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# Cu, Zn and Se Deficiency of Sucked Veal Calves in the Western Massif Central of France

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**Abstract**: Sucked veal calves maintained under intensive conditions of farming, i.e. housed in individual boxes with muzzles produced a pale and tender meat that is greatly appreciated by the consumers. Recently the European regulations have ordered to modify the housing conditions, to introduce fibrous components in the milk based diet of these calves and to prohibit the use of muzzles. Since, the application of these new practices the farmers noticed the appearance of behavioural troubles, which are able to modify the quality of the meat of the calves. In order to investigate the possibility of a nutritional or biochemical origin for these behavioural modifications, we compared the serum levels of Cu, Zn and Se between a group of 30 Limousine sucked veal calves presenting troubles of behaviour and a control group (60 calves) which did not present any alteration of behaviour. The animals have been divided into 2 groups GT1 and GT2 with different techniques of calving and were compared within these 2 groups. They have been tested at 4 sampling dates (D15, D60, D90 and D120) after birth. The determinations of Cu, Zn and Se levels were performed by Electrothermal Atomic Absorption Spectrophotometry EAAS with a Zeeman background correction. The Cu values at D15 and D120 in the experimental group were significantly lower than those of the control group ($p<0.05$). No significant differences in Cu values were noted between GT1 and GT2 groups throughout the study. It was only at D90 in Zn and Se values that a significant difference ($p<0.05$) appeared for the group GT1 compared to the group GT2. The Zn and Se values of the experimental group were lower than those of the control group throughout the study with a significant difference ($p<0.01$) at D60 for Zn and during all the study for Se. A Cu and Se deficiency associated with sub normal levels of Zn especially at D60 may be involved in the behavioural troubles of calves under the new conditions of farming. The different conditions of farming of groups GT1 and GT2 does not involved in these abnormalities of behaviour. The importance of the maternal supplementation with trace elements during the late pregnancy may partially explain this syndrome. The Cu and Zn levels of the experimental group at D120 can explain the Cu-Zn antagonism.

**Key words:** Sucked veal calves, copper deficiency, zinc subcarence, Se deficiency, calf behaviour

## INTRODUCTION

The consumer is particularly attracted by a pale and tender calf meat. To produce such a pale meat, the sucked veal calves were maintained under intensive condition from birth to slaughtering at the age of 3-5 months at a liveweight of 110-170 kg. Some of them are nourished exclusively with milk of their mother and it acts of a typically French production. They were housed in small boxes of one calve, fastened and muzzled to avoid the ingestion of litter. The application of the recent European regulations on animal welfare obliged the farmers to change their practices: two or 3 calves by box, on straw, without muzzle. The most important change is the ability of the animals to take in fibrous food. This may have some effect on the quality of the meat, particularly the colour. Moreover, since the setting of the new regulations, behavioural troubles appeared, including sucking their

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congener, chewing and tongue playing and urine drinking. These behavioural modifications may cause a potential increase of the intake of iron and induce a partial blockade of the intestinal absorption of copper and zinc (Brewer *et al*., 1985; Hurley *et al*., 1983; Reinstein *et al*., 1984). Excessive intake of one of these elements may result in a deficiency of the other ones.

Recently there has been great interest in the determination of levels of trace elements in biological systems. Although, trace elements comprise less than 0.01% of the total mass of an organism, many are essential for the human and animal nutrition and for normal function (Fisher, 1975). They act indeed in the composition of enzymes or act like co-factors of antioxydant mechanisms (Levander, 1986). In the bovine species, production of milk and meat induced the oxidizing stresses which can lead to health disorders (Miller and Brzezinska- Slebodzinska, 1993; Valko *et al*., 2005). Pathologies associated with trace element deficiencies are abundantly described in the literature (Graham, 1991; Caramia *et al*., 1986; Leichtmannn and Sitrin, 1991; Aggett *et al*., 1985. Nevertheless, the majority of the clinical signs met at the time of trace element deficiency are not pathognomonic. The clinical diagnosis is generally confirmed by milk, urine or blood tests carried out in laboratory. The soil analyses are of little interest because the assimilation of the trace elements by the plants and then by the animals is complex and depends on many factors. Moreover, if relative deficiencies are present, the analysis of fodder will not allow the extrapolation of the trace element level to the animal. A trace mineral is considered essential if its withdrawal from the body induces the same structural and physiological abnormalities regardless of species (Fisher, 1975; Underwood, 1979). These abnormalities are accompanied by specific biochemical changes, which can be prevented or cured, once the deficiency is corrected (Underwood, 1979). Cu, Zn and Se are one of the fifteen trace minerals considered essential in mammalian nutrition (Ullrey *et al*., 1977). In enzymes, they act as a cofactor or as an essential part of the enzyme structure. They participate in ionic interactions affecting cell permeability and mineral matrix and act as direct catalysts in the promotion of reactions (Smart *et al*., 1981). These biochemical functions are frequently associated with metalloenzyme complexes or with metal ion activated enzymes (Fisher, 1975).

Cu deficiency in cattle is known to result in a loss of coat colour, anaemia, boen and cardiovascular abnormalities, low fertility, growth retardation and an increased susceptibility to infection (Underwood and Suttle, 1999). Zn, a constituent of many important enzymes, is one of the most important trace elements in the ruminant. Zn deficiency has been reported under practical conditions in cattle (Dynna and Havre, 1963), sheep and goats (Parasteriadis, 1973). In addition to its anti-oxidant activity, known as a cofactor of glutathion peroxydase (GPx), Se is involved in the general metabolism. Indeed, the conversion of thyroid hormone T4 to T3 is catalyzed by a selenoenzyme. Furthermore, it is also engaged in the prevention of cancers, in spermatogenesis and immunity (Whanger, 2004). The general roles of Cu and Zn in animal nutrition may be understood by evaluating physiologic changes associated with deficiency states of these essential elements. Deficiencies are associated with antagonisms and interactions from other elements and from chelating organic compounds as well as with primary deficiencies due to insufficient dietary levels (Fisher, 1975). In addition, interactions between mineral elements during pregnancy are affected by their respective levels in the mother's blood and the rate of transfer from mother to foetus. This is well known for Zn and Fe (Graham *et al*., 1994). The placental rate of transfer of Cu in humans (Nagel *et al*., 1986); Takacs *et al*., 1984) and rodents (McArdle and Erlich, 1991; Romeu *et al*., 1986) is naturally low and altered by Fe and Zn excess. Dietary imbalances in the mother may affect the foetus before any metabolic abnormality could be detected in the mother because of the relatively greater length of exposure of the foetus to this stress. Such an imbalance could specifically affect foetal Cu.

The early growth represents one of the most critical periods in life for the Cu supply because the rapid growth increases the Cu requirement, whereas diets based on milk provide low amounts of this element (Lönnerdal, 1996). Although, deficiency is the most prominent concern during this stage of life, there is also a high risk of toxic effects associated with the unability to handle higher Cu exposure because of immature liver function.

The aim of this study was to investigate the level out of the trace elements Cu, Zn and Se of sucked veal calves within 2 groups of calves GT1 and GT2. And to compare the statute of healthy calves to those obtained in breedings in which behavioural problem occurred; and to determine if the modified behaviours can be due to a disorder of metabolism of trace elements whether or not involving interactions between them.

## MATERIALS AND METHODS

**Animals**: The study took place in 10 farms in the western Massif Central (France). One hundred healthy calves were randomly divided into 2 groups: an experimental group of 30 calves (with behavioural troubles) and a control group

of 70 calves. The data concerning the non food oral behaviour which we described as troubles of behaviour including sucking their congener, chewing and tongue playing and urine drinking were collected in each farm by 3 types of observation. On the one hand by the stockbreeders, who had a recording card of the observations of the behaviours of their calves. This card had a double entry: an individual entry for each calf and an entry by date of observation. In addition, a device made up of a camera connected to a computer registered the monitoring carried out by the stockbreeders. This camera was installed in 3 different farms during 4 months. The device was installed in front of the calf boxes and functioned all day long. Finally, the observations of the technician during his interventions (blood test, measures of turn of chest) made it possible to collect information on the deviating behaviours. The calves selected for the study were born in July 2006. They were fed milk of their mothers twice a day. The calves were raised and studied in 10 farms, which is an average of 8±2 animals in each farm. At the same time, during the study, the stockbreeders were classified into 2 groups according to the techniques of breeding. The analysis of the constitution of the technical groups shows a homogeneous distribution of the stockbreeders and number of calves. Two groups called GT1 and GT2 differ on several points: strategy of iron administration, the diagram of supplementation in vitamins, trace elements and minerals, the level of isolation into the boxes compared to the possible sources of iron, the tendency to use the muzzle; different methods of breeding to reach a high quality product. GT1 consists of a mode of intensive breeding and very followed, while GT2, rather exclusive, whose stockbreeders are less attentive. Thirty eight percent of calves belonged to the technical group GT2 and 62% to the GT1. The various characteristics of these 2 groups of stockbreeders were classified as described in Table 1.

**Blood samples:** Blood samples were collected from all calves at 4 sampling dates (D15, D60, D90 and D120) after birth to assess trace elements levels in serum. They were collected via jugular venipuncture into $K_3$EDTA tubes specifically designed for trace mineral analysis ($K_3$EDTA-Metall-Analytik Monovette®, Sarstedt, Nümbrecht-Rommelsdorf, Germany). They were transported to the laboratory on ice. Less than 12 h after sampling, the tubes were centrifuged at 3000 rpm for 15 min at 4°C. The serum that was not used for fresh analysis was collected and stored in 5 mL polypropylene tubes (PP 55.1578, Sarstedt, Nümbrecht-Rommelsdorf, Germany) at -20°C for later analysis.

Table 1: The criteria conditions for breeding techniques of the two groups of farmers

| GT1 | GT2 |
|---|---|
| Systematization of Fe intake | more punctual Fe Intake |
| Systematization intake of hépatoprotectors | more punctual hépatoprotectors intake |
| Supplements Vitamins and oligoelements ++++ | Supplements Vitamins and oligoelements + |
| Level of isolation of boxes / Fe sources optimal | Isolation of boxes/ Fe sources more random |
| High tendency to use muzzle | Weak tendency to use the muzzle |

**Serum Cu, Zn and se measurements**

**Standard solutions and regents for trace elements measures:** All the chemicals used were of the highest purity available and all the glassware and plastic ware were nitric acid-washed and rinsed with ultrapure water obtained using a Milli-Q® advantage A10 ultrapure water purification system (Millipore, Molsheim, France).

**Serum Cu determination:** A standard solution of Cu was made with $CuSO_4$, $5H_2O$ (Merck Schuchardt-VWR, Fontenay sous Bois, France) dissolved in ultrapure water.

**Serum Zn and Se determination:** Commercial standard solutions of Zn and Se were used (Merck Schuchardt-VWR, Fontenay sous Bois, France) and diluted with 0.5% solution of Nitric acid 65% (Merck-VWR, Fontenay sous Bois, France).

**Serum samples:** The serum sample were deproteinized by diluting it 1: 200 for Cu with a 0.5% solution of Nitric acid 65% (Merck-VWR, Fontenay sous Bois, France), 1: 4 for Zn with a 10% solution of Nitric acid 65% and 1: 3 for Se with a 0.5% solution of TritonX100 (Merck-VWR, Fontenay sous Bois, France). A Pd solution (Merck-VWR, Fontenay sous Bois, France) at 0.5 g $L^{-1}$ was used as chemical solution of modification for Se measurements. The resulting Cu, Zn and Se concentrations were analysed by electrothermal atomic absorption spectrophotometry EAAS, following the procedures of Sabe *et al.* (2002) and the Association of Official Analytical Chemists (1990). For all analyses control standard solutions were run at the start, during and at the end of sample runs to assure continued accuracy. For most samples metal concentrations were well within the range of control standard concentrations. However, the accuracy (and potential contamination) of samples with very low concentrations meant that those with concentrations below a certain threshold were recorded as 'trace' concentrations, given that zero measurements are difficult to demonstrate. Overall, 350, 185 and 170 samples were analysed for each of the 3 metals Cu, Zn and Se, respectively.

Table 2: Furnace programme for Cu and Se determination in serum samples of calves

| Step | Temperature (°C) | Ramp (°C/sec) | Duration (sec) | Air flow ($mLmin^{-1}$) |
|---|---|---|---|---|
| Drying | 90 | 3 | 20 | 300 |
| | 120 | 5 | 10 | 300 |
| Ashing | 1100 | 50 | 20 | 300 |
| Atomising | 2100 | 0 | 3 | 0 |
| Cleaning | 2500 | 0 | 3 | 300 |

Table 3: The general parameters of analyses of the three trace elements Cu, Se and Zn in the serum of calves with the AAS

| | Serum Cu | Serum Se | Serum Zn |
|---|---|---|---|
| Mode instrument | furnace | Furnace | flame |
| Runner | FS90plus | FS90plus | - |
| Type of flame | - | - | air-$C_2H_2$ |
| Wavelength (nm) | 324.8 | 196.0 | 213.9 |
| Time of analysis (sec) | 3.0 | 3.0 | 4.0 |
| Mode of measurement | absorbance | absorbance | absorbance |
| Running lamp a | 60% | 60% | 75% |
| Number of measurements | 3 | 3 | 2 |

**Equipment:** A single-beam Atomic Absorption Spectrophotometer (Unicam 989QZ AA 6800 Spectrophotometer, Thermo Fisher Scientific, Waltham, USA) with a Zeeman background correction (Unicam GF90 furnace, Thermo Fisher Scientific, Waltham, USA) equipped with an FS-90 plus furnace autosampler was used in all the analysis. The measurements of serum Cu and Se concentration were performed using a hollow cathode Cu lamp for Cu concentration and hollow cathode Se lamp for Se concentration running at 15 mA, at a wavelength of 324, 8 nm for Cu and 196, 0 for Se. Serum Zn concentration was performed using a flame. Pyrolytically coated graphite tubes with built-in L'vov platform, argon as a purge gas at a flow rate of 200 mL $min^{-1}$ and stopped flow conditions during the atomisation were used. The Furnace programme for Cu and Se determination in serum samples of calves is shown in Table 2. The general parameters for analysis the 3 trace elements in the serum of calves are shown in Table 3. All samples were analysed at least 3 times.

**Statistical analysis:** The data is presented in figures as means±SEM. Statistical analysis of the results was carried out by One-way ANOVA analysis of variance using MINITAB version 13. A $p<0.05$ value was accepted as statistically significant (Ozdamar, 1999).

## RESULTS

The evolution of serum Cu, Zn and Se levels during the study of the 4 groups of calves is presented in Fig. 1-3.

Concerning serum Cu, no significant difference was observed between the 2 technical groups GT1 and GT2

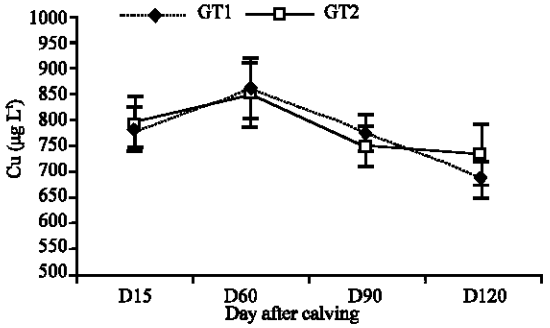


Fig. 1a: Variations of the serum Cu concentration (µg $L^{-1}$) in GT1 and GT2 group of calves during all the study

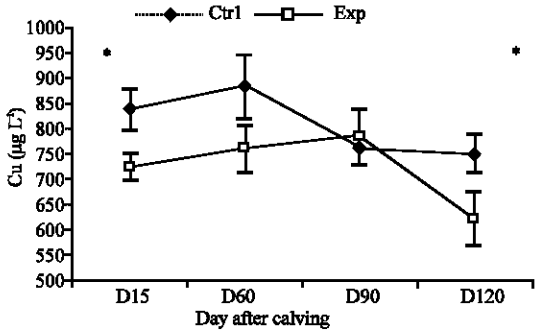


Fig. 1b: Variations of the serum Cu concentration (µg $L^{-1}$) in control and experimental group of calves during all the study. Results are means±SEM. The asterix indicates a significant difference ($p<0.05$) between the 2 groups

throughout the study. Concentrations are in limit of subcarence. We observed a significant difference in the same group GT1, between D60 and D120. Serum Cu was of 867±59 µg $L^{-1}$ at D60 and 690±34 µg $L^{-1}$ at D120 (Fig. 1a).

A significant difference ($p<0.05$) between the control group and the experimental group was found at D15 (840±43µg $L^{-1}$ vs 725±29µg $L^{-1}$). However, it had to wait D120 before to see again a significant difference ($p<0.05$) between these same groups of calves (750±35µg $L^{-1}$ for the control group vs 619±52µg $L^{-1}$ for the experimental group). The experimental group has remained below the threshold of failure (800 µg $L^{-1}$ Mee and Rogers (1996) and Enjalbert *et al.* (2006) throughout the study (Fig. 1b).

Concerning the serum concentration of Zn, there is no subcarence in any cases of figure. The Zn serum showed a different trend from the serum Cu. It was only at D90 that a significant difference ($p<0.05$) appeared for the group GT1 compared with the group GT2. The content of Zn was 1157±110 µg $L^{-1}$ for GT1 at D90 and

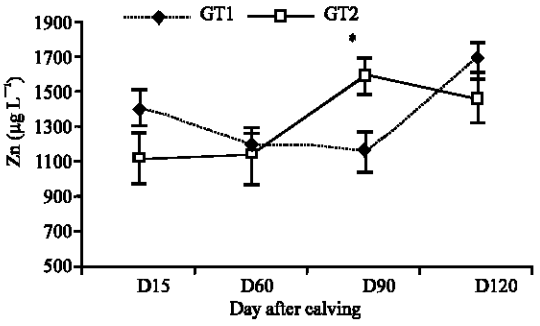


Fig. 2a: Variations of the serum Zn concentration (µg $L^{-1}$) in GT1 and GT2 group of calves during all the study

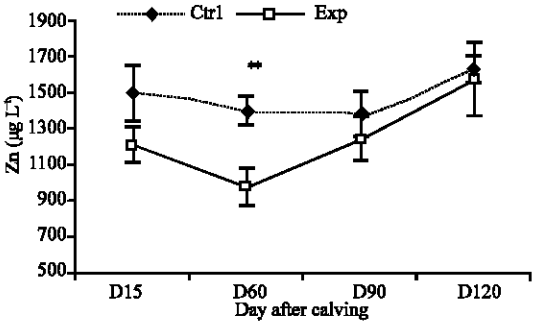


Fig. 2b: Variations of the serum Cu concentration (µg $L^{-1}$) in control and experimental group of calves during all the study. Results are means±SEM. One asterix indicates a significant difference ($p<0.05$) between the 2 groups. Two asterix indicates a significant difference ($p<0.01$) between the 2 group

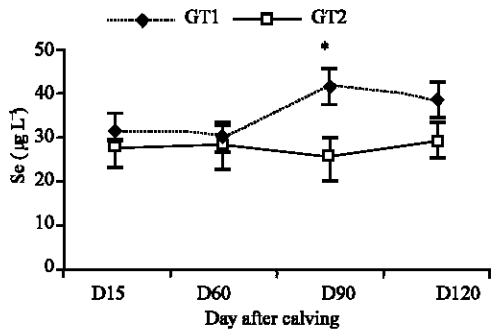


Fig. 3a: Variations of the serum Se concentration (µg $L^{-1}$) in GT1 and GT2 group of calves during all the study

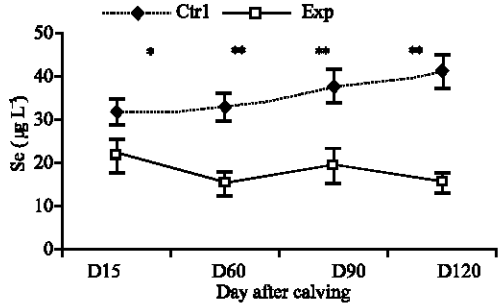


Fig. 3b: Variations of the serum Se concentration (µg $L^{-1}$) in control and experimental group of calves during all the study. Results are means±SEM. One asterix indicates a significant difference ($p<0.05$) between the 2 groups. Two asterix indicates a significant difference ($p<0.01$) between the 2 groupss

1587±113 µg $L^{-1}$ for GT2 at D90. Zn concentrations in serum were significantly different in the group GT1 between D15 and D120 ($p<0.05$; 1398±106 µg $L^{-1}$ vs 1684±93 µg $L^{-1}$) and between D60 and D120 (1193±77 µg $L^{-1}$ vs 1684±93 µg $L^{-1}$; $p<0001$) and between D90 and D120 (1157±110 µg $L^{-1}$ vs 1684±93 µg $L^{-1}$; $p<0.01$) and in the group GT2 between D15 and D90 (1125±143 µg $L^{-1}$ vs 1587±113 µg $L^{-1}$; $p<0.05$) and between D60 and D90 (1130±166 µg $L^{-1}$ vs1587±113 µg $L^{-1}$; $p<0.05$) (Fig. 2a).

The serum concentrations of Zn were lower for calves of the experimental group compared to calves of the control group throughout the study. There was a significant difference ($p<0.01$) at D60 between the control and experimental group (1384 ±81µg $L^{-1}$ vs 962±102µg $L^{-1}$). A significant difference ($p<0.05$) was observed between D60 and D120 for calves of the experimental group (1384±81µg $L^{-1}$ vs 1627±76µg $L^{-1}$) and between D60 and D120 for calves of the control group (962±102µg $L^{-1}$ vs 1570±199µg $L^{-1}$) (Fig. 2b).

As for the values of serum sélénémie, no significant difference between the 2 groups techniques GT1 and GT2 except D90 ($p<0.05$) with a sélénémie mean serum 38±3.7 µg $L^{-1}$ for GT1 and 28.8±4, 5 µg $L^{-1}$ for GT2. Se concentrations in serum group GT1 are slightly higher than the group GT2 throughout the study (Fig. 3a). Likewise for contents of Se the experimental group compared to the control group, they were significantly lower in the experimental group compared to the control group (for D15 $p<0.05$ and at D60, D90 and D120 $p<0.01$). Although, the values observed between the 2 groups were significantly different, they are all below the threshold of deficiencies which is about 70µg $L^{-1}$ in cattle (Rollin, 2003) (Fig. 3b).

## DISCUSSION

It was very difficult to have a good evaluation of the trace elements in young calf and the limit between normality and subcarence was very fuzzy for these 3 values Cu, Zn and Se. It is interesting to note that the differences between GT1 and GT2 are weak, whereas the methods of breeding are very different and in the same time, we note that the results (2nd column Fig. 1-3) between control group and experimental group present a tendency at divergence. This implies an independence of the methods of breeding in the assertion of the differences between control and experimental.

Trace elements deficiency occurs in cattle either as a primary or secondary problem. In a primary deficiency, there is a decreased level of trace elements in the diet. In a secondary deficiency, there is a failure of their absorption or utilization caused by an imbalance or excess of other elements in the ration (Graham, 1991). The level of dietary calcium, cadmium, zinc and the chemical form of copper in the diet also influence copper availability. As there is little placental transfer of copper, the calf is dependent on the copper in the colostrums as its initial source. Colostrum is the main source of minerals for newborn calves after parturition. As reported by Van Saun *et al*., 1989), the decline in maternal serum selenium at 8, 5 mo may be related to losses to colostrums or rapid fetal growth. As shown in Fig. 1, from 0-40 days after the birth, the level of trace elements is related to the statute of the mothers which can be deficient in trace elements, that's why we can explain the low levels of serum Cu with significant difference between the 2 groups. Kume *et al.* (1993) have studied the effect of parity on colostral mineral concentrations of Holstein cows and the value of colostrums as a mineral source for newborn calves. Colostral Fe, Cu and Mn were much lower than proper intakes for newborn calves. Mineral status of newborn calves does not depend only on mineral intake from colostrums but also on placental mineral transfer from the dam during gestation (Enjalbert *et al.*, 2002; Van Saun *et al*., 1989). As shown in Fig. 1.b, the significant difference ($p<0.05$) in serum concentration of Cu observed at D15 between the experimental group and the control one may not be linked to a deviation of behaviour. According to Abi-Rizk *et al.* (2008), the recordings of video capture confirm that the deviations do not exist at this age, they appear only later: the average age at appears of these deviations depend on the type of deviance. The first deviant behaviour observed is urine drinking that appears on average around 68 days and lasts about 2 weeks. The polyphagie appears to be slightly later, about 78 days and stay for 8 days. The last observed behaviour: the rolling the tongue, occurs at a later age or approximately 106 days and stay about 11 days. The standard deviation of age at onset of these deviations is quite high which suggests a high variability. On the other hand the evaluation group and therefore farming method tends to show that techniques for raising group GT1 seem to be more favourable to the development of deviant behavior (120 deviances) compared to that used by the GT2 (64 deviances) (Abi-Rizk *et al.*, 2008). In addition, the introdution or not of the muzzle, can not also explain the Cu deficiency because no significant difference was observed between the groups GT1 and GT2 during all the study, as shown in Fig. 1a. Hence the initial hypothesis on the origin of serum Cu deficiency is due to the placental and colostral transfer from deficient mothers in some trace elements.

Bostedt *et al.* (1990) determine the iron and copper values in the blood plasma of newly born calves. The copper values increase linearly and significantly from the birth onwards until the end of the first week, then remain on a high level. Our results on Fig. 1b show a linear increase of serum Cu and high level at D90 for the experimental group, then a decrease at D120. The level of copper in milk drops significantly 48 h after calving. After weaning there is a significant decrease in the absorption of copper from the small intestine. Generally less than 30% of the copper consumed is absorbed.

High levels of iron over extended periods of time have an influence on copper availability. Ground water or ingestion of soil, during period of high grazing are common sources of iron. Ruminants are often exposed to high iron intakes through ingestion of water, soil or metal barriers that are high in iron. Analyses on serum Fe by AAS were done and there were very high compared to the normal (data not shown). A number of studies indicate that addition of 250-1, 200 mg of iron (from ferrous carbonate)/kg of diet greatly reduces copper status in cattle (Barone *et al.*, 1998; Bremner *et al.*, 1987). High dietary iron did not affect copper status in young preruminant calves, which suggests that a functional rumen is needed for iron to interfere with copper metabolism. It is unclear whether the antagonistic effects of iron and molybdenum on copper are additive (Mills *et al.*, 1985).

Copper deficiency produces clinical signs which are generally related to its role as a catalyst or as an essential component of various metallo enzymes or metal activated enzyme systems (Arredondo and Núñez, 2005; Underwood, 1981). Copper is essential for erythrocyte production and the maintenance of their integrity in the circulation. In copper deficiency there is an impairment of iron release from the reticuloendothelial cells, because of

a decreased activity or production of ceruloplasmin and ferroxidase II. Thus, the iron is not available for erythrocyte production. The affected calves are usually stiff and lame with a marked swelling at the distal metatarsal and metacarpal physics. The biochemical lesion is a decrease in amine or lysyl oxidase; enzyme responsible for cross linkages of collagen and thus the stability and strength of bone (Hidiroglou, 1979). The clinical signs shown by the calves with Cu-deficiency, were, namely, poor weight gain/weight loss, poor haircoat, pale mucous membranes, anemia and neonatal ataxia (Tiffany *et al*., 2002). Impaired immune competence is the likely result of Cu deficiency in cattle (Boyne and Aurthur, 1981; Xin *et al*., 1991). Cu deficiency has a direct impact on the ability of cattle to mount a normal response to viral infection. Calves with Cu deficiency have lower percentages of lymphocytes than control calves and tend to show a decreased cytokine response to disease challenge (Gengelbach *et al*., 1997). In copper deficiency, there is a breakdown in the conversion of tyrosine to melanin because of reduced amino oxidase activity. Myocardial degeneration and fibrosis associated with acute heart failure have been described. This has been associated with a reduction in cytochrome oxidase. The scouring associated with copper deficiency has been also related to the depletion of cytochrome oxidase in the small intestine resulting in partial villus atrophy. Cu-deficiency contributed to the development of a general matrix osteoporosis and overgrowth of the epiphyseal cartilage (Suttle and Angus, 1978).

No figures of subcarence concentrations in serum Zn were observed. This means that if we find any significance between 2 groups, we are not certain of the role of this trace element. A significant difference was mainly an indicator role, but not a diagnosis one. Numerous factors will influence the zinc level in blood, making serum or plasma determinations an unsuitable method for diagnosing zinc deficiency. Hemolysis of a blood sample increases the zinc level; stress to the animal and physical illness decrease the plasma zinc levels (Underwood, 1979). According to researchers', the zinc in blood could be fixed at the rubber of the cover of propylene tubes or vice versa and thus consequently, proportioning could be distorted. Comparing to the references, the serum Zn values seem to be higher for the 2 groups of calves because some of our sample were hemolysis.

The reduction of serum Cu of calves was concomitant with an increased serum Zn. This observation is consistent with the well-described Cu-Zn antagonism, occurring at the absorption site and at the intracellular level (Carroll *et al*., 2004; Bremner *et al*., 1981). The role of the placenta during maternal excessive Zn exposure indicates that this organe may be affected by the maternal mineral intake and hence alter the transport of the essentials mineral to the foetus (Mills, 1985). As shown in the introduction, deficiencies are associated with antagonisms and interactions from other elements and from chelating organic compounds as well as with primary deficiencies due to insufficient dietary levels (Fisher, 1975). As shown in Fig. 1 and 2, for the experimental group at D120, low level of Cu serum (619±52 μg $L^{-1}$) concominant with a high level of Zn serum (1570±199 μg $L^{-1}$) with ratio at 0.4 in comparison with the control group at the same date, with a ratio of 0.65.

At the foetus state compared to the adult, Se in blood is mainly present in the pool erythrocyte, with a smaller proportion in the serum (Van Saun *et al*., 1989; Kirk *et al*., 1995). This trend is clearly visible after birth, with newborn calves with literacy rates serum Se lower than those of adults, although the concentration of Se in whole blood is similar. As shown by Van Saun *et al*. (1989), in contrast to foetal hepatic selenium being consistently higher than maternal hepatic selenium, foetal serum selenium was consistently lower than maternal serum selenium. This maternal difference may potentially increase the concentration gradient between dam and foetus to facilitate placental transport. Shariff *et al*. (1984), determined bidirectional placental transfer of selenium with an efficiency exchange from dam to foetus of 2% and for foetus to dam, 1% suggesting the possibility that serum selenium is the fraction of maternal blood responsible for placental transfer. The maternal serum selenium pool is also hypothesized to supply selenium for colostrums, as suggested by the rapid decline in maternal serum selenium values the last month of gestation (Van Saun *et al*., 1989). The Se concentration in the serum remained low in young animals during the feeding period as the milky Se concentration in the milk is generally low. However, the form of Se ingested by the mother varies greatly content Se in the milk, with more Se in milk when the mother consumes a source of Se Organic (Knowles *et al*., 1999; Ortman and Pehrson, 1999; Pehrson *et al*., 1999; Juniper *et al*., 2006). The serum concentration of Se increases then as soon as calves are eating solid foods, as long as they were not deficient Se. The calves, introduced for this study, were fed only on the milk from their mothers twice daily. No solid foods were attributed into their ration except for some multivatiminic and mineral additives. This will explain the lower values of serum Se which are all under the threshold of deficiency which is 70 μg $L^{-1}$ in cattle (Rollin, 2003). Se deficiency contributes to the emergence of growth and also to the influence of a lack of conversion of thyroxine

(T4) in the tri-iodothyronine (T3) (Arthur *et al*., 1988; Graham, 1991; Larsen and Berry 1995). The Se concentration in the blood of newborn calves are correlated with that of their mothers (Hidiroglou *et al*., 1987; Kincaid and Hodgson 1989; Awadeh *et al*., 1998; Enjalbert *et al*., 1999). During the last quarter of gestation, large amounts of Se are transferred from mother to the foetus (Van Saun *et al*., 1989). The status Selenic calf depends more on the placental transfer that transfer via the colostrum (Enjalbert *et al*., 1999). If the mother doesn't take at least 3 mg Se per day (an operation between 0.2 and 0.3 ppm, ingesting MS) in the last trimester of gestation, serum Se at the mother is reduced (Abdelrahman and Kincaid, 1995). The low concentration observed in Se of the mother at calving, due to the transfer of Se to the foetus, gradually increases during the first month of lactation (Miller *et al*., 1995). As shown in Fig. 3a with a linear increasing of serum Se level for GT1 and in Fig. 3b for the control group. It was not the same for GT2 with lower concentrations than GT1 during all the study due to the difference on supplementation in vitamins, trace elements and minenal which is lower than GT1 and the lower level of isolation into the boxes compared to the possible sources of iron and the lower tendency to use the muzzle.

## CONCLUSION

The mineral balance of calves is a fundamental pillar of the performances of growths. It thus appears interesting to improve or optimize the growth of calves to carry out certain measurements making it possible to evaluate the proportioning of blood minerals to highlight subcarences, deficiencies or excesses and thereby adapting as well as possible the strategies of mineral complementation of the animals. In addition the data collected in each breeding is added to a base of bench-mark data specific to the calves, which will make it possible to refine knowledge in this field to provide to the stockbreeders more precise councils for the production of sucked veal calves.

However, according to the literature, it would seem that the mineral reserves of new borns are constituted mainly during gestation (transplacental transmission) and also by coclostral way. The colostrum seems to be concentrated much more on the mineral level than the milk which presents less important fluctuations. The complementation and the deficiency statute of the mothers during gestation associated with a good colostrale transfer with calf as soon as possible after the birth seem to be fundamental factors to optimize the level of the mineral reserves of young calves. We can conclude that the status of serum Cu, Zn and Se in the newborn depends on the status in trace elements of the mother because low levels of this element in the mother produce low levels in the cord blood.

We can wonder about the difference between the 2 groups control and experimental at the birth whereas there is not between groups GT1 and GT2. The authors seek to explain this paradox, in particular by a differential transfer of trace elements and placenta, perhaps related to the foetal suffering at the end of gestation.

To treat deficiencies in trace elements, the aim is to maintain adequate blood levels either by increasing the dietary intake or by parental trace elements injections. When deciding on the method and amount of supplementation the practitioner must determine whether a primary or secondary deficiency exists. And finally, evaluation of trace element status can be difficult because many disease states will alter blood analytes used to evaluate nutrient adequacy. Proper dietary and animal evaluation, as well as response to supplementation, are necessary before diagnosing a trace element deficiency.

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