

# Fortification of dairy goats' products with various selenium sources



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## SUMMARY

The aim of this study was to evaluate the effects of two dietary selenium (Se) sources in dairy goat's milk and cheese. Twenty-one goats were allocated to 3 dietary treatments: control (C) with 0.07 mg of Se/kg dry matter (DM); Se yeast (SeY) with 0.14 mg of total Se/kg DM; and sodium selenite (SeNa) with 0.14 mg of total Se/kg DM supplementation. Individual blood and milk samples were collected to determine the Se content. Three cheese wheels were made from each group at three different time, and the Se content was determined. The enumeration of dairy microorganisms was also performed. The SeY group showed a significantly higher milk Se content ( $P < 0.05$ ) than the SeNa group with 44.71 vs 39.29  $\mu\text{g/l}$ , respectively. Both values were also significantly higher ( $P < 0.01$ ) than that of the group C (31.19  $\mu\text{g/l}$ ). The SeY group showed a significantly higher Se carry-over value (31.29%,  $P < 0.05$ ) than the SeNa group (26.95%). Both values were significantly ( $P < 0.01$ ) lower than in the C group (49.66%). Significant differences were also observed in cheese Se content among the 3 groups. The average Se content in cheeses from groups C, SeY, SeNa was 230  $\mu\text{g/kg}$ , 353  $\mu\text{g/kg}$  and 306  $\mu\text{g/kg}$ , respectively. Se yeast supplementation influenced Se concentration in goat's milk and cheese but, unlike other authors, we also observed an increase of Se concentration in milk and cheese supplemented with SeNa, although to a smaller extent. Our results indicate that Se yeast supplementation seems to be the best fortification source for dairy goat's products. In several countries the selenium intake is considered to be low in the human diet, the consumption of Se-enriched products could represent a good way to prevent the deficit in the Se intake currently reported in many countries.

## KEY WORDS

Selenium, dairy goats, milk, cheese, carry-over.

## INTRODUCTION

Selenium (Se) is an essential trace element in animal nutrition and has multiple actions in animal production, fertility and disease prevention. The National Research Council<sup>1</sup> recommended a dietary Se concentration of 0.3 mg/kg dry matter (DM) in ruminants to prevent deficiency. In several countries Se intake is considered to be low or marginal as reported in the report Vitamin and Mineral Requirements in Human Nutrition, by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO)<sup>2</sup>.

The WHO/FAO/International Atomic Energy Agency (IAEA) report<sup>3</sup> recommends a daily Se intake of 50-200  $\mu\text{g/day}$  taking into account the possible influence of dietary factors on Se metabolism and the effect of individual variations on Se requirements. Dairy products could be a possible source to increase Se intake, since Se supplementation in livestock diets may enhance Se content in products, such as milk and cheese. Some authors proposed to fortify dairy

milk with different Se sources, such as inorganic and organic forms. In general the most widely used source of Se is inorganic sodium selenite (SeNa), which, however, does not seem to lead to an increase of Se in goat's milk<sup>4,5</sup>, yet in dairy cow's milk it has been observed that the Se content increases in relation to the Se level in the diet<sup>6</sup>. Pechova et al. (2008)<sup>7</sup> tested different organic Se sources in goats and found that only Se yeast was mainly excreted in milk. In dairy cows the supplementation with Se yeast led to a higher concentration of this element in milk compared to SeNa supplementation<sup>8</sup>. Little information is available about the effect of inorganic and organic Se sources on goat's milk cheese. Some authors proposed Se fortified milk<sup>5,7</sup> obtained by supplementing different types of Se, however no complete studies have been carried out on goat's milk and cheese. Se carry-over in goat's milk has never been evaluated so far, while in dairy cows Moschini et al. (2009)<sup>6</sup> showed that milk Se carry-over decreased progressively, as the SeNa content in diets increased (from 26.00% in the control group to 9.34% in diets with the highest Se content).

The aim of this study was to compare the effect of Se yeast and SeNa supplementations in dairy goats' diet on Se carry-over in milk and Se content in cheese. The effects of these supplementations on milk yield, milk characteristics and enumeration of dairy microorganisms (*Lactobacilli* and lactic acid *cocci*) were also observed.

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## MATERIALS AND METHODS

### Animals and diets

The research protocol and the animal welfare approach were in accordance with the guidelines included in the European Council Directive for animals used for experimental and other scientific purposes<sup>9</sup>. The trial involved 21 secondiparous *Camosciata delle Alpi* lactating goats (initial live weight, days in milking and daily milk production: 55.44±1.55 kg, 128.3±2.9 days and 2.98±0.15 l respectively) for an experimental period of 45 days. During the trial all animals received the same basal diet offered twice a day. The diet included two types of hays (alfalfa hay and natural meadow hay), corn meal and a commercial mixed feed. Table 1 shows the quantity of feed offered, feed chemical composition and Se content. Representative samples of hays and concentrate feeds were collected weekly and pooled for the analysis during the trial. The diets were balanced according to the goats' requirements for energy, protein and minerals in accordance with INRA (1989)<sup>10</sup>, taking into consideration their body weight and daily milk production. Fresh potable water was available *ad libitum*. Goats were machine-milked twice a day (at 7:00 a.m. and 6:00 p.m.).

### Experimental design

The animals were randomly allotted into three homogeneous groups in terms of live weight, parity and milk yield. During the trial the animals were fed and kept in single pens to evaluate individual feed intake. The daily individual quantities of hays and concentrate were previously weighted. Eventual orts were collected before the morning feed administration and weighed. After fifteen days of adaptation period group C was used as control group (diet Se only: 0.20 mg/head/d, equivalent to 0.07 mg/kg DM), the selenium yeast (SeY) and sodium selenite (SeNa) groups received 0.20 mg of Se/head/d supplementation of yeast enriched with Se (Sel-Plex, Alltech, Lexington, KY, USA) and sodium selenite, respectively. During the experimental period, each animal of the SeY and SeNa groups received a single oral dose of selenium preparation, once a day after the first meal in the morning. The total dietary Se content of the supplemented groups, SeY and SeNa, was equivalent to 0.14 mg/kg DM.

### Sample collection periods and laboratory analyses

In the beginning (T0) and at the 15<sup>th</sup> (T15), 30<sup>th</sup> (T30) and 45<sup>th</sup> (T45) day of the experimental period, individual milk

yield was recorded. At the same time, individual blood and milk samples were collected before the first meal in the morning to determine Se content and milk composition. Blood samples were taken from *v. jugularis*: two 10-ml Li-heparin treated tubes (VacutainerR). The first Li-heparin treated tube of whole blood was stored at -20° C; the second tube was centrifuged (3500 x g for 15 min at 10° C), and plasma fraction decanted and stored at -20° C. Blood, plasma and milk samples, stored at -20°C, were used to evaluate Se content. Bulk tank milk from each group was also collected to evaluate the raw milk hygienic profile. The enumeration of total mesophilic bacteria was performed on Agar Plate Count (APC) (Oxoid, Milano, Italy) during the experimental trial (T0, T15, T30 and T45). APC plates were incubated at 30°C for 72 h.

At T15, T30 and T45, three cheese wheels were made with milk from each group. The milk from each group was separately thermised (65°C, 15 s) and cooled to 37°C. Then, an autochthonous mesophilic milk-starter culture (*Lactobacillus paracasei* and *Lactococcus lactis* subsp. *lactis*) was added to the milk and after 30 min it was coagulated with liquid commercial calf rennet (20 ml/100 l) with a coagulation time of 45 min after the rennet addition. The curd was manually cut, transferred into perforated moulds and pressed to drain the whey. The cheeses were then dry-salted and ripened for 28 days at 10°C and 90% relative humidity. The enumeration of mesophilic *lactobacilli* and lactic acid *cocci* was performed on curd and cheeses at 28 days of ripening us on MRS agar and M17 agar (Oxoid), respectively. At the end of the ripening time (28 days), cheese samples were collected and stored at -20°C until Se content determination.

Feedstuffs were analysed to measure dry matter, crude protein, ether extract, ash, neutral detergent fibre (NDF) by Martillotti et al. (1987)<sup>11</sup>. The NDF was analysed with a heat-stable amylase with correction for residual ash, according to Mertens (1997)<sup>12</sup> without sodium sulphite. Milk fat and proteins were measured in fresh samples by Milkoscan (Mod. FT6000, Foss Electric Hillerød, Denmark) and the somatic cell count (SCC) was determined using Fossomatic (Mod. Fossomatic 5000, Foss Electric Hillerød, Denmark). SCC data were expressed as Linear Score (LS). The Se content in feeds, blood, plasma, milk and cheese was determined by the mineralization of 1g of each sample in presence of 4 ml (16 M) HNO<sub>3</sub> and 2 ml (9.8 M) H<sub>2</sub>O<sub>2</sub> within a closed-vessel heating block system. The solution was further diluted with water and Se was determined by inductively coupled plasma

**Table 1** - Offered basal diet: feed quantity and chemical composition (as fed).

	Alfalfa hay	Natural meadow hay	Corn meal	Mixed feed
Offered feeds, kg/head/d	1.0	1.0	0.4	0.7
Dry matter, %	92.04	92.10	91.02	90.86
Crude protein, %	13.99	10.62	6.94	16.71
Fat, %	1.35	1.61	3.52	3.71
Ash, %	8.38	7.99	1.08	7.10
NDF, %	45.55	54.81	11.58	23.88
Selenium, g/kg	0.050	0.069	0.080	0.070

NDF, neutral detergent fibre.

**Table 2** - Milk yield, chemical parameters and somatic cell count (SCC) of milk.

	Main effects			SEM	P		
	C	SeY	SeNa		Tr	T	Tr x T
Milk yield, l	3.13	2.83	2.74	0.028	0.564	0.142	0.026
Fat, %	3.97	3.83	4.14	0.063	0.396	0.002	0.383
Protein, %	3.85	3.81	3.78	0.022	0.954	0.003	0.242
SCC, Linear Score	5.7	6.2	6.1	0.06	0.457	<0.001	0.228

C, control diet; SeY, Se yeast diet; SeNa, Na selenite diet; Tr, treatment effect; T, time effect.

mass spectrometry (ICP-MS Elan 6100, Perkin Elmer, Norwood, MA).

Se carry-over in milk was calculated as follow:  $CO = 100 \times [\text{Total Se milk excretion (mg)}] / [\text{Total Se intake from feeds (mg)} + \text{Se supplementation (mg)}]$ .

### Statistical analysis

Data were processed with GLM using the MIXED procedures of SAS (1999)<sup>13</sup>:

$$Y_{ijkl} = \mu + A_i + B_{ji} + C_k + (AC)_{ik} + e_{ijkl}$$

$y_{ijkl}$  = observation;  $\mu$  = overall mean;  $A_i$  = fixed effect of the  $i^{\text{th}}$  diet ( $i=1$  to 3);  $B_{ji}$  = random effect of the  $j^{\text{th}}$  subject ( $j=1$  to 7) in  $i^{\text{th}}$  diet;  $C_k$  = fixed effect of the time ( $k=1$  to 3);  $(AC)_{ik}$  = the interaction between diet and time;  $e_{ijkl}$  = residual error.

## RESULTS AND DISCUSSION

During experiment the meal offered was completely consumed by the animals, irrespective of treatment.

### Milk production and characteristics

The main chemical bromatological parameters and somatic cells in milk (Table 2) were not affected by the different sources of administered selenium. However, the chemical bromatological parameters showed significant differences in relation to time, as is usually observed during a regular lactation curve. During the experimental period (0-45<sup>th</sup> day) the average ( $\pm$  standard error) milk yield was  $2.9 \pm 0.08$  l/d with fat and protein contents of  $3.98 \pm 0.08\%$  and  $3.81 \pm 0.05\%$ , respectively, and the SCC milk content of  $5.6 \pm 0.1$  LS units.

Total mesophilic bacteria mean values were always below the threshold set by EU Regulation 853/2004 (<500.000 cfu/ml) for milk from species other than cattle and intended for the production of raw milk dairy products. These data highlight proper hygienic management of animals, milking routine and milk storage.

### Total selenium in whole blood and plasma

Before the trial, the Se concentration in blood and plasma of each individual goat was determined and no significant differences were found among the 3 groups (Fig. 1A, B). The Se content of 0.07 mg/kg DM in the basal diet ensured a Se plasma level of 100-140  $\mu\text{g/l}$ . This value was 1.25-1.75 higher than the limit value for plasma (80  $\mu\text{g/ml}$ ), which is a deficiency state index<sup>14</sup> that indicated a sufficient Se status for the animals used in this trial.

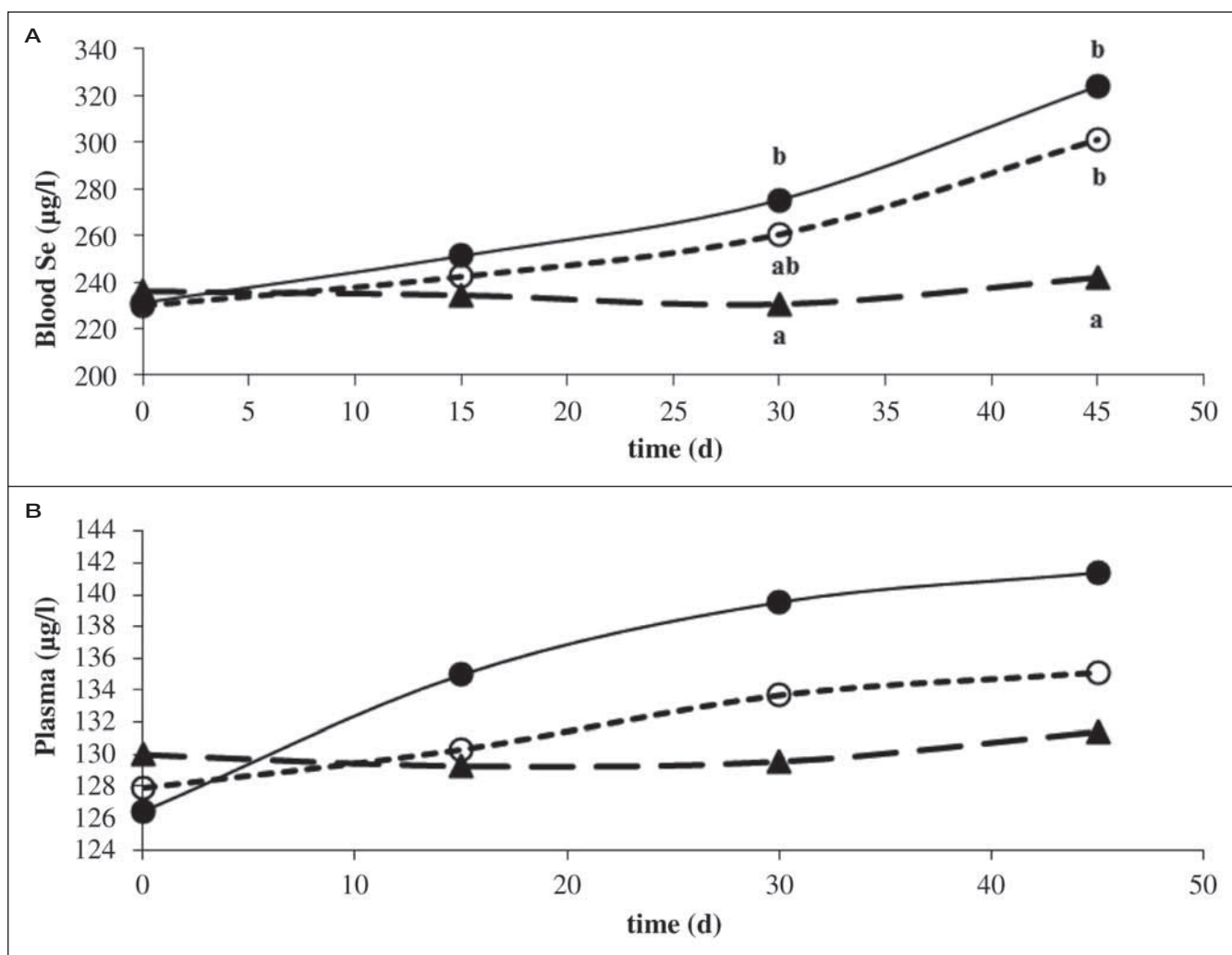
In SeY and SeNa groups the selenium supplementation led to a significant increase of the Se content in blood ( $P=0.011$ ), but not in plasma ( $P=0.414$ ), moreover no differences were found between the SeY and SeNa groups for the same blood parameters (Table 3). Figure 1 A and B show a progressive increase over time of the Se content in blood and plasma. In the blood, the Se content was significantly different in the SeY groups vs the C group ( $P<0.05$ ) at T30 and T45 in the goats of the two groups supplemented with Se vs the C group. The overall total Se content in blood (Table 3) showed a significant treatment x time interaction. The Se content in the blood of the control group (235.7  $\mu\text{g/l}$ ) was similar to that found by Petrera et al. (2009) (5) (232.3  $\mu\text{g/l}$ ) and higher than that reported by Pechova et al. (2008)<sup>7</sup> (about 183  $\mu\text{g/l}$ ) despite the content of Se supplemented in the diet was lower. These differences, found in the blood of animals fed diets not

**Table 3** - Overall values of selenium content in blood, plasma, cheese and milk and selenium carry-over.

	Main effects			SEM	P		
	C	SeY	SeNa		Tr	T	Tr x T
Blood, $\mu\text{g/l}$	235.7 <sup>a</sup>	283.5 <sup>b</sup>	268.2 <sup>b</sup>	2.815	0.011	<0.001	0.007
Plasma, $\mu\text{g/l}$	130.09	138.67	133.05	0.319	0.414	<0.001	0.158
Milk, $\mu\text{g/l}$	31.19 <sup>a</sup>	44.71 <sup>c</sup>	39.29 <sup>b</sup>	0.444	<0.001	<0.001	<0.001
Cheese, $\mu\text{g/kg}$	230 <sup>a</sup>	353 <sup>c</sup>	306 <sup>b</sup>	7.080	<0.001	<0.001	0.022
Ingested, mg/d	0.196 <sup>a</sup>	0.395 <sup>b</sup>	0.394 <sup>b</sup>	0.209	<0.001	0.886	0.232
Excretion in milk, mg/d	0.098	0.124	0.106	1.790	0.144	<0.001	<0.001
Carry-over in milk, %	49.66 <sup>b</sup>	31.29 <sup>a</sup>	26.95 <sup>a</sup>	0.579	<0.001	<0.001	<0.001

C, control diet; SeY, Se yeast diet; SeNa, Na selenite diet; Tr, treatment effect; T, time effect.

<sup>a,b,c</sup> the means without common letters differ significantly at  $P<0.05$ .



**Figure 1** - Total Se in blood (A) and plasma (B) during the supplementation period in dairy goats that received diets containing either no Se additional (C: ▲ dashed line), or Se as Se-yeast (SeY: ● continuous line) or Se as Na selenite (SeNa: ○ dotted line). a,b: at the same time the means without common letters differ significantly  $P < 0.05$ .

supplemented with selenium, could be attributed to the different selenium absorption in different diets feed used in the trials carried out by authors cited above, as suggested by Spears (2003)<sup>15</sup>.

As found by Petrer et al. (2009)<sup>5</sup>, the Se concentration in the whole blood was not affected by the Se source. This result confirmed what was assumed by Juniper et al. (2006)<sup>16</sup> who reported a non-response when the total amount of Se ingested is above the threshold at which a response would be recorded. This is the case in our trial, as well as in the trials carried out by Knowles et al. (1999)<sup>17</sup> with dairy cows and Petrer et al. (2009)<sup>5</sup> with goats.

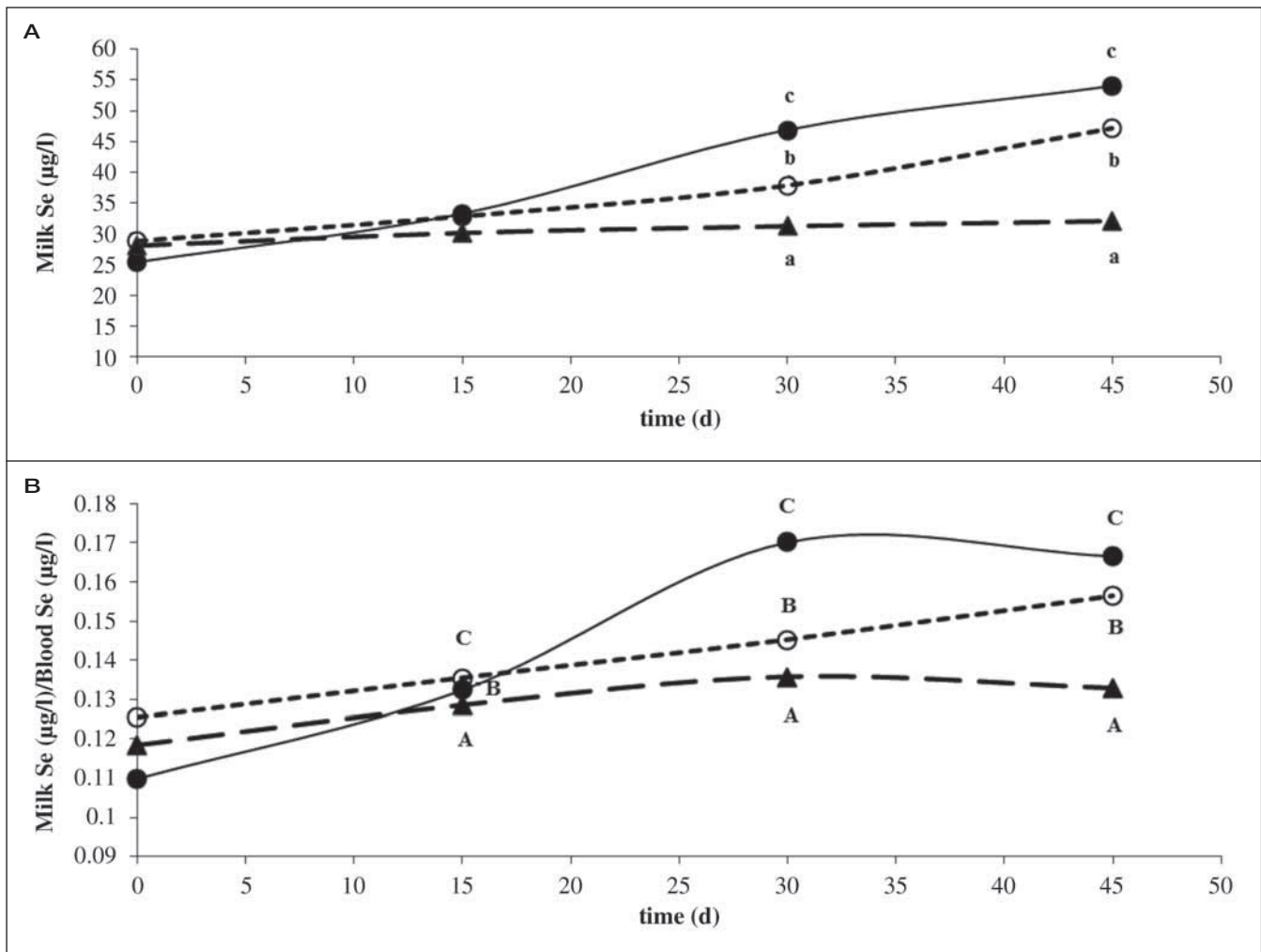
### Total selenium in milk and cheese

At the beginning of the trial, before the supplementation, the Se content in the milk of the 3 groups was about 27 µg/l. The data about the Se yeast content in milk (Table 3) were significantly higher ( $P < 0.05$ ) when compared to C and SeNa groups. SeY group showed the highest milk Se content ( $P < 0.05$ ) while SeNa group showed a higher ( $P < 0.05$ ) milk Se content if compared to the control group (Table 3) according to Petrer et al. (2009)<sup>5</sup>. The Se content in milk (Figure 2A) increased with a trend similar to that of blood. At T30 and T45 the SeY group had a higher ( $P < 0.05$ ) selenium con-

centration than SeNa and C groups and the SeNa group had a significantly higher value ( $P < 0.05$ ) than C group. The addition of inorganic Se to the diet led to a significant increase in Se in goat's milk. As to this aspect, other authors reported different data. Petrer et al. (2009)<sup>5</sup> and Pechova et al. (2008)<sup>7</sup> have found no increase of Se in goat's milk. Ortman and Pehrson (1999)<sup>18</sup>, with a supplementation of 3 mg/head/d of SeNa, of the basal diet of dairy cows, found a significant increase in the milk Se content of approximately 20% compared to the control group. Moschini et al. (2009)<sup>6</sup> supplemented the dairy cows' diet with two different levels of SeNa and observed an increase of Se concentration in milk only with the highest level (4.3 mg/head compared to 2.2 mg/head). We observed that the addition of SeNa led to a progressive and steady increase which was already significant from the 30<sup>th</sup> day. Petrer et al. (2009)<sup>5</sup> found an increase compared to the control group starting from the 80<sup>th</sup> day of supplementation. In Pechova et al. (2008)<sup>4</sup> long-term supplementation of SeNa selenite had no significant impact on Se concentration in milk.

In our trial, the Se yeast supplementation led to an increase of Se concentration in milk from T30. Petrer et al. (2009)<sup>5</sup> found a progressive and significant increase in the milk Se content in the group supplemented with Se yeast. Pechova et





**Figure 2** - Total Se in milk (A) and ratio between total Se in milk and total Se in blood (B) during the supplementation period in dairy goats that received diets containing either no Se additional Se (C: ▲ dashed line), or Se as Se-yeast (SeY: ● continuous line) or Se as Na selenite (SeNa: ○ dotted line). a,b,c, The means without common letters differ significantly at  $P < 0.05$ ; A,B,C: at the same time the means without common letters differ significantly at  $P < 0.01$ .

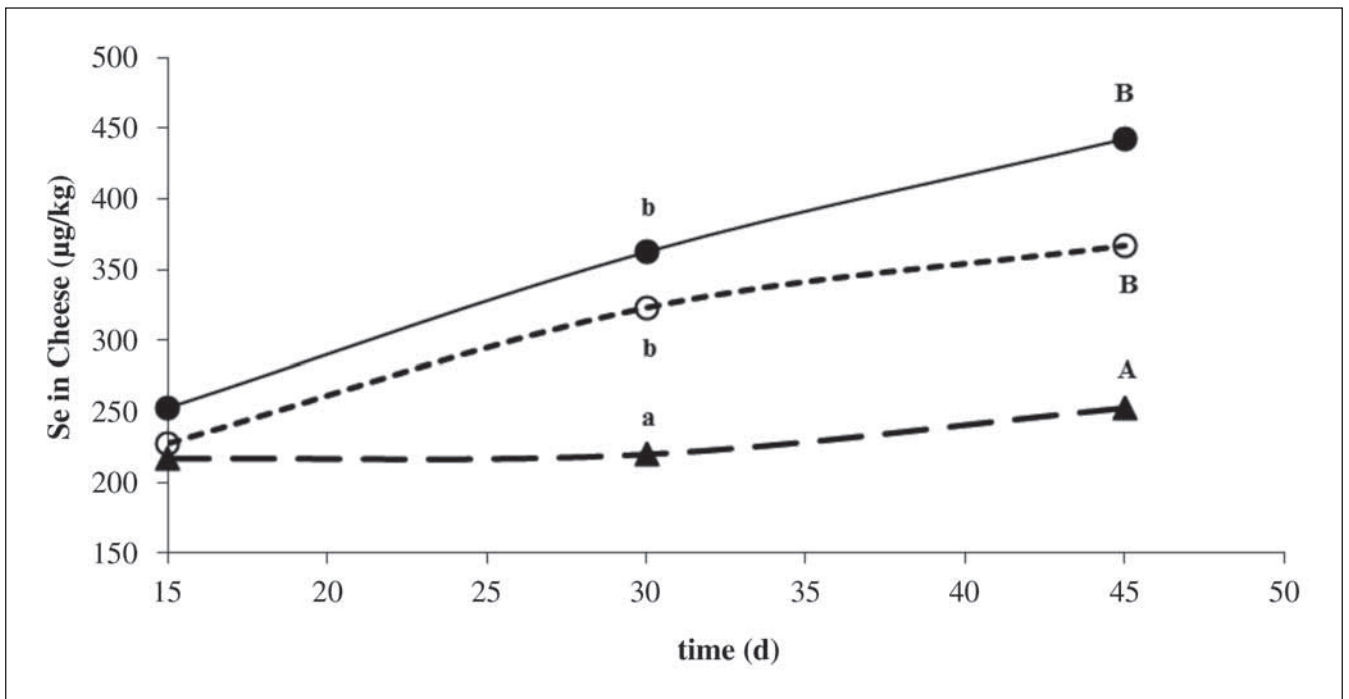
al. (2008)<sup>7</sup> administered Se yeast in lactating goats in a 20-day trial and found a progressive increase in the Se content, which more than doubled from the 6<sup>th</sup> day if compared to the initial level. The ratio between milk and blood Se content tended to increase in the treated groups, but it was more marked in the SeY group mainly at T30 (Figure 2B). This ratio indicated that the amount of Se which was transferred from blood to milk was 15.6% in the SeY group and 14.6% in the SeNa group, while in the C group the transfer amounted to 13.2%. In the ratio between milk and blood, Se concentration is considered to be an index of efficiency of the Se transfer from blood to milk. We found that the Se supplementation showed a significant correlation ( $P < 0.01$ ) between the Se concentration in blood and in milk. Also, Petrera et al. (2009)<sup>5</sup> reported a significant relation ( $P < 0.001$ ) between the Se content in blood and in milk.

The Se content in the different cheeses (Table 3) reflected the Se content in milk with a significant difference ( $P < 0.05$ ) among the 3 groups, as for the content in blood and milk the interaction treatment  $\times$  time in cheese was significant. The trend of the Se content in cheese (Fig. 3) showed significant differences at T30 and T45 of cheese making. During the cheese making process, Se got concentrated about 7.5 to 8 times in the three experimental groups.

As reported by Knowles et al. (1999)<sup>17</sup> in dairy cattle, we observed that also in goats the response in terms of Se content in cheese mirrored the results found in whole milk. Table 4 report the enumeration of mesophilic *lactobacilli* and lactic acid *cocci* in curds and cheeses at 28 days of ripening times. Our results revealed that the organic and inorganic Se supplementation did not lead to a significant difference in dairy microorganisms in curd and cheese samples.

### Selenium carry-over in milk

The amount of Se ingested daily (Table 3) by treated animals was about twice as much the amount ingested by the animals in the control group (0.395, 0.394 vs 0.196 mg). The total amount of Se excreted daily in milk (Table 3) was not significantly different among the three groups ( $P = 0.144$ ). In relation to the amount of Se ingested and excreted, the carry-over (CO) in milk (Table 3) was higher ( $P < 0.001$ ) in the C group (49.66%) compared to the SeY (31.29%) and SeNa (26.65%) groups. The value of CO of the two supplemented groups showed no significant difference. Also, Moschini et al. (2009)<sup>6</sup> supplementing the diets of lactating cows with two different levels of SeNa found a higher CO in the control group compared to the supplemented groups. The CO of supplemented groups was also calculated consi-

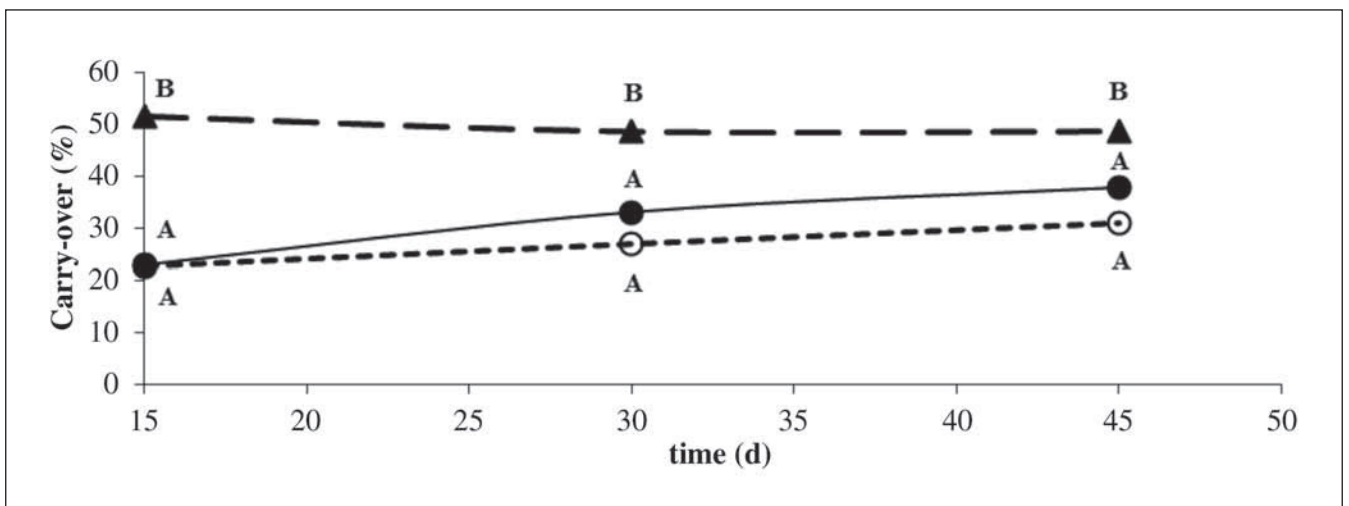


**Figure 3** - Total Se in cheese: no Se additional (C: ▲ dashed line), Se as Se-yeast (SeY: ● continuous line), Se as Na selenite (SeNa: ○ dotted line). a,b,c, The means without common letters differ significantly at P<0.05. A,B,C: at the same time the means without common letters differ significantly at P<0.01.

**Table 4** - Mesophilic lactobacilli and lactic acid cocci (log cfu/ml) in curd and cheese.

	Main effects			SEM	P		
	C	SeY	SeNa		Tr	T	Tr x T
Lactobacilli:							
Curd	7.05	7.36	7.57	0.081	0.012	<0.001	0.103
Cheese	8.35	8.49	8.76	0.077	0.242	0.156	0.213
Lactic acid cocci:							
Curd	7.02	7.22	7.22	0.051	0.765	<0.001	<0.001
Cheese	8.13	8.31	8.39	0.084	0.453	<0.002	0.771

C, control diet; SeY, Se yeast diet; SeNa, Na selenite diet; Tr, treatment effect; T, time effect.



**Figure 4** - Carry-over in milk during the supplementation period in dairy goats that received diets containing either no Se additional (C: ▲ dashed line), or Se as Se-yeast (SeY: ● continuous line) or Se as Na selenite (SeNa: ○ dotted line). A,B,C: at the same time the means without common letters differ significantly at P<0.01.

dering the difference between total Se excretion in milk of the treated goats in the SeY and SeNa groups and the average value of Se excreted in milk of C group, and the differences were expressed on the supplemented Se. The CO in the SeY group was about three times higher than in the SeNa group, 13% vs 4%. The Se CO in milk seems to be reduced by increasing the quantity of supplemented Se (Moschini *et al.*, 2009)<sup>6</sup> and, on the basis of our results, in relation to the different selenium source (inorganic or organic).

Our results revealed that a Se supplementation, either in the inorganic or organic form, had no significant impact on milk yield and milk characteristics.

## CONCLUSION

Our results indicated that the supplementation with organic and inorganic selenium sources in the diet of lactating goats led to a significant increase of Se in milk and cheese. Unlike other authors, we observed that also a supplementation with SeNa led to a significant increase of Se in the dairy products, even if the increase was significantly lower compared to Se yeast supplementation. The carry-over of Se in milk decreased as the amounts of supplemented Se increased, but this effect was less significant with Se yeast compared to SeNa. Different Se sources did not affect the enumeration of dairy micro-organisms in goat's cheese.

The increase of Se in dairy products, which can be obtained especially using a Se yeast supplementation, could help to prevent the deficit in Se intake, as currently reported in many countries.

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