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ORIGINAL ARTICLE

Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study

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Summary

A retrospective study using analysis of plasma copper and zinc, and erythrocyte glutathione peroxidase from 2 080 dairy and beef cow herds was conducted to evaluate the relationship between trace-element status and production, reproduction and health in cows and their calves. Classification of the herd status as deficient, marginal, low-adequate or high-adequate was based on the lower tercile of individual values. Odds ratios for each disorder in herds were calculated by multivariable stepwise logistic regression. Inadequate copper status was not associated with adult disorders, but was an important risk factor for poor calf performance or health. Selenium deficient status was associated with most studied disorders in cows, and both deficient and marginal herd status were strongly associated with poor health of calves, particularly with increased risks of myopathy and infectious diseases. Zinc insufficiency was strongly associated with low milk production and impaired locomotion in dairy herds, and was also associated with diarrhoea and poor growth in calves. Because a low-adequate status increased the risk of many disorders in adults and calves, we propose to classify herds as deficient and marginal when the lower terciles of plasma zinc concentration are below 12 and between 12 and 14 μmol/l respectively.

Introduction

Trace elements deficiencies have been observed and studied in France for more than 30 years. First studies (Lamand and Perigaud, 1973) showed that hays were deficient in copper, cobalt, zinc and selenium, and frequently deficient in manganese and iodine. Recently, maize silage, which is one of the most common forages for dairy cows, has been shown to have very low concentrations of trace elements (Beguin and Dagorne, 2003). Recommendations for trace element supplementation first focused on the prevention of reduced productivity and clinical signs of deficiencies, but the role of trace elements in immunity has been emphasised in further studies which have been reviewed recently (Gaylean et al., 1999; Spears, 2000).

The mineral supplementation of diets is almost ubiquitous in lactating adults in dairy herds, but is much less routine in dairy heifers and dry cows, or in beef herds. Moreover, the trace mineral status of animals depends not only on dietary allowance, but also on the efficiency of digestion and storage, which both can be affected by interactions with other food constituents. Hence, trace elements deficiencies are often suspected by veterinary surgeons in low performance herds, and when assessed, a deficient status is considered as the likely cause of disorders.

However, most of these herd disorders have a multifactorial origin, and the importance of individual trace mineral deficiencies as risk factors has not been evaluated in commercial herds. This retrospective study has been based on a data set of analysis in dairy and beef herds over several years. The objectives were to estimate the relationship between copper, zinc and selenium status of herds, and production, reproduction and health, and to assess the accuracy of thresholds for classification of herds.

Materials and methods

Origin of data

Data from 10 325 animals in 2 080 commercial herds in France and Belgium (997 dairy herds and 1083 beef cow herds), were recorded over five years (1998 to 2002). No information on the intakes of copper, zinc and selenium was collected. Samples from healthy animals were collected and sent by veterinary surgeons. At least three animals per herd were tested (average 5.0). Case herds justified investigations by productive, reproductive or health abnormalities. Up to five disorders could be indicated for each herd (average 1.5). The 177 herds for which investigations were requested without any disorder were taken as control herds; such investigations can be requested for routine control of status within a framework of preventive medicine. Among these 177 herds, assessments of copper, zinc and selenium status were performed in 125, 131 and 158 herds respectively.

Choice of criteria, analysis of samples and selection of data

Zinc status was assessed by plasma zinc analysis. Plasma zinc is the most widely used indicator of zinc status in animals, but other indices have been proposed. However, bone, which is the main storage site, can only be sampled postmortem, and hair zinc concentration cannot be considered as a sensitive diagnostic criterion (Underwood and Suttle, 1999).

Copper status was assessed by plasma copper analysis. Hair copper as an indicator of copper status is too slowly responsive to intake and lacks reference standards (Kincaid, 1999). Liver copper is an accurate indicator of copper depletion, but sampling is difficult and cannot be performed on large populations of animals.

Selenium status was assessed by erythrocyte glutathione peroxidase activity (eGSH-Px).

The methods of determination have been already described for copper (Enjalbert et al., 2002), and eGSH-Px (Enjalbert et al., 1999). The determination of plasma zinc was done with a similar procedure to plasma copper. Copper and zinc were analysed in the same laboratory, and eGSH-Px was assayed in a different laboratory.

High blood values of plasma copper are usually the reflect of inflammation or infection, not of dietary supply above recommendations (Underwood and Suttle, 1999), so that individual plasma copper values over 18 μmol/l were not considered in this study. Because infection reduces plasma zinc (Shankar and Prasad, 1998), individual plasma zinc values below 10 μmol/l were not considered when associated with high plasma copper.

Criteria for classification of herd status

Herds were classified as having deficient, marginal or adequate status according to the lower tercile of individual values observed for each parameter. For copper and zinc status, we used the threshold values proposed by the review of Kincaid (1999). Because of important variations between laboratories, there is no consensus among authors for eGSH-Px cut-off points, so that we based our thresholds on the values of serum selenium concentrations proposed by Pehrson et al. (1999) from a review of literature, and the relationship observed between eGSH-Px and serum selenium in our laboratory. This relationship (eGSH-Px $= 2.88 \times$ selenium $+0.60$, $r^2 = 0.69$) was established with 112 samples from steady-state animals, ranging from 7 to 378 units of eGSH-Px/g of Hb, and from 5 to 103 μg of selenium/l of serum.

Because herds with nearly one-third of animals having marginal or deficient status were considered adequate with this tercile-based classification, we divided the adequate status in low-adequate and high-adequate status. The chosen thresholds led to a mean percentage of marginal plus deficient animals around 16% in low-adequate herds and 3% in high-adequate herds.

Threshold values are shown in Table 1. Because only 15 herds were zinc deficient, they were considered together with marginal herds.

Determination of odds ratios for each abnormality

For each disorder studied, odds ratios were calculated with Systat (Version 9, SPSS Chicago, IL, USA), using multivariable stepwise logistic regression model with a backward selection procedure where the significance to remove was 0.10. Copper, zinc and selenium status were the independent variables and disorders were the dependent variables. With

Table 1 Thresholds for classification of herds in categories of nutritional status according to the lower tercile of individual values

Status	Deficient	Marginal	Low-adequate	High-adequate
Plasma copper* (μmol/l)	<8	8–11	11–13	13–18
Plasma zinc* (μmol/l)	<8	8–12	12–14	14–21
eGSH-Px† (units/g of Hb)	<75	75–150	150–220	220–600

*Adapted from Kincaid (1999).
†Erythrocyte glutathione-peroxidase activity.

this backward selection procedure, only significant ($p < 0.10$) effects are in the final regression step, so that odds ratios of other independent variables are not calculated.

Results and discussion

The distribution of lower terciles of control and case herds for each abnormality is shown in Table 2. Odds ratios for adult and calf disorders are presented in Tables 3 and 4 respectively.

Copper status

None of the disorders observed in adult cows could be attributed to low plasma copper. Tolerance of adult cattle to plasma copper values between 3 and 9 μmol/l has already been outlined (Underwood and Suttle, 1999). In our study, copper deficiency assessed by a lower tercile of plasma copper below 8 μmol/l did not increase the risk of low fertility or other reproduction disorders. Suttle (1993) previously reported that infertility is rarely due to copper deficiency. Poor fertility can be due to a lot of nutritional imbalances, particularly energy deficiency in dairy (Jorritsma et al., 2003) and beef (Petit and Agabriel, 1993) cattle, which could hide possible but small effects of copper deficiency. Moreover, plasma copper alone is less accurate than the caeruloplasmin to copper ratio for identification of copper responsive infertility (Kendall et al., 2003), which suggests that infertility is more largely related to secondary deficiencies due to excess molybdenum than to primary deficiencies. The earliest sign of copper deficiency is depigmentation and harsh hair (Underwood and Suttle, 1999), but this relationship was not observed in the present study.

Table 2 Distribution of the lower tercile of plasma copper, plasma zinc, and erythrocyte glutathione-peroxidase activity (eGSH-Px) in control vs. case herds (number of herds, lower quartile, median and upper quartile)

	Plasma copper (μmol/l)		Plasma zinc (μmol/l)		eGSH-Px (units/g of Hb)	
Control	125	11.1, 12.6, 13.8	131	12.8, 14.3, 15.6	158	135, 231, 389
Case						
Adults general and production disorders						
Poor hair condition*	86	10.3, 12.1, 13.6	86	11.3, 13.0, 15.0	84	58, 213, 381
Poor body condition*	58	10.6, 12.2, 13.2	59	11.1, 12.5, 13.8	64	53, 141, 270
Low milk production†	117	11.6, 12.7, 13.8	124	10.9, 11.8, 12.8	129	95, 210, 367
Reproduction disorders						
Low fertility*	672	10.6, 12.5, 13.6	691	12.2, 13.5, 14.8	711	73, 205, 379
Abortion*	60	9.9, 11.2, 13.3	63	11.5, 12.5, 13.6	71	38, 93, 259
Retained placenta*	131	9.9, 12.0, 13.8	134	11.5, 12.7, 14.0	158	45, 107, 266
Adult other health disorders						
Metritis*	61	11.2, 12.7, 13.8	66	11.9, 13.4, 14.5	71	59, 162, 374
Mastitis†	126	12.3, 13.2, 14.3	131	12.4, 13.3, 15.0	153	161, 255, 403
Impaired locomotion†	89	11.8, 12.9, 14.1	101	11.2, 12.5, 13.7	102	143, 260, 418
Calves production disorders						
Growth retardation‡	99	4.2, 7.6, 10.3	99	11.1, 12.1, 13.2	101	32, 57, 110
Calves other health disorders						
Perinatal mortality*	190	6.0, 9.2, 11.2	191	11.6, 13.0, 14.1	233	26, 52, 96
Diarrhoea*	437	8.0, 10.2, 12.3	452	11.7, 12.9, 14.2	505	34, 65, 121
Vaccination failure‡	130	6.1, 9.2, 11.4	132	11.3, 12.5, 13.6	144	39, 51, 98
Myopathy‡	63	9.1, 10.1, 11.7	62	12.0, 13.5, 14.6	96	18, 33, 61
Heart failure‡	45	4.4, 8.7, 10.9	46	11.5, 12.7, 13.8	51	27, 63, 100

*Beef and dairy herds.
†Dairy herds.
‡Beef herds.

Table 3 Odds ratios for adult disorders associated with status of herds

Herd status	Number of case herds	Copper			Zinc		Selenium		
		Deficient	Marginal	Low adequate	Deficient or marginal	Low adequate	Deficient	Marginal	Low adequate
General and productive disorders									
Poor hair condition*	78				4.19[d]		2.80[b]		
Poor body condition*	54				3.89[d]		3.55[c]		
Low milk production†	114				44.00[d]	6.53[d]			
Reproduction disorders									
Low fertility*	643				1.80[a]		2.46[c]		
Abortion*	59				3.32[c]		6.34[d]	2.59[b]	
Retained placenta*	126				6.42[d]	1.98[b]	5.93[d]		
Other health disorders									
Metritis*	59				2.67[b]		2.67[b]		
Mastitis†	117				3.15[a]	2.40[b]		3.25[a]	2.49[a]
Impaired locomotion†	84				16.99[d]	3.88[c]			

[a]$p < 0.10$; [b]$p < 0.05$; [c]$p < 0.01$; [d]$p < 0.001$.
*Beef and dairy herds.
†Dairy herds.

Table 4 Odds ratios for calves disorders associated with status of herds, estimated through measurements on cows

Herd status	Number of case herds	Copper			Zinc		Selenium		
		Deficient	Marginal	Low adequate	Deficient or marginal	Low adequate	Deficient	Marginal	Low adequate
Production disorder									
Growth retardation†	96	10.88[d]			6.09[c]	3.23[b]	5.30[c]		
Health disorders									
Perinatal mortality*	180	3.98[b]			3.82[c]		30.77[d]	5.42[d]	
Diarrhoea*	427	3.63[b]	1.76[a]		3.03[c]	1.65[a]	13.48[d]	3.63[d]	
Vaccination failure†	129	5.05[c]					15.37[d]	2.72[b]	
Myopathy†	60						77.5[d]	7.29[b]	
Heart failure†	44	9.41[c]	2.45[a]				5.56[d]		

[a]$p < 0.10$; [b]$p < 0.05$; [c]$p < 0.01$; [d]$p < 0.001$.
*Beef and dairy herds.
†Beef herds.

Low plasma copper in cows increased the risk of health disorders or growth retardation in calves. The relationship between diarrhoea and copper deficiency has been mainly described in animals grazing on molybdenum-rich pastures. In the present study, copper deficiency in cows increased the risk of diarrhoea in calves, even in calves whose dams had been vaccinated against the main viral and bacterial agents responsible for neonatal enteritis. The faetus has priority for copper when the dam is copper deficient so that newborn calves from copper deficient cows have a normal copper status (Enjalbert et al., 2002). Consequently, diarrhoea and perinatal mortality could be related to impaired immune function of the dams, decreased immune transfer or decreased immune function of young animals. However, the effect of copper status on immunoglobulin content of colostrum has not been extensively studied, although Ward et al. (1997) failed to show a clear relationship. Moreover, studies on the effect of copper status on humoral immune response in cattle (Spears, 2000) or the risk of diarrhoea in calves (Naylor et al., 1989) did not lead to consistent conclusions.

In the present study, perinatal mortality was associated to deficient copper status. Cardiac enlargement and fragility, known as 'falling disease', can be due to copper deficiency in cattle and results in sudden death (Lamand and Périgaud 1973; Mills, 1987).

Growth retardation in calves from copper deficient cows has already been described, but in most experiments, copper deficiency was secondary to dietary molybdenum excess so that separation of the deleterious effects of molybdenum excess and copper deficiency was difficult. Discussing the relative effects of copper deficiency and excess molybdenum, Underwood and Suttle (1999) concluded that growth retardation observed with excess dietary molybdenum is presumably related to compromise copper-dependent functions. In France, copper deficiency is a primary rather than a secondary deficiency due to excess molybdenum. The high odds ratio for growth retardation associated with deficient copper status in this study is consistent with this conclusion. Similarly, long term copper supplementation of beef cows having initial serum copper around 10 μmol/l has already been shown to significantly increase average daily gain of calves (Naylor et al., 1989). Wittenberg and Devlin (1987) explained poor growth of calves by the low milk yield of dams fed molybdenum-enriched diets. However, in our study, copper status was not associated to low milk production in dairy herds. Growth retardation in copper deficient calves could also relate to anaemia or tissue mitochondrial degeneration (Mills, 1987).

Zinc status

Odds ratios for productive disorders were high in this study, particularly for milk production in dairy cattle, and even for a low-adequate status. It is probably related to loss of appetite, which is the earliest clinical sign of zinc deficiency (Underwood and Suttle, 1999). In addition to lack of appetite and low feed efficiency (Engle et al., 1997), increased risk of poor growth of calves in beef herds could be related to lower milk production by dams with marginal or deficient status. The importance of zinc status on milk production has not been extensively studied previously, and the effect of supplemental zinc on milk production, alone or combined with other trace elements, has been reported to be low (Corbellini et al., 1997) or absent (Uchida et al., 2001).

Hair loss and rough coat is a known effect of zinc deficiency in cattle (Lamand and Perigaud, 1973). Zinc deficiency is also involved in lameness or hoof deformation (Lamand and Perigaud, 1973; Corbellini et al., 1997), and zinc supplementation has been shown to improve hoof quality scores (Kessler et al., 2003). This relationship was observed in this study where zinc deficiency was associated to impaired locomotion.

The role of zinc in several immune mechanisms has been extensively studied in humans, showing that even moderate deficiencies can rapidly diminish antibody- and cell-mediated responses (Fraker et al., 2000). In cattle, Spears (2000) outlined that, as a whole, studies on the effects of marginal deficiency in ruminants do not carry definitive conclusions regarding immunity. However, early immunological effects of zinc deficiency have been demonstrated in heifers (Engle et al., 1997), and enhanced humoral immune responses have been demonstrated in calves after zinc supplementation (Prasad and Kundu, 1995). In the present study, the relationship between zinc status and the risk of infectious diseases was significant for diarrhoea in calves and metritis and mastitis in adults. The risk of metritis is highly related to the risk of retained fetal membranes, and in this study, marginal or deficient zinc status increased the risk of retained placenta. To our knowledge, the relationship between retained placenta and zinc status has only been explored by Campbell and Miller (1998), who did not observe a significantly decreased incidence of this trouble after zinc supplementation of dairy cows. However, in their experiment, the basal diet provided more than 1 g of zinc per day, and plasma zinc before supplementation was around 14 μmol/l, suggesting that cows had adequate zinc status and would not benefit from supplement.

In the same way, zinc is not usually considered as strongly related to fertility in female ruminants (Underwood and Suttle, 1999), but metritis can delay fecondation and could explain our observed relationship between zinc deficiency and fertility. Low serum zinc concentration has already been associated with abortion (Graham et al., 1994).

Selenium status

Muscular degeneration in calves is the most specific manifestation of selenium deficiency. Odds ratios associated with myopathy were high in the present study in deficient herds. Because myocardial necrosis can cause sudden death in selenium deficient calves (Cawley and Bradley, 1978), the odds ratio for heart failure also was significant. The importance of selenium in immune function in cattle has been outlined in recent reviews (Gaylean et al., 1999; Spears, 2000). In the present study, deficient and marginal status of cows increased the risk of calf diseases related to low immunity. This result is consistent with previous observations in cattle: Sanders (1984) observed that selenium supplementation increased vaccination efficacy in late pregnant cows against *Escherichia coli* and BVD in a herd with a high incidence of diarrhoea. Similarly, selenium injection improves production of antibodies against *E. coli* in dairy cows (Panousis et al., 2001), or *Pasteurella haemolytica* in steers (Droke and Loerch, 1989). Access to high-selenium salt improves the concentration of immunoglobulins in the colostrum of beef cows (Awadeh et al., 1998).

In dairy cows, a lot of studies have focused on the relationship between selenium and mastitis (Erskine, 1993). Subsequently selenium allowance in dairy herds is usually consistent with recommendations for mastitis prevention. For this reason, most dairy herds in this study had adequate selenium status and the low number of herds with marginal or deficient status resulted in poor significance for odds ratio associated with mastitis. Moreover, high eGSH-Px values in dairy herds with mastitis (Table 2) suggest that selenium supplementation might have been increased before blood sampling in herds with observed mastitis. Thus, the lack of apparent relationship between selenium and mastitis in this study could be biased by the routine use of selenium supplements in herds after appearance of mastitis. For the same reason, the lack of observed relationships between selenium status and impaired locomotion and poor milk production were difficult to interpret in dairy herds.

Abortion and perinatal mortality are not usually associated with selenium deficiency in cattle in the literature, but our study showed greatly enhanced risks, even in herds with marginal status. Decreased immunity in selenium deficient dams can increase the risk of infectious abortion. Similarly, myocardial necrosis and heart failure in calf foetuses due to selenium deficiency have been suggested as a possible cause of abortion (Orr and Blakley, 1997). Moreover, selenium deficiency can alter iodine metabolism in cattle (Arthur et al., 1988; Awadeh et al., 1998), and iodine deficiency is known to result in abortion, perinatal mortality and growth retardation in young animals (Underwood and Suttle, 1999).

Increased risk of retained placenta when cows are selenium deficient has been outlined long ago (Trinder et al., 1969), and was associated with a deficient status in the present study. In addition to immune dysfunction, this can explain the increased risk for metritis in deficient herds.

Consistent with this study, selenium deficiency has already been reported to be associated with delayed conception, poor fecondation or cystic ovaries in cows (Corah and Ives, 1991), possibly via impaired plasma progesterone concentrations (Kamada and Hodate, 1998).

Thresholds for classification of herds and practical implications

Regarding selenium and zinc status, herds classified as low-adequate in our experiment did not exhibit significant risks for most disorders, which means that only herds with a lower tercile under 11 μmol of copper/l of plasma or 150 units of eGSH-Px/g of Hb needed copper or selenium supplementation respectively. Moreover, correction of copper status in dams is mainly justified for performance and health of the calves.

Herds with low-adequate zinc status were at higher risk of production, reproduction and health disorders in both cows and calves, compared with herds with a high-adequate status. We suggest to raise the threshold classifying herds according to plasma zinc, and to state herds as marginal when the lower tercile of plasma zinc is between 12 and 14 μmol/l. Furthermore, in our study, herds classified as having either deficient or marginal zinc status had greatly increased risks for some production and health disorders. Because most of these herds were classified as marginal, so that we propose to state herds as deficient when the lower tercile of plasma zinc is under 12 μmol/l.

Conclusions

Overall, this study suggests that copper, zinc and selenium deficiencies are risk factors for impaired production, reproduction and health in both beef and dairy herds in France. Copper deficiency only had effects on calves; zinc and selenium deficiencies also affected adults. Some high odds ratios (e.g. selenium deficient status associated with myopathy) could be expected from literature data, but some others (e.g. zinc inadequate status associated with low milk production in dairy herds) were less consistent with literature. Moreover, this study suggests that plasma zinc concentrations higher than 14 μmol/l can be required for optimal performance. A larger set of data could ascertain these relationships, possibly using different thresholds for dairy and beef herds.

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