, Hillman RB, Pearson EJ. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *J Am Vet Med Assoc* 1983; 183: 559–561.
30. Thebault A. L'acétonémie des vaches laitières. *L'Hebdo Vétérinaire* 2005; 153: 24–29 (in French).