

Oxidative stability and quality of raw Saanen and Alpine goats milk

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Abstract

The milk composition and concentration of thiobarbituric acid reactive substances (TBARS) and superoxide dismutase (SOD) activity in fresh and refrigerated raw goat milk of Saanen and Alpine breed at the breeding period were investigated. Low average milk fat, SNF and lactose content were determined in both breeds with no significant differences between breeds regarding the milk yield and milk composition. The significantly higher somatic cell count (SCC) was detected in Saanen goats. The SOD activity and TBARS concentration were significantly higher 14 h after the collection and cold storage in both breeds of dairy goats with no difference between the breeds. In the Alpine goats the TBARS concentration was negatively correlated with urea concentration, and in the milk of the Saanen goats the TBARS was positively correlated with proteins and SCC. The higher SCC in the Saanen goats may have caused higher production of secondary oxidative products catalysed by the enzyme superoxide dismutase from polymorphonuclear granulocytes present in the udder, or by some other enzymes that participate in oxidation of the milk constituents. The energy shortage and protein surplus in the diet of the dairy goats may have induced the higher degree of lipid mobilisation and a consequent increase of polyunsaturated fatty acids (PUFA) that are easily prone to oxidation and formation of the secondary oxidative products.

Keywords: goat, raw milk, milk components, peroxidation, quality

Zusammenfassung

Oxidative Stabilität und Qualität der Rohmilch von Saanen- und Alpenziegen

Untersucht wurde die Milchezusammensetzung, die TBARS-Konzentration (Thiobarbitursäurereaktive Substanzen) und die SOD-Aktivität (Superoxiddismutase) in frischer und gekühlter Rohmilch von Saanen- und Alpenziegen während der Fortpflanzungszeit. Zwischen beiden Rassen bestanden keine signifikanten Unterschiede bei der Milchleistung und der Milchezusammensetzung, wobei ein relativ niedriger Fett-, Laktosegehalt und ein niedriger Wert für die Trockensubstanz nachgewiesen wurden. Bei den Saanenziegen ergab sich ein höherer somatischer Zellgehalt (SCC). 14 Stunden nach der Milchgewinnung und Kühllhaltung waren in der Milch die TBARS-Konzentration und die SOD-Aktivität signifikant höher, wobei zwischen den Rassen keine Unterschiede ermittelt wurden. Eine negative Beziehung fand sich zwischen dem TBARS-Wert und dem Harnstoffgehalt bei den

Alpenziegen, positiv war bei den Saanenziegen die Beziehung zwischen dem TBARS-Wert und dem Eiweiß- sowie SCC-Gehalt. Der höhere SCC-Gehalt war möglicherweise die Ursache für eine höhere Erzeugung von sekundären Oxidationsprodukten verursacht durch die Superoxiddismutase und den im Euter vorhandenen polymorphkernigen Granulozyten oder anderen Enzymen die an der Oxidation der Milchinhaltsstoffe teilnehmen. Energiemangel und Eiweißüberschuss in der Ernährung von Milchziegen verursacht möglicherweise die höhere Lipidmobilisation und folglich die Erhöhung von mehrfach ungesättigten Fettsäuren welche leicht oxidieren und sekundäre Oxidationsprodukte bilden.

Schlüsselwörter: Ziege, Rohmilch, Milchinhaltsstoffe, Peroxidation, Qualität

Introduction

The health benefit of goat milk to human health has been proclaimed from the ancient times and has been extensively discussed but not scientifically improved in the matter under notice (HAENLEIN 2004)). The fatty acid composition in milk has drawn respectable attention, especially some fatty acids, such as caprylic and capric acid, and more recently conjugated linoleic acid (CLA) regarding their impact to human health (BADINGA and GREEN 2006, SORYAL *et al.* 2003, PFEUFFER 2001). The production and market expansion of goat's milk around the European Union shows the availability of goat's milk production in accordance with all regulatory standards (EUROPEAN COMMISSION 1995, STRZALKOWSKA *et al.* 2006). It is questionable whether refrigerated raw goat's milk could retain high quality standards prior to processing in dairy industry. The milk constituents are greatly influenced by the breed, physiological stage (PETERS and LAES-FETBACK 1995, STRZALKOWSKA *et al.* 2004, GORECKI *et al.* 2004), stage of lactation (WOJTOWSKI *et al.* 2001, FAHR *et al.* 2001, STRZALKOWSKA *et al.* 2001), parity (STRZALKOWSKA *et al.* 2004), climate conditions (KIJORA *et al.* 2002) and nutrition (LEIBETSEDER 1996, KIJORA *et al.* 2002, FAHR *et al.* 2001, WALISIEWICZ-NIEBALSKA *et al.* 2004) of dairy goats. The unsaturated fatty acids (UFA) in raw milk may favour oxidative changes and oxidative reactions which may reduce the nutritive quality of milk and milk products (KONDYLI *et al.* 2005). Some indigenous enzymes are considered important for the oxidative stability of milk, such as superoxide dismutase (SOD) which inhibits the lipid oxidation (KORYCKA-DAHL and RICHARDSON 1979, LAMBERTSEN and CHRISTIANSEN 1997).

The main objective of the present research was to compare and evaluate the milk composition and oxidative stability of fresh and refrigerated milk in two breeds of dairy goats reared under the same farm conditions at the breeding season. The relationship between milk components, somatic cell count and oxidative stability of raw goat milk has been hypothesized.

Material and methods

The research was conducted on two breeds of dairy goats, seven each of Saanen and Alpine goats, managed and kept on the same farm in an indoor/outdoor farming system. The farm was located in the inland part of Croatia, in Zagreb County where 21 breeders with 750 goats (male and female), mainly of the Alpine and Saanen breeds, were

registered (CROATIAN AGRICULTURAL AGENCY 2006). At the experimental farm, the animals were kept indoors in a loose housing system, and outdoors, in a 6500 m² paddock. In both breeds the feeding strategy was substantially the same. The daily ration consisted of mid-mature meadow hay, pasture and concentrate mixture consisted of 15% wheat bran, 10% barley grains, of 40% of corn grains, 15% soybean meal, 15% sunflower meal, 3% Ovisan (Sano, GmbH) 1% Multisan Nektar (Sano, GmbH), 1% Camisan (Sano, GmbH). Hay and water was offered *ad libitum*, and concentrate mixture twice daily, at the milking time. Both breeds were in the end of lactation (210 DIM). Alpine goats were in the fourth and the Saanen goats in the first parity. The goats were hand-milked at 7 a.m. and 7 p.m. The milk production at the sampling periods was in average 1-2 litres/day (l/d) in both breeds. For the purpose of milk component analysis, 40 ml of individual milk samples was collected at the morning milking period during the five days (70 milk samples) and transported in a cooler-box at 4°C to the laboratory. At the time of milk sampling (September 2006) the daily average air temperature was 22°C, which was above the average annual temperature for that period of the year. The milk component analysis was performed 2 h after the milk sample collection, and measurement of SOD (superoxide dismutase) activity and TBARS (thiobarbituric acid reactive substances) concentration was performed, 2 h and 14 h after the collection and cold storage of samples in laboratory refrigerator at the 4°C. The milk constituents protein, fat, lactose, and solids-non fat (SNF) were measured by automatic analyser (MilcoScan 4000, Denmark) and the somatic cell count (SCC) was determined by Fossomatic 5000 (Denmark). The milk urea concentration was measured by the colorimetric method using the commercial kit (Herbos Dijagnostika, Sisak, Croatia). Total SOD activity was measured using the commercial kit (RANSOD, »Randox«, Ireland). Determination of thiobarbituric acid reactive substances (TBARS) was performed according to the method of TROTTA *et al.* (1982). The absorption peak was measured at 532 nm and concentration was calculated using a molar extinction coefficient of $1.5 \cdot 10^5$ (PLACER *et al.* 1966). Total SOD activity and TBARS concentration were expressed per litter of milk.

The data were analysed using one-way MANOVA (Statistica 7.1, STATSOFT 2005). The results were presented as mean \pm standard deviation. The correlations between parameters were calculated. A multivariate test of significance was performed using the Wilks lambda test. The significance for all data was declared at $P < 0.05$.

Results and discussion

The daily milk yield at the sampling period was lower from the average annual breed production (Table 1) with respect to parity which was, according the Annual Report of Croatian Agricultural Agency (CROATIAN AGRICULTURAL AGENCY 2006) 2.22 l/day for the first parity in Saanen goats, and 2.38 l/day, at the fourth parity in Alpine goats, respectively. Despite the difference in breed and parity, in the present study the milk components were not significantly different between two experimental groups of dairy goats (Table 1). The milk protein content was higher than 2.6%, which is congruent with the results of KOZAIČINSKI *et al.* (2004), but somewhat lower than reported by the CROATIAN AGRICULTURAL AGENCY (2006). The milk fat content was rather low in both breeds of dairy goats in the study, and lower than the average

milk fat content in the Annual Report (CROATIAN AGRICULTURAL AGENCY 2006) and the result of KOZAČINSKI *et al.* (2004) and STRZALKOWSKA *et al.* (2007) (Table 1). Among other milk components, there is some evidence of higher deviation in milk fat content during the lactation cycle, especially due to dietary changes (PLOUMI *et al.* 1998, POLJIČAK-MILAS and MARENJAK 2007, SANZ SAMPELAYO *et al.* 2007). In dairy goats, as well as in other ruminants, low milk fat syndrome might occur mostly as an effect of changes in rumen biohydrogenation processes. The essential strategy for prevention of milk fat depression, especially if fat supplements, fresh grass or oilseeds are present in the ruminant's diet, is adequate nutritional composition (WALISIEWICZ- NIEBALSKA *et al.* 2004). In the present study, an inverse relation of milk fat and milk protein, and milk urea and milk protein in the majority of experimental goats may reveal an energy shortage and protein surplus in the diet of dairy goats (Figure 1).

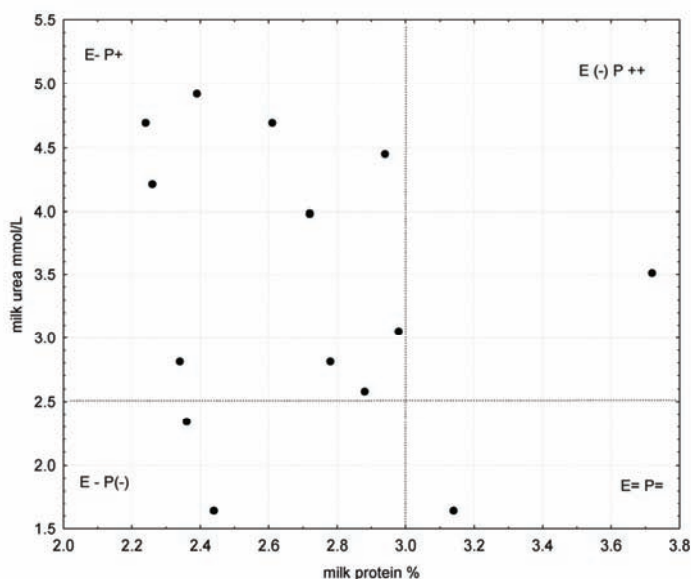
Table 1

Average milk composition and somatic cell count (SCC) in two breeds of dairy goats at the research (mean \pm standard deviation)

Milchzusammensetzung und Gehalt somatischer Zellen in der Milch der beiden untersuchten Populationen

Milk components	Alpine	Saanen
Fat, %	1.85 \pm 0.5	2.10 \pm 0.23
Protein, %	2.62 \pm 0.34	2.78 \pm 0.49
Lactose, %	4.02 \pm 0.18	4.11 \pm 0.29
SNF, %	7.42 \pm 0.45	7.66 \pm 0.65
Urea mmol/l	3.61 \pm 0.14	3.15 \pm 0.86
SCC, $\times 1\ 000$	180.28 \pm 194.13 ^a	1 122.43 \pm 1 048.57 ^b
Milk yield, l/d	1.70 \pm 0.05	1.80 \pm 0.10

SNF solids-non fat, SCC somatic cell count, Data with the different superscript^{ab} are significantly different at $P < 0.05$.



E- P+ energy shortage and protein surplus, E(-) P++ mild energy shortage and intensive protein surplus, E- P(-) energy and mild protein shortage, E= P= equable nutritional energy and protein

Figure 1

Milk protein and urea interrelationship

Beziehung zwischen Milchprotein und Milchharnstoff

The lactose content was lower than in STRZALKOWSKA *et al.* (2004) and KOZAČINSKI *et al.* (2004) studies, and lower SNF content was also determined (Table 1). Usually, the lactose content positively correlates to milk yield and negatively correlates to lactation period according to VOUTSINAS *et al.* (1990) and PLOUMI *et al.* (1998) studies. The stage of lactation as a factor of difference between two the breeds could be omitted, but the stage of lactation could be taken into account, while lactose content is expected to be lowest in late lactation (VOUTSINAS *et al.* 1990). Furthermore, an insufficient energy supply in the ratio may cause a decrease in milk lactose content due to the lower supply in digestible carbohydrates that are an important source for the lactose synthesis in the mammary gland.

The concentration of milk urea ranged over 3.3-3.9 mmol/L, with lower average value for Saanen goats (Table 1), but was higher than reported by ARIELI *et al.* (2005). Milk urea is very often used for monitoring the nutritional status (energy/protein relation in the diet) in lactating dairy cows (MARENJAK *et al.* 2000) and there is some evidence of applicability in lactating ewes and goats (BED SANDOR NAGY *et al.* 2006, KIJORA *et al.* 2002). The higher milk urea concentration in the present research could be related to a protein surplus or concentration effect due to lower milk production. Elevated milk urea and losses in milk production could be related to improper balancing of rumen degradable proteins (RDP) and inefficient use of nitrogen, while goats are more efficient in nitrogen recycling in low crude protein diet than cows (PLOUMI *et al.* 1998). Certainly, other factors such as the environmental temperature and water consumption are very important factors that might influence the milk urea concentration and other milk constituents (SAHLU *et al.* 2004).

The average SCC (somatic cell count) in Alpine goats in the present study was lower than the allowable maximum level in most European countries. On the other hand, average SCC in Saanen goats was higher than recommended (Table 1) by Croatian and European Standards (EUROPEAN COMMISSION 1995, OFFICIAL GAZETTE 2000). However, in contrast to dairy cows, individual milk samples for evaluating total SCC in terms of detection of udder infection are less reliable for dairy goats (HAENLEIN 2002, STRZALKOWSKA *et al.* 2006). From the WILSON *et al.* study (1995), as much as 90% of the differences in goats' SCC were not due to intramammary infection. Many factors, other than intramammary infections, can influence the SCC in dairy goats, and as opposed to dairy cows, a higher allowable maximum SCC is permitted for raw goat milk. The stage of lactation usually influences the SCC in goats, but also age, level of milk production and breed may cause significant variations in SCC. HAENLEIN (2002) stated that hand milking may also elevate SCC. Moreover, the reproductive cycle and induction of oestrus were related to higher SCC, suggesting that the increase of SCC could be brought about by the oestrogen-induced proliferation and exfoliation of epithelial cells (MORONI *et al.* 2007) which might cause the average higher somatic cell count in Saanen goats in the present research. Therefore, it can be considered some other non-pathological factors that might have influenced the results of SCC analyses in the milk of Saanen goats in the present study, particularly nutrition, stage of lactation and the beginning of oestrus.

The results of SOD activity and TBARS concentration 2 and 14 h after collection and cold storage were presented in Table 2. The oxidative stability and formation of secondary oxidative products in milk samples were not significantly different between two breeds of

dairy goats, although, to some extent, higher SOD activity and TBARS concentration were detected in the Saanen goats. The superoxide dismutase can serve as antioxidant agent in biological systems and foodstuffs, but under certain conditions might have peroxidative ability (KORYCKA-DAHL and RICHARDSON 1979). Determination of SOD activity and TBARS concentration in milk samples, after 14 hours of storage at 4 °C, revealed a significant increase of secondary oxidative products and decrease of superoxide dismutase activity in both breeds of dairy goats, with rather lower formation of TBARS in the Saanen goats (Table 2).

Table 2

SOD activity and TBARS concentration in raw milk samples of Alpine and Saanen goats 2 and 14 h after collection (mean \pm standard deviation)

SOD-Aktivität und TBARS-Konzentration in der Rohmilch der Alpen- und Saanenziegen 2 und 14 Stunden nach der Milchgewinnung

Breed	Alpine	Saanen
SOD 2h, U/L	3 028.86 \pm 690.41 ^a	3 199.14 \pm 2 010.37 ^a
SOD 14h, U/L	2 155.29 \pm 431.36 ^b	2 486.27 \pm 1 301.44 ^b
TBARS 2h, μ mol/L	2.25 \pm 0.86 ^a	2.73 \pm 1.46 ^a
TBARS 14h, μ mol/L	3.39 \pm 0.41 ^b	3.12 \pm 0.76 ^b

SOD superoxide dismutase, TBARS thiobarbituric acid reactive substances, Different superscripts^{ab} indicate differences of means at 2 and 14 hours being significant at $P < 0.05$.

Off-flavours in milk and milk products are very often an effect of oxidation of fatty acids, linoleic acid especially, through the presence of superoxide dismutase (SOD) and hydrogen peroxide (H₂O₂), therefore the oxidative stability of milk products is closely related to raw milk quality (KELLY and FOX 2006). On the other hand, SOD activity in fresh raw milk might have been dependent on the overall antioxidant status of the milk-producing animal. Higher oxidative stability in animal products may be expected under certain nutritional conditions such as natural or synthetic antioxidant agents, enzymes or minerals (SMET *et al.* 2005).

In the present research the TBARS concentration was very positively correlated to milk protein and SCC in the Saanen goats (Figure 2 and 3) and negatively correlated to milk urea concentration in the Alpine goats (Figure 4). The products of secondary oxidation might have originated from the SOD activity of polymorphonuclear granulocytes that are normally found in uninfected udders of dairy goats, but also are increased in infected ones, linearly with an increase of SCC. Therefore it could be presumed that positive correlation of SCC and TBARS concentration in Saanen goats was partly initiated from the hydrogen peroxide formation of neutrophile granulocytes, which might generate free radicals and oxidative changes of milk. The presence of numerous enzymes and higher SCC may be evidence of lower hygienic quality and technological properties of the raw milk. The highly negative correlation of TBARS and milk urea concentration in Alpine goats might be considered a result of nutritional imbalance and possible abounded lipid mobilisation to compensate the negative energy status of the experimental dairy goats. That could possibly affect the milk fatty acid composition and the higher percentage of PUFA in milk may encourage a higher degree of lipid peroxidation and consequently the formation of secondary oxidative products in milk. Low milk fat, higher milk urea concentration and high SCC in the Saanen goats, in particular, pointed to potential nutritional imbalance. A high grain diet might have been related to higher SCC in the Saanen goats, and lower production of milk fat and lactose in both experimental breeds of dairy goats.

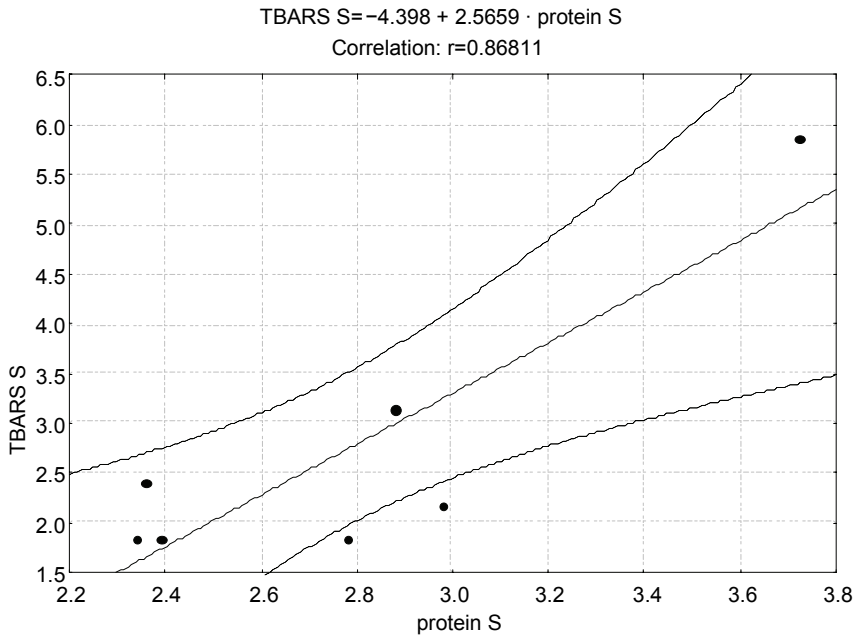


Figure 2
 Milk protein and TBARS concentration in Saanen goats
Milchprotein und TBARS-Konzentration bei Saanen Ziegen

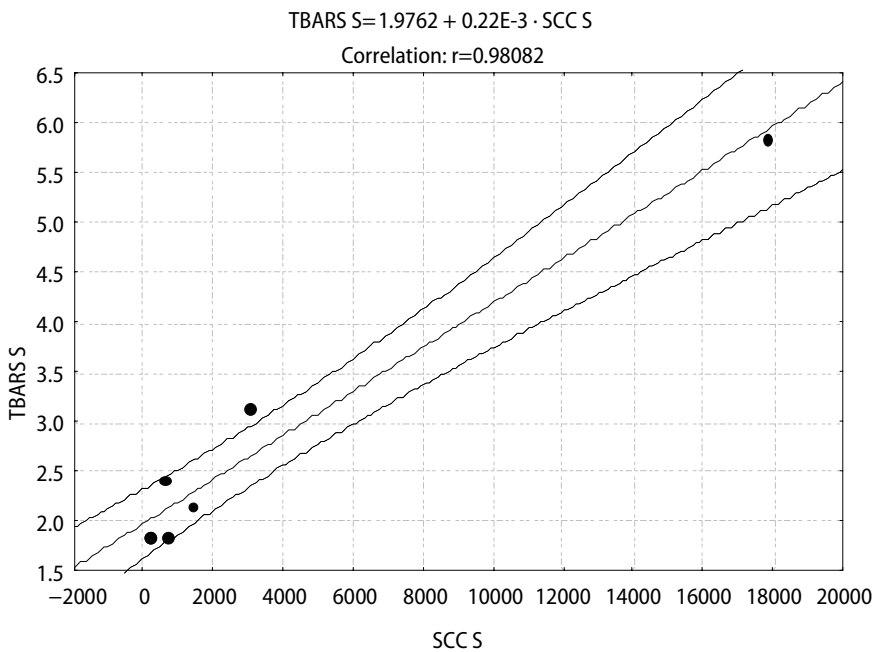


Figure 3
 Somatic cell count and TBARS concentration in Saanen goats
SCC-Gehalt und TBARS-Konzentration bei Saanenziegen

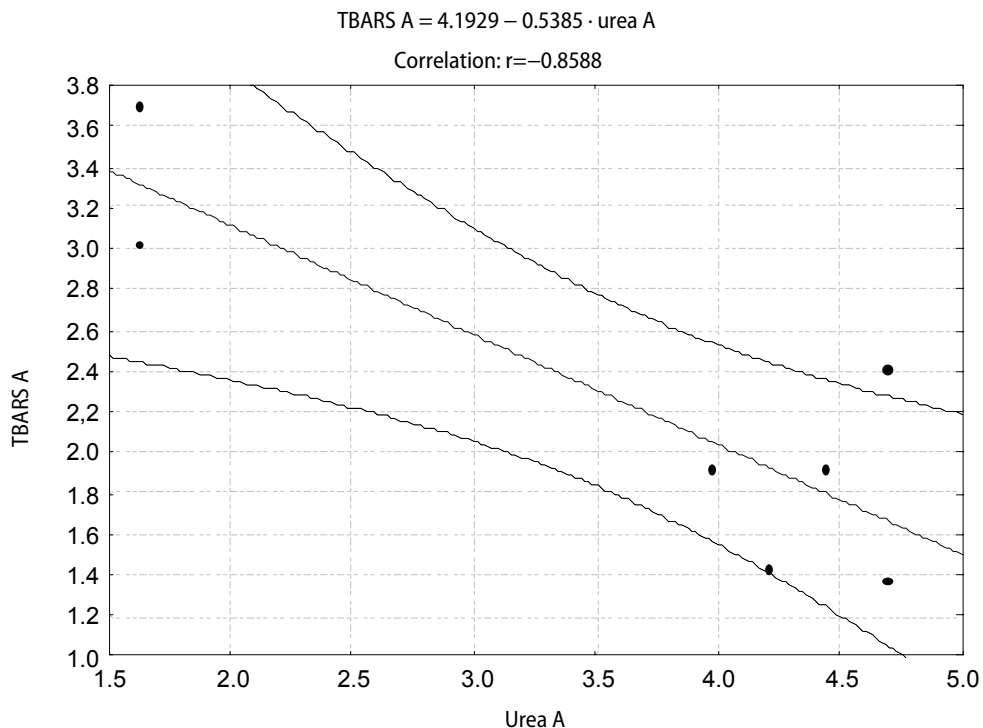


Figure 4
Milk urea and TBARS concentration in Alpine goats
Milchharnstoff und TBARS-Konzentration bei Alpenziegen

From the results of our study we may conclude, that the stage of lactation was a less important factor of difference in milk composition. We may also suspect that initiation of oestrus might have influenced the food intake and consequently a nutritional imbalance occurred. The initial oxidative stability of raw milk decreased in both breeds of dairy goats, after 14 h of cool storage, with more pronounced formation of secondary oxidative products in multiparous Alpine goats.

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