SHORT COMMUNICATION

Blood biochemical parameters of crossbred Istrian x East Friesian dairy ewes: relation to milking period

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ABSTRACT

The research was conducted to investigate the serum biochemistry values for crossbred Istrian x East Friesian dairy ewes and additionally to test the effects of the milking period on biochemistry values. The values could be used for monitoring metabolic status and breeding program.

Biochemical values for crossbred Istrian x East Friesian dairy ewes were derived from one hundred and twenty ewes raised in the Mediterranean region of Croatia. The milking period was divided into early, mid and late milking periods in order to examine the effects of the milking period on biochemical values. The concentrations of calcium, phosphorus, magnesium, blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, globulin, total lipids, triglycerides, cholesterol, beta-hydroxy-butirate as well as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP) and creatine phosphokinase (CK) activities were examined.

Key words: Biochemistry, Dairy ewes, Mediterranean, Milking.

RIASSUNTO

PARAMETRI BIOCHIMICI DEL SANGUE DI PECORE DA LATTE ISTRIANA X FRISONA ORIENTALE: RELAZIONE CON IL PERIODO DI LATTAZIONE

Lo scopo della ricerca è stato quello di studiare i parametri biochimici del siero in pecore da latte di incrocio Istrian a Frisona Orientale, nonché testare gli effetti del periodo di lattazione sui parametri stessi. I valori potrebbero essere usati per monitorare lo stato metabolico e i programmi di accoppiamento. I valori biochimici dell’incrocio Istrian x Frisona Orientale sono stati analizzati in 120 pecore da latte allevate nella regione mediterranea della Croazia. Gli animali sono stati divisi in tre gruppi in base al periodo di lattazione: fresche, medie e in lattazione avanzata, per esaminare gli effetti del momento di lattazione sui parametri biochimici. Sono state esaminate le concentrazioni di calcio, fosforo, magnesio, azoto ureico (BUN), creatinina, glucosio, proteine totali, albumine, globuline, lipidi totali, trigliceridi, colesterolo, beta-idrossi-butirato, nonché l’attività di aspartato aminotransferasi (AST), alanina aminotransferasi (ALT), gamma-glutamiltransferasi (GGT), fosfatasi-alcalina (ALP) e creatinina-fosfataasi (CK).

Parole chiave: Biochimica, Pecore da latte, Mediterraneo, Lattazione.
Introduction

The breeding of dairy sheep suitable for the Mediterranean region of Croatia was first described by Mikulec et al. (2000). The base for genetic development was the indigenous Istrian sheep described by Rako et al. (1957) as the combined milk-meat-wool type with average production of 100L during the milking period. In order to improve milk production in Istrian sheep, ewes were crossbred with high producing East Friesian rams. Nevertheless many authors observed the poor viability of East Friesian cross sheep of over 50% East Friesian (Ricordeau and Flamant, 1969; Katsaounis and Zygoyiannis, 1986); therefore the aim of the program was to improve milk production without affecting resistance of animals and to produce a dairy sheep breed that could be successfully raised in large flocks on Mediterranean pastures.

The metabolic profile was first established as a tool for assessing metabolic status and helping in the diagnosis in dairy herds by Payne et al. (1970). Subsequently, many studies have applied the metabolic profile test to improve feeding management, detect health problems and prevent production disorders. The normal values may be influenced by many factors such as age or reproduction status that have been described in sheep by many authors (Alonso et al., 1997; Dubreuil et al., 2005; Roubies et al., 2006). The establishment of referential values is an important basis for the interpretation of clinical and laboratory data (Alstrom et al., 1975).

The aim of this research was to investigate values for the most commonly used biochemical parameters in crossbred Istrian x East Friesian dairy ewes. Since production of sheep milk or cheese is limited to the machine-milking period we wanted to test the effects of the milking period on serum biochemistry. These data could be used for monitoring the metabolic status and long-term success of the breeding program.

Material and methods

One hundred and twenty crossbred Istrian x East Friesian dairy ewes (Mikulec et al., 2000) were used in the trial during the milking period starting from the 30th to the 180th day of lactation (season 2005/2006). The milking period was divided into early (30th to 80th day of lactation), mid (81st to 130th day of lactation) and late (131st to 180th) milking periods. All animals were multiparous, aged between 2.5 and 4.5 years. The ewes were milked twice daily (at 06.00h and 17.00h) in a double 24 stall parallel milking parlour. Ewes were randomly selected from a herd numbering 534 animals.

The animals were fed as follows: 1 kg/ewe/day of concentrate with lucerne hay ad libitum and allowed to graze from 09.00h to 16.00h on rotational mixed grass pasture. Samples of the feed were collected throughout the experimental period, ground and analysed according to AOAC procedures (AOAC 1995) (Table 1). Fresh water was available ad libitum.

Individual milk yield was recorded weekly during two consecutive milkings (06.00 and 17.00h) by using recording jars in the milking parlour. At the same time, individual milk samples were taken from each ewe at each milking. Milk fat, protein, and lactose contents were measured by near infrared spectrophotometer using a Milkoscan FT 120 (Foss Electric, Denmark).

Blood samples were collected biweekly by puncture of the jugular vein, with the addition of heparin as an anticoagulant, prior to morning feeding. The blood plasma...
was separated by centrifugation at 1500 g for 10 minutes and stored at -20ºC for a maximum of 60 days until assayed according to metabolic profile (Payne et al., 1970). The concentrations of calcium, phosphorus, magnesium, urea, creatinine, glucose, total protein, albumin, globulin, total lipids, triglycerides, cholesterol, beta-hydroxybutirate and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP), creatine phosphokinase (CK) were all assayed by an automatic analyser (Olympus AU 600, Diamond Diagnostics, USA). Reagents for all analyses were obtained from Randox Laboratories (Ardmore, Antrim, UK). Enzyme assays were done at 37ºC, and alkaline phosphatase activity was measured at pH 10.5. The values for globulin were calculated.

All values were analysed using SAS® software (SAS, 1991). The normality of the distribution was verified with skewness and kurtosis tests, and Shapiro and Wilks test. The data not presenting Gaussian distribution (Triglycerides, AST, CK and Mg) were normalised by log transformation. Subsequently, the raw or transformed data were subjected to ANOVA procedures (General Linear Model). Post hoc multiple comparisons were adjusted for the p values by using the Tukey-Kramer procedure. The biochemical values were calculated as the data lying between 5th and 95th percentile. Differences were considered at a significance level of $P<0.01$.

Results and discussion

The average values of milk yield and chemical composition are presented in Table 2. The concentrations of milk fat, protein and lactose were within values published for crossbred Istrian x East Friesian dairy ewes (Maek et al., 2005). Table 3 gives the summary of normality diagnosis and Table 4 gives the biochemical values for the early, mid and late milking period and the total means derived from all the samples.

The total mean concentrations of electrolytes were within the limits of most published literature for sheep (Kaneko et al., 1997; Dubreuil et al., 2005; Roubies et al., 2006). The concentration of calcium varied significantly during the milking period with the highest value in the late and the lowest

| Table 1. Chemical composition (% of dry matter) of concentrate mixture, mixed grass pasture and lucerne hay |
|-----------------|-----------------|-----------------|
| Parameter       | Concentrate mixture | Mixed grass pasture | Lucerne hay |
| Ash %           | 7.5              | 7.0              | 7.2          |
| Crude fat       | 3.0              | 3.8              | 2.4          |
| Crude protein   | 18.3             | 9.1              | 16.1         |
| Crude fibre     | 7.7              | 33.5             | 33.3         |
| NDF             | 16.9             | 59               | 51.4         |
| ADF             | 7.1              | 36               | 35.7         |
| Ca              | 0.67             | 0.38             | 1.24         |
| P               | 0.77             | 0.30             | 0.32         |
| Mg              | 0.25             | 0.14             | 0.33         |
in the mid milking period, however they were always within the reference values. Phosphorus values were significantly higher in the early milking periods with respect to the mid or late milking periods. The values of magnesium were constant during the milking period, which was in contrast with the results of Brito et al. (2006) who observed higher levels in late lactation. The concentration of magnesium was slightly higher than results from Dubreuil et al. (2005) but these authors tested only non-pregnant ewes.

The total mean values for blood urea nitrogen was lower than in some reports (Brito et al., 2006; Yokus et al., 2006; Roubies et al., 2006) but very similar to other authors (Bedo et al., 1997; Balikci et al., 2006). According to the literature, serum urea values are always strongly correlated to the protein energy ratio of the diet (Carlsson and Pehrson, 1994) so it is very difficult to compare results because authors rarely provide chemical composition of diet fed in their trials. The concentration of blood urea nitrogen was the highest in early lactation, decreased in mid and then increased again in the late milking period. A similar pattern was described by Bedo et al. (1997) and explained by the increased NDF content of grass, which caused a decrease in protein to energy ratio and lower milk urea values. The milking period also influenced creatinine values that were significantly lower in the early milking period with respect to the mid or late periods. The total mean concentrations for creatinine were within the limits of Dubreuil et al. (2005) and Yokus et al. (2006) but lower than reported by Roubies et al. (2006). The increase in creatinine value during the mid and late milking period was probably caused by the stabilization of live weight and occurrence of the maximum muscle mass and a high rate of protein turnover (Caldeira et al., 2007).

Glucose levels were similar to values in literature (Kaneko et al., 1997; Brito et al., 2006; Yokus et al., 2006; Roubies et al., 2006) and highest in the early milking period with a decrease later in the mid and late milking periods. A somewhat similar pattern was also described by Bedo et al. (1997) with significantly increased values in the 2nd month of lactation, then a significant decrease in the 3rd and 4th month and a non-significant increase in the 5th month. Variations in glucose levels were probably caused by the changes in feed intake during the trial, which could be expected in grazing animals.

| Parameter                  | Early | Mid | Late   |
|----------------------------|-------|-----|--------|
| Number of ewes             | 120   | 118 | 110    |
| Milk yield (g/day)         | 1158 ± 130 | 791 ± 84 | 526 ± 56 |
| Milk composition:          |       |     |        |
| Fat %                      | 7.22 ± 0.86 | 7.92 ± 0.46 | 8.53 ± 0.78 |
| Protein "                  | 5.24 ± 0.51 | 5.43 ± 0.62 | 6.60 ± 0.89 |
| Lactose "                  | 4.58 ± 0.27 | 4.59 ± 0.24 | 4.07 ± 0.19 |

2 Values represent means ± SD.
The total protein concentration significantly varied during the milking period and variations were caused by variable levels of globulin fraction. In contrast, albumin levels were constant during the milking period. Nevertheless, all three values were within the published reference ranges (Kaneko et al., 1997; Roubies et al., 2006). Similar results were presented by Brito et al. (2006) who also observed constant levels of albumin and variable, but not significant, levels of globulin during lactation. The globulin concentrations observed during milking were probably related to increased exposure to different antigens, although animals were constantly examined for clinical and subclinical mastitis and all ewes with symptoms were excluded from the trial.

Levels for cholesterol and total lipids were higher than those observed by Nazifi et al. (2002), but levels for triglycerides were similar to those observed by the same author. Both triglycerides and total cholesterol levels significantly increased in the late milking period. The increase of lipid values in the late milking period coincided with low milk production and decreased requirements for substances needed for the milk fat synthesis. In contrast beta-hydroxy-butyrate values decreased successively during the milking period coinciding with decreased requirements for milk production.

The total mean serum activities of AST, ALT and CK were very similar to published values by most other authors (Kaneko et al.,

| Table 3. Summary of normality diagnosis |
|----------------------------------------|
|                                         |
| Calcium mmol/L | 0.551 | 0.014 | 0.329 |
| Phosphorus "   | -0.362| -0.981| 0.198 |
| Magnesium "    | 3.488 | 15.467| 0.000 |
| Blood urea nitrogen " | -0.566| 0.441 | 0.105 |
| Creatinine µmol/L | 0.035 | -0.840| 0.393 |
| Glucose mmol/L  | 0.425 | -0.130| 0.283 |
| Total Protein g/L | 0.163 | -0.118| 0.690 |
| Albumin "       | -0.300| 0.304 | 0.196 |
| Globulin "      | 0.437 | -0.222| 0.177 |
| Total lipids "  | 0.373 | -0.122| 0.359 |
| Triglycerides mmol/L | 2.204| 8.058 | 0.000 |
| Cholesterol "   | 0.170 | 0.121 | 0.712 |
| Beta-hydroxy-butyrate mmol/L | 0.155| -0.062| 0.493 |
| Aspartate aminotransferase U/L | 1.194| 1.029 | 0.007 |
| Alanine aminotransferase " | -0.175| -0.717| 0.540 |
| Gamma-glutamyl transferase " | 0.309| -0.151| 0.711 |
| Alkaline phosphatase " | 0.276| -0.446| 0.437 |
| Creatinine phosphokinase " | 1.565| 2.249 | 0.000 |

a Values<0 indicate left-asymmetry, values>0 indicate right-asymmetry, values=0 indicate normality.

b Values<0 indicate flatness, values>0 indicate distribution in peak, and values=0 indicate normality.

c If p≥0.05, the normality hypothesis is not rejected.
The total mean activity of GGT was higher than the reference range published by Kaneko et al. (1997) but similar to values reported by Yokus et al. (2006), Dubreuil et al. (2005) and Smith, (2002). The activities of AST, ALT and GGT did not differ significantly during the milking period, while the activities of CK and ALP varied significantly. The lowest value for the activity of CK was in the mid milking period. Nevertheless, the serum activities were always similar to the published values mentioned and probably caused by stress and manipulation with the animals. In contrast the ALP activity values decreased successively during the milking period. A similar, but non significant, decrease in the ALP activity was reported by Sato et al. (2005) in cows. Those authors observed higher levels of serum ALP in lactation periods than in dry periods and it was explained by

| Table 4. Mean and 5th to 95th percentiles of serum biochemical values during milking period. |
|---------------------------------------------------------------|
| Parameter            | Milking period                               |
|                     | Early | Mid   | Late  | Total mean |
|                     | Mean  | Percentiles | Mean | Percentiles | Mean  | Percentiles | Mean  | Percentiles |
| **Electrolytes:**   |
| Calcium (mmol/L)    | 2.65  | (2.44-2.77) | 2.54 | (2.47-2.62) | 2.78  | (2.55-3.01) | 2.66  | (2.47-2.97) |
| Phosphorus (µmol/L) | 1.81  | (1.54-2.18) | 1.62 | (1.32-2.01) | 1.62  | (1.29-1.86) | 1.68  | (1.32-2.04) |
| Magnesium           | 1.13  | (0.97-1.53) | 1.07 | (0.96-1.17) | 1.11  | (1.01-1.21) | 1.10  | (0.97-1.21) |
| **Chemistry:**      |
| Blood urea nitrogen (mmol/L) | 7.83  | (6.19-9.30) | 6.38  | (4.39-8.04) | 7.53  | (5.67-9.14) | 7.24  | (4.83-9.17) |
| Creatinine (µmol/L) | 75.6  | (66.2-84.4) | 84.6  | (75.6-91.2) | 82.6  | (68.0-98.0) | 80.7  | (68.0-95.9) |
| Glucose (mmol/L)    | 3.41  | (2.99-3.86) | 2.97  | (2.52-3.42) | 2.99  | (2.80-3.30) | 3.13  | (2.74-3.80) |
| Total Protein (g/L) | 71.2  | (61.3-84.0) | 69.8  | (62.4-78.6) | 73.7  | (67.0-82.0) | 71.6  | (62.3-82.7) |
| Albumin             | 32.2  | (26.8-36.6) | 32.9  | (29.4-36.0) | 32.7  | (29.3-35.5) | 32.6  | (27.7-37.6) |
| Globulin            | 39.0  | (30.5-50.6) | 36.9  | (31.1-43.6) | 41.0  | (35.0-47.5) | 39.0  | (31.2-48.7) |
| Total lipids        | 3.35  | (2.49-4.48) | 3.18  | (2.43-3.87) | 3.25  | (2.48-4.04) | 3.25  | (2.45-4.23) |
| Triglycerides (mmol/L) | 0.26  | (0.12-0.36) | 0.24  | (0.17-0.32) | 0.30  | (0.16-0.49) | 0.27  | (0.14-0.44) |
| Cholesterol         | 1.88  | (1.51-2.33) | 1.87  | (1.49-2.27) | 1.98  | (1.68-2.42) | 1.91  | (1.51-2.35) |
| Beta-hydroxy-butyrate | 0.56  | (0.40-0.84) | 0.57  | (0.27-0.80) | 0.51  | (0.35-0.68) | 0.55  | (0.34-0.80) |

| Enzymes1:          |
| Aspartate aminotransferase (U/L) | 157  | (112-253) | 148  | (94-223) | 155  | (89-254) | 153  | (93-265) |
| Alanine aminotransferase | 14.7 | (10.0-17.0) | 18.0 | (9.2-22.8) | 16.4 | (7.4-24.6) | 16.4 | (7.0-24.6) |
| Gamma-glutamyl transferase | 57.5 | (30.2-80.0) | 53.8 | (35.4-73.2) | 48.1 | (32.6-67.0) | 53.3 | (30.4-78.8) |
| Alkaline phosphatase | 254+ | (123-374) | 228+ | (142-343) | 180+ | (88-301) | 220  | (100-366) |
| Creatinine phosphokinase | 134+ | (88-223) | 92+  | (67-130) | 142+ | (89-233) | 122  | (71-237) |

a, b, c: Values within rows with different superscripts are significantly different (P<0.01) by the Tukey-Kramer procedure.

1 Measured at 37ºC.

1997; Smith, 2002; Dubreuil et al., 2005; Yokus et al., 2006). The total mean activity of GGT was higher than the reference range published by Kaneko et al. (1997) but similar to values reported by Yokus et al. (2006), Dubreuil et al. (2005) and Smith, (2002). The activities of AST, ALT and GGT did not differ significantly during the milking period, while the activities of CK and ALP varied significantly. The lowest value for the activity of CK was in the mid milking period. Nevertheless, the serum activities were always similar to the published values mentioned and probably caused by stress and manipulation with the animals. In contrast the ALP activity values decreased successively during the milking period. A similar, but non significant, decrease in the ALP activity was reported by Sato et al. (2005) in cows. Those authors observed higher levels of serum ALP in lactation periods than in dry periods and it was explained by
increased bone specific ALP and liver ALP activities in lactation periods and by the suggestion that ALP originating from the mammary gland could influence serum ALP values (Sato et al., 2005).

Conclusions

In our investigation biochemical values for crossbred Istrian x East Friesian dairy ewes did not differ significantly from reference values reported for other breeds. Milking period had a significant influence on several biochemical parameters, however the results suggest the necessity for more detailed investigations of other variables and interactions, such as sex, season, gestation and dry period, in addition to those observed in this study. In our opinion these results could be used for the assessment of the adaptation of crossbred ewes to large flock conditions and monitoring of breeding success.

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REFERENCES

Alonso, A.J., Deteresa, R., Garcia, M., Gonzalez, J.R., Vallejo, M., 1997. The effects of age and reproductive status on serum and blood parameters in merino sheep breed. J. Vet. Med. A. 44:223-231.

Alström, T., Glasbeck, R., Hjelm, M., Skandsen, S., 1975. Recommendation concerning the collection of reference values in clinical chemistry and activity report. Scand. J. Clin. Lab. Invest. 35:3-44.

AOAC, 1995. Official methods of analysis.16th ed. Association of official analytical chemists, Arlington, VA, USA.

Balikci, E., Yildiz, A., Gurdogan, F., 2006. Blood metabolite concentration during pregnancy and postpartum in Akkaraman ewes. Small Rumin. Res. (In press).

Bedo, S., Nikodemusz, E., Gundel, K., Nagy, Z., 1997. Relation of plasma concentration of urea, glucose and total protein to milk levels of urea, lactose and protein of grazing ewes during lactation. Arch. Tierz. 40:265-275.

Brito, M.A., Gonzalez, F.D., Ribeiro, L.A., Campos, R., Lacerda, L., Barbosa, P.R., Bergmann, G., 2006. Blood and milk composition in dairy ewes from southern Brazil: variations during pregnancy and lactation. Ciência Rural 36:942-948.

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I., Portugal, A.V., 2007. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Ruminant Res. 68:242-255.

Carlson, J., Pehrson, B., 1994. The influence of the dietary balance between energy and protein on milk urea concentration. Experimental trials assessed by 2 different protein evaluation systems. Acta Vet. Scand. 36:245-254.

Dubreuil, P., Arsenault, J., Belanger, D., 2005. Biochemical reference ranges for groups of ewes of different age. Vet. Rec. 156:636-638.

Kaneko, J.J., Harvey, J.W., Bruss, M.L., 1997. Clinical biochemistry of domestic animals. 5th ed. San Diego Academic Press., San Diego, CA, USA.

Katsaounis, N., Zygoiannis, D., 1986. The East Friesland sheep in Greece. Res. Dev. Agric. 3:19-30.

Mašek, T., Mikulec, Ž., Valpotić, H., Kulič, L., Mikulec, N., Strakova, E., Serman, V., Mas, N., 2005. Effects of live yeast cells on production results of Croatian crossbred dairy sheep. pp 105-110 in Proc. 6th Int. part. Conf. Kubrt’s dietetic days, Brno, Czech Republic.

Mikulec, K., Sušić, V., Mikulec, Ž., Serman, V., 2000. Breeding of dairy sheep for the Mediterranean region of Croatia. Options mediterraneennes. Serie A: Seminaires Mediterraneennes. 43:79-86.

Nazifi, S., Saeb, M., Ghavami, S.M., 2002. Serum lipif profile of Iranian fat-tailed sheep in late pregnancy, at parturition and during the post parturition period. J. Vet. Med. A. 49:9-12.

Payne, J.M., Dewsally, M., Manston, R., Faulks, M., 1970. The use of a metabolic profile test in dairy herds. Vet. Rec. 87:150–158.

Rako, A., 1957. Istrian dairy sheep. Stočarstvo. 10:23-29.

Ricordeau, G., Flamant. J.C., 1969. Croisements entre les races ovines Préalpes du Sud et Frisonne (Ostfriesisches Milchschaf). II. Reproduction, viabilité, croissance, conforma- tion. Ann. Zootech. 18:131-149.
Roubies, N., Panousis, N., Fytianou, A., Katsoulos, P. D., Giadinis, N., Karatzias, H., 2006. Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. J. Vet. Med. A. 53:277-281.

Sato, J., Kanata, M., Yasuda, J., Sato, R., Okada, K., Seimiya, Y., Naito, Y., 2005. Changes of serum alkaline phosphatase activity in dry and lactational cows. J. Vet. Med. Sci. 67:813-815.

Smith, B.P., 2002. Large animal internal medicine. 3rd ed. Philadelphia, Mosby, USA.

SAS, 1991. SAS User's Guide: Statistics, version 6. Institute Inc., Cary, NC, USA.

Yokus, B., Cakir, U.D., 2006. Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biol. Trace Elem. Res. 109:255-266.