The clinical significance of measuring copper concentration in blood plasma of Dutch sheep

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Index

Abstract

For blood plasma copper concentrations in Dutch sheep, reference values, clinical cut-off values, seasonal variation and within flock variation are partly unknown. To determine the clinical significance of blood plasma copper concentration in sheep, a study was conducted with the aims (1) to determine the reference values for copper concentrations in blood plasma, (2) quantify the difference in copper concentrations between sheep suspected of mineral disbalance and sheep unsuspected of mineral disbalance and identify clinical cut-off values for copper intoxication or deficiency, (3) describe the seasonal variation of copper concentrations, and (4) describe the variation in copper values within Dutch sheep flocks. In this cross-sectional study, 1,698 blood plasma samples of Dutch sheep were obtained through convenience sampling, of which 1,270 samples were sent in with a suspicion of a mineral disbalance. The samples were sent in between 1999 and 2012. A mixture model was used to determine clinical cut-off values. The 95 % reference interval for copper concentration in blood plasma of Dutch sheep in this study was 3.6 µmol/L – 21 µmol/L. Sheep suspected of a mineral disbalance had significantly higher copper values in blood plasma than sheep unsuspected of a mineral disbalance. In April and June, significantly higher copper values in blood plasma were found compared to the other months of the year ($p < 0.05$). The within-farm variation in copper values was greater than 10.0 µmol/L. No clinical cut-off values could be obtained through the mixture model. Altogether, these findings suggest there is some value in measuring the copper concentration in sheep, but when interpreting these results, season should be taken into account, and multiple samples should be taken within a flock in order to validly assess the copper status of the flock as a whole.

Introduction

Copper is an essential trace element in the metabolism of the sheep. It is a component of enzymes such as cytochrome C oxidase, which is necessary for electron transport during aerobic respiration. Also, in ceruloplasmin, an enzyme used for transport of iron and oxidation of Fe^{2+} to Fe^{3+} 6-8 molecules of Cu are incorporated (Nederbragt, van den Ingh,

and Wensvoort 1984). Other functions in which copper is an essential element is connective tissue formation (lysyl oxidase), melanin synthesis (tyrosinase), and immune defense (superoxide dismutase). Furthermore, copper can function as a structural component in macromolecules acting as a coordination center (Herdt and Hoff 2011; Reece et al. 2015; Sinclair et al. 2013). The liver is the storage room for copper, leading to fairly even concentrations of copper in the blood. When the liver overflows with or is completely devoid of copper, clinical diseases as respectively copper toxicosis and copper deficiency can manifest (Reece et al. 2015). However, not all animals have the same level of sensitivity towards copper. Ruminants in particular are sensitive to varying copper concentrations, especially concerning copper toxicity. Monogastric animals have the capability of excreting copper via the bile, while ruminants do not possess this quality to the same degree. Domestic sheep (*Ovies aries*) tolerate even less copper than goats and are the species most susceptible to copper toxicosis (Reece et al. 2015).

Copper toxicosis in sheep has been researched worldwide. In Australia, Howell et al. have done extensive research on the effects of detrimental levels of copper in feed on sheep in the 1990s (Howell et al. 1991; Howell and Kumaratilake 1990). In the United Kingdom, copper poisoning is widely described in multiple articles. Especially the susceptibility of North Ronaldsay sheep to copper is thoroughly determined (Haywood et al. 2005; Maclachlan and Johnston 1982; Simpson et al. 2006). However, conditions and breeds can differ greatly in other countries, making findings difficult to translate to the situation in the Netherlands. For Dutch sheep, reference values as well as clinical cut-off values for copper concentrations in blood plasma are unknown, making it difficult for veterinary practitioners to differentiate between normal copper values in blood plasma and levels, which are too high. Also, seasonal variation and variation within flocks in copper concentrations in blood plasma have not been described in scientific literature for Dutch sheep.

Copper intoxication and deficiency

Copper intoxication in sheep can be displayed by a large set of symptoms, depending if the sheep is suffering from acute or chronic intoxication. It is thought that acute copper toxicosis is less common than chronic copper toxicosis. Acute copper toxicosis usually manifests after ingestion or parenteral administration of excessive copper in anthelmintics, footbaths, mineral supplements, or improperly formulated rations (Ortolani, Antonelli, and De Souza Sarkis 2004). The symptoms of acute intoxication are severe gastroenteritis, which is characterized by abdominal pain, diarrhea, emesis, anorexia, dehydration, and shock (Bozynski et al. 2009). These clinical signs usually lead to death, however a sheep can survive the acute phase of copper toxicosis. Unfortunately, after acute copper intoxication, hepatic necrosis, hemolysis and hemoglobinuria may arise, which is called subacute copper toxicosis. Within a few days of exposure, sheep, which initially survived the acute phase, may still die because of subacute copper intoxication (Ortolani et al. 2004; Puls 1994).

Chronic copper toxicosis arises from a long history of nutritional imbalances between copper and other trace elements (such as zinc or molybdenum), defects in hepatic metabolism or altered biliary and urinary excretion of copper (related to high dietary intake of copper). Diary goats have been poisoned by supplementation of trace minerals that were

formulated for dairy cattle and it is suggested this may also play a role in copper intoxication in sheep (Smith, Metre, and Pusterla 2020). Chronic copper toxicosis is a massive accumulation of copper in the liver which leads to, at first, subclinical hepatocellular damage. This is the prehemolytic stage of chronic copper toxicosis. The hemolytic stage follows, leading to acute hepatocellular degeneration and necrosis associated with copper-induced oxidative damage (Smith et al. 2020). The step from prehemolytic stage to hemolytic stage is defined by the sudden release of vast quantities of copper from hepatic stores. The release of copper from copper-containing lysosomes can be triggered by a stressful event such as transportation, pregnancy, hierarchal change, lactation, strenuous exercise and/or handling, administration of oxidative drugs, or disease and malnutrition (Bozynski et al. 2009; Smith et al. 2020). The sudden increase in blood copper concentration causes formation of methemoglobin and Heinz bodies, lipid peroxidation of erythrocyte membranes and intravascular hemolysis of damaged erythrocytes. This causes a severe acute anemia and affected animals usually show signs of depression, anorexia, rumen stasis, thirst, weakness, recumbency, hemoglobinuria, icterus and may die within 1 to 2 days (Mendel, Chłopecka, and Dziekan 2007; Oruc, Cengiz, and Beskaya 2009; Puls 1994; Smith et al. 2020; Soli 1980).

On the other side, a deficiency of copper has as a consequence an impairment of growth, reproduction, connective tissue development, and pigmentation. Other clinical signs can be diarrhea, changes in wool quality, anaemia, spontaneous fractures, lameness and demyelinization (enzootic ataxia in sheep). Within copper deficiency is a distinction between primary copper deficiency and secondary copper deficiency. Primary means an abnormally low amount of copper in the diet, secondary means that copper absorption or metabolism is affected, which is associated with high dietary levels of molybdenum, sulfates, zinc, or iron. Because of a very low concentration of ceruloplasmin, iron is not adequately mobilized from the liver, leading to an anaemia (Nederbragt et al. 1984).

Differences in breeds

70 percent of the sheep kept in the Netherlands are of the Texel breed. The other 30 percent consists of Zwartbles sheep, Friesian Milksheep, different types of heath sheep, Swifter, Suffolk and Flemish milk sheep (Veehouderij Wageningen 2002). In 1983, researchers determined that Texel sheep are most susceptible to copper poisoning, compared to Flemish milk sheep, Finnis landrace and several crossbreds when offered ratios with high copper and low copper (van der Berg, Levels, and van der Schee 1983). It is thought that because Texel sheep originate from the Wadden island Texel, which has poor copper concentrations in its pastures, Texel sheep are predisposed to have an enhanced absorption of dietary copper (van der Berg et al. 1983; van der Schee, van den Assem, and van der Berg 1983). Drents heath sheep is more susceptible to a copper deficiency although the reason is unknown. Unfortunately, copper reference values per breed are still lacking.

Diagnostics

The concentration of copper in several tissues of the sheep can provide an indication of the nutritional copper status of the sheep. Examples are liver, blood, kidney, muscle and milk (MacLachlan et al. 2016). The liver represents the storage pool of copper and reflects

the long-term availability of dietary copper. Depletion of hepatic copper concentration is therefore the most sensitive sign of inadequate copper consumption. A liver biopsy sample as small as 50 to 75 mg of fresh tissue weight can give a reliable determination of the copper concentration (Herdt and Hoff 2011). Also, kidney samples can provide an insight in the copper status of the sheep. However, studies referring to copper levels in kidneys, took samples from kidneys taken from abattoirs, which also goes for muscle samples (MacLachlan et al. 2016). Moreover, liver samples are quite invasive techniques and are not practiced regularly by veterinarians. Therefore, blood samples are widely used in practice to give an indication of the copper levels in the sheep. In sheep kept for milk production, milk samples are also an option. Puls described in his book (Puls 1994) a reference window for copper in milk (0.2 – 1.5 ppm). Nonetheless, there is very little research done on copper values in milk and its validation. In addition, obtaining milk samples would only be practical in lactating sheep, making it an option with limited use in sheep farming. For all the reasons stated above, this paper focuses solely on blood plasma samples, its (dis)advantages and its relevance in the field.

Blood plasma copper concentration

Although blood samples are taken frequently for the evaluation of copper status, the regulation of blood copper concentrations is under homeostatic control. This means that even though the liver, functioning as storage for copper, can have a low concentration of copper, blood copper concentration levels can be within normal range (Herdt and Hoff 2011; Puls 1994; Smith et al. 2020). The relationship between liver and blood copper concentrations is curvilinear. In cattle, at hepatic concentrations greater than 30 to 50 μg/g dry weight, there is little correlation between liver and blood concentrations. Low blood (serum or plasma) concentrations are generally not observed until liver concentrations decrease to less than approximately 25 μg/g DW (Mulryan and Mason 1992). In sheep, this effect has also been described (van der Berg et al. 1983). Furthermore, the proportion of blood copper that becomes incorporated in the clot is not consistent among animals. This situation does not reduce the usefulness of assessing blood concentrations for herd-level assessment of copper status, but does emphasize the need for sampling a sufficient number of animals (preferably 10 to 15 animals) (Herdt and Hoff 2011). And, despite the curvilinear relationship, reference values and even copper concentrations for copper deficiency and intoxication have been described by Puls (1994): 1.6 μmol/L – 15.7 μmol/L for copper deficiency and 55.9 μmol/L – 314.7 μmol/L for copper intoxication.

Aims

The aim of this study is to determine the clinical significance of measuring blood plasma copper concentrations in sheep. In order to do so (1) the reference values for copper concentrations in blood plasma will be determined, (2) the difference in copper concentrations between sheep suspected of mineral disbalance and sheep unsuspected of mineral disbalance will be quantified and clinical cut-off values for copper intoxication or deficiency identified, (3) the seasonal and or yearly variation of copper concentrations, and (4) the variation in copper values within flocks will both be described.

Materials and Methods

Description of the study population and samples

In total, 1,698 sheep were tested for blood copper concentration between 1999 and 2012. Two submissions were excluded because in the description it suggested a goat was tested, instead of a sheep. A total of 1,270 blood samples were sent to the veterinary laboratory Royal GD (GD) for a suspicion of a mineral disbalance in the sheep. Other reasons for submission are given in table 1.

Table 1. Reasons and distribution of blood copper sampling in Dutch sheep.

Reason for submission		***Number of samples***
Unsuspected of mineral disbalance	Test study Royal GD	21
	Pilot study Royal GD	141
	Pilot study for third parties	174
	Routine	49
	Herd health monitoring	43
Suspicion of mineral disbalance		1,270
Total		1,698

Field samples obtained by veterinary practitioners at suspicion of mineral disbalance were send to GD. The Trace element package determines zinc, copper, selenium and iodine levels in blood plasma. Other sampling reasons were: pilot studies, routine, and herd health monitoring. This information was listed on the submission form. Pilot study or routine samples usually came from sheep without suspected mineral disbalance, e.g. ‘unsuspected’ or ‘normal’ or ‘control’. For sheep suspected of mineral disbalance 1,270 blood samples were admitted, the other reasons for submission comprise of 428 samples.

Analytical methods

Blood samples were taken by a qualified veterinarian. Samples for trace mineral assessment were placed into tubes with heparine. The concentration of the trace minerals is determined using inductively coupled plasma mass spectrometry (ICP-MS) according to Quarles et al. (2020).

Statistical tests

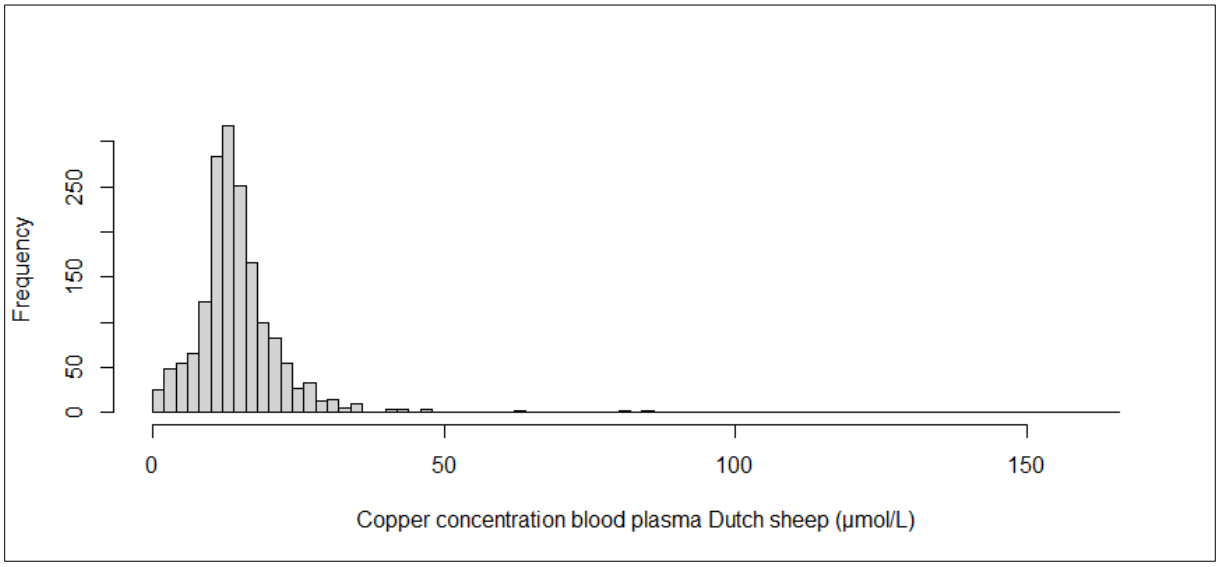
Statistical analyses were conducted using the R statistical package, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), with packages ‘xlsx’, ‘tidyverse’, ‘gdata’ and ‘DescTools’. To determine if the data is distributed normally, a *Shapiro-Wilk normality* test was performed ($p < 0.0001$). Differences in copper concentrations between sheep suspected of mineral disbalance and sheep unsuspected of mineral disbalance were analyzed using Mann-Whitney *U* test. To determine the variation within farms the difference between the highest and lowest blood copper value was calculated per farm. Only farms with 3 or more blood samples submissions (n = 130) were taken into account. A mixture model was used in R (package ‘mixtools’) to determine clinical cut-off values, with 3 underlying distributions on the raw data (n = 1,968). Mixture models give descriptions of subgroups, which may not be observed *per se*.

Results

1. *Reference values*

The mean copper concentration was 14.9 µmol/L (standard deviation 9.5 µmol/L, the median was 13.6 µmol/L and the values ranged from 0.9 µmol/L to 166.0 µmol/L. See figure 1 for a distribution of the copper concentrations in blood plasma in Dutch sheep.

Figure 1. Blood plasma copper concentrations (µmol/L) in Dutch sheep, suspected and unsuspected of mineral disbalance (n=1,698).



The 95% reference values of the copper concentrations in blood plasma of the unsuspected sheep were 3.6 µmol/L – 21.0 µmol/L.

2. *Copper concentrations unsuspected versus suspected and clinical cut-off values*

See figure 2 for the boxplots of copper concentrations in blood plasma for sheep suspected of a mineral disbalance and sheep unsuspected of a mineral disbalance.

Figure 2. Blood plasma copper concentration (µmol/L) in Dutch sheep displayed as samples of unsuspected sheep (n = 428), and sheep suspected of mineral disbalance (n = 1,270).

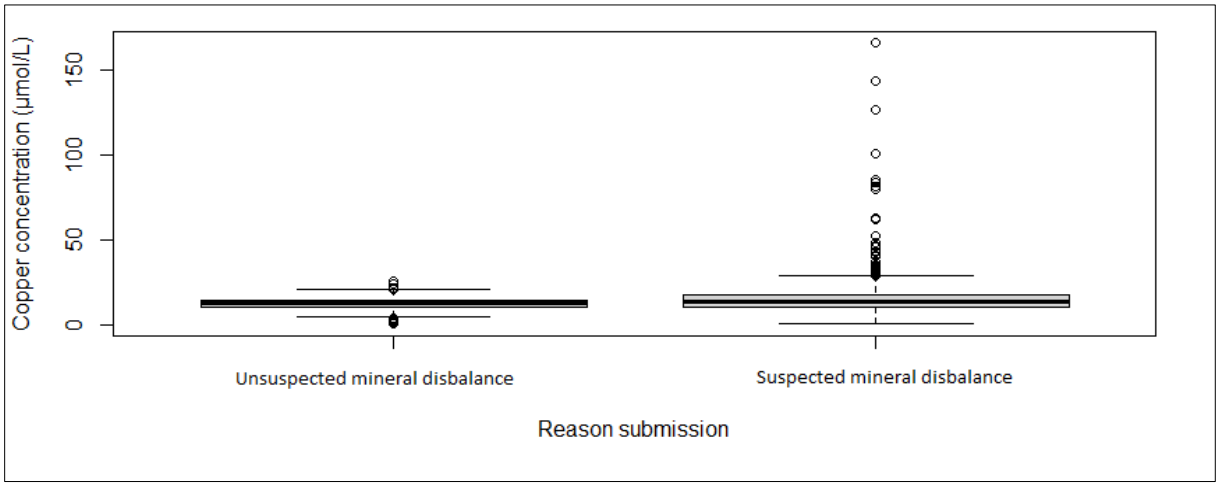


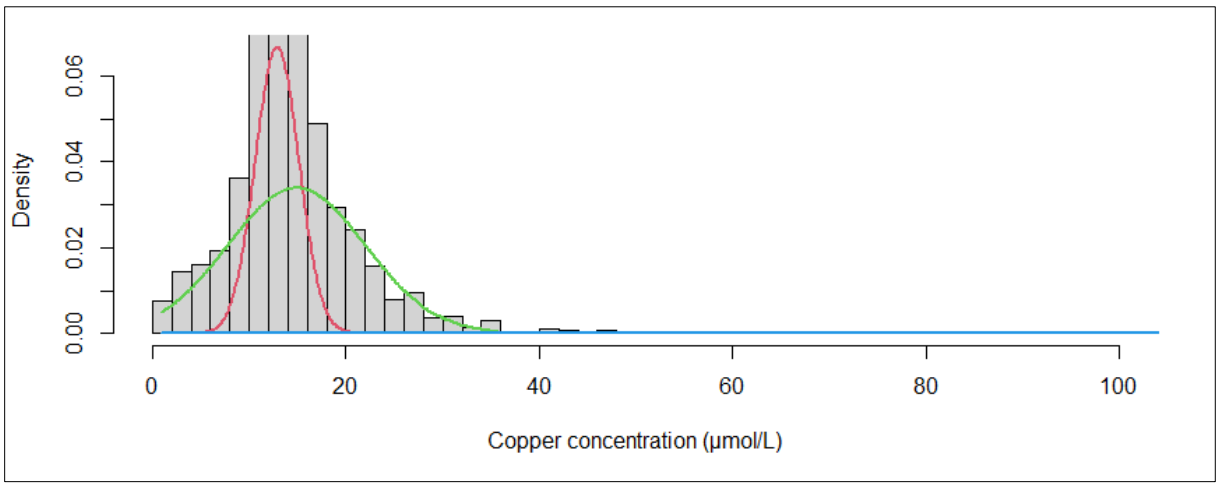
Table 2. Descriptive statistics of Dutch sheep suspected of a mineral disbalance and unsuspected individuals.

	Suspicion mineral disbalance (n = 1,270)	***Unsuspected (n= 428)***
Mean copper value *(µmol/L)*	15.5	13.0
Standard deviation *(µmol/L)*	10.7	3.8
Reference interval *(2.5 -97.5 percentile, µmol/L)*	2.5 – 34.1	3.6 – 21
Median *(µmol/L)*	14	13

On average, sheep with a suspected mineral disbalance had higher copper values than unsuspected sheep ($P < 0.001$), as shown in table 2.

A mixture model was used to determine a clinical cut-off value for copper deficiency and a clinical cut-off value for copper intoxication. See for the result figure 3.

Figure 3. Mixture model with 3 distributions of copper concentration in blood plasma of Dutch sheep, where each line (red, blue, and green) represents a theoretical subgroup in the data.

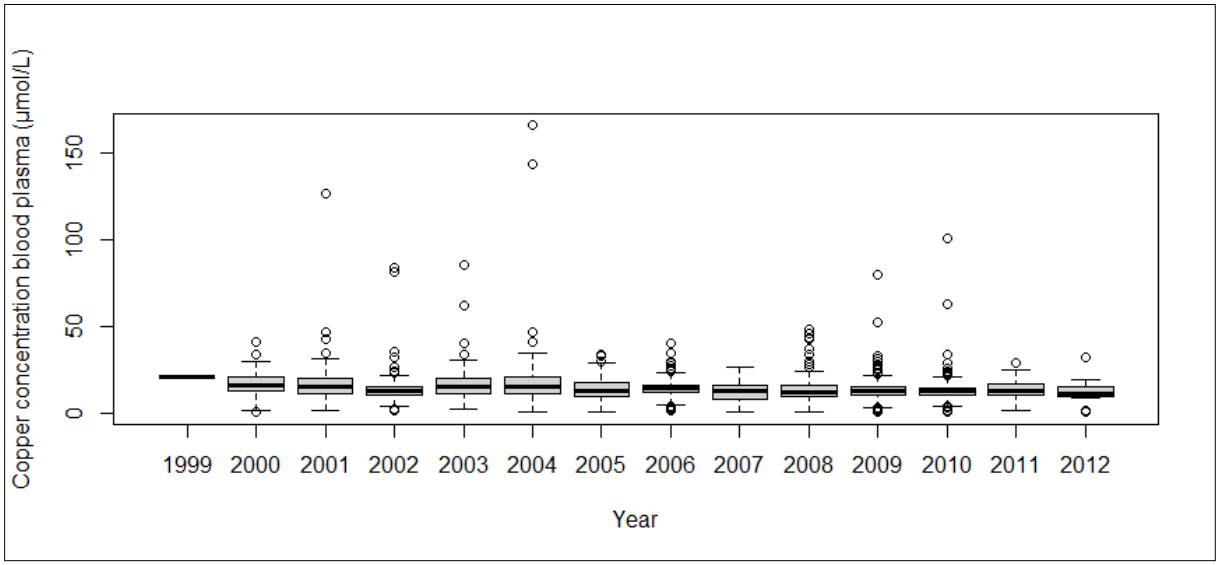


The mixture model did not distinguish separate distributions and therefore could not be used to determine a cut-off value to distinguish between sheep with too high, too low or normal plasma copper concentrations.

3. *Variation throughout the years and seasons*

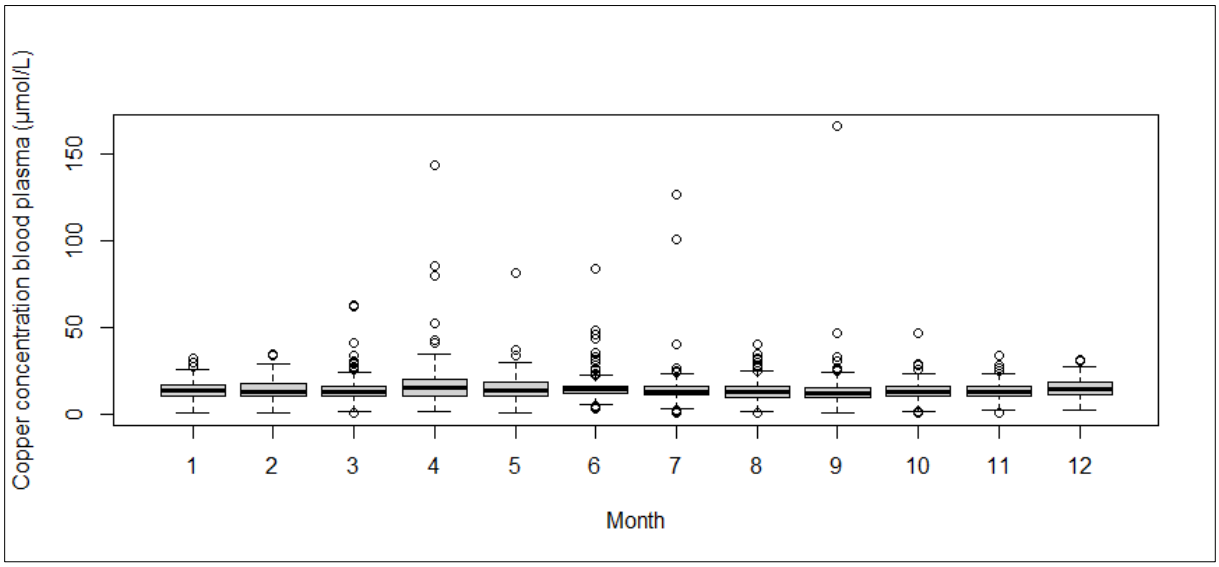
Figure 4 shows the distribution of copper values in blood plasma throughout the years 1999 to 2012. There were no significant differences between copper values over different years ($p > 0.05$).

Figure 4. Blood plasma copper concentrations (µmol/L) of Dutch sheep between 1999 and 2012.



A significant difference was found in the copper values between months. The months April and June had significantly higher copper values in blood plasma (resp. $p < 0.001$ and $p < 0.05$). Figure 5 shows an overview of the copper values between months.

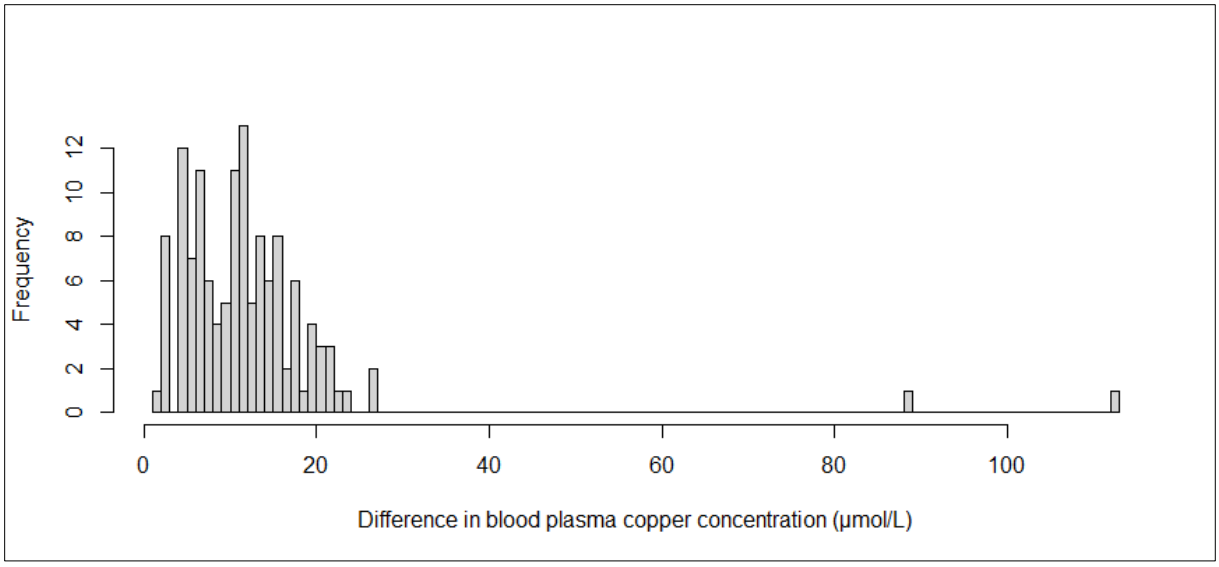
Figure 5. Monthly distribution of blood plasma copper values (µmol/L) in Dutch sheep.



4. Variation within farms

We looked at the range of copper values in each farm. Only farms with 3 or more sample submissions were included to determine the difference between the highest and the lowest copper concentration within the flock. See figure 6 for an histogram depicting the calculated differences.

Figure 6. Within farm variation depicted as difference between the highest and lowest blood plasma copper concentrations (n = 130 farms which had ≥ 3 measurements per farm).



As made visible in figure 6, the majority of farms (n = 70) have a calculated difference between highest and lowest copper concentration higher than 10 µmol/L.

Discussion

The objective of this study was to evaluate the clinical significance of measuring copper values in blood plasma of Dutch sheep. We determined 95% reference values, but could not obtain clinical cut-off values for copper intoxication and deficiency. Also, a significantly higher copper concentration in blood plasma of sheep with a suspected mineral disbalance in comparison with sheep unsuspected of a mineral disbalance was determined. We also observed that during April and June, significantly higher copper concentrations were found in the blood plasma of Dutch sheep. Furthermore, a large variation in copper values was found within farms in the Netherlands.

We found a copper concentration 95% reference interval of 3.6 µmol/L – 21.0 µmol/L. This is in accordance with other reference intervals, determined in Europe, especially the reference interval the GD determined. See table 3 for other reference values determined for copper concentrations in blood of sheep. A possible explanation for the lower 2.5% reference value in comparison with reference values from other studies, could be the large population of Texel sheep in The Netherlands. Texel sheep are susceptible for a copper intoxication (van der Berg et al. 1983; van der Schee et al. 1983), which as a consequence could mean that sheep owners deliberately feed ratios low in copper. This could lead to lower blood plasma copper concentrations and therefore a lower reference value. The reference values of the GD are rather similar, because they are partly based on the same dataset.

Table 3. Reference values for copper in blood plasma, transcribed to µmol/L.

	Copper value blood	*Location*	*Breed*	*Reference*	*Study design*
1	11.0 – 31.5 µmol/L	Canada	-	(Puls 1994)	-
2	11.8 – 26.8 µmol/L (80% reference interval)	USA and Canada	-	(Herdt and Hoff 2011)	Cross sectional
3	7.7 – 23.1 µmol/L (winter)	Slovakia	Merino and East Friesian	(Kovacik et al. 2017)	Cohort study
4	13.0 – 20 µmol/L	Germany	Mutton Merino and Blackhead Suffolk crossbreed	(Humann-Ziehank et al. 2001)	Case - control study
5	5.2 – 18.8 µmol/L	The Netherlands	-	GD	Cross sectional

Sheep suspected of a mineral disbalance had significantly higher copper values in blood plasma than sheep unsuspected of a mineral disbalance. However, in reference values both groups largely overlapped. The significant difference between both groups can be explained by two explanations found in literature. Firstly, the sheep suspected of a mineral disbalance are indeed suffering from a chronic copper intoxication, leading to higher copper concentrations in the blood plasma, which has been described in numerous articles (Bozynski et al. 2009; Herdt and Hoff 2011; Hoffmann 2009; MacLachlan et al. 2016; Maclachlan and Johnston 1982; Mendel et al. 2007; Mulryan and Mason 1992; Puls 1994). Secondly, the sheep suspected of a mineral disbalance could be suffering from inflammation. During a generalized inflammatory response, blood copper concentrations rise, because of an upregulation of ceruloplasmin, functioning as an acute-phase protein (Herdt and Hoff 2011). This would explain a slight increase in copper concentration in the suspected sheep group. Unfortunately, the information regarding the clinical status of the sampled sheep is limited, so we could not differentiate between the two explanations. Overall, this finding suggest that symptoms fitting a mineral disbalance have a slight predictive value for higher Cu levels in blood plasma.

Unfortunately, the mixture model did not determine clinical cut-off values for copper deficiency and copper intoxication. However, our data is not ideal for determining clinical cut-off values. More accurate clinical information about the sampled sheep, normally distributed sample means, equal sample size or a bimodal distribution would make the use of mixture models for finding clinical cut-off values more successful. In this dataset, a selection bias applies, since mainly suspected animals are tested. Furthermore, as described in literature, the liver functions as a storage pool for copper, keeping copper concentrations in blood on a relatively equal level (Laven and Smith 2008; Puls 1994; Reece et al. 2015; Smith et al. 2020), which would make it more difficult to find clinical cut-off values. Although, this research points out that some effects are visible (significant difference between suspected and unsuspected sheep and seasonal variation), there is too much unexplained variation in this dataset to determine clinical cut-off values. Besides, in this dataset, the sheep suffering from acute copper toxicosis (with high copper concentrations in blood) are lacking, because they

usually die within hours or days of ingesting the excessive amounts of copper (Bozynski et al. 2009; Ortolani et al. 2004). These signs may be the signal to take blood samples of the remaining sheep and submitting them to the GD for a copper concentration determination. This leads to a dataset in which the extreme copper concentrations are missing, giving a more moderate picture.

We found that the blood plasma copper concentrations of Dutch sheep are significantly higher in April and June. In New Zealand this seasonal difference has been found as well, where in spring the mean Cu levels in liver increased and in winter decreased. The researchers in New Zealand did not offer an explanation for this finding, however they rule out the possibility of gestation playing a factor, since this seasonal effect is also reported in yearlings. An induced Cu deficiency stemming from high molybdenum (Mo) intake is unlikely as well, according to the researchers, because the Mo concentrations in pasture were consistently low (Grace et al. 2004). In Slovakia, researchers reported a significantly higher accumulation of copper in the blood clot in spring, corresponding with our findings. The researchers ascribe it to the impact of polluted areas, high in copper and other heavy metals. However, in The Netherlands, the pastures are notoriously low in copper (Brolsma 2019). For the situation in The Netherlands, this finding could be related to the way sheep are managed. It is general practice to keep sheep during the lambing period indoors, which coincides with the winter months, and several weeks after lambing, with the start of spring (March / April / May), the animals are put on pasture. Leading up to lambing, the sheep are fed concentrates, which can range from specialized sheep concentrates, to cattle or goat concentrates. Sheep concentrate feeds are low in copper (mostly no added copper, so only copper in ingredients), but cattle and goat concentrates are not. Copper concentrations in cattle and goat concentrates are around 20 mg/kg feed. In contrast to sheep, other ruminants such as cattle and goats are less predisposed to copper intoxication (Poppenga and Spoo 2009). In literature is described that copper poisoning can occur in sheep after two months of daily dosing 3.5 mg/kg of copper, or after several months when feeding a ration that contains 20 mg/kg copper (Reece et al. 2015). This information would suggest that during the winter months, sheep might be fed with concentrates with high concentrations of copper. The copper stacks in the liver, but only after several months, at the end of winter, the higher amounts of copper in the liver might lead to higher concentrations in the blood plasma.

Another explanation could be selection bias, where in spring more animals suspected of a copper intoxication are tested, leading to a higher copper concentration mean in these months. With the use of creepfeeders on pastures, it is general practice to feed lambs concentrates during springtime. This could lead to more lambs tested, because of a suspicion of a copper intoxication. These lambs are then over-represented in the dataset. For practitioners and farmers this finding suggest that if a low copper value is found in spring, this might be out of the ordinary and should be looked into. For winter this works the other way around, if a high copper value is found. Copper values should therefore be interpreted according to the season they are found in.

The variation within farms in this study was rather high. The majority of Dutch farms in this study (n = 70) had a copper concentration difference higher than 10 µmol/L between their highest and lowest copper value in blood plasma. This has a consequence that, with a German reference window of 13.0 – 20 µmol/L (Humann-Ziehank et al. 2001), on a Dutch farm can be sheep with copper concentrations within reference and sheep with too high or too low copper concentrations. This is an interesting finding, because dietary copper intake is

relatively equal among sheep within flocks, unless there are separate (production) regimes for example in milk sheep farming. Herdt and Hoff (2011) also described variation among animals within flocks, large enough to obscure dietary influences. They do not offer a cause to this variation within flocks, however they argue that larger number of animal must be tested to classify flocks as copper deficient or intoxicated (Herdt and Hoff 2011). Although, we adopt this advice, our study design in this matter was not ideal for describing the variation within flocks, because the samples can range from dates between 1999 and 2012. In figure 6, samples from different seasons and years and perhaps some sheep tested multiple times, are treated as if they come from different sheep at the same moment in time. As stated before, there is a significant difference between the seasons in copper concentrations in blood plasma, which could lead to large variations over time and not a large variation within flocks at one moment in time.

For future research, our recommendation would be to investigate the cause for seasonal differences in copper concentrations in The Netherlands. An observational study towards different feed regimens throughout the seasons and blood plasma copper concentrations, could give us a better insight in where these variations come from. Furthermore, a study towards individual copper concentrations differences among sheep within flocks and its explanation would be our proposal.

Conclusion

In conclusion, although no clinical cut-off values could be obtained, these findings suggest there is some clinical value in measuring copper concentrations in blood plasma of Dutch sheep. Veterinary practitioners should keep in mind that, because of significantly higher copper values in spring, the season in which the blood sample is taken, will affect the chances of finding copper concentrations outside the reference interval. Furthermore, because of high variation within farms, one blood sample is insufficient to give a reliable outcome for the entire flock and multiple blood samples should be taken to make a trustworthy diagnosis. The amount of blood samples would, naturally, be dependent on the size of the flock, however, at least five should give a decent overview of the copper status in the flock.

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