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Evaluation of concentration of heavy metals in animal rearing system

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ABSTRACT

Animal manure is one of the diffusion routes of heavy metals and metalloids into the environment, where the soil can accumulate them. Heavy metals and metalloids can then be released into groundwater sources, be absorbed by crops, and enter the food chain with negative effects for human and animal health. The aim of this study was to evaluate the concentration of heavy metals and mineral nutrients from modern animal rearing systems in order to develop effective strategies to increase the sustainability. Samples of feed ($n=24$: $n=16$ from swine, $n=8$ from cattle), faeces ($n=120$: $n=80$ from swine, $n=40$ from cattle) and water ($n=8$), were collected from eight typical intensive swine and cattle farms located in northern Italy. All samples were analysed for the humidity and the principal components. The samples were also dried, mineralised, and analysed by ICP-MS to detect the following elements: Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, and Pb. The swine diets represented the highest amounts of Zn and Cu, with an average concentration for the finishing and weaning phases of Zn: 1737.9 ± 301.3 ; 821.7 ± 301.3 ; Cu: 133.8 ± 11.6 ; 160.1 ± 11.6 mg/kg as fed, respectively. The faecal content reflected the heavy metal composition from feed. The average content of cattle diets of Zn and Cu did not result higher than the maximum permitted levels. We observed that the swine manure represented the sources of Zn and Cu output into the environment. The Zn and Cu content should be monitored strictly in line with agroecology principles.

HIGHLIGHTS

- Evaluation of the role of zinc and copper in animal production.
- Increase the sustainability of farms.
- Consider the feed as major route of HMs in livestock.

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Introduction


Heavy metal and metalloid (HMM) pollution has become a serious problem in agriculture (Bhargava et al. 2012). The soil accumulates HMMs without apparent toxic effects; however, HMMs can be released into water, be absorbed by crops, and enter the food chain in high concentrations, with negative effects for human and animal health (Jarup 2003). The major causes of HMM diffusion into the environment include the geological characteristics of soil, industrial and anthropic activities, atmospheric deposition, sewage sludge, animal manure, agrochemicals, and inorganic fertilisers (Nicholson et al. 2003). The strategy for the minimisation of HMM effects is to

reduce the environmental and human exposure to these elements.

Animal production thus represents a possible source of HMMs and also a key link in the food chain. The major HMMs input in pig livestock is represented by feed, which should be controlled in order to prevent the excessive spread of HMMs into the environment (Adewole et al. 2016). Thus, HMMs should be monitored strictly in line with the modern principles of agroecology and in order to increase sustainability (Dumont et al. 2013).

HMMs can enter animals' diet as both contaminants or undesirable substances, and also as essential nutrients (Fink-Gremmels 2012; Hejna et al. 2018). For

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contaminants such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As), the maximum levels in certain foods have been established by regulation (European Parliament and of the Council 2002). In cases where the threshold is exceeded, they are not permitted in food and feedstuff. On the other hand, minerals such as cobalt (Co), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn) are part of the numerous enzymes that coordinate many biological processes and consequently should be used to supplement animals' diet in accordance with the authorised levels (European Parliament and the Council 2003; Lopez-Alonso et al. 2012).

The bioavailability of these minerals that can influence their presence in the manure ranging from 10% to 80% is influenced by factors such as animal status (age, production, health, and physiological status), the chemical composition of the mineral source (organic vs. inorganic), and the diet composition. Nutrients that are not retained by the animal or exported in livestock products are excreted through manure, composed of animal faeces and urine (García-Vaquero et al. 2011; Fink-Gremmels, 2012; Bastianelli et al. 2015).

The manure is frequently used as a valuable organic fertiliser containing a broad range of nutrients such as nitrogen, phosphorus, potassium, as well as micronutrients (Nicholson et al. 2003; Moral et al. 2005; Chardon et al. 2012; Jakubus et al. 2013). HMMs output from intensive animal production varies considerably in relation to countries and farming system (Hejna et al. 2018). According to Nicholson et al. (2003) in England and Wales, the Zn and Cu output was higher in swine manure compared to cattle and poultry manure (650.00, 470.00 mg/kg DM, respectively). Moreover, Hölzel et al. (2012) reported the concentration of Zn in swine slurry ranged from 93.00 to 8239.00 mg/kg DM in Germany (Bavaria). Shi et al. (2019) reported higher average concentration of Zn and Cu in swine manure compared to cattle and sheep manure in China (1313.00, 514.70 mg/kg, respectively). Through the manure, a large number of metallic ions can be absorbed into the soil, thus interfering with the quality of cultivation or increasing soil pollution (Lopez-Alonso et al. 2012; Jakubus et al. 2013; Rossi et al. 2013; Rossi, Dell'Orto et al. 2014a; Gul et al. 2015; Liu et al. 2018).

Recently, the EU has banned the inclusion of pharmacological levels of ZnO (EMA 2017) in animal feed after 2022 because overall benefit–risk balance for additives containing ZnO remains negative. Due to recent legislation and lacking of the data about the

HMMs pollution in Lombardy region, it is important to determine the situation in the field to monitor the Italian situation in the major part of intensive animal production. Thus, the aim of this study was to evaluate the concentration of heavy metals and mineral nutrients from feed, water, and faeces in modern swine and cattle rearing systems in northern Italy in order to develop effective strategies to increase the sustainability.

Material and methods

Farm selection and sample collection

In this study, both pig and cattle randomly selected farms were considered. A total of four typical intensive swine farms (F1, F2, F3, and F4) and four typical dairy cattle farms (F5, F6, F7, and F8) located in northern Italy were included. All swine farms (F1–F4) lead to the Consortium of Parma. The breeding management including the organisation of safeguard, economic policy, and quality control was developed according to Consortium regulations. Commercial swine farms selected to the survey consisted of a closed system with a number of sows ranging from 200 to 600, and farrowing, weaning, and finishing phases. In each swine farm, dry feed, maize-, and soybean-based meal was supplied *ad libitum* in the growing phases and was formulated according to specific nutritional requirements for the production phase (NRC 2012). Pregnant sows were fed complete dry feed (from 2 to 2.5 kg/head/day). Lactating sows on each farm were fed *ad libitum* in order to obtain the maximum attainable consumption of nutrients. Water was supplied through nipple drinkers.

All indicated cattle farms (F5–F8) lead to the Grana Padano Consortium. The Italian Friesian dairy cattle farms enrolled in this study consisted of modern free-stall barns, with animals housed on padded mattresses or different types of bedding materials (sawdust or shavings, straw). These cattle farms consisted of a number of lactating cows ranging from 200 to 600, and calves, and heifers phases. The cows were fed a homogenous total mixed ratio (TMR) in feed lanes. The feed was composed of dry forage mixed with milled raw materials, which provide adequate nutrient intake for the milk production to meet the needs of dairy cows. Calves were fed commercial feed (dry forage and commercial milk replacement diet) individually. Water was available *ad libitum* through automatic waterers.

For each farm, the animal welfare, housing conditions, and health status were registered and assessed (Council Directive 2008; Grandin 2017).

All samples of feed and faeces were collected in the 10-day sampling procedure at the same hour in order to reduce the variability among periods. The feed samples were collected in order to guarantee the representativeness of samples according to the AOAC procedure (965.16). A total of 16 feed samples (500 g each) from F1 ($n=4$), F2 ($n=4$), F3 ($n=4$), and F4 ($n=4$) were collected from different phases of production on swine farms (gestation, $n=4$; farrowing, $n=4$; weaning, $n=4$; finishing, $n=4$). Two different sampling procedures were adopted depending on the feed storage system used on the considered farms. The sampling from feed storage was adopted in order to reduce the variability between different feed distributions in the selected farms. In particular, where silos were used, ten subsamples were collected from at least 10 regions of silos which were then bulked together and thoroughly mixed to obtain a composite sample of approximately 500 g. Where the feed was kept in 25-kg bags, the commercial diets were sampled by taking grab subsamples from at least 10 regions of bags (50 g) within three-fourths of the depth. Individual grab subsamples were bulked and thoroughly mixed, and a composite sample of approximately 500 g was created.

A total of eight feed samples (500 g each) from F5 ($n=2$), F6 ($n=2$), F7 ($n=2$), and F8 ($n=2$) were collected from different phases of cattle farms (calves, $n=4$; lactation, $n=4$). The commercial diet of calves and the TMR of lactating cows were sampled during the feeding period. At sampling, at least 10 subsamples were collected, taking care to prevent any spilling. Individual grab subsamples were bulked together and mixed thoroughly before a composite sample of approximately 500 g. All collected feed samples were placed in an airtight nylon bag, devoid of air, sealed tightly, and identified by a serial number.

A total of 80 faeces samples (50 g/each) from F1 ($n=20$), F2 ($n=20$), F3 ($n=20$), and F4 ($n=20$) were collected from different phases of swine farms (first week of gestation, $n=20$; farrowing, $n=20$; weaning, $n=20$; finishing, $n=20$). The fresh faecal samples from the first week after insemination and from lactating sows were collected individually from inside the cages. The fresh faecal samples of weaning and finishing pigs were collected from five different areas of the pen floors.

A total of 40 faecal samples (50 g/each) from F5 ($n=10$), F6 ($n=10$), F7 ($n=10$), and F8 ($n=10$) were collected from different phases of cattle farms (calves, $n=20$; lactation, $n=20$). The fresh faecal samples from calves were collected individually from the cages. The fresh faecal samples from lactating dairy cattle were collected from different parts of fresh pats on the pen floor. Each sample was placed into a 50-mL polyethylene sterile tube, labelled and sealed immediately in order to avoid contamination. All the faecal samples were kept separately until the lab analysis.

Eight water samples from each selected farm were collected into 50-mL polyethylene sterile tubes, labelled and sealed immediately. All collected feed and faeces samples were transported in controlled conditions (4 °C). Feed samples were then analysed immediately, and faecal samples were frozen and stored at -20 °C for further analysis.

Chemical composition of feed and faecal samples

Each of feed samples (from F1 $n=4$, from F2 $n=4$, from F3 $n=4$, from F4 $n=4$; from F5 $n=2$, from F6 $n=2$, from F7 $n=2$, from F8 $n=2$) was mixed thoroughly and analysed for humidity as well for principal components (10 g/DM each), such as crude protein (CP), crude fibre (CF), ether extract (EE), and ashes according to the Association of Official Analytical Chemists (AOAC 2005; European Commission 2009). Dry matter (DM) was obtained by inserting mixed feed samples in preweighed aluminium bags and dried in a forced air oven at 105 °C for 24 h (AOAC 2005 method, proc. 930.15; European Commission 2009). All dried feed samples were then ground with a laboratory mill (Cyclone Sample Mill, Model 3010-019, pbi International, Milan, Italy). CP was measured according to the Kjeldahl method (AOAC 2005 method, proc. 2001.11). CF was determined by the Filter Bag technique (AOCS 2005 method, proc. Ba 6a-05). EE was determined by the Soxhlet method, with prior hydrolysis (European Commission 2009). Ashes were measured using a muffle furnace at 550 °C (AOAC 2005 method, proc. 942.05).

Individual swine faecal samples ($n=5$ per each phase, each farm) collected from the same farm (F1, F2, F3, F4) and phase (gestation, farrowing, weaning, finishing) were thawed and combined to create one mega sample per each phase (from F1 $n=4$; from F2 $n=4$; from F3 $n=4$; from F4 $n=4$). Individual cattle faecal samples ($n=5$ per each phase, each farm) collected from the same farm (F5, F6, F7, F8) and phase

(calves; lactation) were thawed and combined to create one mega sample per each phase (from F5 $n = 2$; from F6 $n = 2$; from F7 $n = 2$; from F8 $n = 2$). The faecal samples were analysed for the evaluation of DM, CP, CF, EE, and ashes following the procedure described above.

Evaluation of minerals in feed, faecal, and water samples by ICP-MS

Dried feed and faecal samples (0.3 g/DM each) were mineralised by the ultrawave single reaction chamber Microwave Digestion System (MULTIWAVE 3000; Anton Paar GmbH, Graz, Austria) in Teflon tubes filled with 10 mL of HNO₃ (65% concentrated) by applying a one-step temperature ramp (at 120 °C in 10' and maintained for 10). The mineralised samples were cooled for 20 min, and the homogenous sample solutions were transferred into the polypropylene test tubes. Feed samples (250 µL) were then diluted 1:40 with a standard solution containing an internal standard (100 µL) and H₂O (9.75 mL), while faecal samples (100 µL) were diluted 1:100 with standard solution containing an internal standard (100 µL) and HNO₃ (0.3 M, 10 mL).

An aliquot of 2 mgL⁻¹ of an internal standard solution (⁷²Ge, ⁸⁹Y, ¹⁵⁹Tb) was added to the samples and calibration curve to obtain a final concentration of 20 µgL⁻¹. All samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Bruker Aurora M90 ICP-MS, Bremen, Germany) in order to detect the following elements: Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, and Pb. The accuracy and precision of the results obtained using ICP-MS were evaluated using internal reference materials supplied by LGC Standards Company: sewage sludge (LGC 61812); poultry feed (LGC7173); and wastewater (SPS-WW2 1). The typical polyatomical analysis interferences were removed using the Collision-Reaction-Interface (CRI) with an H₂ flow of 75 mL/min through a skimmer cone.

Statistical analysis

In order to evaluate any statistically significant differences among the means, all data were analysed using Proc Glimmix of SAS software (9.4. SAS. Inst. Inc., Cary, NC, USA). The analysis accounted for the fixed effects of phases. Means were considered different when $P \leq .05$ and tended to differ if $0.05 < P \leq .10$. Tukey-Kramer studentized adjustments were used to separate treatment means within phases.

Results and discussion

Chemical composition of feed and faecal samples

The collected diets showed a nutrient composition in line with the specific animal nutritional requirements (NRC 2001, 2012) for both swine and cattle feed within different phases. Nutritional requirements are different according to the animal's physiological stages which were considered during the formulations of the rations (Jha and Berrocoso 2015; Patience et al. 2015; Wang et al. 2018).

The humidity content of the swine diets ranged from 8.83 ± 1.60 for weaning, to $11.13 \pm 1.66\%$ as f.w. for the farrowing phase (Table 1). Swine are monogastric and are usually reared on conventional high energy and protein rich rations (from 14.89 ± 0.45 for gestation to 15.78 ± 0.40 g/100 g DM for the weaning phase) in order to guarantee the productivity. Although economic and environmental factors have compelled nutritionists to develop low-protein diets, proteins are the most important component in the swine diet (Miller 2004; NRC 2012). Traditionally swine diets are formulated on the basis of crude proteins, which refer to the nitrogen content of the feedstuff per 6.25. The CPs of the analysed diet ensured the essential nutritional requirements for growth and gain efficiency.

As well as swine, the calves need high protein content in their diet, the calves received dried feed with a higher level of crude proteins of milk origin compared

Table 1. The average chemical composition (on DM basis, excluded humidity) of feed from swine farms (F1 $n = 4$, F2 $n = 4$, F3 $n = 4$, F4 $n = 4$) per phase: gestation, farrowing, weaning and finishing, and cattle farms (F5 $n = 2$, F6 $n = 2$, F7 $n = 2$, F8 $n = 2$) per phase: calves and lactation.

Composition	Swine				Cattle	
	Gestation	Farrowing	Weaning	Finishing	Calves	Lactation
Humidity (% as f.w.)	11.02 ± 1.70^a	11.13 ± 1.66	8.83 ± 1.60	9.81 ± 1.28	11.42 ± 1.18	49.69 ± 3.38
Crude protein (%)	14.89 ± 0.45	15.34 ± 0.41	15.78 ± 0.40	15.16 ± 0.15	20.35 ± 0.18	13.86 ± 0.82
Nitrogen (%)	2.38 ± 0.07	2.45 ± 0.07	2.52 ± 0.06	2.42 ± 0.02	6.16 ± 0.03	2.22 ± 0.13
Crude fibre (%)	5.70 ± 0.50	5.63 ± 0.38	4.20 ± 0.37	4.24 ± 0.12	6.16 ± 0.20	15.39 ± 0.60
Lipids (%)	4.33 ± 0.74	5.11 ± 0.16	5.26 ± 0.31	5.13 ± 0.35	3.35 ± 0.69	3.23 ± 0.27
Ash (%)	7.81 ± 0.50	7.88 ± 0.62	6.57 ± 0.42	6.50 ± 0.42	7.95 ± 1.15	7.02 ± 0.31

^aData are presented as means and standard error of the means (SE).

Table 2. The average chemical composition (on DM basis, excluded humidity) of faeces from swine farms (F1 $n=4$, F2 $n=4$, F3 $n=4$, F4 $n=4$) per phase: gestation, farrowing, weaning and finishing, and cattle farms (F5 $n=2$, F6 $n=2$, F7 $n=2$, F8 $n=2$) per phase: calves and lactation.

Composition	Swine				Cattle	
	Gestation	Farrowing	Weaning	Finishing	Calves	Lactation
Humidity (% as f.w.)	72.78 ± 1.75 ^a	71.65 ± 1.73	70.13 ± 2.46	73.52 ± 1.10	80.03 ± 3.12	85.80 ± 0.25
Crude protein (%)	12.98 ± 1.04	11.51 ± 0.74	19.48 ± 1.53	19.02 ± 1.56	20.61 ± 3.57	14.15 ± 0.74
Nitrogen (%)	2.08 ± 0.17	1.84 ± 0.12	3.12 ± 0.24	3.05 ± 0.25	3.30 ± 0.57	2.26 ± 0.12
Crude fibre (%)	17.39 ± 1.45	18.31 ± 1.36	15.28 ± 1.69	14.96 ± 0.76	18.89 ± 2.21	25.20 ± 0.99
Lipids (%)	4.02 ± 0.89	3.88 ± 0.73	6.86 ± 0.72	8.23 ± 1.26	4.00 ± 1.34	1.87 ± 0.29
Ash (%)	18.64 ± 2.68	17.96 ± 1.01	15.95 ± 1.60	14.12 ± 0.61	12.90 ± 0.59	12.26 ± 0.55

^aData are presented as means and standard error of the means (SE).

to cows (20.35 ± 0.18 vs. 13.86 ± 0.82 g/100 g DM, respectively; NRC 2001). On the other hand, the cow diet, represented by a total mixed ratio (TMR), showed a higher percentage of humidity (49.69 ± 3.38 vs. 11.42 ± 1.18% as f.w., respectively; Leonardi et al. 2005; Eastridge 2006) and fibre (15.39 ± 0.60 vs. 6.16 ± 0.20 g/100 g DM, respectively; Table 1). Fibre is an important nutrient for maintaining the functional parameters of the rumen and thus for sustaining animal health (Terré et al. 2013).

Faeces represent the final product of the digestive process and mineral bioavailability of the nutrients from the diets (Bastianelli et al. 2015). Increasing humidity content was observed in the swine faeces. The humidity content ranged from 70.13 ± 2.46 for weaning to 73.52 ± 1.10% as f.w. for the finishing phase. The faecal samples contained a high amount of water. Faeces resulting from the digestive process thus contained a higher amount of water (Le Goff and Noblet 2001; Van Vliet et al. 2007). The protein content (CP) of swine faeces ranged from 11.51 ± 0.74 for farrowing to 19.48 ± 1.53 g/100 g DM for the weaning phase. The high percentage of crude fibre in swine faeces is related to the monogastric physiology, which is not able to digest cellulose. The CF content (on DM basis) of faeces was three or four times higher than in the feed (5.63 ± 0.38 vs. 18.31 ± 1.36 g/100 g DM for the farrowing phase; Table 2). Moreover, the ash content (on a DM basis) increased significantly compared to the swine feed (6.57 ± 0.42 vs. 15.95 ± 1.60 g/100 g for weaning). This was related to the low absorption of minerals from the gut (Adewole et al. 2016).

The high percentage of crude fibre in cattle faeces is related to the ruminant physiology (6.16 ± 0.20 vs. 18.89 ± 2.21 g/100 g for the calf phase). The amount of fibre in cattle faeces is a result of incomplete digestion or indigestible components (Bargo et al. 2002; Indugu et al. 2017; Table 2). In addition, the chemical composition resulting after the digestion process of the ruminants has been shown to have a higher percentage of ash compared to cattle feed. This is related, as in the

case of swine farms, to a low nutrient bioavailability (Warly and Fariani 2017).

The amount of water consumed by the livestock is influenced by the animal species, growth stage, feed intake, environmental temperature, and the choice of feeding equipment. The method used to cool animals and wash barns can also increase the input of water in the manure.

Evaluation of HMs and minerals in feed, faecal and water samples by ICP-MS

Minerals are an essential part of the diet in sustainable animal production for the fulfilment of nutrition requirements and to maintain the appropriate animal growth. However, animal diet may also contain contaminants.

Our study showed that undesirable elements represented by arsenic (As), cadmium (Cd), lead (Pb), cobalt (Co), nickel (Ni), and molybdenum (Mo) did not exceed the threshold levels for feed established by regulation (European Parliament and of the Council 2002) for all the selected feed samples of swine and cattle farms. Thus, feed samples did not represent any apparent risk for the intensive swine and cattle production systems (Supplementary Table 1). These results are in line with other studies (Mendoza-Huaitalla et al. 2010; Zhang et al. 2012; Wang et al. 2013; Adamse et al. 2017).

Iron (Fe) and manganese (Mn) did not exceed the threshold limits for feed in swine and cattle feed (European Parliament and the Council 2003). The average content of selenium (Se) in swine diets slightly exceeded the maximum permitted level (European Parliament and the Council 2003). This is probably not related to the mineral supplementation, but is a natural variation of the nutrients in raw materials (Gaudré and Quiniou 2009; NRC 2012). Inversely, Se did not represent a risk for cattle feed and did not exceed the thresholds (European Parliament and the Council 2003). On the other hand, the contents of sodium (Na), magnesium (Mg), potassium (K), calcium (Ca),

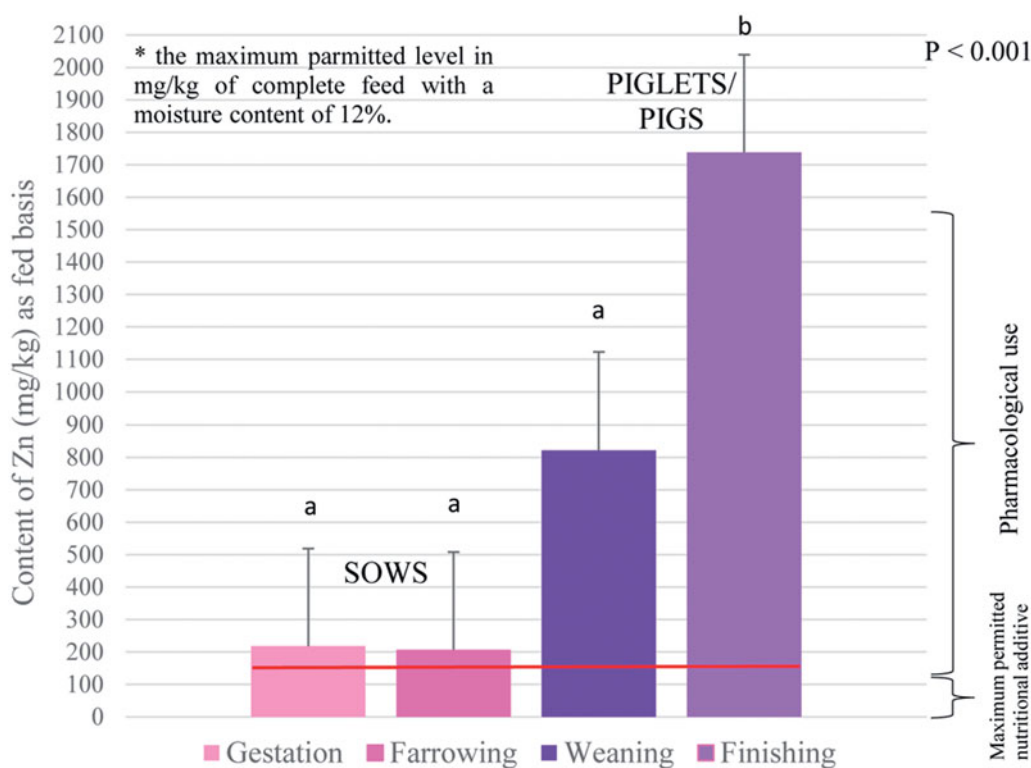


Figure 1. The average concentration of zinc content in feed from different swine phases (gestation, weaning, farrowing, finishing) in considered swine farms (F1–F4) located in northern Italy. The red line represents the maximum permitted level of zinc as nutritional additive in feed for pigs (150 mg/kg; EC N° 1831/2003; EU 2016/1095). The average humidity content (% as f.w.) in swine feed (with SE): 11.02 ± 1.70 for gestation; 11.13 ± 1.66 for farrowing; 8.83 ± 1.60 for weaning; 9.81 ± 1.28 for finishing. Data are presented as least-squares means and SEM.

and chromium (Cr) met the range of nutritional requirements for both swine and cattle species (NRC 2001, 2012).

Content of Zn and Cu in swine and cattle farm feed samples

A different situation was found in relation to zinc (Zn) and copper (Cu) (Figures 1 and 2). The diets considered showed high levels of zinc, in particular for both the weaning and finishing phases (821.7 ± 301.3 ; 1737.9 ± 301.3 mg/kg as fed, respectively). In our results, the wide range of dosage was similar to the feed from weaning phase in England (from 212.00 to 2350.00 mg/kg DM: Nicholson et al. (1999)). Moreover, Mendoza-Huaitalla et al. (2010) reported in China the highest Zn average value in weaning phase from swine feed samples (1497.14 mg/kg, respectively). In contrary, Zhang et al. (2012) observed the Zn concentration in swine feed in China from 37.37 to 598.32 mg/kg. The estimated contents of Zn were significantly higher than 150 ppm, corresponding to the maximum permitted level as nutritional additives (European Parliament and the Council 2003; EU 2016).

This is probably related to the pharmacological use of Zn, after veterinary prescription, to control enteric disorders which often appear during the growing phase. In fact, Zn is usually used to guarantee livestock productivity by controlling enteric pathogen bacterial infection as well as to enhance the integrity of the immune system (Luo et al. 2009; Hu et al. 2012; Liu et al. 2018). It is also crucial in these phases to reduce post-weaning diarrhoea and enhance animal growth performance related to the role of Zn in intestinal integrity (Zhang and Guo 2009; Pearce et al. 2015).

After the antibiotics ban in 2006 in Europe (European Parliament and the Council 2003), there has been an increased use of high dosages of zinc oxide (ZnO). However, excessive exposure with higher concentrations of Zn has been linked to an increase in antimicrobial resistance, change in microbiota, accumulation of ions in vital organs, and environmental issues (Lyubenova and Schröder 2011; Dumont et al. 2013; Rossi, Fusi et al. 2014b). The EU has thus banned the inclusion of pharmacological levels of ZnO after 2022 (EMA 2017). In line with the major topics of agroecology, considering the needs of animals (health, welfare and nutrition productivity) and farmers

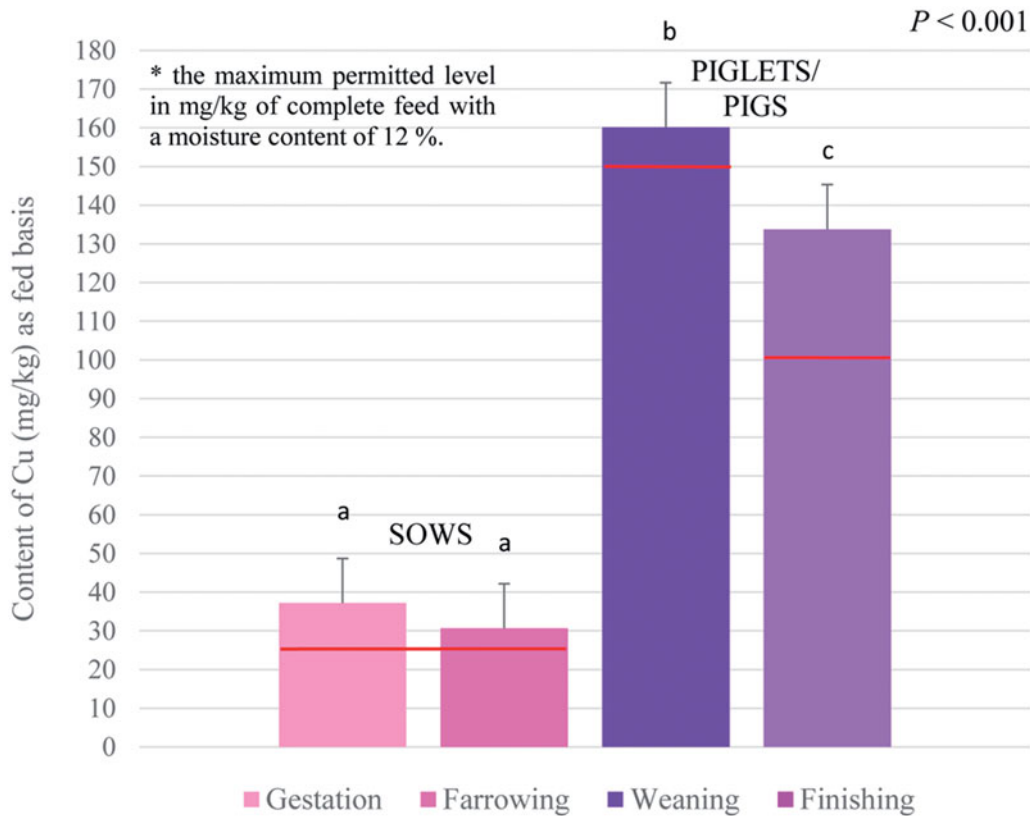


Figure 2. The average concentration of copper content in feed from different swine phases (gestation, weaning, farrowing, finishing) in considered swine farms (F1–F4) located in northern Italy. The red line represents maximum permitted level of copper as nutritional additive in feed for pigs (weaning – 150 mg/kg, finishing – 100 mg/kg; above 12 weeks – 25 mg/kg; EC N° 2018/1039). The average humidity content (% as f.w.) in swine feed (with SE): 11.02 ± 1.70 for gestation; 11.13 ± 1.66 for farrowing; 8.83 ± 1.60 for weaning; 9.81 ± 1.28 for finishing. Data are presented as least-squares means and SEM.

(profitability and productivity) together with the environment, alternative solutions are required to control enteric disorders and implement the sustainability of pig livestock.

In pig diets, the amount of Cu in all phases resulted higher than maximum permitted level although at lower concentrations compared to Zn (Figure 2; weaning: 160.09 ± 11.55 ; finishing: 133.75 ± 11.55 mg/kg as fed, respectively). The results were in line with Nicholson et al. (1999), which reported in England the Cu concentration in feed for the weaning phase from 121 to 190 mg/kg DM. Moreover, Dai et al. (2016), in Wisconsin (USA), reported the similar average Cu concentration in pig feed (169.90 mg/kg). In contrary, in China, according to Wang et al. (2013) the average Cu concentration in pig feed was lower (36.90 mg/kg). Furthermore, compared to our results, Mendoza-Huaitalla et al. (2010) reported higher mean values of Cu in the weaning and finishing feed (240.29 mg/kg; 192.71 mg/kg, respectively).

In the sows, Cu was supplemented in the diet in order to maintain the fertility and improve reproductive performance. The most critical inclusion was

observed in the finishing phase, where the average value was 30% higher than the maximum permitted level. The inclusion of Cu in young animals' rations is common practice to improve growth performance. It is related to the bacteriostatic and bacterial properties of Cu, which may reduce the bacterial population in the intestine and protect against oxidative stress (Luo et al. 2009; Liu et al. 2018). Excessive exposure to high concentrations of Cu has been linked to the increase of the antimicrobial resistance and influence on the environment (Dumont et al. 2013). The EU thus recently decided to reduce (from 170 to 100 mg/kg for up to four weeks after weaning) the maximum level of Cu in animal feed (Commission Implementing Regulation 2018). When the ration is supplemented with minerals, it is important to consider any elements that may be contained naturally in the diet that could influence the total amount of the elements found in the manure.

Analysed water samples for Zn elements from swine farms for F1 were as follows: 0.0001 mg/L; F3: 0.0553 mg/L; F4: 0.2540 mg/L and for Cu F1: 0.0029 mg/L; F3: 0.0748 mg/L; F4: 0.2117 mg/L. These

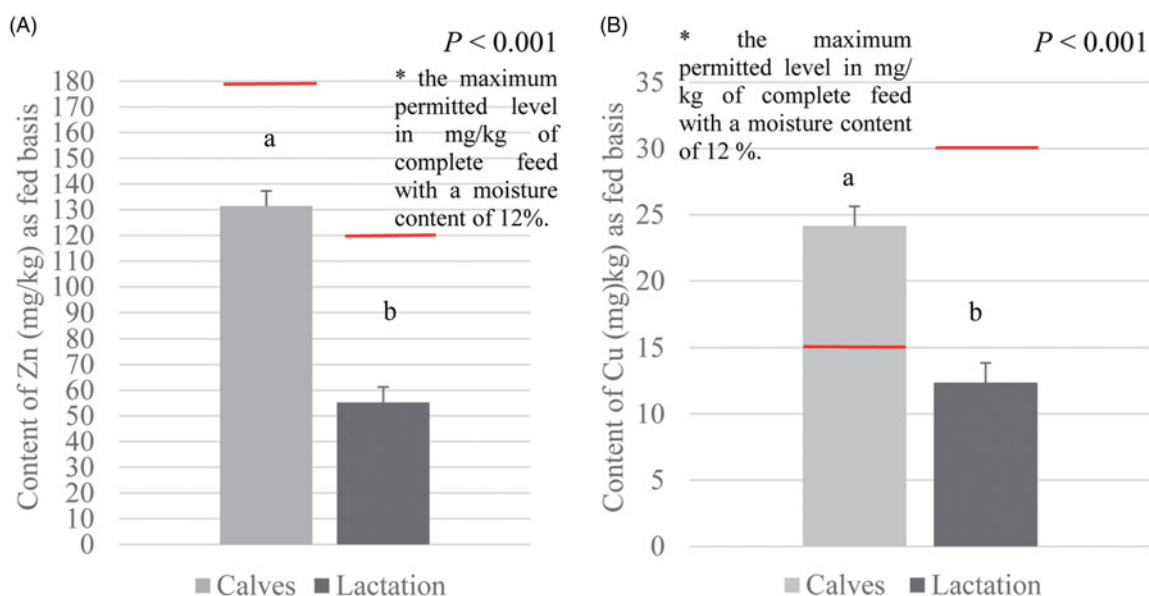


Figure 3. The average concentration of Zn content (A) in feed with the red line represents maximum permitted level of zinc as nutritional additive in feed for cattle (calves – 180 mg/kg; other – 120 mg/kg; EC N° 1831/2003, 2016/1095) and for Cu content (B) in feed with the red line represents maximum permitted level of copper as nutritional additive in feed for cattle (bovines before rumination – 15 mg/kg; other bovines – 30 mg/kg; EC N° 2018/1039) from different cattle phases (calves, lactation) in considered cattle farms (F5–F8) located in northern Italy. The average humidity content (% as f.w.) in cattle feed (with SE): 11.42 ± 1.18 for calves; 49.69 ± 3.38 for lactation. Data are presented as least-squares means and SEM.

results did not exceed the legal thresholds for drinking water (for Zn: 3 mg/L according to Decreto legislativo (2006) and for Cu: 2 mg/L according to 1998/83/EC).

In order to increase the sustainability of livestock, some aspects should be recognised. Bioavailability should be considered because the absorption of minerals from the gut is not complete and animals excrete from 70% to 95% of Cu and Zn. The mineral absorption is mostly influenced by the chemical form of salts used as additives in the diet (Suttle 2010). In order to increase mineral absorption, and decrease the excretion, other sources of mineral additives should be considered (Liu et al. 2018). In addition, nutrient absorption is influenced by factors such as the interaction with other compounds in the diet (García-Vaquero et al. 2011; Fink-Gremmels 2012; Hejna et al. 2018). Mineral-binding factors, naturally present in cereals and grains, such as the salt form of phytic acid (phytates), may also limit the absorption of minerals (Jondreville et al. 2007; Bohn et al. 2008).

As we observed in intensive animal production systems, especially in swine feed, the common strategy is to use the maximum tolerable permitted levels of microelements such as Zn and Cu in the complete diet. This tendency is above the amount required for the animals and should not be a nutritional target. In the light of those findings, it is possible to reduce the amount of excreted nutrients such as Zn and Cu in

manure by (i) avoiding nutrient overformulation, (ii) feeding at optimal rather than maximum permitted levels, (iii) implementing a feed efficiency strategy, and (iv) controlling Zn and Cu concentrations in raw materials before creating the complete diets (Sims et al. 2005; Mendoza-Huaitalla et al. 2010; Suttle 2010; Dumont et al. 2013).

In order to maintain the sustainable production of swine livestock, the diet formulation should be integrated into total production systems. The overall management of animal production is crucial in order to maintain animal performance and reproduction with the minimal excretion of minerals. Moreover, feed efficiency, through advanced genetic techniques, could further improve the environmental conditions and enhance the processing of feed (Sims et al. 2005; Suttle 2010).

The data showed that the Zn and Cu concentration in cattle feed were not largely used and did not result higher than the maximum permitted level (Figure 3). In our study, higher concentration of Zn and Cu was observed in the calves phase (131.36 ± 5.97 ; 15 ± 1.48 mg/kg as fed, respectively). Compared to our results, Zhang et al. (2012) reported higher average Zn concentration in China for dairy feed in small-, medium-, and large-headed size farms (156.28; 101.78; 114.90 mg/kg DM, respectively). Moreover, the average Cu concentration in dairy feed from small- and large-

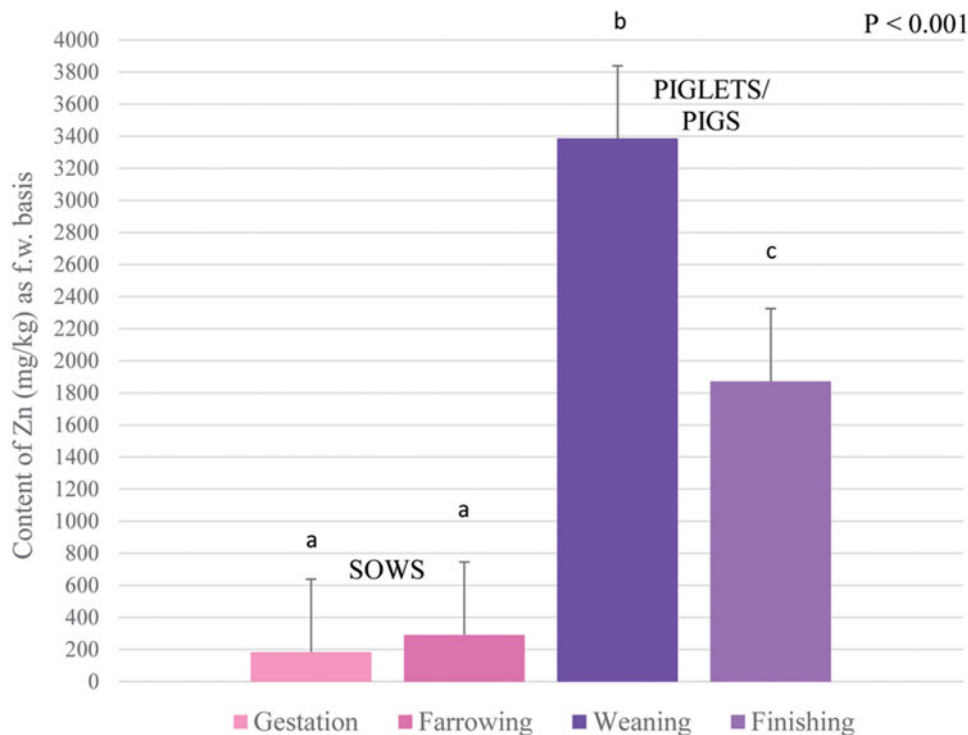


Figure 4. The average concentration of Zn content (mg/kg of f.w. – fresh weight) in faeces from different swine phases (gestation, weaning, farrowing, finishing) in considered swine farms (F1–F4) located in northern Italy. The average humidity content (% as f.w.) in swine faeces (with SE): 72.78 ± 1.75 for gestation; 71.65 ± 1.73 for farrowing; 70.13 ± 2.46 for weaning; 73.52 ± 1.10 for finishing. Data are presented as least-squares means and SEM.

heard size farms in China was higher than in our work (32.12; 25.98 mg/kg DM, respectively; Zhang et al. 2012). Also Dai et al. (2016) reported in USA higher average Cu concentration (37.80 mg/kg). The established thresholds (European Parliament and the Council 2003) for Zn and Cu in cattle feed were also not exceeded (Figure 3).

The water samples analysed regarding Zn elements from cattle farms were for F6: 0.0553 mg/L; F7: 0.2540 mg/L; F8: 0.0031 mg/L and for Cu F6: 0.0748 mg/L; F7: 0.2117 mg/L; F8: 0.0024 mg/L which did not exceed the legal limit for water (for Zn: 3 mg/L in accordance with Decreto legislativo (2006) and for Cu: 2 mg/L in accordance with 1998/83/EC).

Zn and Cu content in faecal samples for swine and cattle farms

The results showed that swine faeces had a higher Zn concentration in the weaning and finishing phases in line with the same feed phases in the swine diet (Figure 4; 3385.20 ± 454.00 ; 1871.60 ± 454.00 mg/kg as f.w., respectively). In general, a wide range of Zn content was observed because many factors can alter the Zn excretion by faeces (the diet, the management, the different regulations). Li et al. (2018) reported high

average Zn concentration in the solids residue of swine manure (17467.00 mg/kg), and Xu et al. (2013) observed a wide range of this element (183.42 to 1126.25 mg/kg) in pig manure in China. According to Yang et al. (2017), the range of Zn concentration in the swine compost (containing swine slurry and straw) ranged from 11.80 to 3692.00 mg kg⁻¹ DW in China farms. In Europe, the situation was similar with our results and with outcomes from China. In Bavaria (Germany), Zn from swine manure ranged from 93.0 to 8239.0 mg/kg DM (Hölzel et al. 2012). In Portugal, Alvarenga et al. (2015) reported high concentration of Zn (2033.0 ± 255.6 mg kg⁻¹) in pig slurry. In contrary, Nicholson et al. (2003) and Luo et al. (2009) observed the lower average Zn concentration of swine slurry in England (650.0 mg/kg DM) and in China (843.30 mg/kg). Higher concentration of Zn in swine faeces is related to the low mineral absorption from the gut. Even if the differences in concentrations of HMMs may be related to several factors, the concentration of Zn and Cu in animal feed and manure was positively correlated (Wang et al. 2013). The highest Zn excretion was observed in the weaning phase. This is probably due to the immature digestion system of the animals or by the pharmacological use of this mineral source to control the enteric disorders that can influence the

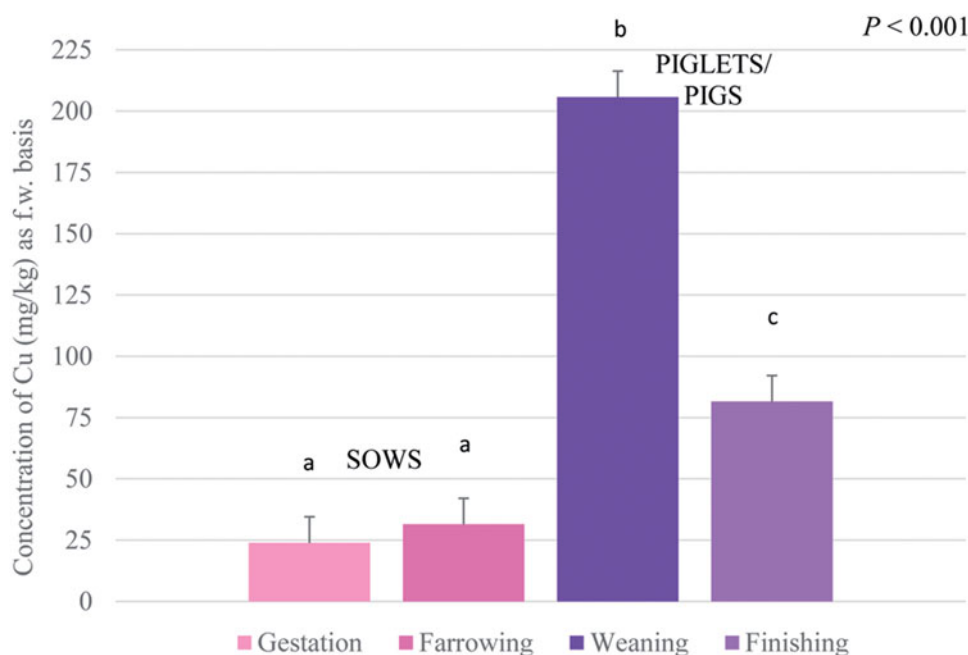


Figure 5. The average concentration of Cu content (mg/kg of f.w. – fresh weight) in faeces from different swine phases (gestation, weaning, farrowing, finishing) in considered swine farms (F1–F4) located in northern Italy. The average humidity content (% as f.w.) in swine faeces (with SE): 72.78 ± 1.75 for gestation; 71.65 ± 1.73 for farrowing; 70.13 ± 2.46 for weaning; 73.52 ± 1.10 for finishing. Data are presented as least-squares means and SEM.

manure content (Hu et al. 2012; Liu et al. 2018). Moreover, due to the wide use of Zn at a pharmacological level, in the weaning and finishing phases, swine fed with 2000 ppm of Zn excreted approximately 10 times more Zn in faeces than pigs fed the basal diet containing 165 ppm of Zn.

The Cu content was more concentrated in faeces compared with the feed. The average Cu concentration was the highest in the weaning phase in swine faeces (205.72 ± 10.61 mg/kg as f.w.; Figure 5). Our results reported lower Cu concentration in faeces compared with the concentration from different literatures. The average Cu concentration in pig manure in Bavaria (Germany) ranged from 22.40 to 3387.60 mg/kg DM (Hölzel et al. 2012). Alvarenga et al. (2015) reported in Portugal high concentration of Cu (510.3 ± 30.8 mg kg⁻¹) in a digested pig slurry. Moreover, high average Cu concentration for swine slurry was also observed in England (470.00 mg/kg DM; Nicholson et al. 2003). Furthermore, in China according to Xu et al. (2013), high average Cu concentration in pig manure was reported (418.42 mg/kg); and Yang et al. (2017) ranged the Cu concentration in the swine compost samples from 3.55 to 916.0 mg kg⁻¹ DW.

Nevertheless, the Zn and Cu content in animal faeces may be altered not only by the feed input. For instance, the therapeutic treatments of animals, ingestion of the soil, livestock bedding, hoof disinfection

tanks, corrosion of containment structures, and the water could also be sources (Nicholson et al. 2003).

Our results showed that Zn and Cu were the most critical HMMs in the swine faeces and that their concentration in the manure was strictly related to the feed level. In order to increase environmental sustainability, the Zn and Cu input should be controlled considering the quality and the quantity of mineral supplements using novel additives (Bolan et al. 2003; Nicholson et al. 2003; Luo et al. 2009; Mendoza-Huaitalla et al. 2010; Zhang et al. 2012; Jakubus et al. 2013). Moreover, the different situation should be considered, because some metals are naturally present in the raw materials, which integrate the feed diets rations. Thus, they can influence the HMMs concentration in the feed. In line with the major topics of agroecology, considering the needs of animals (health, welfare, and nutrition productivity) and farmers (profitability and productivity) together with the environment, multidisciplinary strategies about feed formulations are required.

Conversely, cattle and calves faeces did not exceed regulation thresholds in intensive animal production systems in terms of the Zn and Cu elements (Figure 6).

Conclusions

Our results showed that nutrition plays a pivotal role in the sustainability of intensive animal rearing system.

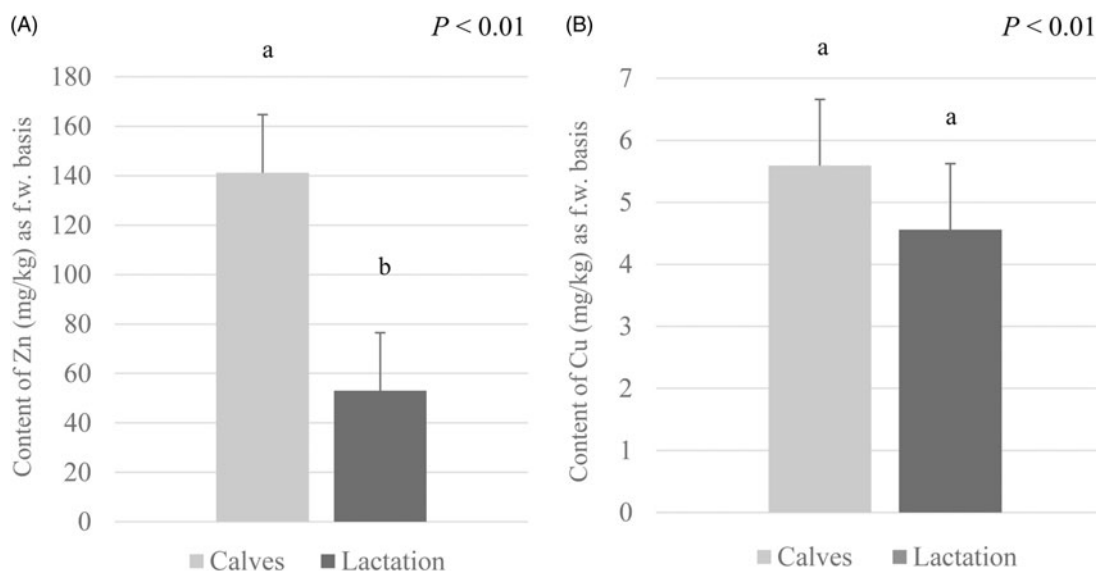


Figure 6. The average concentration of Zn content (A) and Cu content (B) in faeces (mg/kg of f.w. – fresh weight) from different cattle phases (calves, lactation) in considered cattle farms (F5–F8) located in northern Italy. The average humidity content (% as f.w.) in cattle faeces (with SE): 80.03 ± 3.12 for calves; 85.80 ± 0.25 for lactation. Data are presented as least-squares means and SEM.

In fact, HMMs in animal manure were mainly influenced by the feed. Nevertheless, the contaminants did not result as a problem in swine and cattle diets, and the most critical elements were zinc and copper probably due to their use in controlling the enteric disorders in growing piglets. In line with agroecology principles, strategies should be adopted to reduce the flux of Zn and Cu intake from swine production. In particular, the different strategies should be implemented to discover the innovative approach to control the enteric disorders. Attention should be paid to use the additives with higher bioavailability levels considering that the raw materials can influence on total HMMs amount. Moreover, the use of optimal doses rather than maximum permitted levels of microelements should be introduced due to avoiding the nutrient overformulation.

Disclosure statement

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