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Research Article

The effect of diet composition on the digestibility and fecal excretion of trace minerals in horses

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ABSTRACT

This study investigates the correlation between dietary intake of these trace minerals and their fecal excretion rates, aiming to enhance our understanding of equine mineral metabolism and improve dietary recommendations. The criteria for selection included healthy horses aged 4 to 14, weighing 400 to 600 kg, with a good body condition score (BCS, 3/5) across 14 yards in the Netherlands. Data on yard type, size, and location, as well as horse demographics (age, sex, breed, weight), were collected. Each horse's diet was analyzed concerning daily nutritional intake and requirements according to National Research Council (NRC). Fecal samples were randomly collected from stables (n=14) for dietary analysis and stored in three labeled jars. Samples, approximately 200 g each, were frozen at -20°C and later analyzed for cobalt (Co), copper (Cu), manganese (Mn), and zinc (Zn) using inductively coupled plasma mass spectroscopy (ICP-MS). Among the 14 horses, all were geldings, with one being a cold blood and another a Welsh pony, while the rest were warmbloods. Their median age was 10 years, mean body weight (BW) 506 ± 82.3 kg. Most horses grazed, averaging 8.6 ± 8.5 h/day. All received commercial concentrate feed, with nine also receiving supplements. Energy intakes varied, with ten horses consuming more than required. Trace mineral intake was classified by the NRC; two horses had high Cu intake. Manganese intake exceeded NRC recommendations significantly (618.6 ± 125.1 mg vs. 1403.8 ± 312.7 mg), while Co intake was 2.3 ± 1.6 mg against a requirement of 0.8 ± 0.2 mg. Manganese excretion in feces was highest (459.1 ± 386.4 mg/day), followed by Zn (58.3 ± 46.0 mg), Cu (2.7 ± 3.2 mg), and Co (1.5 ± 0.4 mg). This study emphasizes the need for tailored diets to prevent excess mineral intake in horses, which mainly originates from concentrate feed and supplements. Further research with a larger sample size is necessary for a deeper understanding.

INTRODUCTION

Maintaining optimal levels of trace minerals is paramount for the health and performance of horses (Jackson, 1997). Trace minerals, including Co, Zn, Co, and Mn play indispensable roles in various physiological processes essential for equine well-being. These encompasses Cu; including proper coat and hoof health Zn; skin health, wound healing, and immune function Co; the synthesis of vitamin B12 crucial for energy metabolism and nerve function Mn; contributes to bone formation, cartilage development, and enzyme function (NRC, 2007). Despite their significance, achieving and maintaining the delicate balance of trace minerals in equine diets is challenging. Horses acquire minerals primarily through their diet, with excess amounts often excreted through feces (Coenen, 2013). This excretion pattern underscores the intricate relationship between dietary intake and fecal excretion rates, which can be influenced by factors such as mineral form, interactions among supplemented minerals, and individual variations in mineral metabolism. Deficiencies in trace minerals can have profound implications for equine health. Cu deficiency, for instance, may manifest as poor coat quality, joint issues, and anemia, while Zn deficiency can lead to skin problems, impaired wound healing, and compromised immunity (Baltaci et al., 2019). Co and Mn deficiencies similarly contribute to anemia, decreased energy levels, and skeletal abnormalities. Therefore, ensuring adequate intake of these trace minerals is imperative for safeguarding equine health and preventing associated health complications. There exists a positive linear relationship between dietary mineral intake and fecal excretion (Coenen, 2013). Supplementation of trace minerals in horse diets is common practice, yet it often exceeds dietary requirements, resulting in elevated fecal excretion rates (Harper et al., 2009). Studies have shown that higher plasma concentrations of certain minerals, such as Zn, may not always correlate with the highest dietary intakes, highlighting the complex dynamics of mineral metabolism in horses (Cymbaluk and Christensen, 1986). Moreover, the environmental impact of excessive mineral excretion in horse manure cannot be overlooked. Minerals excreted in feces due to oversupply from supplements can disrupt the agricultural cycle, affecting soil health and potentially contaminating water sources (Hsu et al., 2000). Understanding the mobility of trace minerals in horse manure and their subsequent impact on the environment is crucial for sustainable equine management practices. Consideration of mineral forms in horse ration formulation is essential, as interactions between dietary compounds and mineral availability can influence overall mineral absorption (Kaya Karasu et al., 2018). While organic trace mineral compounds are often presumed to offer superior bioavailability compared to inorganic salts,

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this assumption remains uncertain in horses. Assessing true mineral digestibility provides a more accurate estimation of bioavailability, aiding in the formulation of balanced equine diets. Despite advancements in understanding mineral nutrition in other species, research specific to horses is limited, particularly regarding the effect of mineral form on availability. Studies investigating plasma responses to different mineral forms have shown varying degrees of availability, emphasizing the need for further research in this area (Wichert et al., 2002).

This study aims to bridge existing knowledge gaps by assessing the correlation between dietary intake of trace minerals (Co, Cu, Mn, and Zn) in horses and the subsequent excretion rates observed in feces. By elucidating the relationship between dietary mineral intake and fecal excretion, this research seeks to enhance our understanding of equine mineral metabolism, inform more precise dietary recommendations.

MATERIALS and METHODS

The selection criteria included a healthy horse aged 4 to 14, a gelding weighing 400 to 600 kg, in good health, and in good body condition score (BCS, 3/5) in the 14 yards in the Netherlands. Data were gathered regarding geographical location, yard size (number of horses), yard type, and demographic details of the horses (body weight (BW), breed, age, and sex). Each horse's daily level of activity was recorded, as well as information about their dietary intake (grazing duration, forage intake, concentrate intake, supplements with types, brands, and quantities). Feces samples were collected from the same yards, and the ration was calculated for horses.

Ration calculation

The nutritional requirements and total diet of each horse were assessed based on their daily intake of nutrients. The nutrient intake for crude protein (CP), crude fiber (CF), starch, energy, and micro minerals such as Zn, Co, Mn, and Co were calculated, an estimate of workload was also calculated and these data were utilized to compare the estimated intake with the estimated horse requirements based on National Research Council recommendation (NRC, 2007). The estimated nutritional content of the pasture and forage given were predicted using published NRC recommendations (NRC, 2007), while the data for feed and supplements was based on publicly available product data. A 500 kg horse was estimated to consume pasture dry matter (DM) of 1.2 g DM/$kg^{0.75}$ (NRC, 2007). The pasture DM intake was estimated for the initial 4h at pasture as 1.5 g DM/kg BW/h and for every hour after the initial 4 hours as 0.9 g DM/kg BW/h based on published estimated intakes (Dowler, 2009).

The metabolizable energy (ME) requirement for horses between 200 and 800 kg was determined based on the calculation by Kienzle and Zeyner. (Kienzle and Zeyner, 2010). The energy requirement calculation included an adjustment for activity level as classifications "light," "moderate," "heavy," and "very heavy"(NRC, 2007). The provision of nutrients for energy, starch, sugar, crude fat, crude protein, energy, crude protein, and trace minerals for Co, Cu, Mn, and Zn were classified as low (<90%), normal (90%–110%), and high (>110) of the requirement compared to the NRC recommendations (NRC, 2007). The mineral intake for trace minerals Co, Cu, Mn, and Zn by diet minus the requirement, which is defined as "potential mineral excretion".

Feces samples collection and analysis

Feces samples were collected as randomly from horse stables in the Netherlands (n=14) to gather data for dietary plans and samples for feces as part of their assignments. The fresh feces samples after horses are defecated immediately collected without bedding contamination to a minimum. Then composited into 3 disposable sample jars that were kept closed to prevent moisture loss. Mix the feces with the plastic spoon. Fill wet feces samples in such a way that all 3 disposable sample jars numbered as A, B and C. Each jars feces capacity is approximately 200 g. Placed the samples at −20 ∘C in the freezer within a maximum of 3 hours. The selected horses' feces analyzed trace minerals Co, Cu, Mn, and Zn which were identified as "actual mineral excretion". The fecal samples were homogenized to achieve consistency, and 1 gram of the sample was weighed to ensure appropriateness for analysis. The samples were analyzed for minerals for Co, Cu, Mn, and Zn using inductively coupled plasma mass spectroscopy (ICP-MS). Total fecal excretion was calculated as a 500 kg horse will generally produce about 25 kg of manure (feces and urine) per day which means approximately %5 BW (Westendorf, 2004).

Data Analysis

Plots and descriptive statistics were examined in the initial analysis of the data. Kolmogorov Smirnov and Levene's tests assessed the data's normal distribution and homogeneity, respectively. Data with a normal distribution were shown as mean ± standard deviation (SD), while data with a skew were shown as median and range. Descriptive statistics were performed on the quantitative data describing percentage responses with 95% confidence intervals. To determine whether the relationship between variables was statistically significant, the chi-squared test and the k-independent test (Kruskal-Wallis) were used. Data were analyzed using SPSS 16.0 (IBM, 2021), with a value of $P < 0.05$ assumed to be statistically significant for all analyses.

RESULTS

Demographics

The horses (n=14) were all geldings. One horse was a cold blood, one was a Welsh pony, and the remaining horses were warmbloods. The median age of the population was 10, ranging from 4 to 22 years. Horses' average BW was 506 ± 82.3 kg. The median BW of the horses was 496.5 kg. The horses were all in BCS 5/9. The majority of the horses (n=9) were sport horses, with the remainder being livery yard horses (n=5).

Forage, Concentrate and Supplement intake

Only two horses did not have access to pasture; the rest were grazing, with an average grazing time of 8.6 ± 8.5 h/day (range, 2-24). Nine horses were grazed with continuous grazing method while four horses were grazed rotational grazing method. Hay (n=11) or haylage (n=4) was fed to all horses. Hay intake averaged was 9 ± 1.9 haylage was 6.7 ± 4.7 kg/d. All horses received at least one commercial concentrate feed, with one horse receiving two different commercial concentrate feeds and four horses receiving three different commercial concentrate feeds. The average intake of concentrate feed was 2.5 ± 1.8 kg/d. Nine horses were fed supplements, two of which received only one supplement, three of which received two supplements, and three of which received three supplements. All trace minerals in supplements (Zn, Co, Cu, and Mn) were not chelated. Vitamin-mineral mixtures were the most commonly used supplement (n=8).

Nutrient Analysis

Horse owners/trainers fed their horses an average of 141.52 ± 25.85 MJ ME/d. The energy intakes varied in comparison to requirements, 10 horses had higher intakes than required with a further 1 horse fed under the recommended energy levels, the rest had optimum intake. The crude protein intake was higher in 13 of the horses compared to NRC recommendations (NRC, 2007), one horse CP intake was lower than the requirement. The CP intake was 3.39 ± 0.71 g/kg BW. The mean CP requirement of these horses was 1.79 ± 0.17 g/kg BW on the base of NRC recommendations (NRC, 2007). The estimated lysine intake was within the normal range for 11 horses, with the remaining horses receiving less than recommended (NRC, 2007). Mean CF intake was 5.7 ± 1.3 g/kg BW, and crude fat intake was 0.7 ± 0.1 g/kg BW, both being within NRC recommendations (NRC, 2007). Mean starch intake was 1.1 ± 0.8 g/kg BW, which is within the NRC recommendation although sugar intake was 2.3 ± 0.4 g/kg BW, both of which were high compared to the NRC recommendations (NRC, 2007).

Potential and measured excretion of trace minerals in feces

The NRC has classified the trace mineral intake as low, normal, and high groups. Based on these categorizations, five horses maintained a normal Cu intake, while seven horses had a low intake, and two horses had a high intake. All horses used in the research were classified in the high-intake Mn group. Eight horses were placed in the high-intake Zn group, while the same number of horses were classified in both the low-intake and the normal-intake Zn groups also. The low-intake Co group had no horses, whereas the normal-intake Co group had one horse, and the high-intake Co group had thirteen horses (Table 1).

The mean weight of the horse was determined to be 508 kg. The mean daily weight of the fresh and dry feces was measured to be 16.3 kg and 4.3 kg, respectively (Table 2). The Cu intake was lower (146.9 ± 83.3 mg) compared to the mean requirements of the NRC recommendations (154.6 ± 31.3 mg). The

Table 1. Trace minerals intakes (number of horses) of horses and mean values compared to NRC recommendations (n=14)

Minerals	Low intake	Normal intake	High intake
Cu, mg	7	5	2
Mn, mg	-	-	14
Zn, mg	3	3	8
Co, mg	-	1	13

Low intake <90%; Normal intake 90-110%; High intake >110 of the requirement compared to NRC recommendations

Table 2. Average horse manure and feces generated per day (n=14)

Weight of horse (kg) (mean ± SD)	*Manure excretion (kg/day) (mean ± SD)	**Fresh feces (kg/day) (mean ± SD)	Feces (DM) (kg/ day) (mean ± SD)
508.9 ± 83.0	25.3 ± 4.1	16.3 ± 4.1	4.3 ± 1.6

*%5 BW is used for manure excretion; ** %26 DM is used for feces (mean ± DM 26.5 ± 7.5)

Table 3. Average minerals requirements, intake and calculated minerals excretion per day per horse according to laboratory results (n=14)

Minerals	*Mean requirement (mean ± SD)	Mean intake (mean ± SD)	Mean excretion by horse per kg wet feces (with lab results) (mean ± SD)	Mean excretion by horse feces per day (with lab results) (mean ± SD)
Cu, mg	154.6 ± 31.3	146.9 ± 83.3	0.1 ± 0.1	2.7 ± 3.2
Mn, mg	618.6 ± 125.1	1403.8 ± 312.7	29.5 ± 23.9	459.1 ± 386.4
Zn, mg	618.6 ± 125.1	755.7 ± 316.1	3.5 ± 2.5	58.3 ± 46.0
Co, mg	0.8 ± 0.2	2.3 ± 1.6	0.09 ± 0.01	1.5 ± 0.4

*requirement calculated on the base of NRC

Mn intake was notably higher (618.6 ± 125.1 mg) compared to the mean requirements of the NRC recommendations (1403.8 ± 312.7 mg). The intake of Co was found to be 2.3 ± 1.6 mg, whereas Co requirements for horses were 0.8 ± 0.2 mg. Mn excretion (459.1 ± 386.4 mg) by horse feces per day was the highest of the mineral excretions, followed by Zn (58.3 ± 46.0 mg), Cu (2.7 ± 3.2 mg), and Co (1.5 ± 0.4 mg) excretion (Table 3).

DISCUSSION

In the present study, nutritional factors that could affect the trace minerals status of horses fecal excretion were evaluated. There are dietary factors include consumption level (relative to requirements), intake of other minerals, and intake of substances that may improve or prevent the mineral's absorption. Furthermore, some factors are associated with the mineral compound used, including its water solubility and chelating properties. (Kirchgessner, 2004). An oversupply of minerals from supplements in horse diets, with possible interactions and interferences (Kaya Karasu et al., 2018), are excreted in manure (Fowler, 2020) and can have a potentially negative impact on the agricultural cycle. According to National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) report (Vos and Janssen, 2008), reducing the amount of Cu and Zn leaching from agricultural soils is particularly challenging in the Netherlands, although there are no restrictions under Regulation (EU) 2018/848 on the organic production of agricultural goods regarding the Cu and Zn content in horse manure (Regulation, 2018). Small changes in daily horse feeding management practices can have positive impacts on the environment, circular agriculture, and horses. Improper management of compost heaps can result in nutrient leaching, raising environmental concerns such as eutrophication. Moreover, high concentrations of trace minerals like Co, Cu, Mn, and Zn can be toxic to plants, microorganisms, and aquatic organisms (Nagpal, 2004; Harford, 2015).

Bioavailability

The bioavailability of a mineral in an animal is a rather elusive entity which may be changing relatively quickly. Factors which affect bioavailability of minerals relating to the animal are species, life stage, health and nutritional status. A mineral's bioavailability may be impacted by nutritional status. The animal's ability to absorb a mineral may enhance during this mineral deficiency whereas it may diminish during excessive intake of this mineral (Kirchgessner, 2004). The bioavailability of minerals can also be impacted by an excess or deficiency of other nutrients. Several interactions between minerals such as Several interactions between minerals such as zinc and copper are commonly assumed to occur in both experimental and domestic animals. (Mertz, 1986; Meyer and Coenen 2002; Kirchgessner, 2004). However, few of these interactions have been confirmed in the target species of horses; they may not occur at all or may differ slightly. For copper it is likely that bioavailability plays a highly important role in the horse. There is no clear consensus on the amounts of copper requirements. (Meyer et al. 1994; Hintz 1996). Variation of bioavailability of copper is an obvious reason for differing evidence on copper requirements. Zn is an effective Cu antagonist. In the horse, however, 500 mg of Zn/kg of diet did not have obvious effects on Cu metabolism (Hoyt et al., 1995). Ascorbic acid, cadmium, iron, and calcium are other potential antagonists of Cu (Meyer et al., 1994). Dietary factors may influence the bioavailability of Zn. Various compounds, including Ca, Fe, and phytate, have been documented as blocking Zn absorption in other species (Mertz, 1986). There is, however, only one study on experimental Zn deficiency in the horse and naturally occurring clinical zinc deficiency has not been unequivocally described so far in the horse. Excess intake of Fe decreased plasma and liver zinc content in ponies (Lawrence et al., 1987).

Mineral source

A 500 kg horse produces around 25 kilogram of manure daily, reaching around 9 tons per year. The manure usually comprises around 5 kg nitrogen (N), 0.9 kg phosphorus (P), and 3.6 kg potassium (K) per ton (Annete, 2013). Supplementing horses with an excessive amount of trace minerals that is higher than their requirements which may results in an increase in fecal output (Harper et al., 2009). However, the accumulation of trace minerals may lead to environmental concerns despite being excreted in smaller amounts (Brugger and Windisch, 2015). Supplementing trace minerals such as Cu, Mn, Zn, and Co with the diet resulted in an increased loss of Cu via feces compared to the control diet, which may lead to raise concerns about water quality (Fowler et al., 2019). In the current study, five horses remained in the normal-intake Cu group, seven in the low-intake Cu group, and two in the high-intake Cu group. The mean intake and mean daily excretion of Cu were found to be 146.9 and 2.7 mg, respectively. The endogenous Cu losses from feces may be established at a rate of 35 µg per kilogram of body weight per day (Coenen, 2013). Zn toxicosis is uncommon in horses because large animals generally have a high tolerance for this dietary mineral (Aragona et al., 2024). Zn use as a feed additive for horses is subject to legal restrictions in the European Union. The maximum amount of Zn allowed for equines' total diet (with 88% DM) is 120 mg/kg (PaBlack et al., 2022). Mineral additives are commonly used in animal feed in excess of the physiological requirements in order to prevent deficiencies and improve animal health. Therefore, this excessive supplementation may lead to more excretion of trace minerals, resulting in soil and surface water contamination (Xiong et al., 2023). Fowler et al (2019) showed that supplementing the diet with organic Zn resulted in higher levels of Zn in the fecal bacteria. Dietary strategies that improve gut bacteria's Zn absorption may help mitigate the effects of Zn leaching into water systems. The current study showed that the mean intake of Zn was higher than the mean requirement of Zn. The mean daily excretion of Zn in horse feces was 58.3 mg. Considering the excretion of 5 kg fecal DM per day, Zn losses via feces in horses fed without additives would be 80 mg/d, but horses fed with organic trace mineral supplements would lose 210 mg/d of Zn or horses fed with inorganic trace minerals would lose 134 mg/d of Zn (Fowler et al., 2019). A minimum daily Mn intake of 0.3 mg/kg BW is recommended. The amount of feces excreted is directly related to the Mn intake (Coenen et al., 2013). A study by Fowler et al. (2019a) indicated that the Mn content of horse fecal compost was 481.23 mg/kg. The current study showed that the mean Mn intake was 1403.8 mg, whereas the mean Mn loss via feces was 459.1 mg. The NRC established the minimal threshold for horses at 0.05 mg of Co kg/d DM, which was lower than the 0.1 mg Co/kg DM recommended in the 1989 NRC guidelines (NRC, 2007). Excessive intake of Co may disrupt normal organ function and result in thyroid dysfunction, cardiotoxicity, as well as heart failure (Mørkeberg, 2013). A study conducted by Semenza (2003) showed that the possibility of tumor growth due to excessive being overexposed of Co intake. Adequate amounts of Co are necessary for the microflora in the large intestine's colon and cecum to synthesize vitamin B12, or cobalamin. Therefore, a decrease in vitamin B12 concentrations in the tissues is most likely the cause of clinical Co deficiency (Mitchell et al., 2007).

CONCLUSION

This study underscores that the examined minerals Zn, Cu, Co, and Mn were consumed by the horses in quantities surpassing their requirements and subsequently excreted in feces. Given that all horses received concentrate feed and additional supplements, these excessive mineral sources likely originated from these dietary components. Thus, individualized diet calculations are crucial to ensure a balanced nutritional intake for the welfare and health of horses, while also mitigating potential environmental impacts. To gain a clearer understanding, further studies with a larger sample size are warranted.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that there is no conflict of interest

Consent for publication

Not applicable.

Author Contributions

Idea, concept and design: G.K.K

Data collection and analysis: G.K.K

Drafting of the manuscript: G.K.K and H.G

Critical review: G.K.K and H.G

Data availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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REFERENCES

Aragona, F., Cicero, N., Nava, V., Piccione, G., Giannetto, C., & Fazio, F. (2024). Blood and hoof biodistribution of some trace element (Lithium, Copper, Zinc, Strontium and, Lead) in horse from two different areas of Sicily. Journal of Trace Elements in Medicine and Biology, 82, 127378.

Baltaci, A.K., Mogulkoc, R., & Baltaci S.B. (2019). The role of zinc in the endocrine system. Pakistan Journal Pharmaceutical Science, 32, 231-239.

Brugger, D., & Windisch, W. M. (2015). Environmental responsibilities of livestock feeding using trace mineral supplements. Animal Nutrition, 1, 113–118.

Coenen, M. (2013). Macro and Trace elements in equine nutrition. In: R.J. Geor, P. A. Harris, & M. Coenen (Eds.), Equine Applied and Clinical Nutrition (pp. 190–228). Saunders: Philadelphia, PA, USA

Cymbaluk, N. F., & Smart, M. E. (1993). A review of possible metabolic relationships of copper to equine bone disease, Equine Veterinary Journal. Supplement, 16, 19-26

Dowler, L. E., Siciliano, P. D. (2009). Prediction of hourly pasture dry matter intake in horses. Journal of Equine Veterinary Science, 29, 354-355.

Fowler, A. L., Brummer-Holder M., &Dawson, K. A. (2020). Trace mineral leaching from equine compost. Sustainability, 12(17), 7157.

Fowler, A. L., Brummer-Holder, M., & and Dawson K. A. (2019a). Trace Mineral Leaching from Equine Compost. Sustainability, 12, 7157.

Fowler, A. L., Brümmer-Holder, M., & Dawson, K. A. (2019). Dietary trace mineral level and source affect fecal bacterial mineral incorporation and mineral leaching potential of equine feces. Sustainability, 11, 7107.

Harford, A. J., Mooney, T. J., Trenfield, M. A. & Van Dam, R. A. (2015). Manganese toxicity to tropical freshwater species in low hardness water. Environmental Toxicology and Chemistry, 3412, 2856-2863.

Harper, M., Swinker, A., Staniar, W., & Welker, A. (2009). Ration evaluation of Chesapeake Bay watershed horse farms from a nutrient management perspective. Journal of Equine Veterinary Science, 529, 401-402.

Hintz, H. F. (1996). Mineral requirements of growing horses. Pferdeheilkunde, 12(3), 303-306.

Hoyt, J. K., Potter, G. D., Greenead, L. W., & Anderson., J. G. (1995). Copper balance in miniature horses fed varying amounts of zinc. Journal of Equine Veterinary Science, 15, 357-359.

Hsu, J. H., & Lo, S. L. (2000). Characterization and extractability of copper, manganese, and zinc in swine manure composts. Journal of Environmental Quality, 292, 447-453.

IBM Corp. Released 2021. IBM SPPS Statistics for Windows. Version 28.0. Armonk, NY: IBM Corp.

Jackson, S.G. (1197). Trace minerals for the performance horse: known biochemical roles and estimates of requirements. Irish Veterinary Journal, 50, 668-674.

Kaya Karasu, G., Huntington, P., Iben, C., & Murray, J. A. (2018). Feeding and management practices for racehorses in Turkey. Journal of Equine Veterinary Science 61, 108-113.

Kienzle, E., Zeyner, A. (2010). The development of a metabolizable energy system for horses. Journal of Animal Physiology Animal Nutrition, 94:, 231-240.

Kirchgeßner, M. (2004): Tierernährung, 11. Auflage, DLG-Verlag

Lawrence, L. A., Ott, E. A., Asquith, R. L., & Miller, G. J. (1987). Influence of dietary iron on growth, tissue mineral composition, apparent phosphorus absorption, and chemical properties of bone. Proceedings of the Nutrition Society, 10, 563-568.

Mertz, W. (1986). Trace Elements in Human and animal nutrition, 5th edition. Beltsville, Maryland Academic press.

Meyer, H. (1994). Kupferstoffwechsel und Kupferbedarf beim Pferd. Übersichten zur. Tierernährung, 22, 363-394.

Meyer, H., Coenen, M. (2002) Pferdefütterung. Parey Verlag, Berlin

Mitchell, L. M., Robinson, J. J., Watt, R. G., McEvoy, T. G., Ashworth, C. J., Rooke, J. A., & Dwyer, C.M. (2007). Effects of cobalt/vitamin B12 status in ewes on ovum development and lamb viability at birth. Reproduction Fertility and Development, 19, 553-562.

Mørkeberg, J. (2013). Blood manipulation: current challenges from an anti-doping perspective. Hamatology, 1, 627-631.

Nagpal, N. (2004). Water Quality Guidelines for Cobalt: Water Protection Section, Water, Air and Climate Change Branch; Technical Report; Ministry of Water, Land, and Air Protection: Victoria, BC, Canada.

Nijs, A. C. M., Driesprong, A., Hollander, H. A., Poorter, L. R. M., Verweij, W. H. J., Vonk, J. A., & Zwart D (2008). Risico's van toxische stoffen in de Nederlandse oppervlaktewateren. RIVM (National Institute for Public and Environment) report 607340001.

NRC (2007). National research council. The Nutrient Requirement of Horses. 6th revised edition Washington, USA. National academic press.

Paßlack, N., Bömmel-Wegmanna, S., Vahjena, W., & Zenteka, J. (2022). Impact of dietary zinc chloride hydroxide and zinc methionine on the faecal microbiota of healthy adult horses and ponies. Journal of Equine Veterinary Science, 110, 103804.

Regulation (EU) 2018/848 of the European Parliament and of the council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007

Semenza, G. (2003). Targeting HIF-1 for cancer therapy. Nature Reviews Cancer, 3, 721-732.

Annete, C. J. (2013). Pastures and pastures management. In: R. J. Geor, P. A. Harris, & M. Coenen (Eds.), Equine Applied and Clinical Nutrition (pp. 332-349). Philadelphia, PA, USA, Saunders press.

Vos, J. H., & Janssen M. P. M. (2008). EU-wide control measures to reduce pollution from WFD relevant substances: Copper and zinc in the Netherlands. RIVM (National Institute for Public and Environment) report 607633002

Westendorf, W. (2004). Horses and manure. Rutgers Equine Science Center Publications: Factsheet 036, Rutgers, New Brunswick

Wichert, B., Kreyenberg, K., & Kienzle, E. (2002). Serum response after oral supplementation of different zinc compounds in horses. Journal of Nutrition, 132(6), 1769-70.

Xiong, Y., Cui, B., He, Z., Liu, S., Wu, Q., Yi, H., Zhao, F., Jiang, Z., Hu, S., & Wang L. (2023). Dietary replacement of inorganic trace minerals with lower levels of organic trace minerals leads to enhanced antioxidant capacity, nutrient digestibility, and reduced fecal mineral excretion in growing-finishing pigs. Frontiers Veterinary Science 10, 1142054.