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Effect of the physicochemical properties of zinc oxide sources on their bioavailability in broilers

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ABSTRACT

The bioavailability of a trace mineral source is related to its intestinal solubility (bioavailability), which in turn is determined by its physicochemical properties. It is still not clear which characteristics are more relevant in affecting solubility and bioavailability of mineral sources. Zinc oxide (ZnO) is a common feed additive used to supplement zinc in the diet of monogastric animals. However, different sources have shown different responses on animal bioavailability. This study hypothesized that different sources of feed grade ZnO have various physicochemical features that lead to distinct bioavailability values. Feed grade ZnO samples collected from the feed industry worldwide and characterized for their physicochemical features were tested in broilers and their zinc bioavailability determined. A total of 135 male Cobb broiler chickens were fed a standard starter diet from day 1 after hatching up to day 7. At day 8, animals were allocated in individual cages and fed one of the following dietary treatments during 15 days: a basal diet with 23.5 ppm of zinc and 7 diets with supplemented ZnO or zinc sulphate (ZnSO₄) at 6 or 12 ppm. Different sources of ZnO showed an effect of Zn solubility in the stomach and Zn supplementation influenced total Zn in the ileum. The bioavailability of the different sources varied from 49 to 160% compared to the ZnSO₄ reference. Aggregate size of particles seems to explain a large variability in the bioavailability of the different sources tested in broilers. In conclusion, physicochemical properties of Zn oxide can partly explain the variability observed in terms of Zn biological value.

INTRODUCTION

Zinc (Zn) is a trace element essential for a proper body function. Present in over 300 metalloenzymes and 2000 transcription factors, Zn is mainly involved in gene expression, immune function and oxidative stress (Underwood, 2010). Poultry diets often do not have the enough amount of Zn to support optimal animal growth and supplementation is necessary (Schlegel et al., 2013). Zn requirement, defined by old studies (NRC, 1994), is 40 ppm. While, genetic companies recommend 110 ppm of Zn in the total (Ross, 2019). This gap between requirements and recommendations requires a further understanding of the mechanism underlying Zn digestion and absorption. Additionally, there is a pressure to reduce the safety margins of this mineral in animal feed to diminish the environmental footprint left by the livestock production, which requests a better adjustment of dietary supply to the animal needs and a better characterization of supplemented sources to understand the mechanisms affecting Zn absorption in the body.

The intestinal uptake of Zn is mainly influenced by the source and interaction with feed components, such as phytate (Schlegel et al., 2013). Zinc sulphate ($ZnSO_4$) and oxide (ZnO) are the most common Zn sources used to supplement diets. Zinc sulphate is a water-soluble source and therefore, taken as reference having a bioavailability of 100 %. Zinc oxide showed a bioavailability varying from 37 % to 105 % in relation to the sulphate counterpart (Edwards & Baker, 1999; Narcy et al., 2015). The manufacturing process of ZnO yields powder with different physicochemical properties (Moezzi et al., 2012), which lead to variable speed of dissolution in vitro. We showed that these different characteristics could explain the variability observed on Zn bioavailability values (Cardoso et al., 2020). The physicochemical characteristics and the dissolution behaviour allowed classifying the various ZnO grade in three classes as ‘Large dense particles’ (LDP), ‘Small aggregated and agglomerated particles’ (SAAP), and ‘Small aggregated particles’ (SAP). Therefore, the objective of the present study was to relate the physicochemical properties of different sources of ZnO used in the feed industry to their in vivo bioavailability.

MATERIAL AND METHODS

Birds and housing

The experimental protocol was approved by the Regional Ethical committee on animal experiment (N°19) and by the French ministry of higher education and research.

A total of 135 one-day-old male Cobb broiler chicken were used in a dose-response experiment to assess the bioavailability of 7 zinc sources. Each treatment consisted of 9 replicates. At day 1, birds arrived at the poultry facility of INRAE Val de Loire (Nouzilly, France), were weighted and housed in floor pens and fed a standard diet until day 7. At day 8, broilers were allocated according to body weight (BW) in individual cages resulting in similar mean BW per treatment. Birds were housed in these individual cages until the end of the experiment at day 22 or 23. Ambient temperature was gradually decreased in average 0.5 °C per day from 31 °C at day 1 to 22 °C at the end of the trial. Feed and water were supplied for ad libitum intake throughout the entire experiment. Birds were weighed at day 1, 8 (to standardize mean BW per treatment) and 22 or 23 of the experiment. Individual feed intake was recorded from day 8 to day 22 or 23. Feed conversion ratio (FCR) was calculated from day 8 to day 22 or 23. Mortality was registered daily throughout the experiment. At the day of sampling, the feed was removed in the morning and animals were weighted. Feed was re-introduced individually 3 hours prior to slaughter to synchronize with bird digestive physiology. Birds were euthanized by pentobarbital injection (1 mL/kg of BW; Laboratoire Vetoquinol SA, Lure, France). A sample of blood was withdrawn and centrifuged to collect the plasma before storage at -20 °C. The gastrointestinal tract of each bird was removed and separated in the different segments: (1) proventriculus and gizzard, (2) duodenum, (3) jejunum and (4) ileum. The content from each segment was collected as well as the right tibia and stored at -20 °C until further analysis.

Experimental Diets

Birds were fed a standard diet from day 1 upon to 7 days of age that covers all nutrients requirements. A basal diet (Table 1) containing corn (62%), potato protein (17%) and starch (13%) was formulated in a way that Zn originated exclusively from plant feedstuffs using a zinc-free premix. Seven different sources of feed grade zinc were thus tested according to a dose-response experiment: (1) Zinc sulfate ($ZnSO_4$) used as a reference zinc source, (2) SAAP-1; ZnO source characterized as small aggregates and agglomerates particles, (3) SAAP-2, (4) SAAP-3, (5) LDP-1; ZnO source presenting large dense particles, (6) LDP-2 and (7) SAP, ZnO characterized as small aggregates and large agglomerates (HiZox[®], Animine, France). The study included 15 dietary treatments consisting of a basal diet, with no zinc added, and zinc sulfate or one of ZnO sources providing at 6 and 12 mg/kg of zinc.

Experimental diets were manufactured by adding the respective zinc source to the basal diet. Prior to this in vivo experiment, different feed grade ZnO samples were collected and characterized according to their physicochemical properties (Table 2). Samples were assessed for particle size, specific surface area, and in vitro dissolution kinetics. The different ZnO sources were then gathered into families and representative samples of each family was selected for this bioavailability study.

All diets met or exceeded nutrient requirements of broilers according to French standards, except for dietary zinc. From day 8 onward, the birds were fed their respective pelleted experimental diets until the end of the experiment.

Prior to experimental feed manufacturing, Zn was analyzed in critical feedstuff (e.g. maize, potato, starch, casein) and supplemented sources. Based on the analyses, experimental diets were optimized according to experimental setup (Table 1). Each zinc source was added to the basal diets at the highest level, and then basal diet were mixed to high level treatment to create the intermediate level of treatments. The Zn concentration of each experimental diet was checked before the start of the trial.

Zinc Analysis

Dry matter was determined for tibia diets and digestive contents (103 °C for 4 h). To assess the soluble and insoluble fraction, the rest of the digestive content was centrifuged and the liquid phase separated from the solid. This step was repeated, with the addition of 5 mL of distilled water prior the second centrifugation. Samples were then ashed at 550 °C and was then solubilized with nitric acid (16 M) and Zn content determined using ICP-OES (Thermo Scientific™ iCAP™ 7000).

Statistical Analysis

Observations were marked as outliers and excluded from the data set before statistical analysis if an observation exceeding the parameter mean by more than two times the standard deviation (SD). The experimental data were analysed by ANOVA (GLM procedure) using R software (3.5.1 edition). The following model was used:

$$Y_{ij} = \mu + \text{Block}_i + \text{Treatment}_j + e_{ij},$$

where Y is the Zinc concentration in tibia ash, μ is the overall mean, Block is the effect of replicated block ($i = 1$ to 9), Treatment is the effect of zinc source and dose level ($j = 1$ to 15) and e is the random error. Treatment means were compared by least significant differences. A p -value < 0.05 was considered statistically significant and $0.05 < p < 0.10$ was considered a trend. The dose-

response relationship between Zn intake and tibia Zn was tested according to the method of Littell, Henry, Lewis, & Ammerman (1997). The slope of the ZnSO₄ source was considered as the reference to estimate the relative bioavailability of ZnO sources. The correlation between physicochemical properties and zinc bioavailability were then determined using the correlation procedure of R software.

RESULTS

Dietary Zinc Levels

The Zn level was determined in each dietary treatment as shown in Table 3. The analyzed zinc level in basal diet was 23.5 mg/kg, as expected. Supplementation of the Zn sources were also in accordance with the expected dose levels. Results described below will refer to supplemental Zn intake of birds throughout the experiment.

Production Performance

Results in feed intake, BW gain and FCR are given on Table 4. Daily feed intake, body weight gain per day from 8 to 22 or 23 days and FCR were not significantly affected by the dietary zinc source and supplemented level among the different treatments. The feed intake varied from 27.5 to 36.8 g per day per bird. The BW gain varied from 37.5 to 42.8 g per bird per day. FCR showed a variation from 1.19 to 1.49.

Tibia zinc content

Results on Zn on tibia dry matter content are described on Table 4. Zinc in the tibia in the control diet was 85.7 mg of Zn /kg of DM. In the supplemented diets, Zn in tibia varied from to 91.16 in the LPD-2 treatment to 120.7 mg of Zn per kg of DM in the treatment supplemented with SAP. The supplementation of either 6 or 12 mg/kg of Zn increased significantly the Zn in the tibia for all treatments ($p < 0.001$) compared with the unsupplemented diet.

Bioavailability of zinc sources

The zinc content in tibia dry matter and the supplemental zinc intake throughout the experiment were used to calculate the relative bioavailability of the respective sources. All sources showed a linear response for both levels of supplementation. The linear slope ratio of sources is shown on Figure 2. A significant difference in bone zinc was observed according to the source and to the supplemental zinc intake ($p < 0.001$). The bioavailability is represented by the slope of the different linear dose-response of each source. The higher the slope, the higher is the bioavailability. At the

opposite, a lower slope indicates a lower response to the supplementation. The slopes and bone relative bioavailability values (RBV) are described on Table 5. The coefficients of relative bioavailability of zinc was estimated considering ZnSO₄ as 100 %. Sources bioavailability had a significant variability, from 49 % for a ZnO source made of large dense particles (LDP) to 160 % for SAP, a ZnO source made of small aggregates and large agglomerates, in relation to ZnSO₄.

Gastrointestinal contents

No differences were observed regarding the supplemented Zn intake in the 3 hours before sampling (Table 7). The Zn intake in the 3 hours prior to euthanasia was not affected by the source of Zn supplemented, but was significantly higher than Zn in the control diet as expected. The supplemented Zn intake varied from 0.31 µg in the SAP treatments to 0.47 µg in the SAAP-3 treatment. The gastric content showed no differences in the total Zn and soluble content for any of the treatments, but a significant difference ($p = 0.011$) in the insoluble part of the gastric content. LDP-2 had the highest content of insoluble Zn in the stomach with 5.9 µg/g DM, as ZnSO₄ the lowest showing 1.5 µg/g DM. Interestingly, LDP-2 showed to have the highest percentage of soluble Zn in the stomach with 29 % of Zn soluble while birds fed the SAAP-3 showed 17 % of soluble Zn. In the ileum, no differences were observed within sources in the different analyzed contents. The soluble part showed to be numerically higher for ZnSO₄ source with 6.8 µg/g DM, which represented 4.7 % of soluble Zn, while birds fed LDP-2 source showed only 4.3 µg/g DM to be soluble in the ileum that characterized 3.0 % of soluble Zn. The control diet, however, showed a significant lower concentration of total Zn ($p < 0.001$) in the ileum, which is expected as the Zn content was lower than the other treatments. The highest Zn content in the ileum was for birds in the SAAP-2 treatments with 146 µg/g DM, while birds in the control diets presented 101 µg/g DM.

Physicochemical correlation

The correlation between physicochemical properties and Zn bioavailability, described on Figure 1, showed a significant impact of aggregate size, density and specific surface area ($p < 0.05$) in tibia Zn. In vitro dissolution kinetics and agglomerate size did not significantly correlate with bone Zn in the present experiment.

DISCUSSION

Zn is an essential micronutrient to ensure a proper body function. Broiler zinc requirement is estimated to be 40 mg/kg (NRC, 1994), however innumerous studies have reported improve in

animal performance when Zn was supplemented (Brooks et al., 2013; Zhao et al., 2016). Therefore, a supplementation in the feed is a current practice in poultry production. It is reported in the literature that zinc deficiency can reduce feed intake and limit growth of animals (Salgueiro et al., 2000). However, this was not observed in the present experiment, which is consistent with previous studies from Edwards and Baker (1999); Mohanna et al. (1999); Narcy et al. (2015); Star et al. (2012); Yan et al. (2016). The short period of the present trial might explain the lack of response to zinc supplementation. Brooks et al. (2013) found a difference between the supplemented sources and the control diet in birds submitted to a Zn depletion over 7 days prior to the beginning of the trial, which was not the case in the present study.

ZnO is a source widely used to supplement the feed of broilers. However, this source is poorly investigated and mainly tested in old studies (Edwards & Baker, 1999). As a non-water soluble source, ZnO is considered to have a low bioavailability when compared to ZnSO₄, a water-soluble compound. The great variability in bone Zn concentrations from the different sources of ZnO observed in this study are in agreement with previous works. Edwards and Baker (1999) evaluated the bioavailability of different sources of ZnO from various manufacturing processes. The study showed a variation from 34 % to 97 % in comparison to ZnSO₄ in which the manufacturing process showed a great effect on the measured relative bioavailability (RBV). The LDP-2 originating from the US, obtained from the Waelz-process, resulting in greenish-brown powder showed the lowest RBV (34 %) (Edwards and Baker, 1999), which was confirmed in the present study with the lowest RBV of 49 %.

To be absorbed in the body, Zn needs to be solubilized in its ionic form, Zn²⁺, to be uptaken by the protein transporters in the enterocytes membrane (Cousins, 2010). Nevertheless, in its ionic form Zn is more vulnerable to interactions with other feed compounds that may form insoluble complexes disabling its absorption (Brugger & Windisch, 2017; Schlegel & Windisch, 2006). Phytate (myo-inositol phosphate), widely present in cereal-based diet of poultry, is an important source of phosphorus and the most important feed component affecting the bioavailability of Zn. Phytate is a negatively charged molecule that has a great affinity for divalent cations, such as Zn²⁺, forming an insoluble complex precluding the uptake of this mineral (Brugger & Windisch, 2017; Schlegel et al., 2013; Schlegel & Windisch, 2006). The stomach is a dynamic environment, where reactions occur permanently. Such reactions take place in the liquid phase: molecules are converted into smaller structures, and complexed again with other compounds. Bioaccessibility, which

represents the soluble fraction of the total amount of a substance, is necessary for potential intestinal absorption. The duodenum is the main site of absorption of minerals. This short segment of the small intestine, comprise the end of the stomach up to the pancreatic duct, where enzymes and bicarbonate are released, increasing the pH. In the duodenum, the pH remains low, favouring the solubilization of minerals and therefore their absorption (Vieira, 2008).

The physicochemical properties of powders were shown to influence their *in vitro* dissolution kinetics, which could explain their different *in vivo* bioavailability. The previous article (Cardoso et al., 2020) related three main criteria according to the physicochemical of ZnO sources: (1) Small aggregates and agglomerates particles (SAAP), showing a fast dissolution kinetics, related to the small particle size of the powders. (2) Large dense particles (LDP), characterized by ZnO sources with low specific area and large size, resulting in a slow dissolution kinetics and (3) Small aggregate particles (SAP), a ZnO source with large agglomerates and large specific surface area that results in an intermediate dissolution kinetics, in which the mechanism is explain in two steps: the fast dissolution in the surface of the powders, followed by the slow diffusion of the ion from the quiescent to the solution media.

Oral absorption is a kinetic process, the transit time in the gastrointestinal tract determines the amount that can be absorbed. A fast dissolution can cause a saturation limiting absorption when the dissolution overcomes the capacity of the gut to absorb nutrient (Sugano et al., 2007), as the absorption of Zn in the duodenum and ileum suggests a saturable carrier-mediated pathway (Liu et al., 2008). Additionally, it is believed that sources with fast dissolution, such as ZnSO₄, release Zn ions in the stomach in a very short time after feed ingestion, interact with feed compounds before they are broken down, forming insoluble complexes which are unable to dissociate. *In vitro* studies failed to assess a relationship between *in vitro* solubility and *in vivo* digestibility when attempting to predict bioavailability of minerals (Anwar et al., 2016). The study performed with calcium, comparing coarse and fine particles, showed that fine particles where more soluble *in vitro*, but with lower digestibility values *in vivo*, while the opposite was observed for coarse particles. Mineral interaction and speed of dissolution and capacity of absorption might be one of the reasons to explain these results. Additionally, the lack of techniques to identify the state of the minerals in the soluble content, might blur the understanding of the mechanisms affecting Zn bioavailability. In the present study, gastric and intestine Zn contents partially explain the relationship between *in vitro* and *in vivo* dissolution kinetics. The high concentration of LDP-2 in the insoluble part of the

gastric content agrees with the *in vitro* dissolution, showing an inert source; indeed, it does not dissolve fast enough and stays longer in the gastric environment. On the contrary, the low content of Zn in the insoluble gastric part of ZnSO₄ and SAAP-1 agrees with a fast dissolution of these sources. Additionally, no difference was observed in the total Zn in the stomach, which suggests that the solubilized portion leaves the faster the gastric compartment, towards the intestine.

A source of Zn that dissociates at a slower rate, is less likely to complex and therefore more ions will be available for absorption in the small intestine. Nevertheless, a source that does not dissociate will remain inert and not available for absorption in the small intestine, which is most likely to explain the low bioavailability of LDP-2. In this case, the absorption is limited by its dissolution rate, whereas the ability of absorption is higher than the capacity of the powder of dissolve. In this scenario, an increase in the amount of powder increases proportionally the absorption, because the total solid surface area is proportional to dose amount (Sugano et al., 2007). As many factors can influence the observation of *in vivo* dissolution kinetics, such as: time and amount ingested by each bird, water ingestion, transit time of the feed, that further studies are necessary to better relate *in vitro* and *in vivo* dissolution kinetics of Zn sources.

A correlation analysis, relating Zn bioavailability in bone and physicochemical properties, suggests that aggregates size, density and specific surface area are the most relevant properties influencing the bioavailability of ZnO powders. Aggregate size showed a negative correlation of 43 %, meaning that the smaller the aggregate size, the larger the Zn content in the bone. Specific surface area, demonstrated a correlation of 29 % to tibia content of Zn. As density and aggregate size are largely correlated, most likely the strong relationship between density and tibia Zn is due to the aggregate size of powders that is related to the density values.

The small size of aggregates and large specific surface area favors the dissolution of ZnO in the gastric media, whereas large particles take a longer time to dissolve, or remains in the insoluble content of the digestive content, being unavailable to absorption, which it showed to be the case for LDP-2. Dissolution kinetics did not show any direct relationship with tibia Zn, but it explained at least partially the Zn content in the gastrointestinal tract, in which LDP summed up to the major insoluble content of gastric Zn.

CONCLUSION

The bioavailability of ZnO is variable according to the physicochemical properties of the sources with aggregate size and specific surface area as the most relevant parameters. *In vitro* dissolution kinetics influenced by the properties of the sources partially explain the fate of ZnO sources in the gastrointestinal tract. However, *in vitro* dissolution property cannot be considered as a unique predictor of Zn bioavailability in broilers. The development of techniques that allow to identify the species of Zn and its complexes in the soluble content present in the digestive tract and the description of the different ZnO used in animal feeding studies will help to compare and elucidate how the physicochemical properties of ZnO powders can affect bioavailability in broilers.

Table 1. Characteristics and dissolution behaviour of ZnO used in the present study.

| | Zinc source | Properties¹ | Speed of dissolution² | Origin |
|---|--------------------|--|---|---------------|
| 1 | ZnSO ₄ | * | +++ | Europe |
| 2 | SAAP-1 | Small aggregates and agglomerates; platelet shaped | +++ | Asia |
| 3 | SAAP-2 | Small aggregates and agglomerates; platelet shaped | +++ | Europe |
| 4 | SAAP-3 | Small aggregates, large agglomerates; 'rod-like' shaped | +++ | Asia |
| 5 | LDP-1 | Large dense particles | + | Europe |
| 6 | LDP-2 | Large dense particles | + | North America |
| 7 | SAP | Small aggregates, large agglomerates; platelet shaped | ++ | Europe |

* Characterization not applicable for ZnSO₄. ¹Properties are described by Cardoso et al. (2020).

²The speed of dissolution described in vitro as: (1) +++: fast dissolution, (2) ++ intermediate dissolution and (3) +: slow dissolution rate.

Table 2. Composition of the basal diet without added zinc sources*

| Item | Basal diet |
|---------------------|-------------|
| Ingredient (%) | |
| Corn | 62.4 |
| Potato protein | 17.2 |
| Starch | 13.0 |
| Dicalcium phosphate | 2.36 |
| Limestone | 0.70 |
| Soybean oil | 2.00 |
| Salt | 0.35 |
| Premix† (Zn-free) | 0.50 |
| Casein | 1.00 |
| Lysine HCL | 0.14 |
| Methionin DL | 0.20 |
| Threonin | 0.08 |
| L-tryptophan | 0.02 |
| Anticoccidiostatic | 0.05 |
| Nutrient (g/kg) | |
| DM | 915 |
| CP | 201 |
| CF | 44.3 |
| AMEn (kcal/kg) | 3213 |
| Phosphorus (mg/kg) | 6.1 (6.6) |
| dig Lys | 12.1 |
| dig SAA | 8.3 |
| Calcium (mg/kg) | 9.5 (10.4) |
| Zinc | 22.1 (23.5) |

*Analyzed values in brackets

†Supplied per kilogram of diet: 12,500 IU vitamin A (retinyl acetate); 4300 IU vitamin D₃ (cholecalciferol); 100 IU vitamin E (DL-alpha-tocopherol); 5 mg vitamin K₃ (menadione); 5 mg vitamin B₁ (thiamine); 8 mg vitamin B₂ (riboflavin); 100 mg vitamin B₃ (PP, niacin); 25 mg vitamin B₅ (Ca panthotenate); 7 mg vitamin B₆ (pyridoxine); 0.3 mg vitamin B₈ (biotin, H); 3 mg vitamin B₉ (folic acid); 0.02 mg vitamin B₁₂ (cyanocobalamin); 550 mg choline; 50 mg Fe (FeSO₄); 16 mg Cu (CuSO₄); 80 mg Mn (MnO); 2 mg I (Ca(IO₃)₂); 0.2 mg Se (Na₂SeO₃).

Table 3. Analyzed zinc levels in each of the experimental diets.

| Treatment | Zinc source | Analyzed zinc level in the diet (mg/kg) | Expected zinc supplementation (mg/kg) | Realized zinc supplementation¹ (mg/kg) |
|------------------|--------------------|--|--|--|
| 1 | Control | 23.5 | 0 | 0 |
| 2 | ZnSO ₄ | 28.4 | 6 | 4.9 |
| 3 | | 34.5 | 12 | 11 |
| 4 | SAAP-1 | 28.5 | 6 | 5 |
| 5 | | 35.4 | 12 | 11.9 |
| 6 | SAAP-2 | 28 | 6 | 4.5 |
| 7 | | 34.4 | 12 | 10.9 |
| 8 | SAAP-3 | 29.7 | 6 | 6.2 |
| 9 | | 36.6 | 12 | 13.1 |
| 10 | LDP-1 | 29.3 | 6 | 5.8 |
| 11 | | 33.6 | 12 | 10.1 |
| 12 | LDP-2 | 29.3 | 6 | 5.8 |
| 13 | | 33.1 | 12 | 9.6 |
| 14 | SAP | 28.9 | 6 | 5.4 |
| 15 | | 34.5 | 12 | 11 |

¹Calculated as the analysed zinc content of experimental diet minus zinc content of the control diet.

Table 4. Daily weight gain and FCR from day 8 to 22/23 days of treatments. a–d Means with different superscript within a column differ significantly ($p < 0.001$).

| Treatment | Zinc source | Diet Zn (mg) | Feed intake (g/day) | Weight gain (g/day) | FCR (g/g) | Tibia Zn (mg/kg DM) |
|------------------|--------------------|---------------------|----------------------------|----------------------------|------------------|----------------------------|
| 1 | Control | 23.5 | 34.36 | 40.25 | 1.38 | 85.69 ^d |
| 2 | ZnSO ₄ | 28.4 | 32.98 | 42.33 | 1.36 | 100.38 ^{abcd} |
| 3 | | 34.5 | 29.23 | 39.56 | 1.44 | 107.55 ^{abc} |
| 4 | SAAP-1 | 28.5 | 31.23 | 37.46 | 1.20 | 101.54 ^{abcd} |
| 5 | | 35.4 | 33.13 | 42.39 | 1.32 | 115.95 ^{ab} |
| 6 | SAAP-2 | 28 | 30.46 | 42.80 | 1.41 | 103.14 ^{abcd} |
| 7 | | 34.4 | 27.50 | 37.57 | 1.39 | 113.45 ^{ab} |
| 8 | SAAP-3 | 29.7 | 31.40 | 40.26 | 1.30 | 102.25 ^{abcd} |
| 9 | | 36.6 | 29.52 | 42.60 | 1.49 | 116.66 ^{ab} |
| 10 | LDP-1 | 29.3 | 30.99 | 40.21 | 1.30 | 102.34 ^{abcd} |
| 11 | | 33.6 | 34.86 | 43.81 | 1.28 | 113.83 ^{ab} |
| 12 | LDP-2 | 29.3 | 30.38 | 42.64 | 1.41 | 91.16 ^{cd} |
| 13 | | 33.1 | 36.76 | 42.82 | 1.19 | 99.94 ^{bcd} |
| 14 | SAP | 28.9 | 30.51 | 39.08 | 1.34 | 100.81 ^{abcd} |
| 15 | | 34.5 | 33.14 | 42.50 | 1.32 | 120.68 ^a |
| | p -value | | 0.4060 | 0.158 | 0.207 | < 0.001 |

Table 5. Relative bioavailability of different ZnO sources

| Source | Slope | SE | RBV Zn % Bone |
|-------------------|--------------|-----------|----------------------|
| ZnSO ₄ | 1.49 | 0.42 | 100 ^{ab} |
| SAAP-1 | 1.96 | 0.39 | 133 ^{ab} |
| SAAP-2 | 1.80 | 0.38 | 120 ^{ab} |
| SAAP-3 | 1.98 | 0.36 | 132 ^{ab} |
| LDP-1 | 1.98 | 0.40 | 131 ^{ab} |
| LDP-2 | 0.71 | 0.40 | 49 ^b |
| SAP | 2.41 | 0.40 | 160 ^a |

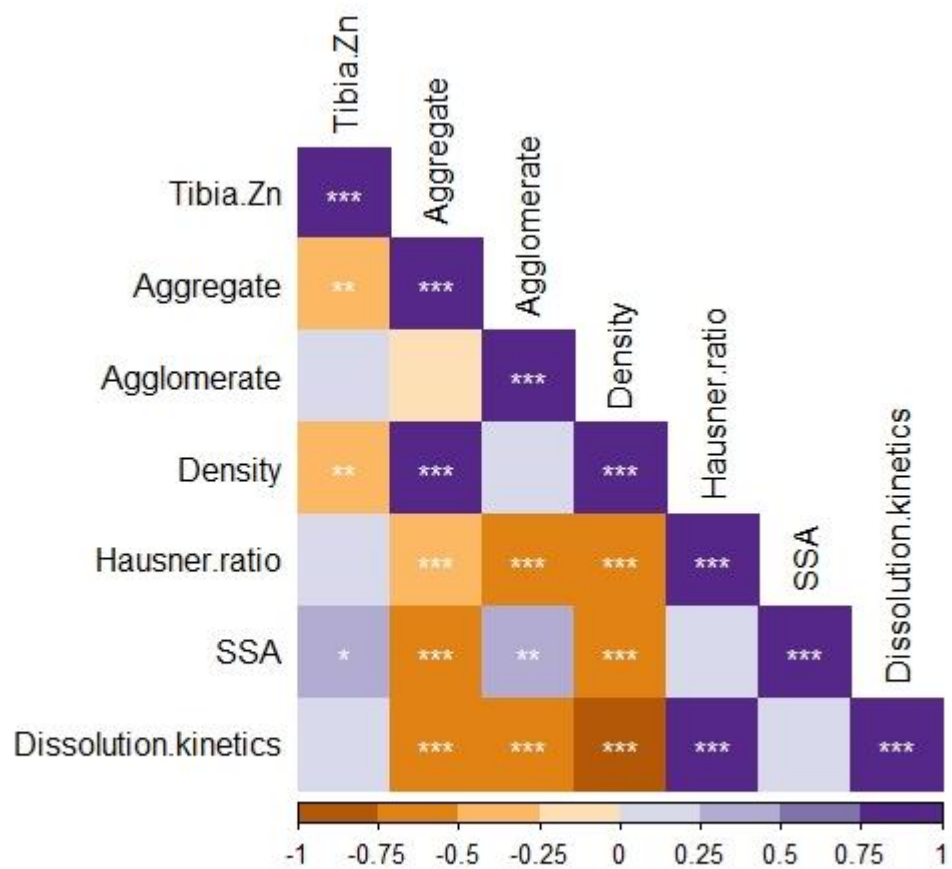


Figure 1: Correlations between tibia Zn and physicochemical properties of ZnO sources
 Significance levels: (***) 0.001, (**) 0.01 and (*) 0.05

Table 7. Zn content in the soluble and insoluble part of gastrointestinal content

| | Proventriculus and Gizzard content | | | | | Ileum content | | | |
|-------------------------|---|--|--------------------------------|-------------------|--------------------------------|---------------------------------|--------------------------------|-------------------|--------------------------------|
| | Supplemented Zn intake 3h (μg) | Insoluble Zn ($\mu\text{g/g}$ DM) | Soluble | | Total | Insoluble ($\mu\text{g/g}$ DM) | Soluble | | Total |
| | | | Zn ($\mu\text{g/g}$ DM) | Soluble Zn (%) | Zn ($\mu\text{g/g}$ DM) | | Zn ($\mu\text{g/g}$ DM) | Soluble Zn (%) | Zn ($\mu\text{g/g}$ DM) |
| Control | 0.00 ^b | 1.9 ^b | 1.3 | 17.4 | 8.56 | 23.4 | 4.6 | 4.41 | 101 ^b |
| ZnSO₄ | 0.43 ^a | 1.5 ^b | 2.4 | 20.2 | 9.75 | 29.8 | 6.8 | 4.69 | 140 ^a |
| SAAP-1 | 0.45 ^a | 1.9 ^b | 1.3 | 18.9 | 9.78 | 31.2 | 6.1 | 4.33 | 140 ^a |
| SAAP-2 | 0.34 ^a | 2.8 ^{ab} | 3.0 | 21.3 | 14.78 | 31.1 | 5.6 | 3.96 | 146 ^a |
| SAAP-3 | 0.47 ^a | 2.3 ^{ab} | 1.8 | 17.1 | 12.21 | 28.0 | 5.5 | 3.92 | 145 ^a |
| LDP-1 | 0.40 ^a | 2.2 ^{ab} | 2.0 | 19.7 | 12.46 | 29.0 | 5.3 | 3.63 | 145 ^a |
| LDP-2 | 0.36 ^a | 5.9 ^a | 4.2 | 29.2 | 13.93 | 26.7 | 4.3 | 3.03 | 145 ^a |
| SAP | 0.31 ^a | 2.7 ^{ab} | 2.8 | 23.9 | 10.87 | 28.9 | 6.4 | 4.64 | 143 ^a |
| <i>p</i>-value | < 0.001 | 0.011 | 0.135 | 0.5608 | 0.361 | 0.3081 | 0.1898 | 0.251 | < 0.001 |

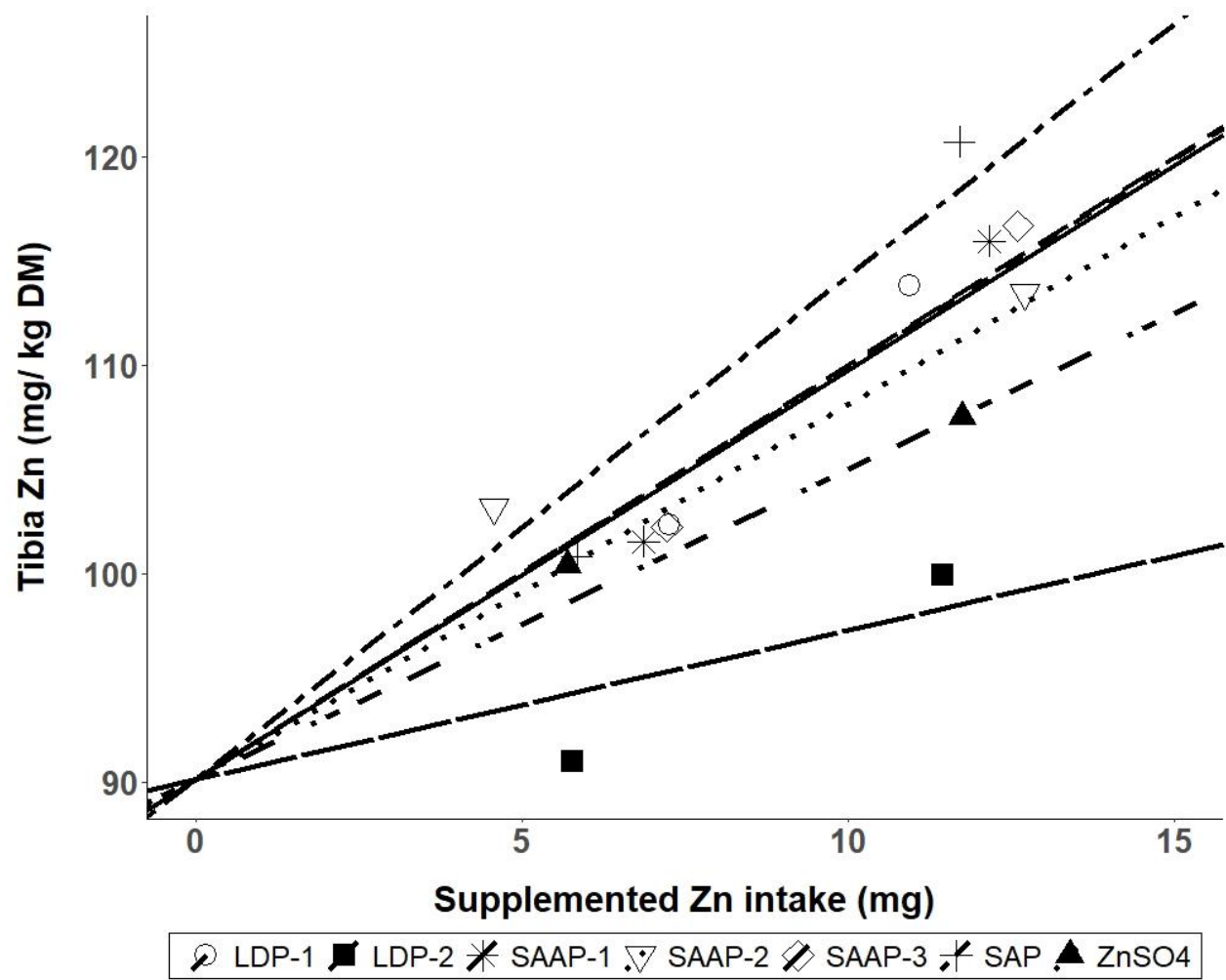


Figure 2: Linear dose response relationship between supplemental zinc intake (mg) and tibia zinc content (mg/kg of DM).

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