Se and I status in pregnant ewes from a pastoral system and the effect of supplementation with Se and I or only Se on wool quality of lambs

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ABSTRACT

Australian Merino ewes and lambs producing fine fibre wool for export are raised in the north-west of Uruguay in pasture-based systems. We studied the status of selenium and iodine in pregnant Merino ewes (10 per treatment) grazing in natural pasture, in natural pasture and supplemented with Se (0.1 mg Se/kg dry matter intake) and I (1 mg I/kg dry matter intake), or in natural pasture and supplemented with Se alone (0.1 mg Se/kg dry matter intake), during the last 30 days of gestation. Further, we evaluated the performance and wool quality of their offspring. Content of Se and I in natural pasture, in the sera of pregnant ewes, and in the wool of their offspring and levels of thyroidal hormones—TSH, T4, and free T3 (FT3)—in the sera of pregnant ewes were determined. The performance of lambs and the commercial parameters of fine fibre wool produced were measured. Results showed normal Se levels in serum (0.12–0.15 mg/l) in the ewes grazing in natural pasture (0.07–0.09 mg/kg DM) during late pregnancy. The observed increase in Se content in the pasture at lambing (0.11–0.16 mg/kg DM) improved serum Se levels (0.216 mg/l); however, the serum levels were not affected by the supplementation.

I content in pasture showed adequate levels (0.50–0.60 mg/kg DM), which were reflected in the blood serum values 30 days prior to lambing (0.197–0.208 mg/l). However, at lambing, the I content in blood serum decreased (0.150 mg/l). Further, the supplementation did not modify the serum I levels (0.163–0.175 mg/l). An increase in FT3 levels in ewes at lambing could be associated with the increase in Se content in pasture and/or the adequate I content in pasture. No effect of supplementation was observed. Lambs showed good results regarding the quality of fine fibre wool and performance after supplementation with Se and I or Se alone and exhibited slightly improved Se and I content in wool. In conclusion, natural pasture provides adequate status in Se and I for the Merino ewes and their offspring without any additional beneficial effects of supplementation with Se and I or only Se.

1. Introduction

Fine fibre wool (16–18 µm diameter) produced in the north-west of Uruguay by Merino ewes and lambs is an important product for export. The most predominant production system for Merino is based on natural pasture feeding, and, at the end of winter, the pregnant ewes are frequently supplemented with concentrate to avoid the loss of body condition (Montossi et al., 1998). Natural pasture is also the main source of trace elements, such as selenium and iodine, for this productive species. The key role of Se and I on the antioxidant defence system in the cells of the thyroid (Schomburg et al., 2006) and on the regulation of thyroid metabolism (Hugejiletu et al., 2013) justify a focus on the

availability of Se and I in pastoral diets. Se is essential for the synthesis of iodothyronine deiodinases, the family of selenoenzymes that are critical for the control of thyroid hormones action at the cellular level (Hefnawy et al., 2014). Deiodinases type 1 and 2 catalyse the activation of tetra-iodothyronine (T4) to the biologically active form, 3-5-3′-tri-iodo-thyronine (T3) (Beckett and Arthur, 2005). In addition, deiodinases type 1 and 3 inactivate T4 to reverse T_3 (Köhrle et al., 2007). The primary role of I is the synthesis of hormones by the thyroid gland, affecting the functions that thyroidal hormones regulate, such as energy metabolism, thermoregulation, reproduction, growth and development, circulation, muscle function (Herdt and Hoff, 2011), and, in sheep, the growth of fibre (Todini, 2007). An Se deficiency induces a significant reduction in

plasma T$_3$ with the corresponding increase in T4 and a reduction in the activity of hepatic deiodinase 1 (Köhrle et al., 2007), while the levels of thyroid-stimulating hormone (TSH) remains constant. However, thyroidal stores of T4, T3, and I decrease with Se deficiency; moreover, they are far more depleted with both Se and I deficiency (Arthur et al., 1999). Sheep are particularly sensitive to Se and I deficiency (Grace and Knowles, 2012), and, in lambs born to Se deficient ewes, depletion occurs 30–60 days after lambing (Arthur et al., 1999). Depletion of I could be expected after 1 or 2 weeks of dietary I deficiency with the fast growth of lambs (Arthur et al., 1999; Guyot and Rollin, 2007). Indeed, adequate amounts of both I and Se are required for optimal thyroid metabolism. In contrast, it seems that a high level of plasma thyroid hormones is associated with increased fibre length, decreased fibre diameter, and higher percentage of active secondary follicles (Todini et al., 2005). Thus, a deficiency in thyroid hormones (T3 and T4) could affect hair fibre production in animals that have continuous hair growth throughout the year, like the Merino sheep (Todini, 2007). Se deficiency also reduces wool growth (Herdt and Hoff, 2011).

The supply of Se and I from pastures is a determinant for Se and I status in ewes and lambs fed grass. Indeed, there is a close relationship between the Se and I status of pregnant ewes, particularly in early and mid-pregnancy, and the Se and I content in the plasma of the offspring (Erdogan et al., 2017; Hefnawy et al., 2008, 2014; Muñoz et al., 2008). Consequently, a low burden of both minerals in ewes will reduce the performance in offspring. Wool is an indicator of trace elements that accumulate during growth and may reflect the trace element status of the sheep at the time of wool-fibre development; consequently, it could affect wool quality (Hynd, 1994; Hynd and Masters, 2002).

In Uruguay, Piaggio et al. (2000) and Pittaluga (2018) reported different degrees of mineral deficiencies in natural grasses. They showed that the lowest level of Se (0.01–0.02 ppm) appeared in the north-west and in the winter. According to Suttle (2010), this level would not be enough to cover minimal dietary requirements for Se (0.03–0.06 μg/g dry matter) even when an adequate vitamin E intake is ensured. However, Se intake can vary depending on the basal diet (silages with high sulphur) or the presence of legumes (Saha et al., 2016). Furthermore, the soil type and the proportion of Gramineae and legumes could explain the great variability in their availability (White and Rewell, 2007). However, when lambs grazing *Pennisetum* or *Lolium,* both low in Se, were supplemented with Se, an unsatisfactory response was still obtained, possibly because of the lack of another factor. It could be hypothesised that I could probably be the other necessary mineral (Van Ryssen et al., 1999). Thus, an I content in pasture of less than 0.1 mg/kg dry matter causes goitre in lambs (Mason, 1976) and an increase in T4 levels in the serum (Contreras et al., 2002). As with Se, I content in pasture can also present important variabilities and, frequently, Se and I were deficient in sheep fed on grass inland versus those reared in coastal areas (Sivertsen et al., 2014). Variability in Se and I depends on season, rainfall (Sivertsen et al., 2014), soil type, and pH (Reis et al., 2017) and well as the plant species (Sivertsen et al., 2014; Reis et al., 2017). These factors affect the uptake of Se and I content in edible plants, which subsequently affects their nutritional availability for animals and humans (Reis et al., 2017). Besides, there are some iatrogenic plants containing thiocyanates, such as *Panicum* and *Paspalum*, or containing glucosynolates, such as *Cichorium* (Mufarrege, 2007), that could be responsible for I deficiency in grazing sheep, whereas Se deficiency causes a decrease in T3 levels and an increase in T4 in blood (Contreras et al., 2002). Simultaneously, a deficiency in I is also observed (Berger, 2008). Thus, a synergic action between Se and I is observed, mainly in sheep, affecting the metabolism of the animals (Vouduri et al., 2003). Consequently, when animals or humans present deficiencies in Se or I or both, supplementation with either one of these elements individually is not efficient enough to compensate the deficiency (Van Ryssen et al., 1999) or improve the thyroid function (Contempre et al., 1991; Hotz et al., 1997; Thompson et al., 2009).

Uruguay, particularly its north-western region, is considered a country that has natural grasses deficient in I (Nores, 1944; Nores and Rossi Stajano, 1948; McDowel and Conrad, 1978; Pereira et al., 1988) and containing low levels of Se (Pittaluga, 2018). However, no information regarding the Se and I status of sheep produced on pasture in Uruguay has yet been reported. Furthermore, no information could be sourced, to the best of our knowledge, on the effects of supplementation of Se and I in ewes on their blood content or on the wool quality produced by their offspring. The present study was planned to address this lack of information, and the investigation focused on the Merino sheep and its wool quality, a valuable product for Uruguay.

Thus, this research aimed to evaluate the I and Se status and the effects of supplementation of Se and/or I in pregnant Merino sheep in a pastoral system and on the quality of wool produced by their offspring. The Se and I content in pasture, in serum of pregnant ewes and in wool of born lambs was determined, as well as hormones (TSH and T4) and FT3 in the serum of the pregnant ewes.

2. Material and methods

2.1. Animals

Thirty pregnant Australian Merino ewes, aged 4–6 years, with a body weight of 45 kg, at 120 days of gestation, were used in the experiment. The body condition score was 3, according to Jefferies (1961). Ten ewes were assigned to one of three experimental groups receiving different diets, as described in 2.2. All pregnant ewes were grazed in natural pasture and were supplemented with brown rice bran at a rate of 250 g ewe^{-1} day^{-1} with free access to water. The forage mass (kg dry matter/ha) was estimated, according to the Haydock and Shaw (1975) method, 30 days before lambing and at lambing. After lambing, ten lambs from each experimental group were used for the study of offspring responses.

Animals used in this study were maintained in the facilities and environment of the Experimental Station of the Faculty of Agronomy (Udelar) in Salto, Uruguay, following the regulations of the University's ethics committee, the Honorary Commission for Animal Experimentation (CHEA). Protocol used in this investigation was approved by the Ethical Commission for the Use of Animals (CEUA, Faculty of Agronomy) following the regulations of the CHEA (Udelar).

2.2. Experimental diets

Thirty pregnant ewes were grazed on natural pasture comprising mainly *Paspalum notatum, Axonopus affinis, Piptochaetium montevidiense,* and *Stipa* sp. At 30 days prior to parturition, the sheep were allocated (10 each) to one of three groups as follows: ewes fed only pasture (Pasture), ewes fed pasture and supplemented with Se and I (Pasture + Se + I), and ewes fed pasture and supplemented with Se but not I (Pasture + Se). Ewes individually received mineral supplementation (30 g animal^{-1} day^{-1}) in the early morning (during August–September) during the 30 days prior to parturition. The supplementation received by each ewe was planned to supply 120 μg of Se ewe^{-1} day^{-1}, as a moderate dose (0.1 mg Se/kg dry matter intake) or 1.4 mg of I ewe^{-1} day^{-1} (1 mg I/kg dry matter intake), taking into account the presence of goitrogenic components in the pasture (Suttle, 2010). Mineral supplementation consisted of an experimental salt containing: Se 4 ppm and I 47 ppm (Pasture + Se + I) or Se 4 ppm, without I (Pasture + Se). Both experimental salts also contained: zinc 16000 ppm, magnesium 2800 ppm, copper 975 ppm, sulphur 734 ppm, manganese 358 ppm, cobalt 42 ppm, and potassium 15 ppm.

After lambing, ten lambs (five males and five females) born in each of the three corresponding experimental groups of ewes (Pasture, Pasture + Se + I, and Pasture + Se) were used for the study of offspring responses. The body weight of the lambs was recorded at birth and at weaning (3 months of age). At 1 year of age, the lambs were sheared and a sample of wool was obtained for analysis. The lambs were maintained on the same

pasture together with their mothers, during suckling, after weaning, and until shearing age.

2.3. Blood serum of ewes and wool samples of lambs

2.3.1. Ewes

Blood samples were collected from the jugular vein of ewes from each treatment (Pasture, Pasture + Se + I, and Pasture + Se), before and at the end of the mineral supplementation. Blood was coagulated and centrifuged at 2500 rpm for 5 min. The subsequent serum was frozen at -20 °C, until further analysis.

2.3.2. Lambs

A sample (200 g) of wool was collected on the fleece in the middle of the left flank and divided into two sub-samples. One sub-sample was sent to the Secretariado Uruguayo de la Lana (SUL) laboratory for analysis of fleece characteristics and the other was washed with deionised water, dried at 40 °C, and stored for subsequent mineral determinations.

2.4. Analytical determinations

Se and I were measured in samples of pasture, experimental salt, serum of pregnant ewes, and the wool of lambs. The hormones T4, FT3, and TSH were measured in blood serum of pregnant ewes.

2.4.1. Preparation of solutions and standards

Sub-boiling distilled HNO_3 1 M, prepared with HNO_3 65%, puriss. p.a. (84378, Merck, Germany); HCl 6 M, prepared with HCl 37%, EMSURE, puriss. p.a. (30721, Merck, Germany); $Mg(NO_3)_2$, in 17% HNO_3 magnesium matrix modifier 1% (63043, puriss. p.a. for graphite furnace-AAS, Fluka, Chemika, Switzerland); and $Pd(NO3)_2$ in 15% HNO_3 palladium nitrate modifier 1% (B0190635, puriss. p. a. for graphite furnace-AAS, Perkin Elmer, Germany) were used for sample preparation and analysis. Millipore-MilliQ distilled deionised water, with a resistivity of 18 MΩ cm, was used throughout. Glassware was soaked in dilute (50 ml/l) distilled nitric acid and, then, rinsed thoroughly in distilled deionised water.

2.4.2. Preparation of samples

Samples of pasture (1 g, from a larger sample previously dried to 50 °C for 48 h and ground), experimental salt (1 g), and wool (2 g; previously washed, dried to 50 °C, and chopped) were dried to 105 °C in an oven, with circulating air, to obtain a constant weight. Subsequently, the samples were ashed in a covered crucible at 550 °C in a muffle furnace, with temperature ramp (Thermolyne, USA) for 16 h, to obtain a white residual ash. The ash was subjected to an acid digestion process in an Erlenmeyer flask, covered with a micro glass-ball, with 6 M HCl and 1 M HNO3 on a hot plate, filtered with ashless Whatman filter paper, and diluted to a 20 ml (pasture and salt) or 10 ml (wool) final volume with distilled deionised water (Shen et al., 2015). Samples of 1 g of serum were prepared, following procedures described by Antunovic et al. (2010), in acid digestion with HNO3 1 M and HCl 6 M and a final volume of 10 ml prepared with distilled deionised water. Calibration solutions of Se (35, 70, 140, and 280 µg Se/L) were prepared immediately before use by dilution (with 0.2% distilled HNO3 65% in distilled and deionised water) of a 1000 µg Se/L, HNO_3 2% standard solution for AA (certified, N93000149, Perkin Elmer, USA). For I measurements, calibration solutions (0.02, 0.1, 1.0–10 mg I/L) were prepared with potassium iodate (KI, puriss. p.a. 99%, B1264843, Merck, Germany) in KOH (puriss. p.a. 99%, Merck, Germany) and distilled deionised water.

2.4.3. Measurements of Se and I

Se measurements in acidic aqueous dilution (blanks, samples, and calibration curve) were performed with an atomic absorption spectrophotometer (AAS Perkin Elmer, Analyst 300), equipped with a deuterium lamp background corrector, a Perkin Elmer HGA 800 graphite furnace,

and a Perkin Elmer AS-800 autosampler (Stephan et al., 2008; Cabrera et al., 2010). The determinations were conducted using matrix modifiers based on magnesium nitrate and palladium nitrate (Butcher, 2017). Argon (99% purity) was used as a carrier gas, and a selenium HCL lamp was used as a light source. All determinations were performed in triplicate. Detection limit was 10 µg/l (IUPAC, 1998; calculated as 3 × standard deviations of blanks + average of 10 blanks), and precision was 5.1% (calculated as RSD, %, of 10 measures of 35 µg/l).

I was measured in the blank, samples, and calibration curve solutions using ion exchange chromatography (Dionex Integrion HPIC system, Thermo Fisher Scientific, USA), as described by Błażewicz et al. (2014). The identification of I was based on the retention time, and quantification was performed by integrating the peak area. The sample peaks of each sample were compared to those produced by the calibration solution. Detection limit was 20 µg/l (EPA, 2016; calculated as 2.8 × standard deviations of blanks + average of 10 blanks).

2.4.4. Measurements of TSH, FT3, and T4

For determinations of TSH, FT3, and T4, Tosoh-AIA reagents (ST AIA-PACK TSH, ST AIA-PACK FT3, and ST AIA-PACK T4, respectively) and AIA900 equipment, (Tosoh's Automated Immunoassay system, USA) were used. The minimal detectable concentration was estimated to be 0.03 µIU/ml TSH, 0.5 pg/ml FT3, and 0.5 µg/dl T4. The minimal detectable concentration is defined as the concentration of TSH, FT3, or T4 that corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 30 replicate determinations of a zero calibrator. Precision for TSH and FT3 assays was 4.8 % and 4.3 % for T4 (calculated as RSD, %, determined by the duplicate assay of three controls in 20 separate runs. The means of each run were used to calculate the standard deviation and coefficient of variation).

2.4.5. Measurements in wool of lambs

Characteristics of the fleece of lambs were determined using standardised methods (IWTO Red Book Specifications, 2015). Mean fibre diameter (MFD, µm) and the coefficient of variation of fibre diameter (CVfd, %) were measured following the specifications of IWTO-12-2012 and measured using a Sirolan-Laserscan Fibre Diameter Analyser (AWTA, AU). Fibre length was measured following the technical rule IWTO-17-2011, with an Almeter Wool Fibre Lenght (AWTA, AU). Color was measured following specification IWTO-56-2014, in clean wool, as yellowness (Y-Z) and brightness (Y) tristimulus values using a Lab Scan XE (dual-bean spectrophotometer, HunterLab, USA), with illuminant D65/10° and converted to a C/2°.

2.5. Statistical analysis

Data are presented as the mean ± SEM. For analysis of the effect of diet on Se, I, and hormone levels in serum, repeated ANOVA measures were used, with diet and days prior to and at lambing as the main effects. To compare the effect of diet on lamb performance, Se and I in wool, and wool quality parameters, one-way ANOVA was used. Differences between means were detected using a Tukey-Kramer test with a significance level of $p < 0.05$. Differences between days prior and at lambing in trace minerals content in pasture were analysed using Student's t-test ($p < 0.05$).

3. Results and discussion

3.1. Content of Se and I in natural pastures

Se content in pastures grazed by pregnant ewes (Table 1), resulted in moderate deficiency level (0.07–0.09 ppm) at 30 days prior to lambing, whereas at lambing, content of Se was suboptimal (0.11–0.16 ppm) for the requirements of sheep (0.2 ppm; Meschy, 2010). Differences between 0 and 30 days prior to lambing for Se content in pasture were significant

Table 1
Pasture yield, grass cut height, iodine (I) and selenium (Se) content in the natural pasture offered to ewes grazing in the Basalto Region in the northwest of Uruguay.

Scale-point[a]	Days prior to lambing							
	30				0			
	Pasture yield (kg DM)	Grass cut height (cm)	I (mg/kg DM)	Se (mg/kg DM)	Pasture yield (kg DM)	Grass cut height (cm)	I (mg/kg DM)	Se (mg/kg DM)
1	380	4.5	0.60 ± 0.10	0.07 ± 0.01b	242	3	0.60 ± 0.13	0.11 ± 0.01 a
2	2122	8.4	0.56 ± 0.12	0.09 ± 0.01b	2745	8	0.60 ± 0.09	0.16 ± 0.02 a
3	3133	18.3	0.58 ± 0.10	0.09 ± 0.01b	3282	17.7	0.58 ± 0.10	0.14 ± 0.01 a
4	4867	23	0.50 ± 0.11	0.07 ± 0.01b	6808	26	0.56 ± 0.11	0.16 ± 0.01 a

Data are mean ± SEM of three samples. DM = Dry matter. a,b: means significant difference between 30 and 0 days prior to lambing by Student test (p < 0.05).

[a] Extracted from "The comparative yield method" by Haydock and Shaw (1975). It is based on a comparative yield method, which estimates the pasture yield from the yields of random quadrats rated to a set of reference quadrats preselected to provide a scale which is available for reference throughout sampling. In this work four reference quadrats were selected (considering height and density of pasture), which constitute the yield scale against which the yields of sample quadrats are rated.

(p < 0.05). This content could be enough for the growth of lambs but not for lactation of ewes (Rock et al., 2001). The increasing requirements of ewes in Se/vitamin E in late gestation and during lactation (NRC, 2007) and a marginal intake of these nutrients in this period could cause seasonal differences in Se status, mainly at the end of winter. The intake of Se from pastures was around 0.120 mg/day 30 days prior to lambing and 0.216 mg/day at lambing, considering a dry matter intake of 1.44 kg ewe^{-1} day^{-1}. With Se supplementation, the intake was near 0.120 mg of Se ewe^{-1} day^{-1}, with the total of Se intake consistent with FDA rules that limits Se to 0.7 mg sheep^{-1} day^{-1} to avoid toxicity. However, for I, the content in pasture could be suboptimal (0.50–0.60 ppm) at 30 days prior to lambing and at lambing (0.56–0.60 ppm), because a minimal level of 0.50–0.80 ppm is necessary for pregnancy and lactation (NRC, 2007; Meschy, 2010). Those levels are, however, enough for the growth of lambs according to Meschy (2010) and Phillips et al. (2014)). Intake of I from pastures, in our study, was 0.792 mg ewe^{-1} day^{-1} prior to lambing and 0.840 mg ewe^{-1} day^{-1} at lambing. With I supplementation, total I intake was around 2.2 mg ewe^{-1} day^{-1}; moreover, this amount seems adequate to satisfy the needs of ewes (Phillips et al., 2014) and avoid the effect of goitrogenic plants that are common in this region. The content of Se found in natural pasture in our study was higher than that reported by Pittaluga (2018), which could be explained by the great variability in the north-west region (Basaltic Region), as reported previously (Ungerfeld, 1998; Berretta, 1998; Pigurina et al., 1998). Content of I in natural pastures results was higher than that expected, probably owing to the influence of factors, such as soil pH in this particular area, predominant botanical species, rainfall, or environmental temperature (Sivertsen et al., 2014; Reis et al., 2017).

3.2. Content of Se and I in blood of pregnant ewes

The content of Se in the blood serum of ewes (Table 2) was within the adequate range (0.120–0.150 mg/L) for adult sheep (Aitken, 2001) and within the reference range of 0.120–0.500 mg/L (Menzies et al., 2005). Levels of blood sera Se at the beginning of supplementation were similar for all diets, and an increase was observed near the end of gestation; these differences were significant for each experimental group (p < 0.05; Table 2) except the Se + I group. The supplementation did not influence this increase because the ewes grazing only in natural pasture had a similar increase in Se in blood serum. The higher Se content in pasture (Table 1) at the beginning of the trial was probably influenced by the onset of spring and the vegetative development of the grasses. Judson et al. (2011) found similar results in Merino sheep in Australia. I in the blood serum of ewes decreased at lambing, as previously reported by Trávníček et al. (2010), probably because of a large transfer of I from the blood serum to mammary glands and milk (Anke, 2004).

3.3. TSH, T4, and FT3 in blood serum ewes

To determine the value of pasture I and the criteria to predict I status

Table 2
Selenium (Se) and iodine (I) status in serum of pregnant Australian Merino ewes reared on natural pasture and supplemented, 30 days prior to lambing, with selenium and iodine or selenium alone.

Diet	Serum selenium (µg/ml)		Serum iodine (µg/ml)	
	Days prior to lambing		Days prior to lambing	
	30	0	30	0
Pasture	0.128 ± 0.023a	0.216 ± 0.032b	0.208 ± 0.017a	0.150 ± 0.016b
Pasture + Se + I	0.122 ± 0.031a	0.140 ± 0.013b	0.197 ± 0.036a	0.175 ± 0.020b
Pasture + Se	0.114 ± 0.022a	0.221 ± 0.041b	0.199 ± 0.021a	0.163 ± 0.027b
Main effects				
Diet	ns		Ns	
Days	p < 0.004		p < 0.05	
	0.121 ± 0.014 a	0.195 ± 0.019 b	0.201 ± 0.014 a	0.162 ± 0.012 b

Data are mean ± SEM of 10 ewes for each diet. Selenium and iodine data in serum were analyzed by repeated measure ANOVA with diet and days prior to or at lambing in ewes, as main effects. ANOVA one way was used to compare between days prior to birth for each diet. [a,b] Values with different letters mean significant differences at p < 0.05 by Tukey-Kramer post hoc test; ns = non significant.

(Clark et al., 1999), blood serum TSH, T4, and FT3 concentrations were measured in ewes 30 days prior to and at lambing (Table 3). No significant differences were observed for TSH or T4 in the blood serum of ewes

Table 3
Serum concentration of TSH, free T3 and total T4 in pregnant Australian Merino ewes reared on natural pasture, and supplemented 30 days prior to lambing with selenium (Se) and iodine (I) or selenium (Se) alone.

Diet	TSH (µIU/ml)		FT3 (pg/ml)		T4 (µg/dl)	
	Days prior to lambing					
	30	0	30	0	30	0
Pasture	0.057 ± 0.01	0.055 ± 0.01	2.24 ± 0.13a	2.80 ± 0.15b	4.64 ± 0.18	4.68 ± 0.21
Pasture + Se + I	0.055 ± 0.01	0.083 ± 0.02	2.26 ± 0.24a	2.86 ± 0.18b	4.83 ± 0.81	4.90 ± 0.25
Pasture + Se	0.055 ± 0.01	0.071 ± 0.01	2.25 ± 0.33a	3.07 ± 0.16b	4.44 ± 0.35	4.91 ± 0.44
Main effects						
Diet	ns		ns		ns	
Days	ns		p < 0.001		ns	

Data are mean ± SEM of 10 ewes for each diet. Data were analyzed by repeated measure ANOVA with diet and days prior to or at lambing as main effects and post hoc Tukey-Kramer test (p < 0.05) when differences were significant. ANOVA one way were used to analyze differences between times 0 and 30 days for each diet. [a,b] Mean with a different superscript letter means significant difference at p < 0.05 by Tukey-Kramer post hoc test; ns = non significant.

grazing natural pasture compared to those supplemented with Se and I or Se alone (Table 3). However, for FT3, an increase was observed at lambing for all groups ($p < 0.05$), probably because of the increase in Se content observed in pasture. Se is essential for normal thyroid hormone metabolism and the conversion of T4 into the more active FT3 via catalysis by enzyme type 4-deiodinase (Hefnawy et al., 2014). Higher intake of Se from pasture and supplementation likely resulted in a significantly higher blood serum FT3 level. In the bovine breed, Se deficiency in steers and heifers was characterised by decreased FT3 and increased T4 serum concentration (Arthur et al., 1992). Vouduri et al. (2003) reported that thyroid metabolism in sheep appears different in species that have a significant thyroidal deiodinase activity. Animals with significant thyroidal type I deiodinase can produce T3 from T4, whereas sheep have to synthesise it directly. This is perhaps why sheep appear more resistant to the effects of Se deficiency on thyroid hormone metabolism in comparison to rats, humans, or cattle. In the present investigation, it was noted that the TSH levels were adequate in ewes grazing pasture prior to and at lambing. However, in animals supplemented with Se and I or Se alone, a non-significant increase was observed (Table 3), which would be an expected response according to Clark et al. (1999) and Pereyra et al. (2014), who observed that I supplementation increased TSH values.

3.4. Body weight and wool quality in lambs

There were no significant effects ($p < 0.05$) on live weights of lambs at birth or at weaning among the treatments (Table 4). The lambs presented adequate weights at birth and were weaned at the best condition of live weight gain for a typical pastoral system, as reported by Thompson et al. (2011).

Content of Se in lamb wool (Table 5) showed an adequate level of this trace element in lambs from ewes grazing in natural pasture, and the critical values (under 0.027 mg/kg dry matter of wool) reported by Grace and Clark (1991) were not observed in our study. The satisfactory Se concentration in wool samples could be explained by an adequate Se concentration in natural pasture for Merino ewes and lambs. As the recommendation for sheep in trace minerals is related to the physiological state, there is limited specific information accounting for the different breeds (Suttle, 2005, 2010). Grace and Clark (1991) have reported Se content of 0.027–0.502 mg/kg dry matter in wool, and Ramirez-Perez et al. (2000) observed values of 0.205 mg/kg dry matter. The dietary Se, the breed, and other factors affecting the level of wool Se content (Davis et al., 2006, 2008) could probably explain this large range. For example, for bovine breeds (feedlot steers), Cristaldi et al. (2005) reported increased Se in the hair when dietary Se content was high. Furthermore, sheep could be a sensitive productive species to Se deficiency according to Gabbedy (1971). When there is a deficiency of Se in the diet, animals mobilise tissues reserves, but when Se is adequate, wool accumulates Se, just as in other tissues such as muscle. Wool, as well as hair, could reflect the status of trace elements in ruminants; this status could have a direct effect on wool follicle development (Lee and Grace, 1988). An adequate content of Se in hair or wool may suggest an adequate long-term Se status (Thomas, 2004). In our study, wool in lambs could be an indicator of the adequate Se level in natural pasture in

Table 4

Live weights at birth and weaning (three months old) of lambs, offsprings of Australian Merino ewes reared on natural pasture, and supplemented 30 days prior to lambing with selenium and iodine, or selenium alone.

Diet of pregnant ewes	Birth live weight (kg)	Weaning live weight (kg)
Pasture	4.21 ± 0.21	16.02 ± 0.18
Pasture + Se + I	4.12 ± 0.16	16.21 ± 0.17
Pasture + Se	3.89 ± 0.20	15.07 ± 0.19

Data are mean ± SEM of n = 10 lambs.
No significant differences between treatments (p < 0.05).

Table 5

Selenium and iodine content of wool of lambs, offsprings of Australian Merino ewes reared on natural pasture, and supplemented 30 days prior to lambing with selenium (Se) and iodine (I) or with selenium (Se) alone.

Diet	Wool selenium (mg/kg DM)	Wool iodine (mg/kg DM)
Pasture	0.063 ± 0.031	0.016 ± 0.003
Pasture + Se + I	0.092 ± 0.033	0.020 ± 0.002
Pasture + Se	0.093 ± 0.022	0.024 ± 0.002
	ns	ns

Data are mean ± SEM of 10 lambs for each diet. Selenium and iodine data in wool were analyzed by ANOVA one way to compare between diets. No difference between diets were detected at p < 0.05. DM = dry matter, ns = non significant.

the north-west of Uruguay. Besides, the mineral supplementation with Se and I or only Se provided here improved the blood serum content of Se. However, the differences observed in wool were not statistically significant ($p > 0.05$) (Table 5). This observation supports the hypothesis that Se in pastures was enough to meet the requirements of this breed of sheep produced in Uruguay.

I content in wool showed values ranging from 16–24 µg/kg dry matter, with an increase in the groups supplemented with Se and I or Se only; however, this increase was not significant ($p > 0.05$; Table 5). The content of I in hair is generally associated with I status (Prejac et al., 2014). However, Pozebon et al. (2017) encountered a great variability (<50 µg/kg to 500 µg/kg) in individual values, probably because of dietary differences or environmental conditions. The same authors proposed another possible explanation for this variability. They explain that part of the observed variability could be attributed to the differences in inter- and intra-laboratory reproducibility of results caused by the lack of standardisation of laboratory methodologies and procedures, such as washing, decomposition, and other operative procedures (Pozebon et al., 2017). Although values obtained in our study seem to be low, it must be noted that there is no information for comparison regarding the nutritional requirement for Australian Merino sheep. The effect of nutrition on wool growth of grazing sheep has long been taken into account, with periods of poor pasture growth or low quality reflected by a reduction in total fleece growth per animal and per unit area of grazed land (Campbell, 2006). In our study, the values obtained for these parameters in the pastoral system seem to be adequate (Campbell, 2006) considering the season and the place (Southern hemisphere) where the study was conducted (Table 6).

A very good diameter of fibre wool (14.37–15.25 µm) as well as a good colour (lightness, 69.21–69.87) was obtained in all dietary treatments (CRILU, 2015). However, the supplementation of ewes in the last days of gestation did not improve these parameters (Table 6). A possible explanation for this result could be that the adopted supplementation was too late in the gestational moment, decreasing the effect on the overall parameters studied. However, McGregor and Butler (2016) showed that

Table 6

First weight fleece and fleece characteristics of lambs, offsprings of Australian Merino ewes reared on natural pasture and supplemented, 30 days prior to lambing, with selenium (Se) and iodine (I) or with selenium alone.

Diet	Clean Fleece Weight (kg)	MDF (µm)	CVfd (%)	Fibre Length (cm)	Wool Fibre Color	
					Y = brightness	Y-Z = yellowness
Pasture	1.28 ± 0.06	15.25 ± 0.34	20.85 ± 0.76	7.46 ± 0.26	69.21 ± 0.45	0.02 ± 0.54
Pasture + Se + I	1.43 ± 0.10	14.36 ± 0.40	20.54 ± 0.76	7.61 ± 0.41	69.76 ± 0.77	-0.3 ± 0.54
Pasture + Se	1.31 ± 0.10	14.37 ± 0.25	19.92 ± 1.15	8.00 ± 0.33	69.87 ± 0.53	-0.26 ± 0.54

MDF = Mean diameter fibre; CVfd = coefficient of variation of fibre diameter. Data are mean ± SEM. No significant differences between groups by ANOVA one way (p < 0.05).

the diameter of fibre wool in Merino is an allometric property and that it is affected by the live weight of the sheep. Consequently, it is highly probable that as the live weight and the live weight gain of lambs with different treatments was the same over the first year of life, a difference in the diameter of fibre wool could not be expected. Another explanation was that the levels of these trace elements, present in the pasture, have been sufficient to respond to the requirement in Se and I of the ewes. In addition, it cannot be ruled out that the bioavailability of Se and I was enough to satisfy the requirements of Australian Merino sheep in the pasture-based production system used here.

Finally, the Australian Merino sheep raised on natural pasture in the north-west of Uruguay presented adequate levels of trace elements Se and I in the blood serum of ewes and the wool of their offspring. However, the supplementation with both Se and I or Se alone did not produce the expected effect, that is, the increase in wool quality of the offspring of the supplemented Merino Australian ewes.

4. Conclusions

Se and I status of pregnant Australian Merino ewes grazing natural pasture, before any supplementation, seem to be adequate, as reflected by the content in blood and the body conditions and performance of their offspring. It could also be proposed, hypothetically, that the good wool quality of the offspring could be because of this status. These favourable effects were present even when the content of both elements, considering the nutritional requirements, was suboptimal or slightly marginal in the pasture during the last month of pregnancy.

While previous investigation indicated that the pasture could be deficient or marginal in Se and I, in the present investigation, we found that the content of Se and I was adequate. Consequently, when supplements of Se and I or Se alone were provided during the last 30 days of gestation, no effects were detected in the blood parameters or the subsequent wool quality of lambs over the following 12 months. Maybe an earlier supplementation during pregnancy could have a better effect on the evaluated parameters. Of course, the most adequate moment to introduce this supplementation must be determined. However, the lack of specific knowledge regarding their mineral requirements, such as Se and I, of the Australian Merino breed, make the accurate determination of the most adequate supplementation procedure difficult. This is a challenge to be addressed by scientists in sheep nutrition and wool production of the Australian Merino breed.

Declarations

Author contribution statement

M.H. Guerra: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.C. Cabrera: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

D. Fernández Abella, A. Saadoun: Performed the experiments.

A. Burton: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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