The effects of injectable sodium selenite on immune function and milk production in Sardinian sheep receiving adequate dietary selenium

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Abstract – The aim of this preliminary study was to determine the effects of selenium (Se) injection on Se status, cell-mediated immunity (CMI), milk yield and milk somatic cell count (MSCC) of ewes fed adequate amounts of Se, and on Se status, passive immunization and CMI of their offspring. Thirty days before lambing, 36 Sardinian ewes were assigned to one of three groups. One group (NT) was not treated; a second group (BL) was given 5 mg of Se on day 30 before lambing; a third group (BLL) was given 2.5 mg of Se on day 30 before lambing and at lambing. Selenium was given intramuscularly as sodium selenite. Selenium status was assessed by measuring glutathione peroxidase activity of erythrocytes (GSHpx-E). The CMI was measured by determining the increases in double skinfold thickness after intradermal injection of phytohaemagglutinin (PHA). Compared to their NT counterparts, ewes belonging to BL and BLL groups and their offspring had significantly higher GSHpx-E ($P < 0.01$). The GSHpx-E values of lambs were positively related to those of their mothers ($P < 0.0005$). Ewes of group BL had a greater ($P < 0.01$) response to PHA 6 h after injection than ewes of the NT group. Lambs born to BL and BLL ewes had a greater ($P < 0.0001$) response to PHA 24 h after injection. Responses of ewes and lambs to PHA 24 h after injection were positively related ($P < 0.05$). Serum immunoglobulin at 10 days of age did not differ significantly among the three groups of lambs. Compared to the NT group, milk yield on day 70 of lactation was significantly higher in BL ewes ($P < 0.05$). The MSCC was not affected significantly by Se injection. Immunoresponsiveness and milk yield might represent additional and appropriate criteria to consider when re-evaluating Se requirements of dairy sheep. © Inra/Elsevier, Paris.

selenium / ewe / lamb / immunity / milk

Résumé – Effets du sélénite de sodium injectable sur l'immunité et la production de lait de brebis sardes supplémentées en sélénium. L'objectif de cette étude préliminaire était de déterminer les effets d'une administration de sélénium (Se) sur la teneur en Se, l'immunité à médiation cellulaire (IMC), la production de lait et la teneur en cellules somatiques dans le lait (CSL) chez des bre-

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bis supplémentées en Se, et sur la teneur en Se, l'immunité passive et la IMC de leurs nouveau-nés. Trente jours avant la mise bas, 36 brebis de race sarde ont été réparties en trois groupes. Un groupe (NT) n'a pas été traité ; le second groupe (AMB) a reçu 5 mg de Se au jour 30 avant la mise bas ; le troisième groupe (AMBMB) a reçu 2,5 mg de Se au jour 30 avant la mise bas, puis le jour de la mise bas. Le Se a été administré par voie intramusculaire sous forme de sélénite de sodium. La teneur en Se a été évaluée par la mesure de l'activité glutathione peroxydase des erythrocytes (GSHpx-E). La IMC a été mesurée par l'augmentation de l'épaisseur du pli cutané après injection intradermique de phytohémagglutinine (PHA). Comparées au groupe NT, les brebis appartenant aux groupes AMB et AMBMB et leur progéniture présentaient une valeur de la GSHpx-E significativement plus élevée ($P < 0,01$). Les valeurs GSHpx-E des agneaux étaient corrélées positivement à celles de leurs mères ($P < 0,0005$). Comparées au groupe NT, les brebis du groupe AMB avaient une réponse plus élevée ($P < 0,01$) à la PHA six heures après l'injection. Les agneaux nés des brebis AMB et AMBMB avaient une réponse plus élevée à la PHA ($P < 0,0001$) 24 h après injection. Les réponses des brebis et des agneaux à la PHA 24 h après injection étaient corrélées positivement ($P < 0,05$). Les immunoglobulines sériques à 10 jours d'âge ne différaient pas entre les trois groupes d'agneaux. Comparées au groupe NT, la production de lait au jour 70 de lactation était significativement plus élevée chez les brebis AMB ($P < 0,05$). La CSL n'était pas affectée par l'administration de Se. La mesure de la capacité de réponse immunitaire et de la quantité de lait produit pourraient permettre l'évaluation des besoins en Se du mouton laitier. © Inra/Elsevier, Paris.

sélénium / brebis / agneau / immunité / lait

1. INTRODUCTION

Selenium (Se) deficiency was shown to exert negative effects on milk yield and milk somatic cell count (MSCC) of dairy cows [6, 18], and on the immune response of calves [13].

With regard to small ruminants, a positive correlation between Se status and milk yield was reported for dairy goats [1]. In Se-deficient Sardinian ewes, the severity of the deficiency was positively related to the MSCC [15]. In lambs, prolonged exposure to a diet deficient in both Se and vitamin E was responsible for a decline in lymphoproliferative responses to phytohaemagglutinin (PHA) and other phytolectins [21].

Furthermore, previous studies have also shown that levels of Se and vitamin E (alone or combined) above the generally accepted requirements enhance the humoral or cell-mediated immunity (CMI) in several species [5, 7]. With regard to ruminants, it has been reported that a dietary allowance of Se above the requirements during pregnancy improves Se status of the newborn calves and is likely to enhance calf viability and immune response [20].

However, the effects of Se at levels higher than those recommended to avoid symptoms of deficiency, i.e. sub-toxic doses [10], upon Se status, milk yield, MSCC and immunological parameters of dairy sheep and their offspring have not been established.

It should be noted, however, that the injection of Se compounds in sheep fed Se-supplemented diets represents a very common and empirical practice carried out in several dairy sheep units located in Se-deficient areas of central-west Italy.

Therefore, the aim of this preliminary study was to establish the influence of Se injection in dairy ewes fed adequate amounts of Se on Se status, CMI, milk yield and MSCC of ewes, and on Se status, passive immunization and CMI of their offspring.

2. MATERIALS AND METHODS

2.1. Ewes, lambs and treatments

The experiment was performed in a commercial dairy sheep unit located in Tuscany on 36

pluriparous (third or fourth lambing) Sardinian ewes and their offspring.

Thirty days before the expected lambing (32.8 ± 5.2 days before the actual lambing) the 36 ewes were divided into three groups of 12 each according to body weight (approximately 50 kg), parity, single or twin bearing (approximately 160 % of the expected lambing rate) and Se status. The three groups were thus treated as follows: the first group was not treated (NT), the second group was given 5 mg of Se on day 30 before lambing (BL), and the third group was given 2.5 mg of Se on day 30 before lambing and at lambing (BLL). Selenium [Farmaceutici Gellini, Aprilia (LT), Italy] was given intramuscularly as sodium selenite. The doses of Se to be injected were chosen according to the positive effects upon Se status, colostrum and milk production that were exerted by approximately the same amount of Se (10 mg per 100 kg of body weight) when administered, even if in association with vitamin E, to late pregnant dairy cows [6]. With regards the BLL group, the division of the 5 mg dose of Se was chosen to ascertain the existence, even if at very preliminary stages, of possible dose- or time-dependent effects of sub-toxic doses of Se excess.

A total of 49 lambs were studied from birth until 30 days of age: 16 were born to NT ewes (NT lambs), 15 were born to BL ewes (BL lambs) and 18 were born to BLL ewes (BLL lambs). During the whole study, lambs were allowed to suckle their mothers without any time restriction.

2.2. Diet

The 36 ewes were fed a clover–oats mixture of pasture and meadow hay on an ad libitum basis. The Se content of both pasture and hay was marginal (from 0.03 to 0.05 mg/kg dry matter). Before and after lambing, the ewes were also fed 0.3 and 0.5 kg, respectively, of a commercial concentrate [Purina Italia s.p.a., Pieve Emanuele (MI), Italy] containing 0.17 mg/kg dry matter of Se as sodium selenite.

2.3. Selenium status

The Se status of both ewes and lambs was assessed by measuring the glutathione peroxidase activity of erythrocytes (GSHpx-E) by using a commercial kit (Randox, Crumlin, N. Ireland).

The Se status of ewes was assessed at 30, 10 and 2 days before expected lambing (32.8 ± 5.2, 11.3 ± 1.9, 2.8 ± 1.1 days before actual lambing, respectively) and on days 7, 14, 35 and 70 after lambing.

The Se status of lambs was established at 10 and 30 days of age.

2.4. Passive immunity

Total serum immunoglobulin of lambs was assessed at 10 days of age. Blood samples were collected and centrifuged for 15 min at 3 500 *g*, and serum was stored at –20 °C until analysis. Total serum protein concentration was determined, using a commercial kit (Boehringer Mannheim Biochemica, Milan, Italy). Total serum immunoglobulin concentration was quantified by electrophoresis, according to the method described by Dardillat [2].

2.5. Cell-mediated immunity

Ewes and lambs were skin tested on day 10 before expected lambing and at 10 days of age, respectively. The CMI was measured both in ewes and lambs by determining the increase in double skinfold thickness after an intradermal injection of 250 µg of PHA [Sigma Chemical, St. Louis (MO), USA]. The PHA was diluted in 0.1 mL of sterile PBS [Sigma Chemical, St. Louis (MO), USA] and was injected on a clipped portion of the right side of the neck by using an automatic syringe for intradermal injections (La Veterinaria Strumenti s.d.f., Padova, Italy). Double skinfold thickness was measured by using a constant tension calliper [Mitutoyo Italiana S.r.l., Lainate (MI), Italy] before the injection and at 6, 12, 24 and 48 h after injection in ewes. In lambs, due to technical reasons, double skinfold thickness was measured before injection and only 24 h after injection. However, the ewes' responses to PHA (see below in the results section), which was maximum and substantially firm between 12 and 24 h after injection, indicated the 24th hour as a reliable time to determine a maximum increase in skinfold thickness. Both in ewes and lambs, control injections of sterile PBS alone were used to ensure that the response was specific to PHA. The two skin test sites (PHA and PBS) were separated by approximately 10 cm in both ewes and lambs.

2.6. Milk yield and milk somatic cell count

Milk yield and MSCC were recorded on days 35 and 70 of lactation at the morning milking only. Ewes were hand milked 12 h after the previous evening milking, milk was collected in buckets, and then weighed and sampled for MSCC analysis. Milking of the 36 ewes took approximately 45 min to complete, and was carried out in random order. The MSCC was determined by use of an automatic processor (Fossomatic 180, Foss-Electric, Hillerød, Denmark).

2.7. Analysis of data

Data were analysed by using a repeated measures procedure [17]. Values are expressed as Lsmeans ± SEM, and effects were considered to be significant at $P < 0.05$. Simple correlation coefficients were calculated according to the method of Pearson [16].

3. RESULTS

3.1. Ewes

The average twinning rate was about 158 % and did not differ significantly among the three experimental groups.

Selenium injection significantly affected both changes and mean values of GSHpx-E. During the last 3 weeks of pregnancy, GSHpx-E decreased in NT ewes and increased in BL and BLL ewes (*figure 1*). During lactation, GSHpx-E continued to decrease in the NT group and remained stable in BL and BLL ewes. Following Se injection on day 30 before lambing, both ewes belonging to BL and BLL groups had, at each of the measurements which were carried out either before or after lambing, higher GSHpx-E than did their NT counterparts (*figure 1*). Conversely, the values of GSHpx-E never differed significantly between BL and BLL groups.

Responses of ewes to intradermal injections of PBS alone were not detectable. With regard to intradermal injections of PHA, the only significant difference between the three groups of ewes was a greater response 6 h after the injection in ewes belonging to the BL group when compared to their NT counterparts (*figure 2*). Responses of the BL ewes to PHA 6 h after injection were greater even when compared to those recorded in the BLL ewes, but the difference was not significant. Compared to both the NT and BLL groups, responses of the BL ewes to PHA were also greater 12, 24 and 48 h after injection but, also in these cases, the differences were not significant.

On day 70 of lactation, ewes belonging to the BL group yielded about 54 % more milk than their NT counterparts did (*table I*). This was, with regard to milk yield, the only significant difference found among the three experimental groups of ewes. It should be noted, however, that the amount of milk yielded by ewes belonging to the BL and BLL groups was always remarkably higher than that yielded by the NT ewes: the increase in milk yield observed in the two groups of ewes injected with Se ranged between approximately 38 and 48 % (when

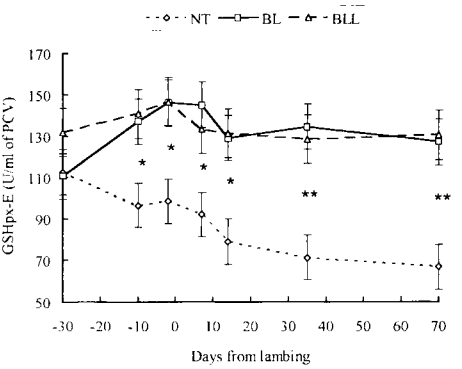

Figure 1. Glutathione peroxidase activity of erythrocytes (GSHpx-E) in non-treated ewes (NT), given 5.0 mg of Se on day 30 before lambing (BL) or 2.5 mg of Se on day 30 before lambing and at lambing (BLL). Significant differences among ewes given Se compared to non-treated ewes : $^{*}P < 0.01$; $^{**}P < 0.001$.

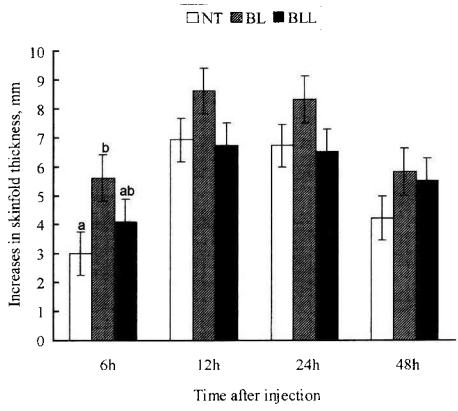

Figure 2. Increases in skinfold thickness after an injection of PHA in non-treated ewes (NT), given 5.0 mg of Se on day 30 before lambing (BL) or 2.5 mg of Se on day 30 before lambing and at lambing (BLL). Values within the same time after injection with different superscripts (a, b) differ significantly ($P < 0.01$).

comparing BL and BLL ewes, respectively, with NT ewes on day 35 of lactation).

No clinical signs of mastitis were detected during the whole experiment, and no significant differences were found among groups for MSCC both at 35 (518 385 ± 87 900; 438 351 ± 98 630 and 492 884 ± 101 220 cells/mL of milk, in NT, BL and BLL ewes, respectively) and 70 days of lactation (407 990 ± 99 510; 527 600 ± 116 980 and 362 609 ± 107 670 cells/mL of milk, in NT, BL and BLL ewes, respectively).

3.2. Lambs

Eight lambs died within the first 10 days of life and were thus not included in the study. The average mortality rate within the first 30 days of life was about 14 % and did not differ significantly among the three groups of lambs.

Compared to their NT counterparts, lambs belonging to BL and BLL groups showed higher values of GSHpx-E both at 10 and 30 days of age (*table II*). The differences in the GSHpx-E values between BL and BLL lambs were not significant.

The GSHpx-E values of lambs were positively related to those of their mothers with a correlation coefficient equal to 0.3 and significant at $P < 0.0005$.

No significant differences among the three groups of lambs were noted with regard to serum Ig at 10 days of age (20.21 ± 6.87, 21.1 ± 8.04, and 20.48 ± 4.34 mg/mL, in NT, BL and BLL lambs, respectively).

Also in lambs, the responses to intradermal injections of PBS alone were not detectable. On the whole, the responses of lambs to PHA were weaker than those recorded for their mothers. However, BL and BLL lambs had a greater ($P < 0.0001$) response to PHA than did their NT counterparts. The increase in double skinfold thickness after the intradermal injection of PHA was equal to 2.2 (± 0.31), 4.1 (± 0.33)

Table I. Lsmeans (± SEM) of milk yield at the morning milking in ewes belonging to NT (non-treated), BL (given 5 mg of Se on day 30 before lambing) and BLL (given 2.5 mg of Se on day 30 before lambing and at lambing) groups.

Days of lactation	Milk yield (g)		
	NT	BL	BLL
35	476.2 ± 107.4	656.8 ± 118.5	703.6 ± 130.1
70	633.2 ± 106.3[a]	976.2 ± 127.4[b]	827.6 ± 130.1[ab]

[a, b] Values in the same row with different superscripts (a, b) differ significantly ($P < 0.05$).

Table II. Lsmeans (± SEM) of glutathione peroxidase activity of erythrocytes (GSHpx-E) in lambs born to NT (non-treated), BL (given 5 mg of Se on day 30 before lambing) and BLL (given 2.5 mg of Se on day 30 before lambing and at lambing) ewes.

Days of life	GSHpx-E (U/mL of packed cell volume)		
	NT	BL	BLL
10	74.3 ± 7.1^{a}	131.5 ± 7.6^{b}	124.6 ± 7.6^{b}
30	61.6 ± 7.1^{a}	114.7 ± 7.6^{b}	121.7 ± 7.6^{b}

[a, b] Values in the same row with different superscripts (a, b) differ significantly ($P < 0.0001$).

and 4.6 (± 0.33) mm in NT, BL and BLL lambs, respectively.

Responses of lambs to PHA were positively related to those of their mothers (at 24 h post-injection) with a correlation coefficient equal to 0.3 and significant at $P < 0.05$.

4. DISCUSSION

The activity of the Se-dependent enzyme GSHpx is considered to be a reliable indicator of the Se status in ruminants [12, 19]. Moreover, it is also widely accepted that values of GSHpx-E are linearly related to Se allowance [4]. In light of these facts, and according to data reported by other authors [12, 22], the values of GSHpx-E recorded in our study in ewes belonging to the NT group, both during pregnancy and lactation, would indicate that ewes of this group were fed adequate amounts of Se.

The decline in GSHpx-E during late pregnancy and lactation has already been reported for Se-deficient Sardinian ewes [15]. The present study demonstrated that even in ewes fed adequate amounts of Se (NT ewes), pregnancy and then lactation were responsible for worsening the Se status. To the best of our knowledge, no data are, however, available documenting changes in Se demand in late pregnant or lactating ewes. In our study, either the injection of 5 mg of Se before lambing or the injection of 2.5 mg of Se before lambing and at lambing prevented a decline in GSHpx-E during the periparturient period and early-mid lactation. Interestingly, the injection of 2.5 mg of Se on day 30 before lambing increased GSHpx-E at the same level recorded in ewes given 5 mg of Se. This shows that 2.5 mg of Se were sufficient to reach a plateau in GSHpx-E. Our study did not permit us to ascertain whether the single injection of 2.5 mg of Se on day 30 before lambing would have determined a lasting increase in GSHpx-E because the BLL ewes were also injected with 2.5 mg of Se at lambing. Conversely, our study demonstrated that the injection of 5 mg of Se on day 30 before lambing was responsible for a lasting increase in the GSHpx-E.

Supplements of Se in excess of those required has already been shown to enhance the immune response in cattle and several non-ruminant species [5, 7, 20]. In our study, as the skin test in ewes was performed before lambing, it must be noted that ewes belonging to the BLL group were given, at the time the skin test was carried out, only 2.5 mg of Se. Therefore, our results indicate that Se injection modifies the responses of ewes to PHA in a dose-dependent fashion. In particular, as the only significant differences in responses to PHA was found between NT and BL ewes 6 h after the injection, it might be assumed that the administration of 5 mg of Se before lambing is responsible for a more rapid cellular response. Furthermore, the GSHpx-E values did not differ between BL and BLL

ewes; therefore, it is assumable that the greater responses of BL ewes to PHA was due to free or non-GSHpx selenium.

A positive correlation between GSHpx-E and milk yield was reported both for dairy goats [1] and cows [6]. For dairy cows, it was supposed that the well-known protective role of GSHpx on membrane integrity might represent at least one of the mechanisms through which Se can increase milk production [9, 11]. Our data on milk yield do not clearly indicate whether Se levels above the generally accepted requirements had significant effects on milk yield of dairy ewes. However, our results and those reported for other species encourage further and larger (on a larger number of animals) studies to ascertain the relationships, if there are any, between Se status and milk yield of dairy ewes.

A previous study reported that in Se-deficient ewes, the severity of the deficiency was positively related to MSCC, in that ewes having the lower values of GSHpx-E had higher MSCC [15]. The results of the present experiment suggest that surplus Se in ewes fed adequate amounts of Se would not exert any beneficial effects on MSCC.

Our data showed that the higher the availability of Se during pregnancy and at lambing the higher were the values of GSXpx-E of lambs during early life. Previous studies reported that GSXpx activities of lambs born to ewes treated orally during pregnancy with 25 mg of sodium selenate were higher than those born to untreated ewes [13]. Analogously, the injection of Se in late pregnant dairy cows was responsible for higher blood GSHpx-E in calves at birth and during the first 4 weeks of life [6]. An increased availability of Se during the intrauterine life [13] or a higher concentration of Se in colostrum or milk [8, 20] would explain the higher GSHpx-E found in our study in lambs born to ewes given supplementary Se before lambing.

To the best of our knowledge, the relationships between the amount of Se given to late pregnant ewes and passive immunization of newborn lambs have not been previously studied. However, the results of the present study on serum Ig concentration of post-colostral lambs confirm those reported for calves born to cows injected or not injected before calving with 10 mg of sodium selenite and 50 IU of d, l- α tocopheryl acetate/100 kg of body weight [6].

Previous studies have demonstrated that the maturation of the immune response in lambs takes about 2–4 months [3]. Therefore, in light of this fact, it is not surprising that, in our experiment, the responses of lambs to intradermal injections of PHA was weaker than that recorded for their mothers. Our data clearly indicated, however, that the improvement of Se status of lambs, obtained by administering Se to their mothers, was associated with a greater cell-mediated immune response. These results support the previous hypothesis that surplus Se during pregnancy may enhance the immune response of newborns [20]. The observation that the lambs' responses to PHA was positively related to those of their mothers is novel. Further studies are warranted to ascertain the relationships, if there are any, between the immune reactivity of ewes and their progeny.

The present preliminary study showed overall positive effects of Se injection in ewes fed adequate levels of Se, and indicates that immunoresponsiveness and milk yield might represent additional and appropriate criteria to consider when re-evaluating Se requirements of dairy sheep. However, further field studies on disease resistance against economically significant pathogens are required before it can be recommended to include immunoresponsiveness among criteria on which to base the establishment of Se requirements. Furthermore, from a practical point of view, this study validates the empirical practice of injecting Se in periparturient ewes fed Se-supplemented diets, and indicates that the single administration of 5 mg of Se on day 30 before lambing may ensure a long-lasting

improvement of Se status and positive effects on milk yield, and upon cell-mediated immunity of both ewes and lambs.

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