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Keywords: Beef calf; Trace mineral supplementation; Passive transfer; Innate immunity; Adaptive immunity; Health status



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## Article

# Effect of Injectable or Oral Trace Mineral Supplementation on Beef Calf Health Status and Growth

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**Simple Summary:** In France, it is common practice to supplement newborn beef calves with Selenium orally (OTMS). However, supplementing the animals with Se, Cu, Zn, and Mn through injection (ITMS) could offer additional benefits. This study monitored 600 newborn calves on two different trace mineral supplementation strategies from birth until D210 to assess their growth and health. The animals on ITMS had a significantly lower risk of developing omphalitis, and the cumulative incidence rate of all health issues was also significantly lower compared to those on OTMS. Additionally, calves supplemented by injection at birth, then on D30 and D60, needed less antibiotic treatment during the follow-up period. Overall, ITMS shows potential in reducing disease risks in newborn beef calves compared to oral Selenium supplementation.

**Abstract:** This study compared the relative effect of two trace mineral supplementation strategies recommended in France for newborn beef calves. 600 calves were supplemented with 20 mg oral selenium (OTM group) at birth (D0) or by injection (ITM group) of a multi-mineral solution (60 mg of Zn, 10 mg of Mn, 15 mg of Cu, 5 mg of Se) on D0, D30 and D60. Mortality and the incidence rate of diseases, including diarrhea, omphalitis, pneumonia, as well as medicinal treatments, were recorded from D0 to D210. The incidence rate of omphalitis was significantly lower in the ITM group than in the OTM group (respectively 11% vs. 17%,  $P = 0.036$ ). The cumulative incidence rate of all health troubles was lower in the ITM group than in the OTM group ( $P = 0.007$ ). Except for pneumonia, incidence of diarrhea (24% vs. 22%), use of oral (7% vs. 6%) or IV rehydration therapy (4% vs. 2%) or use of antibiotics (43.3% vs. 38.0%) and mortality (3% vs 2%) were numerically higher in OTM group than in ITM group (n.s.). In this study, ITM supplementation is as efficient as oral supplementation regarding calves' health status. It reduces the risk of omphalitis at the calf level effectively.

**Keywords:** beef calf; trace mineral supplementation; passive transfer; Innate immunity; adaptive immunity; health status

## 1. Introduction

*For newborn calves to survive and resist diseases, it is crucial that they develop a strong immunity quickly. Achieving early and adequate intake of top-notch colostrum is widely recognized as the single most important management factor in determining health and survival of the neonatal calf [1].* Authors have demonstrated that oxidative stress (OS) impacts the immune responses of newborn calves by affecting calf lymphocyte activation, cytokine expression, and antibody production [2,3]. Oxidative stress can be depicted as an imbalance between oxidants and anti-oxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage [4]. OS results from the excessive accumulation of pro-oxidants, such as reactive oxygen species (ROS) or free radicals, leading to cell membrane disruption and damage to proteins, lipids, and DNA [5–7].

After birth, the calf is exposed for the first time to an oxygen-rich environment once it starts to breathe and this results in an increase in the production of ROS [5], while the calf has a lower serum antioxidant capacity. Concurrently, it acquires passive immunity by consuming colostrum in the first 24 to 36 hours of life [8]. Colostrum is a source of immunoglobulins (Ig) and immune cells, including macrophages, which use ROS-generating systems to kill bacteria [5]. Colostrum is also a source of antioxidants [9] and ROS such as lipids or proteins. Therefore, it has been substantiated that colostrum's oxidative/antioxidative profile significantly affects the calf's oxidative status and serum IgG concentration [5]. These observations underline the importance of improving the calf antioxidant system to prevent diseases in the neonatal and weaning periods, one of the main reasons veterinarians and producers use antimicrobials on farms.

Trace minerals are essential in the calf's health status, performance, and immune system [10–13]. Some trace elements, including selenium (Se) [14,15], zinc (Zn) [16], copper (Cu) [17], and manganese (Mn) [18], are structural components of metalloenzymes involved in ROS neutralization (e.g., superoxide dismutase and glutathione peroxidase). Many studies have also substantiated direct links between trace minerals and enhanced cattle immune function [3,10,12,19]. It was indeed reported that Se, Zn, and Cu support the activity of various immune cells such as lymphocytes [16], neutrophils [14,20,21], macrophages [21], monocytes [22], B and T cells [17,18,20,23,24]. Therefore, authors have suggested that these trace minerals might elicit increased innate and subsequent adaptive immune responses in calves [3]. It has also been hypothesized that Mn may play a significant role in immune function in beef cattle [11].

Deficiencies in trace elements, especially selenium and iodine, are frequently reported in Europe [25]. In France, trace mineral supplementation in beef calves mainly consists of the administration of Se via the oral route. This method is also recommended elsewhere in the world, in other geochemical contexts such as California [26]. However, the digestive absorption of Se and other minerals is deemed to be low [27,28]. Injectable trace mineral (ITM) supplements combining several trace minerals, such as Se, Cu, Zn, and Mn, have recently become available on the market, allowing rapid availability and transport of trace minerals in the blood after injection [11]. Bates and others showed that ITM supplements reduce morbidity and mortality of dairy calves from birth to 140 days [29]. However, to the authors' best knowledge, there are no equivalent data for newborn and pre-weaned beef calves. Therefore, this study was undertaken to investigate the effects of trace mineral supplementations, when administered either orally or subcutaneously, on morbidity, mortality, health status, and growth in beef calves and then to evaluate the potential interest of ITM supplements compared to the oral Se supplementation, which is nowadays the primary way of supplementation in French cow-calf herds.

## 2. Materials and Methods

Twelve cow-calf operations were selected for this study. All participated in the "Bovins Croissance" program (stands for "cattle growth", in French), run by a French technical association promoting optimized beef herd management. All herds were located in the Auvergne area in the center of France. In the selected herds, the farm staff was capable of quickly tagging newborn calves, detecting and treating sick animals, recording health events, and maintaining farm records. Good animal welfare management was an inclusion criterion and complementary feed used in this study were approved for food-producing animals. At the end of the grazing season and before the start of the study (November), pregnant cows were fed a granulated feed supplement containing vitamins A, D<sub>3</sub>, E, C and B<sub>1</sub>, as well as Cu, Zn, Se, Co, I and polyphenol-rich plant extracts (Buffalo Tonic Axion®, Deltavit, France), according to the manufacturer's recommendation.

According to the herd's anticipated calving list, fifty pairs of cows and their calves to be born were enrolled in each farm from December 2020 to December 2021. The animals participating in the study remained in their environment and were intended for commercial purposes. Animals developing diseases during the study were treated according to the farm's standard treatment protocol.

The mineral status of the herds was assessed on pooled blood samples from ten cows selected at convenience, 30 to 45 days before calving, in December. The same animals were bled again in March after calving. Blood samples were analyzed for Cu, Zn, Se, I, GSH-*pxe*, and erythrocyte SOD levels (all tests by IODOLAB, Grézieu-la-Varenne, France).

The 600 selected calves were allocated into two groups of 300 animals, an oral trace mineral (OTM) supplement group and an injectable trace mineral (ITM) supplement group, using a randomization scheme of permuted blocks. Within one day after birth (D0), OTM animals received 20 mg Se (as sodium selenite) as tablets (Orosel®, Octavet, France). In the ITM group, animals were injected a multi-mineral solution (Multimin® Solution for Injection for Cattle, Warburton Technology, Ireland), containing Zn (60 mg/mL), Cu (15 mg/mL), Mn (10 mg/mL), and Se (5 mg/mL), at the labelled dose of 1 mL/50 kgBW at D0, D30 and D60, so that the animals in both groups received in total 20 mg of elemental Se during the study.

The follow-up period consisted in observing animal subjects from birth over 210 days. Farm staff had been trained prior to the start of the study to identify signs of disease and record their observations on scoring sheets. The study protocol did not require any changes in husbandry routines, and calves and dams were managed as usual. Calving difficulty was rated on a scale of 1 to 4, with 1 indicating unassisted birth and 4 indicating veterinary assistance. Diarrhea, omphalitis, and pneumonia were monitored individually and scored as present (1) when they occurred, or absent (0) at the end of the follow-up. Other conditions, if any (otitis, arthritis, interdigital phlegmon, etc.) were scored the same way but considering their very low incidence rate, all were confounded in a unique group (other diseases). The need for rehydration therapy and additional treatments represented an extra workload and was also recorded. It is difficult to determine the exact number of animals that became ill within each group since an animal could be dealing with multiple health issues, such as omphalitis, respiratory infections, and diarrhea. Each temporarily isolated incident requires an intervention on the part of the caregiver, whether the incident occurs on separate animals or on the same one, and health incidents can be counted independently of each other. We, therefore, did not consider the incidence rate (per calf-day at risk) but the cumulative incidence of health troubles for the total population at risk at the start of the observation period (2x300), and calculated a cumulated incidence of common conditions (diarrhea, pneumonia, omphalitis) on the one hand (MR1), and of all conditions on the other hand (MR2).

A technician of the local herd improvement organization “Bovins Croissance” weighed all the calves when they get 4 and 7 month-old. The body weight gain (BWG in g/day) was calculated for these two time points (BWG1, BWG2), respectively.

Passive transfer of immunity (PTI) was evaluated in the two groups between 2 and 7 days after birth by measuring the serum refractive index with an optical Brix refractometer and with an optical clinical refractometer (serum/urine), providing an estimation of IgG concentration (°Bx) and serum total protein concentration (STP, g/L). To distinguish between animals that successfully underwent PTI ( $\geq 8.4^\circ\text{Bx}$ ) and those for which transfer failed ( $< 8.4^\circ\text{Bx}$ ), a refractive index of  $8.4^\circ\text{Bx}$  was been established as the appropriate threshold value [30,31]. Measurement lower than  $< 8.4^\circ\text{Bx}$  was coded as 0, whereas others were coded as 1 (results treated as categorical variable). For STP, 52 g/L was considered the appropriate threshold [32,33].

The statistical tests used are indicated in the results section, when necessary. The Shapiro-Wilk test was used to assess the normal distribution. For all tests used in the present analysis, the significance level was set to 0.05. All calculations by Statgraphics Centurion version XVI.II software (Statgraphics Technologies, Inc., The Plains (VA), USA).

### 3. Results

#### 3.1. Population baseline

The baseline of the study population is reported in Table 1. Six hundred calves were of both sexes with sex ratio male calves:heifers of 106.9. Average body weight at birth was 42.2 ( $\pm 7.8$ kg) and 42.1 ( $\pm 6.7$ kg) for OTM and ITM calves, respectively. Most of the dams ( $> 59.7\%$ ) were at least third

parity beef cows. There was no significant difference between the two treatment groups regarding the parity distribution ( $P=0.184$ ). No assistance has been required for most parturitions (97,0 and 96,0% in the OTM and ITM group, respectively.  $P=0.871$ ). No twin calves were included in the study.

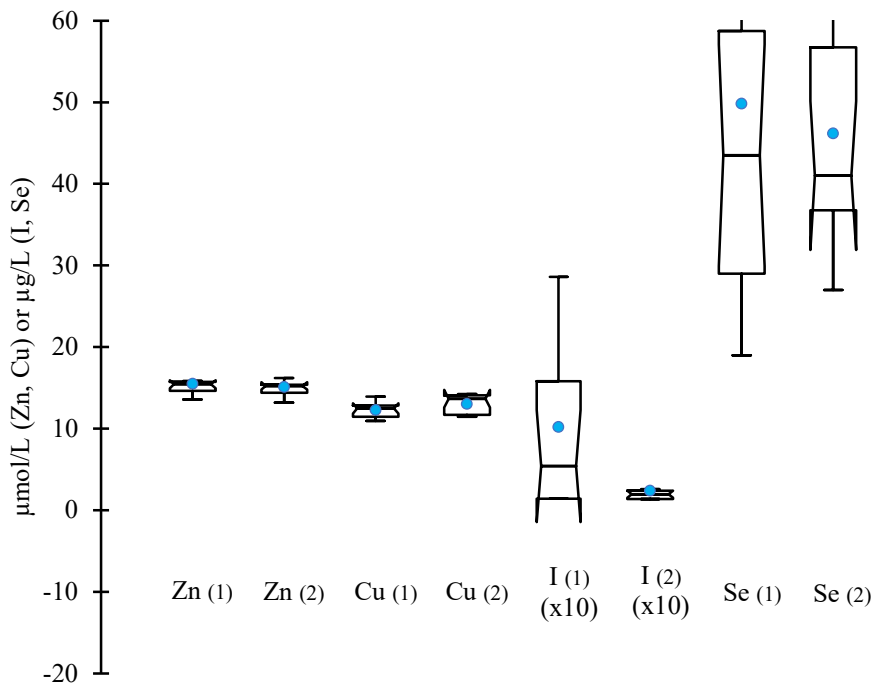
**Table 1.** Characteristics of the study population (dams and calves) per treatment group.

Characteristics	OTM Group	ITM Group	<i>P</i> value <sup>3</sup>
Number of pairs	300	300	
Breeds <sup>1</sup>			0.996
Aubrac	25 (8.3%)	25 (8.3%)	
Salers	118 (39.3%)	117 (39.0%)	
Salers x Charolais	157 (52.3%)	158 (52.7%)	
Sex (calf) <sup>1</sup>			0.567
Female	149 (49.7%)	141 (47.0%)	
Male	151 (50.3%)	159 (53.0%)	
Parity (dam) <sup>1</sup>			0.184
1	63 (21.0%)	46 (15.3%)	
2	58 (19.3%)	66 (22.0%)	
≥3	179 (59.7%)	188 (62.7%)	
Calving difficulty <sup>1</sup>			0.874
1	291 (97.0%)	288 (96.0%)	
2	5 (1.7%)	8 (2.7%)	
3	3 (1.0%)	3 (1.0%)	
4	1 (0.3%)	1 (0.3%)	
Colostrum density (°Bx) <sup>2</sup>	9.13 (±0.95)	9.19 (±0.86)	0.654
Serum Total Protein (g/L) <sup>2</sup>	58.3 (±7.50)	59.0 (±6.39)	0.473
Calf weight at birth (kg) <sup>2</sup>	42.2 (± 7.8)	42.2 (± 6.7)	0.868

<sup>1</sup>Number (percentage) of animals in each category. <sup>2</sup>Mean (± standard deviation). <sup>3</sup>*p* values calculated between treatment groups, using Chi-Square test.

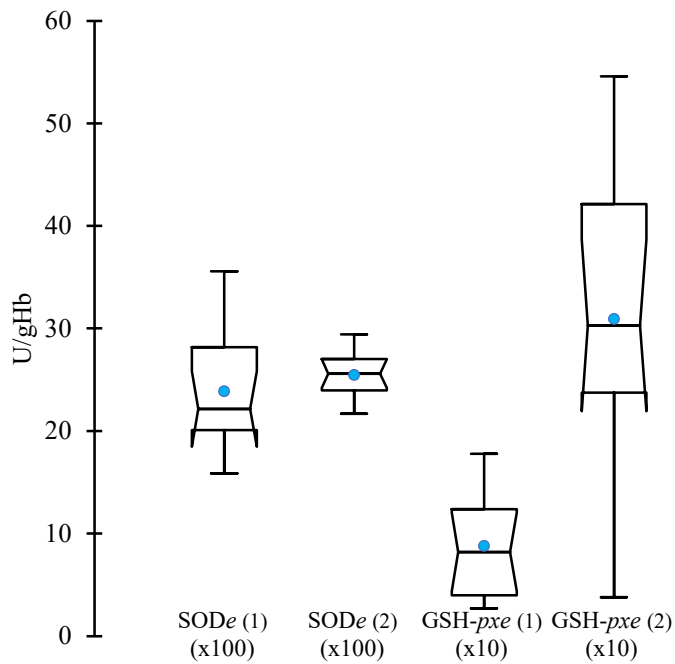
### 3.2. Mineral status

On both test days, before and after calving, the median plasma concentrations of Zn and Cu and their respective 95% confidence interval (CI<sub>95%</sub>) (Figure 1) fell within the typical range reported by the laboratory, with of 13.6-21.0 µmol/L for Zn and 11.8-18.0 µmol/L for Cu. Regarding inorganic I and Se, almost 33 and 41% of herds, respectively, displayed plasma concentrations lower than the typical values (>51 and 40-60 µg/L, respectively). In more than 1 herd out of 3, the Iodine plasma concentration was below the limit of quantification (15 µg/L).



**Figure 1.** Distribution of Zn, Cu, I (inorganic), and Se concentrations in pooled (n=10) plasmas, from twelve beef cow operations, at two (1 or 2) time points (same animals), three months apart. Horizontal segment in boxes represent the median and the two quartiles. The notch represent the 95% Confidence interval of the median. The blue dot stands for the arithmetic mean. Inorganic Iodine concentrations are divided by 10.

On the two test days, the median plasma activity of erythrocyte SOD and its CI<sub>95%</sub> overpassed the typical range reported by the laboratory, generally 1400-2500 U/gHb (Figure 2). Regarding the median GSH-*pxe* plasma activity, 11 values among 12 were below the lowest reference value (155 U/gHb) for the first test. The situation sharply improved on the second test day, with 11 values (out of 12) within the typical range (P<0.05).





**Figure 2.** Distribution of the activity of GSH-*pxe* and SOD-*e* enzyme in pooled (n=10) plasmas, from twelve beef cow operations, at two (1 or 2) time points (same animals), three months apart. Horizontal segment in boxes represent the median and the two quartiles. The notch represent the 95% Confidence interval of the median. The blue dot stands for the arithmetic mean. GSH-*pxe* and SOD-*e* activity are divided by 10 and 100, respectively.

### 3.3. Passive transfer of immunity (PTI)

In both treatment groups, the majority of calves had a successful PTI; failure of PTI ( $^{\circ}\text{Bx} < 8.4$ ), assessed within the first week after calving, was numerically lower ( $\chi^2$ -test,  $P=0.053$ ) in the ITM group (15.1%) than in the OTM group (21.1%). There was a strong correlation between  $^{\circ}\text{Bx}$  and STP in both groups, with a Pearson's coefficient  $r^2=0.893$  and  $0.939$ , in the OTM and ITM group, respectively. Furthermore, the logistic regression in the whole population showed that the incidence rate of diarrhea, death, and antibiotic treatment during the course of the study was strongly correlated with  $^{\circ}\text{Bx}$  ( $P < 0.01$ , all endpoints). There was however no such relationship between the  $^{\circ}\text{Bx}$  and the presence of pneumonia. These observations were confirmed by analyses performed by treatment group ( $P < 0.05$ , all endpoints). Simple regression analysis showed no significant relationship between  $^{\circ}\text{Bx}$  and parity in the whole population ( $P=0.262$ ), neither in the OTM group ( $P=0.181$ ), nor in the ITM group ( $P=0.841$ ).

### 3.4. Relationship between incidence of diseases and $^{\circ}\text{Bx}$

Based on logistic regression analysis of the entire population, a significant correlation was found between  $^{\circ}\text{Bx}$  and three variables: diarrhea ( $P < 0.001$ ), death ( $P < 0.001$ ), and antibiotic treatment ( $P < 0.001$ ). Additionally, one-way ANOVA revealed a significant difference in mean  $^{\circ}\text{Bx}$  between those without diarrhea (score 0) and those with diarrhea (score 1) ( $P < 0.001$ ). There was however no such relationship between the presence of pneumonia and  $^{\circ}\text{Bx}$  ( $P=0.285$ ).

These findings were consistent across both the OTM and ITM groups, indicating a relationship between  $^{\circ}\text{Bx}$  and diarrhea, death, and antimicrobial consumption, and a lack of relationship between PTI and pneumonia.

### 3.5. Diarrhea

The numbers of calves that experienced diarrhea in the OTM and ITM groups were close (n=72 and 66, respectively), and did not differ significantly ( $OR=0.89$ ,  $CI_{95\%}$  [0.69;1.31],  $P=0.628$ ), (Table 2).

**Table 2.** Odds ratio (OR) of clinical events reported by farm staff throughout the study period in the ITM to OTM group and 95% confidence interval.

Variable	OR (ITM/OTM)	95% CI	P value
Diarrhea	0.89	[0.61;1.31]	0.628
Omphalitis	0.59	[0.37;0.94]	0.035
Pneumonia	1.06	[0.66;1.72]	0.902
Other conditions	0.36	[0.14;0.94]	0.037
Death	0.59	[0.21;1.65]	0.447
Oral rehydration therapy	0.85	[0.44;1.63]	0.741
i.v. rehydration therapy	0.54	[0.20;1.47]	0.325

### 3.6. Omphalitis

Only 85 calves out of 600 experienced omphalitis during the course of the study (Table 2). Nevertheless, the odds of developing navel infection were significantly ( $P=0.035$ ) lower in the ITM group (11.0% with 33 calves) compared to the OTM group (17.3% with 52 calves).

### 3.7. Pneumonia

Twelve point three percent ( $n=37$ ) and 13.0% ( $n=39$ ) of the calves experienced pneumonia in the OTM and ITM groups, respectively (Table 2). The percentage of animals (0.67%,  $n=2$ ) that experienced a relapse of pneumonia throughout the study period, was very low and did not differ among treatment groups.

### 3.8. Other conditions

Finally, 5.3% ( $n=16$ ) and 2.0% ( $n=6$ ) of the calves in the OTM and ITM groups (Table 2), respectively, experienced another disease than those described above during the course of the study. ITM animals showed significantly lower odds of getting other diseases than the OTM animals ( $OR=0.36$ ,  $CI_{95\%}[0.14;0.94]$ ,  $P=0.037$ ). Other diseases included arthritis, coccidiosis, undifferentiated fever, meningitis, septicemia, tracheitis, and interdigital phlegmon (Table 3).

**Table 3.** Number (and percentage) of animals having presented another pathology recognized by farm staff throughout the study period, by treatment group.

Other conditions <sup>1</sup>	OTM group ( $n=300$ )	ITM group ( $n=300$ )
Coccidiosis	9 (3%)	4 (1.3%)
Interdigital phlegmon (footrot)	0 (0%)	2 (0.7%)
Arthritis	1 (0.3%)	1 (0.3%)
Fever	2 (0.7%)	0 (0%)
Meningitis	1 (0.3%)	0 (0%)
Septicemia	1 (0.3%)	0 (0%)
Tracheitis	1 (0.3%)	0 (0%)

<sup>1</sup>Number (percentage) of animals in each category.

### 3.9. Cumulative incidence of health troubles

Out of the OTM and ITM groups, the former reported 177 health incidents, while the latter reported 144. This difference of 11.0% in cumulative incidence (MR2) is highly significant ( $n-1 \chi^2$ -test,  $P=0.007$ ). However, focusing only on the three main conditions (MR1 - diarrhea, pneumonia and omphalitis), although the ITM group had a slightly lower number of health incidents, the two groups did not show a significant difference ( $P=0.087$ ).

### 3.10. Death

Death occurred in 3.3% ( $n=10$ ) and 2.0% ( $n=6$ ) of the animals of the OTM and ITM groups, respectively. Odds ratio calculation showed that the odds of dying during the study period decreased for ITM animals compared to OTM ones ( $OR=0.59$ ); however, the difference was not significant. The causes of death and their frequency are detailed in Table 4.



**Table 4.** Causes of death reported by farm personnel during the study, number of animals (and percentage) by treatment group.

Cause of death <sup>1</sup>	OTM group (n=10)	ITM group (n=6)
Diarrhea	4 (40%)	2 (33%)
Coccidiosis	2 (20%)	0 (0%)
Diarrhea and Pneumonia	1 (10%)	0 (0%)
‘Enterotoxaemia’	1 (10%)	0 (0%)
Meningitis	1 (10%)	0 (0%)
Omphalitis and Septicemia	1 (10%)	0 (0%)
Paralysis of unknown origin	0 (0%)	1 (17%)
Sudden death	0 (0%)	3 (50%)

<sup>1</sup>Number (percentage) of animals in each category.

3.11. Rehydration therapy

Among calves affected by diarrhea, clinical status required rehydration therapy by oral or i.v. route in 44.4% (n=32) and 36.3% (n=24), in the OTM and ITM groups, respectively. There were no significant differences between treatment groups regarding the need for rehydration, either orally or i.v. (Table 2).

3.12. Antibiotic treatment

Forty-three point three percent (n=130) and 38.0% (n=114) of calves in the OTM and ITM groups, respectively, required medicinal treatments, most of which were antibiotics (>99%). The need for additional treatments was not statistically different between treatment groups (Kendall’s *t*-b, *P*=0.196).

3.13. BWGs

Average BWG1 was numerically higher in the ITM group than in the OTM group [943.6 g/day (± 259.5) versus 929.1 g/day (± 254.6)], but this difference was not significant (Mann-Whitney Wilcoxon test, *P*=0.454). Later, mean BWG2 was 1052.7 g/day (± 180.9) in the OTM group and 1048.9 g/day (± 193.9) in the ITM group, and again the difference was not significant (*P*=0.826).

4. Discussion

This study was conducted in France to compare two mineral supplementation (TMS) methods for newborn calves, OTMS with a Se-rich *feed supplement* (EU Directive 2002/46/EC), or ITMS with a multi-mineral solution, registered as *veterinary medicinal product* (EU Regulation 2019/6). However, the study’s main drawback is the absence of a control group with untreated calves. The study involved twelve herds and confirmed the subpar levels of iodine and selenium in cow-calf herds in central France. Other authors in France and Europe have already acknowledged this situation [25,34]. Therefore, it is ethically questionable to leave newborn calves without specific supplementation for experimental demonstration purposes, and we have refrained from doing so.

During the last decades, numerous studies have demonstrated the benefits of oral TM supplementation on beef cattle immunity, health and performance [35]. While there is a lot of research

on the impact of selenium status in pregnant cows on the health of their calves, there is limited information on supplementing newborn calves with oral selenium. Only a few papers explore this topic, and most of them lack clinical ambition [36–41] while muscular degeneration in calves is the most specific manifestation of selenium deficiency [42]. Conversely, there is ample literature on injecting cocktail of trace elements into newborn calves, making it difficult to compare results. This suggests a need for more research on the oral supplementation of newborn calves in selenium.

An increase of the immunoglobulin concentration in calves has been demonstrated to be caused by a mineral selenium supplementation of cows before calving [39,43]. Many reports have already substantiated the importance of optimal calf mineral status in improving immune function during the first months of life [29,44–46]. In addition, other researchers have demonstrated that supplemental injections of Zn, Mn, Cu, and Se, increased humoral and cellular immune response and glutathione peroxidase activity in dairy calves [12,13]. Effects of selenium supplementation on the immune system of calves have been documented [40]. High concentrations of Se in serum result an increase of the phagocytic activity of macrophages in 30 day-old calves.

The results of the present study showed that most of the supplemented calves at birth had a good PTI the first week after calving, whatever the type of supplementation they received, even if previous reports stated that Se supplementation in colostrum enhances PTI [41,47]. Moreover, there was no significant difference between the two populations considering the PTI. There was a strong relationship between the incidence of diarrhea, death and antibiotic treatment and failure of PTI, as already recorded in other studies [12,29].

Otherwise, our findings regarding the strong correlation between °Bx and STP confirmed previous results in neonatal dairy calves showing that both endpoints nicely correlate over the first ten days of age [48]. On the other hand, we found no significant relationship between °Bx and parity in this study, while others reported significantly higher IgG concentrations in high-parity cows [49]. Unfortunately, however, there are few studies on beef calves.

Previous studies in dairy calves showed diverse incidence rates of diarrhea but were all in line with our results showing a positive effect of ITM supplement on diarrhea. Feldmann and others demonstrated a beneficial effect of oral zinc supplementation on diarrhea [50]. The frequency of diarrhea found by Bates et al. (2019) in dairy calves over the first 35 days was 4.9% in the multi-mineral ITM animals receiving supplementation within 24 hours after birth and 10.6% in non-treated ones ( $OR=0.44$ ,  $CI_{95\%}$  [0.24;0.82]). Teixeira et al.(2014) reported that ITM-treated dairy calves had decreased odds of diarrhea during the first 50 days of life compared to untreated ones (41.7% vs. 49.7%, respectively,  $OR=0.72$ ,  $CI_{95\%}$ [0.54;0.98]). However, by contrast with other studies, the incidence of diarrhea was not significantly different between groups. Differences observed among studies may result from different methods.

The chance of developing omphalitis was significantly lower in the ITM group, compared to the OTM group. This observation was similar to what observed previously in dairy calves [29]. Eleven percent and 17.3% of the ITM and OTM beef calves, respectively, developed omphalitis in our study, while Bates et al. (2019) documented an incidence rate of omphalitis of 2.6% in the ITM group and 5.0% in the untreated group with an  $OR=0.55$  ( $CI_{95\%}$ [0.39;0.77] within the first 35 days of life. Neither the sex ratio, calving difficulty, nor body weight at birth can satisfactorily explain differences between groups.

The incidence rate of pneumonia was low, without any significant difference between groups. In a paper published by Teixeira et al. (2014), the incidence of pneumonia was much higher (35.2% in the ITM group and 40.0% in the non-treated group) with an  $OR$  of 0.81 ( $CI_{95\%}$ [0.61;1.10]).

During the study, we have chosen to record occurrences of any other disorder than the most frequently experienced by calves (i.e., diarrhea, omphalitis, and pneumonia) as «other diseases». Compared to data reported in dairy calves where only diarrhea and omphalitis [29] or diarrhea, pneumonia, and otitis [12] were observed, beef calves faced other conditions such as coccidiosis, interdigital phlegmon, arthritis, fever, meningitis, septicemia, and tracheitis besides diarrhea, omphalitis, and pneumonia in our study. Surprisingly, the overall cumulative incidence of these minor diseases differed significantly ( $P=0.037$ ) between OTM and ITM animals, whereas frequent

perinatal diseases such as diarrhea and pneumonia did not show significant differences between groups.

The odds of dying for animals treated with ITM decreased compared to those treated with oral Se supplementation; however, this difference was not significant. The size of our study population and the low incidence rate of mortality could explain the lack of difference. Nevertheless, our observations are close to those reported by Bates et al. with an OR of 0.41 (CI<sub>95%</sub>[0.23;0.73]) for mortality in ITM-treated dairy calves compared to untreated ones within the first 35 days of follow-up, and 0.50 (CI<sub>95%</sub>[0.32;0.80]) from birth to 140 days of life [29]. However, Teixeira et al. (2014) reported disappointing results, with a death incidence of 3.8% of the ITM-treated dairy calves and 2.7% of the untreated calves with an OR=1.43 (CI<sub>95%</sub>[0.63;3.33]).

One may explain the discrepancy between studies by i) the fact that in other studies, the ITM group was compared to untreated animals, whereas in our study, the other group received oral Se supplementation, ii) the composition of the ITM used in the studies was different (Zn (60 mg/mL), Cu (15 mg/mL), Mn (10 mg/mL), and Se (5 mg/mL) in the present study and Teixeira et al. study vs. Zn (40 mg/mL), Cu (15 mg/mL), Mn (10 mg/mL), Se (5 mg/mL), and chromium (Cr) (5 mg/mL) in Bates et al. study), iii) the follow-up period was not similar between studies (210 days in our study vs. 140 days in the Bates et al. study and 50 days in the Teixeira et al. study, and iv) the dosage of ITM was different from one study to another (1 mL at D0 and D30 and then 2 mL at D60 in the present study whatever the calf's bodyweight vs. 1 mL at D3 and D30 in the Teixeira et al. study and 1 mL/50 kg at D0, D35 and D70 in Bates et al.).

The comparison of BWG1 and BWG2 between treatment groups did not reveal any significant differences in the present experiment. Se supplementation in calves generally has no influence on calf growth, although positive effects were reported when Se supplementation is performed in deficient calves [28]. Other reports have also described no effect of ITM supplementation on the rate of weight gain in dairy calves from birth to 140 days [29] or in beef calves from birth to 78 days [51], but in those two experiments, ITM group was compared to no oral specific supplementation group. Those observations are in contrast to other experiments where there was a tendency for ITM to increase overall average daily gain in beef calves [52] or in growing heifers [45], nonspecifically supplemented. The effect of ITM on the growth of stressed calves is also controversial. Genther and Hansen (2015) reported no effect of ITM in stressed calves during the growing period. In contrast, Richeson and Kegley (2011) described an increased gain-to-feed ratio in ITM-treated calves compared to their untreated counterparts [53].

The need for antibiotics was 5.3 points lower in the ITM group (38.0% vs. 43.3%), which is notable considering the relatively low incidence of diseases. However, the absence of statistical differences regarding the need for antibiotic therapy between groups may rely on the fact that in our study, we compared ITM- to oral-supplemented animals. Although this result emphasizes the critical role of Se in the maintenance of health status - every animal finally got 20mg Se -, results may have been different if supplemented animals compared to untreated ones; this hypothesis deserves further investigation. In their study, Bittar et al. (2018) suggested that using trace mineral supplementation to enhance natural immunity, and thus to reduce antibiotic usage, should be part of management strategies and preventive-medicine programs [3].

## 5. Conclusions

This study is the first to compare clinical benefits of an injectable multi-trace element (selenium, zinc, copper, manganese) supplementation with an oral Se supplementation in beef calves, two supplementation strategies commonly used in France.

This study again confirm the importance of the passive transfer on the incidence rate of diarrhea and mortality. However, the type of supplementation does not affect the passive transfer of immunity.

ITM supplementation effectively decreased the incidence of calf navel infection compared to Se administered orally. Furthermore, calves receiving a mixture of selenium, zinc, copper, and manganese by injection were less likely to develop diseases other than diarrhea or omphalitis than

those receiving Se supplementation in tablets. Animals supplemented by injection also needed fewer antibiotics than those orally supplemented.

Injectable multi-element supplementation shows critical advantages over oral Se administration. Therefore, animal caretakers should consider it part of procedures for managing health-threatening stressful periods such as the neonatal period and weaning in beef calves.

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