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Effects of Low-Dose Organic Trace Minerals Supplementation on the Mineral Excretion and Physiological Mineral Status in Small Ruminants

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RESEARCH ARTICLE

Abstract

This study investigated the effects of replacing inorganic trace mineral (ITM) supplementations of Mn, Zn and Cu by *iso* or lower doses of organic TM (OTM) on mineral status and mineral excretion of sheep. Following a Latin square design, nine castrated rams were divided to three experimental treatments: supplementation either with ITM (INORG) or OTM (ORG) following the recommended levels, and OTM at a reduced dosage (ORGLow). After an adaptation period, samples of feces, urine and blood were collected for 12 days in metabolic stalls. Serum mineral content showed differences only for Cu, which was significantly higher ($p<0.01$) with ORGLow when compared to INORG and ORG (0.79, 0.74 and 0.69 mg/kg, respectively). Total daily feces mineral excretions were downregulated with ORGLow compared to the INORG or ORG: 77.2, 89.2 and 91.6mg/day; 38.8, 56.0 and 57.0 mg/day; 5.70, 8.15 and 7.06 mg/day for Mn, Zn and Cu, respectively. The results of this study suggest that supplementing sheep with a low dosage of OTM significantly reduces mineral excretion without a negative effect on the physiological mineral status of the animals. Further long-term studies are necessary to assess the mineral mobilization from body storages during supplementation with low dosages of OTM.

Keywords: ruminant; trace minerals, excretion.

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INTRODUCTION

In the current supplementation practice in ruminants, dietary inclusion of trace minerals (TM) such as manganese (Mn), zinc (Zn) and copper (Cu) is of importance to cover the ever-evolving requirements for growth, production, and reproduction. The nutritional feeding systems for ruminants (INRA 2018, NRC 2021) indicate dietary optimum levels of 50, 50 and 10 mg/kg DM (Noziere et al.. 2018; National Academies of Sciences and Medicine. 2021), and regulatory maximum limits of 150, 120 and 35 mg/kg DM for Mn, Zn and Cu, respectively (Trumeau 2014; European Commission and Directorate-General for Health and Food Safety, 2023). However, when it comes to choosing the TM products for dietary inclusion, given the wide range of available trace mineral sources (Vigh et al., 2023b), the task of the animal feed industry specialists is quite challenging. Among the most widely available TM forms for dietary inclusion are the inorganic salts (ITM) such as oxides and sulfates (Ammerman and Goodrich, 1983), however some ITMs have a negative effect on the rumen environment decreasing fermentation activity (Vigh et al., 2023a), dry matter (DM) degradability (Genther and Hansen, 2015), or even lowering specific microbial populations activity, like cellulolytic bacteria (Kišidayová et al., 2018). Another form of TM available for livestock supplementation are the organic trace minerals (OTM), such as glycinates (Byrne and Murphy, 2022), characterized by a higher bioavailability when compared to ITM (Spears et al., 2004; Alimohamady et al., 2019). Nevertheless, even though OTM are included in ruminant diets, there are added more in addition than as a substitute for the standard ITM (Daniel et al., 2020), and often the overall dietary inclusion levels are above the recommended ones (Daniel et al., 2023). From an environmental perspective, supplementation of farm animals with high levels of TM results in an accumulation of metals in the feces and urine, leading to increased emissions, affecting water supply chains, impairment of plant development, as well as an increased occurrence of antimicrobial resistance in farmed animals (Brugger and Windisch, 2015). In the perspective of reducing heavy metal emissions from animal production, a better implementation of TM precision feeding is required (Lu et al., 2017). Based on the hypothesis that a lower intake of a more bioavailable TM forms would lead to better use by the animals and lower emissions to the environment through fecal and urinary excretions, this study aimed to investigate the effects of replacing ITM supplementations by iso dosages or lower dosages of OTM sources (Mn-, Zn- and Cu-glycinate) on nutrient digestibility, mineral excretion, and trace mineral status of sheep.

MATERIALS AND METHODS

The use of castrated rams in metabolic stalls in this study was approved by the Ethics Committee nº052 (France) and the study protocol was registered under the number APAFIS#32360-2021070815571935 v3 by the French Ministry of Scientific Research. This study was carried out at the Applied Zootechnical Research Center ("Centre de Recherche Zootechnique Appliquée" – CRZA) of ADM AN (Montfaucon, France) to evaluate the effect of the form (inorganic or organic) and feed inclusion rate of trace minerals (Mn, Zn and Cu) on the apparent total tract digestibility (ATTD) of nutrients, total trace mineral excretion and blood concentrations measured through a sheep digestibility model (VanValin et al. 2020). The most commonly used ITM sources for ruminant supplementation were identified (Spears, 2003), and included in this study (supplied by SERMIX, Chierry, France): manganese oxide (MnO, 60% - Mn), zinc oxide (ZnO, 72% - Zn), and copper sulfate ($CuSO_4$, 26% - Cu); while for the OTM, the glycinate complexes (Mn-, Zn- and Cu-) were chosen (supplied by SERMIX, Chierry, France), based on their higher bioavailability when compared to inorganic sources (Spears et al., 2004; Byrne and Murphy, 2022).

Experimental design and treatments

Following a Latin square design, nine castrated rams weighing between 55 and 60 kg were divided to three experimental groups (Figure 1): supplementation only with inorganic sources of TM (INORG), supplementation only with organic sources (ORG) at iso dosage of INORG and supplementation only with organic sources at a dosage reduced by 50% (ORG_Low) compared to INORG and ORG. The animals' diet was limited to ~1.0 kg of DM/day and consisted of 40% natural hay (DM-89.8%, CP-7.9 % DM, NDF-64.2 % DM) and 60% complete feed (DM-86.6 %, CP-22.5 % DM, NDF-22.5 % DM). The TM supplements were integrated in the complete feed at levels of 45.0, 70.0 and 5.0 mg/kg DM for Mn, Zn and Cu in the INORG and ORG treatments, and 22.5, 35.0 and 2.5 mg/kg DM for Mn, Zn and Cu in the ORG_Low treatment. Each experimental period (n=3) had a duration of 21 days: 9 days of adaptation followed by 12 days of sample collection (urine, feces, and blood) in metabolic stalls.

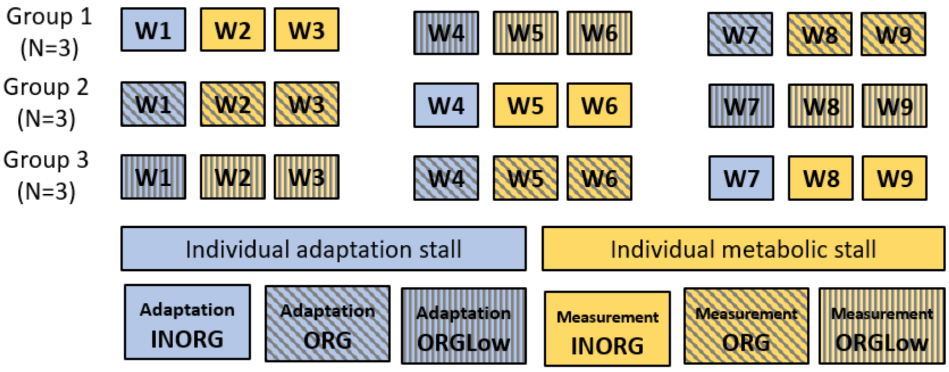

Figure 1. Study setup and timeline.

Animals

The nine castrated rams used in this trial were born in 2021 and were housed in a collective pen with straw bedding, receiving a basal diet consisting of natural meadow hay distributed *ad libitum* and a commercial complete feed (Patrilis Pratic, NOVIAL, Noyelles-sur-Escaut, France) distributed at a level of ~600 g/day/animal. The basal diet was adapted according to the body weight of the animals (40 g of DMI/kg $BW^{0.75}$) to cover their maintenance requirements (Table 1).

Table 1. Maintenance requirements of castrated rams in terms of energy (UFV) and protein (PDI)

BW (kg)	UFV requirement (+10%*)/day	PDI requirement (+10%* g/day
40	0,572	44
50	0,682	51,7
60	0,781	59,4
70	0,88	67,1
80	0,968	73,7

Note: BW – Body weight; UFV – Energy requirements for meat production; PDI – Proteins digestible in the intestine; *Source: INRA 2018, maintenance requirements for dry ewes, increased by 10% (recommendations to cover ram maintenance requirements).

Following the start of the trial, the animals were introduced in individual adaptation pens, and they were fed experimental diets (40% hay and 60% complete feed), divided into two equal meals. The supplemental TM were first added into a vitamin-mineral premix (Table 2), which was next introduced at a level of 0.5 % DM in a pelleted complete feed (Table 3).

Table 2. Composition of the experimental premixes

Composition	INORG	ORG	ORG_Low
Vitamin A (UI/kg)	1 200 000	1 200 000	1 200 000
Vitamin D3 (UI/kg)	240 000	240 000	240 000
Vitamin E (UI/kg)	4 000	4 000	4 000
Vitamin B1 (mg/kg)	1 950	1 950	1 950
Cu (mg/kg)	1 000	1 000	500
Fe (mg/kg)	21 600	21 600	21 600
Mn (mg/kg)	9 000	9 000	4 500
Zn (mg/kg)	14 000	14 000	7 000
I (mg/kg)	1 300	1 300	1 300
Co (mg/kg)	200	200	200
Se (mg/kg)	40	40	40

Table 3. Composition of the experimental complete feeds

Composition (% DM)	INORG	ORG	ORG_Low
Wheat	12.70	12.70	12.70
Corn	10.00	10.00	10.00
Barley	25.09	25.01	24.99
Corn gluten meal	2.00	2.00	2.00
Corn distillers' grain	10.00	10.00	10.00
Soybean oil	0.55	0.60	0.55
Soybean meal	13.80	13.80	13.80
Rapeseed meal	5.60	5.60	5.60
Beets pulp	10.00	10.00	10.10
Sunflower hulls	3.60	3.60	3.60
Sugarcane molasses	3.00	3.00	3.00
$CaCO_3$	2.36	2.39	2.36
NaCl	0.80	0.80	0.80
Experimental premix	0.50	0.50	0.50
Nutritional analysis			
Dry matter (%)	86.6	87.0	87.1
Starch (%)	29.4	29.3	29.3
Crude protein (% of DM)	20.4	20.1	21.1
Crude fiber (% of DM)	9.3	9.5	8.7
Crude ash (% of DM)	8.1	8.1	8.1
NDF† (% of DM)	22.5	23.6	22.8
ADF‡ (% of DM)	12.2	12.7	12.6

Note: †NDF – Neutral detergent fiber; ‡ADF – Acid detergent fiber.

Once the adaptation (9 days) to the experimental diets was realized, the animals were introduced in individual metabolic stalls for a period of 12 days. The three experimental diets were distributed in two equal meals, while daily feed refusals, total feces and urine were collected, weighed, and recorded.

Measurements, samplings, and analysis

The dietary ingredients (hay and complete feed) and fecal samples were oven-dried at 60°C for 72 hours and ground (1.0 mm sieve) before analyzing the organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) content (UpScience, Saint-Nolff, France), and TM (Cu, Mn, and Zn) concentrations (UT2A, Pau, France). A sub-sample of ~10.0 g of fresh feces was used to determine the DM content of the daily collected samples. The OM content of samples was estimated by the difference between DM and ash contents. Daily urine sub-samples (~100 mL) were immediately frozen (-20°C) after collection and sent for TM analysis (UT2A, Pau, France). Daily blood samples were taken during 3 days of each measurement week (2 x 3 samples/animal/measurement period) via a catheter placed on the jugular vein. The blood samples were collected in dry tubes (without heparin) to obtain serum. The serum was obtained by refrigerating (at 4°C for 1 hour) the tubes containing the whole blood followed by a centrifugation at 3 500 x *g* for 15 minutes. The resulting supernatants were stored at -80°C until TM content analysis (UT2A, Pau, France). The apparent total tract digestibility (ATTD) of nutrients (DM, OM, CP, NDF and ADF) was calculated as described by Schalla et al. (2012). The apparent absorption of TM was determined by subtracting fecal mineral content from mineral intake, dividing by mineral intake, and multiplying by 100 (Carmichael et al., 2018). All data was statistically analyzed by ANOVA as a randomized complete design using the General Linear Model (GLM) procedure in R software. Normality assumption was tested for all quantitative parameters (Shapiro-Wilk test), no outliers were identified. Tuckey's test was applied for two-by-two comparisons. Significance was declared at $p<0.05$ and tendencies were declared at $0.06<p<0.10$.

RESULTS AND DISCUSSIONS

Total feces and urine

The feces dry matter content (DM %), total feces (kg DM/day) and total urine (kg/day) output are presented in Table 4. No significant effects of the treatments were detected on feces DM content (%), total feces output (kg DM/day) and total urine output (kg/day). However, the total urine output with the ORG_Low was numerically lower compared to the ORG and INORG treatments (1.82, 2.40 and 2.57 kg/day, respectively).

Table 4. Influence of dietary trace mineral source and supplementation level on feces DM, total feces, and urine output

Treatment	INORG	ORG	ORG _Low	SEM	*p* value
Feces DM %	44.2	44.4	43.9	5.10	0.90
Total feces (kg DM/day)	0.27	0.27	0.26	0.012	0.68
Total urine (kg/day)	2.57	2.40	1.82	0.230	0.23

Note: SEM = Standard error of the mean

Nutrient output and digestibility

The total nutrient output and digestibility data for the 12-days collection period are reported in Table 5. The total output (kg/day) and ATTD (%) of DM, OM, CP, NDF and ADF were not affected by trace mineral source and supplementation level.

Table 5. Influence of trace mineral source and supplementation level on nutrient output and digestibility

Treatment	INORG	ORG	ORG_Low	SEM	*p* value
		Nutrient output, kg/d			
DM	0.27	0.27	0.26	0.012	0.686
OM†	0.20	0.22	0.21	0.015	0.742
CP	0.035	0.037	0.037	0.0016	0.633
NDF	0.14	0.17	0.15	0.011	0.333
ADF	0.10	0.11	0.10	0.007	0.481
		Nutrient ATTD, %			
DM	73.6	73.2	74.6	1.26	0.711
OM*	78.8	77.1	77.9	1.59	0.754
CP	76.9	75.4	75.6	1.09	0.596
NDF	65.7	60.9	63.5	2.52	0.415
ADF	61.3	57.1	61.2	2.96	0.538

Note: SEM = Standard error of the mean; †OM = total DM (kg) – crude ash (kg)

Trace mineral excretion

The influence of trace mineral source and supplementation level on the fecal and urinary excretion (mg/kg DM and mg/day) is reported in Table 6. Considering the Cu concentration (mg/kg DM) in the feces, there were significant ($p<0.001$) differences between the treatments. The feces Cu concentration was significantly lower ($p<0.001$) with the ORG_Low and ORG when compared to the INORG treatment (22.7, 26.6 and 31.6 mg/kg DM for, respectively). The total Cu excretion (mg/day) in the feces was significantly lower ($p<0.001$) with the ORGLow when compared to the INORG and ORG treatments. Furthermore, the total Cu excretion with the ORG treatment was significantly lower ($p<0.05$) compared to the INORG treatment (5.70, 7.06 and 8.15 mg/day for ORG_Low, ORG and INORG treatments, respectively). The Mn concentration (mg/kg DM) in the feces was significantly lower ($p<0.05$) with the ORG_Low treatment compared to the INORG and ORG treatments (332, 368 and 370 mg/kg DM, respectively). The total Mn excretion (mg/day) in the feces was significantly lower ($p<0.001$) with the ORG_Low compared to the ORG and INORG treatments (77.2, 91.6 and 89.2 mg/day, respectively). No significant differences were registered between the ORG and INORG treatments regarding Mn concentration or total Mn excretion in the feces. The Zn concentration (mg/kg DM) in the feces was significantly lower ($p<0.001$) with the ORG_Low treatment compared to the INORG and ORG treatments (162, 221 and 224 mg/kg DM, respectively). The total Zn excretion (mg/day) in the feces was significantly lower ($p<0.001$) with the ORG_Low compared to the ORG and INORG treatments (38.8, 57.0 and 56.0 mg/day, respectively). No significant differences were registered between the ORG and INORG treatments regarding Zn concentration or total Zn excretion in the feces. The Cu concentration in the urine was below the quantification limits (<0.05 mg/kg), regardless of the treatment. Considering the Mn concentration (mg/kg) in the urine, there were significant differences between the treatments. The urine Mn concentration was significantly higher ($p<0.05$) with the ORG_Low treatment compared to the ORG and was numerically higher compared to the INORG treatment (0.066, 0.031 and 0.059 mg/kg for ORG_Low, ORG and INORG, respectively). When taking into consideration the total quantity of urine (kg/day) and urine Mn concentration (mg/kg), there were no significant differences between the treatments regarding the total Mn excretion (mg/day) through urine ($p=0.16$; 0.114, 0.072 and 0.122 mg/day for ORG_Low, ORG and INORG, respectively). Regarding the Zn concentration (mg/kg) in the urine and the total Zn excretion (mg/day) in the urine, there were no significant differences between the treatments.

Table 6. Influence of trace mineral source and supplementation level on fecal and urinary trace mineral content and daily total excretion

Treatment	INORG	ORG	ORG_Low	SEM	*p* value
			Fecal excretion		
Cu (mg/kg DM)	31.6^{b}	26.6^{a}	22.7^{a}	1.26	<0.001
Cu (mg/day)	8.15^{c}	7.06^{b}	5.70^{a}	0.25	<0.001
Mn (mg/kg DM)	370^{b}	368^{b}	332^{a}	11.0	<0.05
Mn (mg/day)	89.2^{b}	91.6^{b}	77.2^{a}	2.70	<0.001
Zn (mg/kg DM)	224^{b}	221^{b}	162^{a}	4.82	<0.001
Zn (mg/day)	56.0^{b}	57.0^{b}	38.8^{a}	1.39	<0.001
			Urinary excretion		
Mn (mg/kg)	0.059^{ab}	0.031^{a}	0.066^{b}	0.0110	<0.05
Mn (mg/day)	0.122^{a}	0.072^{a}	0.114^{a}	0.0252	0.16
Zn (mg/kg)	0.250^{a}	0.272^{a}	0.275^{a}	0.0324	0.87
Zn (mg/day)	0.48^{a}	0.51^{a}	0.46^{a}	0.051	0.77

Note: SEM = Standard error of the mean; $^{a-c}$ means in the same row with different superscripts differ ($p \leq 0.05$).

The influence of trace mineral source and supplementation level on the fecal and urinary excretion and apparent absorption on a percentage of intake basis is reported in Table 7. Fecal excretion of Cu was significantly ($p<0.001$) lower with ORG when compared to INORG and ORG_Low. In addition, the apparent absorption of Cu was significantly ($p<0.001$) higher with ORG when compared to INORG and ORG_Low. Dietary sources and supplementation levels did not influence fecal and urinary excretion (as % of intake), nor apparent absorption of Mn and Zn.

Table 7. Influence of trace-mineral source and supplementation level on fecal and urinary excretion, and trace mineral apparent absorption as a percentage of intake during the 12 days collection period

Treatment	INORG	ORG	ORG_Low	SEM	*p* value
			Fecal excretion		
Cu (%)	82.5^{b}	52.5^{a}	86.1^{b}	2.84	<0.001
Mn (%)	78.0	75.9	73.5	3.51	0.672
Zn (%)	78.0	75.9	73.5	3.61	0.672
			Urinary excretion		
Cu (%)	-	-	-	-	-
Mn (%)	0.10	0.19	0.11	0.089	0.754
Zn (%)	0.82	0.53	0.67	0.145	0.442
			Apparent absorption		
Cu (%)	17.5^{a}	47.5^{b}	13.9^{a}	2.54	<0.001
Mn (%)	21.2	23.5	25.9	2.91	0.376
Zn (%)	18.6	19.0	13.3	2.26	0.202

Note: SEM = Standard error of the mean; $^{a-b}$ means in the same row with different superscripts differ ($p \leq 0.05$).

Mineral status of the animals

The Mn concentration in the serum was below the quantification limit (0.005 mg/kg) in all of the samples, hence no data was available for comparison. Regarding serum Cu (Figure 2), there was a significant ($p<0.001$) difference between the treatments: the ORG and ORG_Low was significantly ($p<0.01$ and $p<0.001$, respectively) higher when compared to the INORG treatment (0.75, 0.79 and 0.70 mg/kg for ORG, ORG_Low and INORG, respectively).

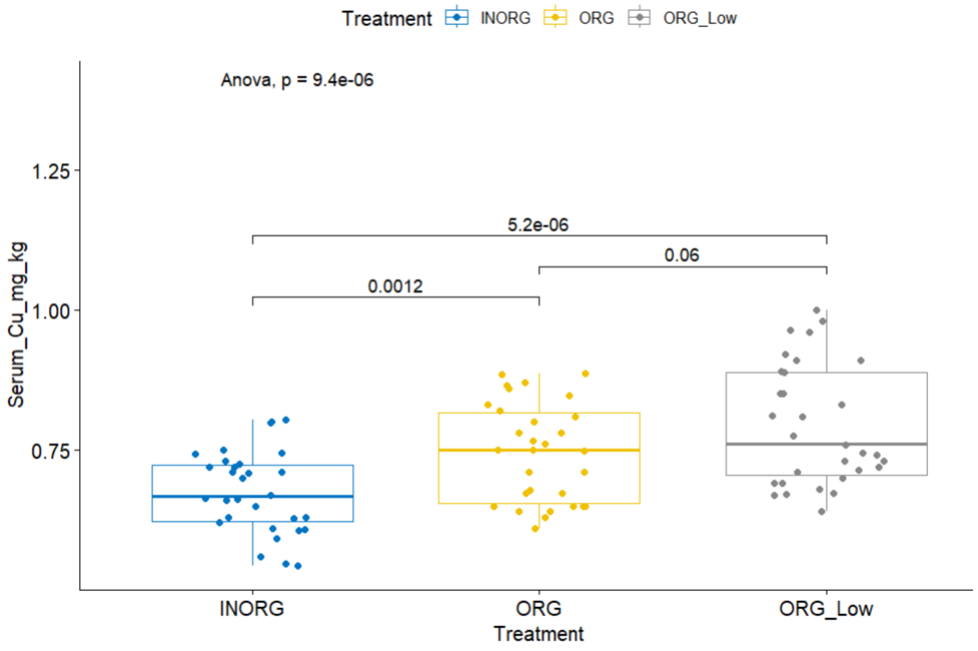

Figure 2. Influence of Cu source and supplementation level on serum Cu concentration.

The Zn serum concentration (Figure 3) showed no significant difference between the treatments. Even though the highest Zn concentration was observed with ORG and the lowest with ORG_Low, there were only numerically different when compared to INORG (0.71, 0.66 and 0.68 mg/kg for ORG, ORG_LOw and INORG, respectively). The serum Zn concentration tended ($p<0.10$) to be higher with ORG when compared to OR_GLow.

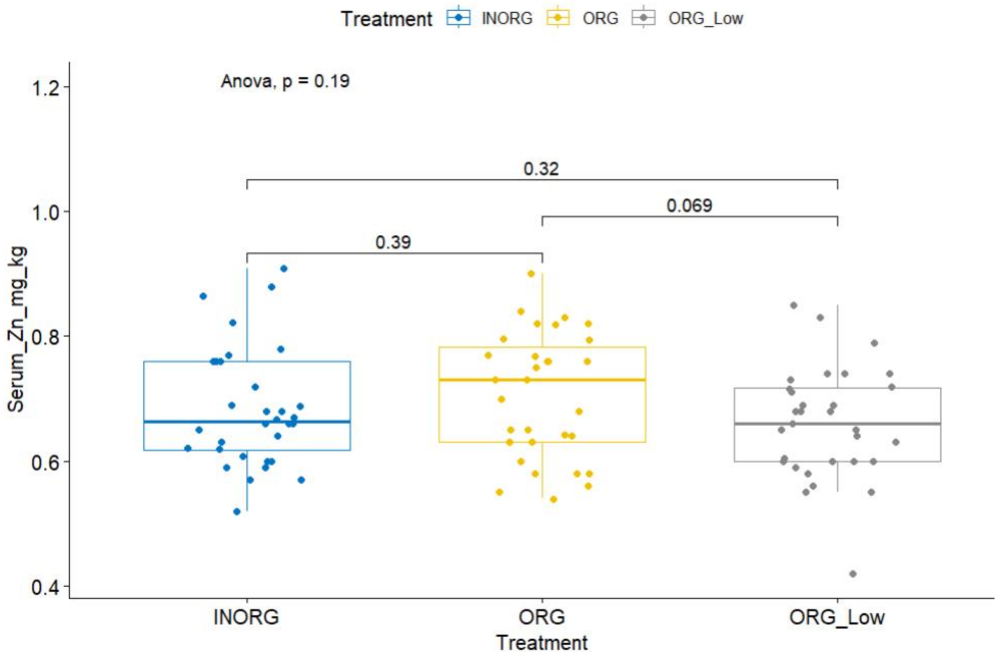

Figure 3. Influence of Zn source and supplementation level on serum Zn concentration.

The daily total fecal and urine outputs recorded in this study were not affected by the trace mineral supplementation, which is in line with the existing literature data (VanValin et al., 2018; Carmichael et al., 2019). The effect of trace mineral supplementation on nutrient digestibility has been reported in previous studies, however their outcomes are inconsistent when comparing ITM and OTM, while studies with reduced dosages of trace mineral supplementation are still lacking. In a recent *in vitro* fermentation study it was shown that a high dosage of ITM decreases DM degradability of forages (Vigh et al., 2023a). Nevertheless, when analyzing the outcomes in the present *in vivo* study, no significant effects were noted on nutrient (DM, OM, CP, NDF and ADF) digestibility when supplementing the animals with inorganic ($CuSO_4$, MnO and ZnO) or organic (Cu-, Mn- and Zn-glycinate) trace minerals. These findings are consistent with the results of VanValin et al. (2018), who showed that supplementing lambs with 40 mg of Zn/kg DM from inorganic ($ZnSO_4$), organic (Zn-methionine) or hydroxy (Zn-hydroxychloride) sources did not affect DM, OM, NDF and ADF digestibility. In another study (Alimohamady et al., 2019), it was found that the effect of Zn supplementation (30 mg/kg DM) on nutrient digestibility in sheep varies between mineral sources: Zn-methionine (OTM) and Zn-proteinate (OTM) increased CP, OM and ADF, but showed no effect on DM and NDF digestibility, while Zn-glycinate (OTM) did not affect nutrient digestibility when compared to a $ZnSO_4$ (ITM) supplementation, which is also consistent with the present study results. Similar to this, Gresakova et al. (2018) found no significant difference in DM, OM, CP, NDF and ADF digestibility when supplementing a high dosage of Mn (150 mg/kg DM) as $MnSO_4$ (ITM) or Mn-glycinate (OTM). In another *in vivo* study with crossbreed Angus steers (Guimaraes et al.. 2022), assessing the effects of trace mineral supplementation at levels of 10, 40 and 60 mg/kg DM of Cu, Mn and Zn as ITM ($CuSO_4$, $MnSO_4$ and $ZnSO_4$), HTM (Cu-, Mn- and Zn-hydroxychloride) or OTM (Cu-lysine, Mn- and Zn-methionine) on nutrient digestibility, the results showed that DM, NDF and ADF was downregulated, while CP digestibility was not affected by ITM when compared to HTM or OTM. These results are not in line with the outcomes of the present study, highlighting the inconsistency and difficulty in assessing the effects of various trace mineral sources on nutrient digestibility in ruminants.

Based on the increasing awareness of potential trace mineral pollutions from livestock production, one of the main strategies to reduce excretion to the environment highlighted in recent studies, is the replacement of inorganic minerals with a more bioavailable organic minerals when supplementing farm animals (Lu et al., 2017; Daniel et al., 2023). Replacing the inorganic sources of Mn, Zn and Cu with reduced amounts of organic sources supplemented to diets in the current study significantly reduced the total amounts of excreted trace minerals without compromising the mineral status of the animals. In a similar study by Creech et al. (2004), supplementing pigs from the nursery phase until the end of the growing phase with a 50 % reduction in the recommended TM levels, and in an organic form (as Mn-, Zn- and Cu-proteinates), resulted in a reduction in fecal concentrations of Mn, Zn and Cu by approximately 50%. The results obtained in the present study are in agreement with the above-mentioned outcomes, given that total fecal excretions (mg/day/animal) were reduced by approximately 25, 30 and 30 % for Mn, Zn and Cu, respectively. Based on the observations made in this study, the urine TM excretion (mg/day) contributed with <1.0% to the total mineral excretion, hence the reduction of Mn, Zn and Cu in the feces contributes the most to the reduction of mineral loss to the environment. These results are consistent with the outcomes of other studies assessing the TM excretion through urine and feces in ruminants (Gresakova et al., 2018; VanValin et al., 2018; Carmichael et al., 2019). Furthermore, considering that urine mineral concentration might be a good indicator of the mineral absorption (Ammerman 1995), and that the urine total TM excretion (mg/day/animal) was not affected by the treatments in this study, it could suggest that the animals were able to better absorb the OTM at a reduced dosage, compared to the ITM at the recommended dosages (Spears, 1996) in order to maintain the normal physiological status (Creech et al., 2004).

Regarding TM status of the animals in this study, serum Cu was significantly higher with the ORGLow ($p<0.001$), while with ORG tended ($p<0.10$) to be higher compared to INORG treatment. Due to the high sensitivity of sheep to Cu (Van Saun, 2023), the serum concentration showed that all the animals had an excessive Cu status (>0.68 mg/kg, (Noziere et al., 2018). Nevertheless, the results obtained in this study indicate that organic Cu has a higher bioavailability compared to inorganic Cu (Byrne and Murphy, 2022). Furthermore, it also confirms that a low dosage of organic mineral is better absorbed by the animals than inorganic Cu at recommended dosage (Creech et al., 2004). Regarding serum Zn, the highest values were obtained with the ORG treatments, indicating the higher bioavailability of the organic form. Furthermore, there were no significant differences between the ORGLow and INORG treatments, suggesting that the low dosage of organic Zn was at least equally valorised by the animals compared to the inorganic Zn at recommended dosage. These findings are consistent with the results of Nocek et al. (2006), who observed no significant effects on health, reproduction performances, milk production and mineral status of dairy cows when organic Mn (as Mn-methionine), Zn (as Zn-methionine) and Cu (as Cu-methionine) were supplemented at a 25 % reduced dosage in comparison to 100 % ITM (as $MnSO_4$, $ZnSO_4$, and $CuSO_4$, respectively) based on the NRC 2001 requirements for Mn, Zn and Cu (Council et al., 2001). The serum Zn concentrations observed in this study was below the normal physiological concentration range (<0.78 – 1.37 mg/kg) for sheep (Noziere et al., 2018), but above the deficiency indicator (serum Zn concertation <0.58 mg/kg), regardless of treatment. This observation could be explained by the high stress occurrence during the metabolic stalls, which may affect Zn metabolism through increasing fecal Zn excretion and decreasing serum Zn concentrations in lambs receiving supplemental Zn, as indicated by VanValin et al. (2020).

CONCLUSIONS

The results of this study suggest that supplementing sheep with a lower dosage of organic trace minerals (Mn-, Zn- and Cu-glycinate) compared to inorganic trace minerals (Mn- and Zn-oxide; Cu-sulfate) at nutritional systems' recommended levels significantly reduces the fecal mineral excretion without compromising the physiological mineral status of the animals. Nevertheless, further long-term studies are necessary to assess the mineral mobilization from body storages during supplementation with low dosage of organic trace minerals.

Author Contributions: A.V., S.P and C.G. conceived and designed the study; A.V. collected the data; A.V. wrote the original draft; A.V., S.P. and C.G. reviewed and edited the paper; A.V. and C.G. acquired the funding; A.V. administrated the project.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee nº052, France and registered under the number APAFIS#32360-2021070815571935 v3, September 30, 2021, by the French Scientific Research Ministry.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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