Meta-analysis of the effect of dietary inclusion of copper on production performance in swine †

[Meta análisis del efecto de la inclusión de cobre en la dieta sobre el comportamiento productivo de cerdos]

Victoria Dessire Tufiño-Loza[1], Renan Patricio Mena-Pérez[1], Diego Rodriguez-Saldaña[2] and Jimmy Rolando Quisirumbay-Gaibor[1*]

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Av. Universitaria, Quito 170129, Ecuador. Email: jrquisirumbay@uce.edu.ec
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad de Cuenca, Av. 12 de Abril y Agustín Cueva, Cuenca 010201, Ecuador.
**Corresponding author*

SUMMARY

Background. Copper is a trace element that is involved in various metabolic reactions (cellular respiration, hemoglobin formation and the development of connective tissue), acting with bacteriostatic and bactericidal properties. It can be toxic and excessively excreted into the environment when used at high doses. There are many studies with different levels of inclusion and times of dietary copper supplementation with variable results, generating uncertainty among nutritionists. **Objective.** To measure the effect of dietary copper inclusion on productive performance in pigs through the use of meta-analysis. **Methodology.** 24 analyzes were run from 28 scientific articles that included a total of 3908 animals. Under the random effects model, effect size, heterogeneity, meta-regressions and publication bias were determined. **Results**. The results showed that copper supplementation improved average daily weight gain (+30.61 g/day, p<0.00001), average daily feed intake (+18.726 g/day, p=0.0001), feed conversion (-0.10009 kg/kg, p=0.00003) and feed efficiency (+0.00597 kg/kg, p=0.0052). High values of heterogeneity (>80%) were found in most of the variables evaluated, so meta-regressions were performed with the moderators: duration and level of supplementation, dietary level of crude protein, zinc, vitamins A and E, and number of repetitions. **Implications**: Optimizing rations with these data maximizes swine performance and mitigates the environmental impact of copper excretion. **Conclusion.** It was concluded that dietary copper supplementation improved productive performance in pigs and these results are affected by other dietary nutrients.
Key words: Nutrition; piglets; fattening; minerals; environment.

.

RESUMEN

Antecedentes. El cobre es un oligoelemento que interviene en diversas reacciones metabólicas (respiración celular, formación de hemoglobina y el desarrollo del tejido conjuntivo), actúa con propiedades bacteriostáticas y bactericidas. Puede ser tóxico y excretarse de manera exagerada al medio ambiente al ser usado a dosis altas. Existe muchos estudios con diferentes niveles de inclusión y tiempos de suplementación alimentaria del cobre con resultados variables generando incertidumbre en los nutricionistas. **Objetivo.** Medir el efecto de la inclusión alimenticia de cobre sobre el rendimiento productivo en porcinos a través del uso del meta-análisis. **Metodología.** Se ejecutaron 24 análisis a partir de 28 artículos científicos que incluyó un total de 3908 animales. Bajo el modelo de efectos aleatorios se determinó tamaño de efecto, heterogeneidad, meta-regresiones y sesgo de publicación. **Resultados.** Los resultados mostraron que la suplementación de cobre mejoró la ganancia diaria de peso (+30.61 g/día, p<0.00001), consumo diario de alimento (+18.726 g/día, p=0.0001), conversión alimenticia (-0.10009 kg/kg, p=0.00003) y eficiencia alimenticia (+0.00597 kg/kg, p=0.0052). Se encontraron valores altos de heterogeneidad (>80%) en la mayoría de las variables evaluadas, por lo que se realizaron meta-regresiones con los moderadores: duración y nivel de suplementación, nivel dietario de proteína cruda, zinc, vitaminas A y E y número de repeticiones. **Implicaciones:** Optimizar raciones con estos datos maximiza el rendimiento porcino y mitiga el impacto ambiental por excreción de cobre. **Conclusiones.** Se concluyó que la suplementación alimenticia de cobre mejoró el rendimiento productivo en cerdos y estos resultados son afectados por otros nutrientes dietarios.
Palabras clave: Nutrición; lechones; engorde; minerales; ambiente.

---

† *Submitted May 10, 2024 – Accepted May 18, 2026.* *http://doi.org/10.56369/tsaes.5618*

ISSN: 1870-0462.
ORCID = V.D. Tufiño-Loza: https://orcid.org/0009-0001-7669-5131**; R.P. Mena-Pérez:** https://orcid.org/0000-0002-4560-4858**; D.F. Rodriguez-Saldaña:** https://orcid.org/0000-0001-7143-4250**; J.R. Quisirumbay-Gaibor:** https://orcid.org/0000-0003-1612-8503

INTRODUCTION

Modern pig production demands an efficient supply of nutrients in the diet in order to cover nutritional requirements and guarantee correct maturation of the digestive system and thus achieve maximum productive performance (Wang *et al.,* 2022). During growth, there are several challenges that the piglet must face to be a good meat producer. Post-weaning diarrhea is one of the main threats to the pork industry, generating large economic losses (Pluske *et al.,* 2018). This event occurs most frequently within the first two weeks after weaning and is characterized by anorexia, dehydration, profuse diarrhea, and loss of body weight in the following stages (grow-finish) (Cao *et al.,* 2022). Mortality can vary between 20-30% in a period of one to two months *(Canibe et al.,* 2022). For many years, nutritional strategies have been used (Tang *et al.,* 2024) to reduce the presentation of post-weaning diarrhea and productive losses, including the use of micro minerals (Huting *et al.,* 2021). Trace minerals act as catalysts in enzymatic and hormonal processes, influencing bone maintenance, growth and development (Sampath *et al.,* 2023). Copper (Cu) is involved in metabolic reactions such as cellular respiration, hemoglobin formation and the development of connective tissue and is absorbed mainly in the duodenum (Manto, 2014).

The Cu nutritional requirements of pigs are 5 to 10 mg/kg; however, Cu can be incorporated at levels that promote growth performance and reduction of post-weaning diarrhea. It is known that the minimum adequate requirement for Cu so that weaned piglets do not show deficiency symptoms and contributes to growth is 5 to 6 mg/kg (Hill, 2023). While the National Research Council of the United States (NRC) (2012) indicated that a level of 10 to 25 mg/kg of Cu should be used for maintenance and 100 to 250 mg/kg to provide effect growth promoter for weaned piglets. Excessive use of Cu in diets can generate resistance in gram-negative bacteria (*Escherichia coli, Pseudomonas syringae*) and gram-positive bacteria (*Bacillus subtilis, Lactococcus lactis*) (Espinosa and Stein, 2021). Additionally, the times (duration) of supplementation, ages, and live weights of the pigs, as well as the levels of nutrients in the diet, have been variable, which generates uncertainty among nutritionists. It is presumed that the dietary inclusion of copper favors productive performance in pigs. The purpose of this research work was to evaluate the effect of dietary copper inclusion on productive performance in pigs using meta-analysis.

MATERIALS AND METHODS

Search for scientific articles

The methodology suggested by Moreira *et al.* (2020) and Palencia *et al.* (2018) was followed for meta-analysis studies in animal science. Between October and November 2022, an electronic search was carried out for scientific articles in journals indexed to the following electronic databases: CAB direct, Elsevier biobase-CABS, Google Scholar, MEDLINE, PubMed, Science Direct (Journal), Scopus, Academic Search Complete. The key words were used: trace minerals, trace elements, copper, diet, feed, nutrition, pigs, piglets, sows and their equivalent in English and Portuguese, without date restrictions; however, the period of years that the search included was between 1957 to 2022, with a total of 129 articles.

Inclusion criteria

Experimental research articles were included in which copper was administered exclusively through diet and with healthy animals. The experiments must have been carried out on pigs, without considering race, sex or country as exclusion factors; as well as, they had to include the number of experimental units per treatment (repetitions), include at least 2 treatments (including the control group: without copper), the sources of copper used and their level of inclusion in the diet, contain at least one of the variables dependents of interest with their respective values of the mean (average), standard deviation (SD), coefficient of variation (CV) or standard error of the mean (SEM).

Based on these criteria, the process of selecting and discarding the articles is shown below. A total of 28 articles were selected (Figure 1), which included a total of 3908 repetitions (Table 1).

Data extraction and processing

A database was created in Microsoft Excel. For each dependent variable, 3 meta-analyses (MA) were executed: a general MA (without considering productive stage: pigs-general) and another considering the productive stage, which was divided into two categories: (a) piglets-breeding and (b) growing-finishing pigs. Quantifying a total of 12 MA.

To address high heterogeneity, a meta-regression analysis was performed using the following moderators: duration and level of copper supplementation, dietary crude protein and zinc levels, vitamins A and E concentrations, and the number of replicates per study.

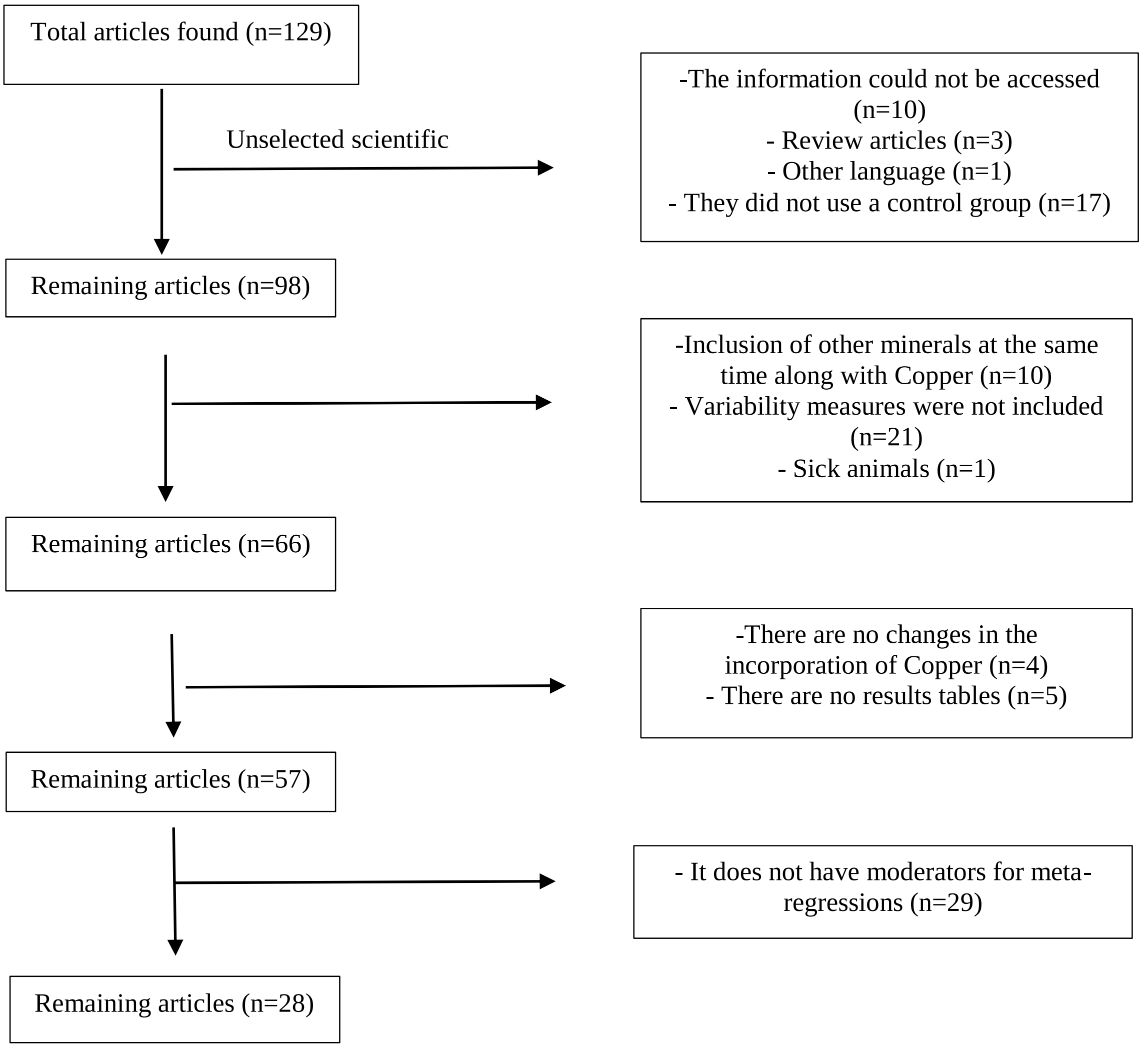

Figure 1. Flowchart for selection and discarding of scientific articles.

Table 1. Sample size for productive performance MA.

Productive performance		n
ADG	Pigs (general)	1361
	Piglets - Rearing	827
	Growth-Fattening	534
ADFI	Pigs (general)	1421
	Piglets - Rearing	635
	Growth-Fattening	786
FC	Pigs (general)	512
	Piglets - Rearing	374
	Growth-Fattening	138
FE	Pigs (general)	614
	Piglets - Rearing	296
	Growth-Fattening	318
	n total	3908

Statistical analysis

MIX 2.0 Pro software was used using the random effects model (Bax, 2016). Tests of effect size (by difference in means), heterogeneity (Inconsistency Index), meta-regressions and publication bias (Egger's Test) were carried out (Higgins and Thompson, 2002) (Egger *et al.,* 1997).

RESULTS AND DISCUSSION

Study Limitations

Despite the methodological rigor employed, this meta-analysis has inherent limitations that should be considered when interpreting the results regarding copper supplementation in swine diets. First, there is a potential risk of publication bias, given that studies with non-significant results often have lower dissemination rates. Furthermore, the detected heterogeneity may stem from variability in experimental designs, the animals' productive stages, and the environmental conditions of each study. Therefore, the validity of these conclusions is tied to the quality of the available primary data and should be interpreted as a synthesis of the scientific evidence reported under those specific conditions.

Summary dependent variables

Table 2 shows the average value of the evaluated production parameters. The general analysis of the average daily gain (ADG) variable demonstrates that pigs supplemented with copper showed greater gain compared to pigs in the control group. In the same way, the analysis of both piglets-rearing and the growing-fattening pigs of the evaluated group had higher ADG. In the case of average daily feed intake (ADFI), the individuals in the general analysis, piglets-rearing and growth-fattening that received copper had a higher feed consumption compared to the control group.

The feed conversion (FC) was lower in all productive stages of pigs (general), piglets- rearing and in the growth- fattening stage compared to the control group. Feeding efficiency (FE) was higher in the treated group in all stages evaluated.

Effect size

The effect size expressed in mean difference (MD) shows how copper supplementation increased ADG by 30.610 g/day (p<0.00001) in pigs (general), 34.959 (p<0.00001) g/day in rearing piglets and 19.602 g/day (p=0.00011) in the growth-fattening stage above the control group (Table 3).

Regarding ADFI, the dietary inclusion of copper caused a considerable increase of 18.726 g/day (p=0.0001) in pigs (general) and 34.666 g/day (p<0.00001) in rearing piglets compared to the control group. On the other hand, in the growth-fattening stage the addition of copper did not favor the evaluated parameter (p=0.49877) and it was the control group that presented a higher value.

Negative values were observed in the FC variable, which expresses that the dietary inclusion of copper makes the pig more efficient in the general analysis (p=0.00003), in piglets-rearing (p=0.00001) and in growth- fattening (p= 0.04354). Thus, the pigs (general) in the treatment group require consuming 100 g less food to produce 1 kg of weight, likewise, 76.48 g less in piglets-rearing and 165.06 g less in the growth- fattening stage compared to the control group.

Table 2. Summary of dependent variables for the MA of the effect of dietary copper inclusion on ADG, ADFI, FC and FE in pigs.

Productive parameter	Meta-analysis	Treatment		Control	
		Mean	SD	Mean	SD
ADG (g/day)	Pigs (general)	477.131	243.487	447.817	248.868
	Piglets - Rearing	374.429	150.003	341.558	132.699
	Growth-Fattening	661.992	270.649	639.082	293.012
ADFI (g/day)	Pigs (general)	912.317	684.722	892.304	704.400
	Piglets - Rearing	511.323	137.345	479.039	131.043
	Growth-Fattening	1306.397	774.814	1298.444	798.034
FC (kg/kg)	Pigs (general)	1.869	0.439	1.960	0.422
	Piglets - Rearing	1.702	0.155	1.761	0.167
	Growth-Fattening	2.242	0.616	2.404	0.483
FE (kg/kg)	Pigs (general)	0.565	0.142	0.552	0.142
	Piglets - Rearing	0.665	0.070	0.644	0.096
	Growth-Fattening	0.455	0.116	0.452	0.115

SD: standard deviation.

Table 3. Effect size of dietary copper inclusion on ADG, ADFI, FC and FE in pigs.

Productive parameter	Meta-analysis	MD	CI		p-value
ADG (g/day)	Pigs (general)	30.610	22.749	38.472	<0.00001
	Piglets - Rearing	34.959	21.741	48.177	<0.00001
	Growth-Fattening	19.602	9.693	29.510	0.00011
ADFI (g/day)	Pigs (general)	18.726	9.291	28.161	0.0001
	Piglets - Rearing	34.666	27.289	42.043	<0.00001
	Growth-Fattening	6.969	-13.225	27.164	0.49877
FC (kg/kg)	Pigs (general)	-0.10009	-0.14702	-0.05316	0.00003
	Piglets - Rearing	-0.07648	-0.10954	-0.04343	0.00001
	Growth-Fattening	-0.16506	-0.32533	-0.00478	0.04354
FE (kg/kg)	Pigs (general)	0.00597	0.00178	0.01016	0.0052
	Piglets - Rearing	0.01634	0.00683	0.02586	0.00076
	Growth-Fattening	0.00216	-0.00142	0.00574	0.2367

MD: mean difference; CI: confidence interval

In the FE parameter, the pigs (general) and suckling piglets supplemented with dietary copper gained respectively 5.9 (p=0.0052) and 16.34 g (p=0.00076) of additional weight from each kg of feed consumed versus the control group. Pigs in the growth-fattening stage gained 2 g of additional weight (p=0.2367).

The present meta-analysis shows in a general way that the dietary inclusion of copper (pigs-general: 5-650 ppm; piglets-rearing: 5 - 300 ppm; growth-fattening pigs: 25 - 650 ppm) favored productive performance since improved ADG, ADFI and FE. The results of the improvement in ADG agree with those presented by Espinosa *et al.* (2017) and Zhou *et al.* (1994), who found that copper supplementation improved productive performance in piglets, associating it with an increase in nutrient digestibility since Cu favors the activity of lipase and phospholipase A enzymes in the small intestine, increasing the absorption of fatty acids and therefore growth (Sampath *et al.,* 2023) (Dove, 1995) . Similarly, in research carried out by Wang *et al.* (2012) the ADG increased significantly by 31.10% more in 21-day-old piglets, fed with diets that included Cu at a level of 100 mg/kg.

The data presented in this research regarding pigs in the growth-fattening stage agree with Feng *et al.* (2007) and Coble *et al.* (2018), who presented in their work that the incorporation of Cu into the diet increased ADG by 8.47% and 2.4% respectively. Associated with improvement in intestinal health and balance in the microbiota of the digestive system (Blavi *et al.,* 2021) (Espinosa *et al.,* 2019).

The data that ADFI and FE were higher for rearing piglets fed diets containing Cu as opposed to piglets fed the control diet agree with the results of Zhou *et al.* (1994) and Wang *et al.* (2012), in which they are 13% and 13.70% higher (p<0.05) respectively. The piglets in the control group had a higher FC compared to those that received copper. Wang *et al.* (2016) and Li *et al.* (2008) showed that FC decreased by 10.58% and 11.1% respectively (p<0.05); demonstrating better use of nutrients.

On the other hand, in the results presented by Espinosa *et al.* (2017) it was found that ADFI increased in pigs in the growth-fattening stage, which is why FE did not improve. Based on these results, they concluded that the improvement in pig growth was due to *ad libitum* access to feed supplemented with Cu, influencing intestinal parameters such as the intestinal microbiome (Holman and Chénier, 2015).

Heterogeneity

Table 4 presents the results of I^2 for each variable analyzed. The FE in pigs (general) and piglets-rearing stage presented a value of 21.48% and 31.7% respectively; that is, low heterogeneity. All other productive parameters showed high percentages of heterogeneity, demonstrating variability among the studies that were part of this meta-analysis. In this way, it was found that through meta-regressions the moderators used affected the productive performance in pigs supplemented with copper.

Meta-regressions

Duration and level of copper supplementation

The research used supplemented copper between 7 to 117 days (pigs-general), 7-45 days (piglets-breeding), 5-117 days (growth-finishing). For each day of copper supplementation in the diet, the daily weight gain in piglets-rearing increased by 5.377 g (p<0.001) compared to those individuals that did not receive copper (Table 5). Similarly, an increase in feed consumption was observed by 5.03696 g/day (p<0.001). However, feed conversion tends to increase by 0.00208 g/g (p=0.02763) for each additional day of supplementation.

Table 4. Inconsistency index of dietary copper inclusion on ADG, ADFI, FC and FE in pigs.

Productive parameter	Meta-analysis	I^2 (%)
ADG (g/day)	Pigs (general)	100
	Piglets - Rearing	100
	Growth-Fattening	100
ADFI (g/day)	Pigs (general)	100
	Piglets - Rearing	99.999
	Growth-Fattening	100
FC (kg/kg)	Pigs (general)	96.528
	Piglets - Rearing	89.413
	Growth-Fattening	98.57
FE (kg/kg)	Pigs (general)	21.481
	Piglets - Rearing	31.7
	Growth-Fattening	0

Table 5. Meta-regression for the duration and level of Cu supplementation.

Productive parameter	Meta-analysis	Duration of supplementation Regression coefficient		Copper level Regression coefficient	
		Slope	p-value	Slope	p-value
ADG (g/day)	Pigs (general)	-0.05264	<0.001	0.09716	<0.001
	Piglets - Rearing	5.37783	<0.001	0.12049	<0.001
	Growth-Fattening	-0.17857	<0.001	0.07787	<0.001
ADFI (g/day)	Pigs (general)	0.12676	<0.001	0.05568	<0.001
	Piglets - Rearing	5.03696	<0.001	0.0118	<0.001
	Growth-Fattening	-0.12987	<0.001	0.06949	<0.001
FC (kg/kg)	Pigs (general)	0.00458	<0.001	-0.00035	<0.001
	Piglets - Rearing	0.00208	0.02763	-0.00053	<0.001
	Growth-Fattening	0.00424	<0.001	-0.00009	0.087
FE (kg/kg)	Pigs (general)	-0.00005	0.28011	0.00001	0.613
	Piglets - Rearing	-0.00052	0.28199	0.00009	0.058
	Growth-Fattening	0.00001	0.88913	0.00001	0.79

For each unit of copper that was increased in the diet, the variables ADG and ADFI in all stages increased ($p<0.001$). For its part, FC in all productive stages decreased by -0.00035 g/g (pigs-general, $p<0.001$), -0.00053 g/g (piglets-rearing, $p<0.001$) and -0.00009 g/g (growth-fattening, $p=0.087$). The copper values in the diet of the experiments used varied between 6 to 650 ppm.

In the case of the level of Cu in the diet, the effects found have shown that added Cu can influence intestinal morphology. Di Giancamillo *et al.* (2018) reported that 150 mg/kg Cu in the diet increased duodenal villus length and reduced crypt depth in piglets relative to diets without added Cu. However, Coble *et al.* (2018) found that the addition of 150 mg/kg Cu did not generate changes in the height of the villi or the depth of the crypts ($p > 0.10$). In contrast, Fry *et al.* (2012) reported that pigs fed 225 mg/kg Cu added in the form of $CuSO_4$ had reduced villus height in the duodenum and proximal jejunum compared to those fed without added Cu. Pigs fed high levels of Cu for a long period of time could be toxic (Espinosa and Stein, 2021, National Research Council, 2012). In this sense, in a study carried out by Veum *et al.* (2004) it was determined that the longer the supplementation time, the greater the response; however, the exact time or clear reason is not defined.

Level of crude protein (CP) and zinc in the diet

For each percentage unit of CP that increased in the diet, the ADG increased in pigs-general (3.0228 g; $p<0.001$), piglets-rearing (22.751 g; $p<0.001$) and growth-fattening (6.502 g; $p<0.001$); as well as, in the ADFI variable in pigs in piglets-rearing (15.021 g; $p<0.001$). Crude protein levels in the diets were 11-22% for general pigs, 17-22% for rearing piglets and 11-21% for growing-fattening pigs. On the contrary, ADFI in pigs-general and growth-fattening decreased ($p<0.001$) (Table 6).

Table 6. Meta-regression for level of crude protein and zinc in the diet.

Productive parameter	Meta-analysis	Crude protein Regression coefficient		Zn level Regression coefficient	
		Slope	p-value	Slope	p-value
ADG (g/day)	Pigs (general)	3.0228	<0.001	0.27495	<0.001
	Piglets - Rearing	22.75141	<0.001	4.19256	<0.001
	Growth-Fattening	6.50179	<0.001	0.29692	<0.001
ADFI (g/day)	Pigs (general)	-3.77082	<0.001	0.53457	<0.001
	Piglets - Rearing	15.02065	<0.001	2.36693	<0.001
	Growth-Fattening	-4.73262	<0.001	0.61797	<0.001
FC (kg/kg)	Pigs (general)	0.04082	<0.001	-0.00116	<0.001
	Piglets - Rearing	0.04101	<0.001	0.00125	<0.001
	Growth-Fattening	0.02315	0.005	-0.00157	<0.001
FE (kg/kg)	Pigs (general)	0.00135	0.015	0.00023	0.148
	Piglets - Rearing	0.00085	0.884	-0.00083	0.187
	Growth-Fattening	-0.00011	0.9	-0.0001	0.648

For Tang *et al.* (2019), crude protein (CP) in the diet is one of the nutrients that affects the gastrointestinal health of weaned pigs. It should be considered that a high level of CP and an imbalance of amino acids in diets predispose to diarrhea, generate enormous protein waste and greater environmental pollution in the pork industry (Xia *et al.*, 2022).

Zinc levels in the diets of the studies used were 73-200 ppm (pigs-general and piglets-rearing) and 80-178 ppm (growth-fattening). For each unit of zinc that was increased in the diet, ADG and ADFI in all productive stages increased (p<0.001). The FC in the pigs-general and grow-fattening stages decreased by 0.00116 g/g (p<0.001) and 0.00157 g/g (p<0.001) respectively (Table 6).

Regarding dietary zinc, Lee *et al.* (2016) observed considerable increases in ADG and FE of pigs supplemented with Zn, associated with the improvement in intestinal barrier function (digestion and absorption of nutrients) (Diao *et al.*, 2021).

The digestibility of Zn and Cu depends on the dietary supplementation of these minerals in the diets, but also on the interaction between these minerals (Ketata *et al.*, 2023). The interaction between Cu and Zn in weaned pigs has been described by Smith *et al.* (1997) who found an increase of 17% in ADG and 14% in ADFI from 3000 ppm of ZnO and 250 ppm of Cu. Excess Zn excreted negatively affects the environment, it can also accumulate in tissues, which has been related to increased cellular stress and lower absorption of other micro and macroelements (Burrough *et al.*, 2019).

Level of vitamin A and E in the diet

Dietary levels of vitamin A (IU/kg feed) were 1500-14000 (pigs-general), 2200-14000 (piglets-rearing) and 1500-11000 (growing-fattening). For each international unit of vitamin A that increased in the diet, the ADG increased in general pigs (0.00158 g/day; p<0.001) and rearing piglets (0.00709 g/day; p<0.001). In rearing piglets, FC tended to decrease 0.00001 g/g (p<0.001) (Table 7).

Vitamin A is an important nutrient to regulate the growth of piglets, because it can favorably affect the intestinal epithelial structure in weaned piglets, producing an increase in the height and surface of the villi counteracting the effects produced by weaning (Wang *et al.*, 2020).

Ayuso *et al.* (2015) observed that pigs fed diets restricted in vitamin A had lower ADG and FE during the growth period. Thus, affirming the data obtained in the present study and the research of Wang *et al.* (2020) that indicates that dietary supplementation of 16 mg/kg vitamin A helped to relieve the stress produced by weaning and led to an increase in significantly from ADG and FE.

Dietary vitamin E levels (IU/kg feed) were 10-3500 (pigs-general and growth-fattening) and 11-1700 (piglets-rearing). For each international unit of vitamin E that was increased in the diet, the ADG in rearing piglets increased by 1.50182 g/day (p<0.001), in the other productive categories it decreased (p<0.001). However, in all cases the FC increased (p<0.001) (Table 7). Vitamin E has been shown to be a powerful antioxidant of fatty acids, as well as regulate the immune system, favoring the productive performance and carcass quality of pigs. (Lauridsen *et al.*, 2021, Lewis *et al.*, 2019).

Number of repetitions

For each repetition that increases, all the variables analyzed in the piglets-breeding category tend to increase: ADG (19.52305 g/day; p<0.001), ADFI (35.73886 g/day; p<0.001) and FC (0.0137 g/day; p<0.001) and FE (0.00953; p=0.053) (Table 8). Obviously, the greater the number of repetitions per

Table 7. Meta-regression for level of Vitamin A and E in the diet.

Productive parameter	Meta-analysis	Vitamin A Regression coefficient		Vitamin E Regression coefficient	
		Slope	p-value	Slope	p-value
ADG (g/day)	Pigs (general)	0.00158	<0.001	-0.00326	<0.001
	Piglets - Rearing	0.00709	<0.001	1.50182	<0.001
	Growth-Fattening	-0.00208	<0.001	-0.00438	<0.001
ADFI (g/day)	Pigs (general)	0.00034	<0.001	0.00183	<0.001
	Piglets - Rearing	0.004	<0.001	0.8478	<0.001
	Growth-Fattening	-0.0052	<0.001	-0.00187	<0.001
FC (kg/kg)	Pigs (general)	0	<0.001	0.00004	<0.001
	Piglets - Rearing	-0.00001	<0.001	0.00003	<0.001
	Growth-Fattening	0.00002	<0.001	0.009	<0.001
FE (kg/kg)	Pigs (general)	0	0.3769	0	0.18487
	Piglets - Rearing	-0.00001	0.28498	0.00016	0.67718
	Growth-Fattening	0	0.69416	0	0.91749

Table 8. Meta-regression for the number of repetitions.

Productive parameter	Meta-analysis	Number of repetitions Regression coefficient	
		Slope	p-value
ADG (g/day)	Pigs (general)	3.14869	<0.001
	Piglets - Rearing	19.52305	<0.001
	Growth-Fattening	-4.35314	<0.001
ADFI (g/day)	Pigs (general)	-2.9345	<0.001
	Piglets - Rearing	35.73886	<0.001
	Growth-Fattening	-9.95333	<0.001
FC (kg/kg)	Pigs (general)	0.02417	<0.001
	Piglets - Rearing	0.0137	<0.001
	Growth-Fattening	0.04479	<0.001
FE (kg/kg)	Pigs (general)	-0.00225	0.0431
	Piglets - Rearing	0.00953	0.053
	Growth-Fattening	-0.00087	0.5613

treatment it would be possible to detect statistical differences. Therefore, Aaron and Hays (2004) highlight the importance of the number of adequate repetitions per treatment when calculating the sample size and design of the experiment. The number of repetitions in each treatment was from 3 to 10 in the investigations that were part of this meta-analysis.

Publication bias

Regarding publication bias, it is observed that the FC pigs-general variable (p=0.00143) had bias, that is, preference was given to articles in which positive results were obtained in productive performance after dietary copper supplementation. In the variables ADG of pigs-general (p=0.897), ADFI (p= 0.796) and FE (p=0.55806) there was no bias (Table 9). In the study carried out by Galiotto Miranda *et al.* (2024) publication bias was not evaluated.

Table 9. Publication bias of dietary copper inclusion on ADG, ADFI, FC and FE in pigs.

Productive parameter	Meta-analysis	Egger test (p-value)
ADG (g/day)	Pigs (general)	0.897
ADFI (g/day)	Pigs (general)	0.796
FC (kg/kg)	Pigs (general)	0.00143
FE (kg/kg)	Pigs (general)	0.55806

The detection of publication bias in the FC (Feed Conversion) variable suggests a potential overestimation of the actual effect size of dietary copper. This asymmetry indicates that studies failing to report significant improvements in feed conversion may have remained unpublished, leading to a cautious interpretation of the magnitude of the performance benefits observed in this meta-analysis.

Comparison between copper sources

The effect size expressed in mean difference (MD) shows how supplemented inorganic copper significantly increased ADG by 34.99 g/day (p<0.00001) in general pigs compared to the control group (Table 10). In the same parameter, organic copper increased by 26.55 g/day (p<0.00001). In the productive piglet-breeding stage, inorganic copper increased by 48.09 g/day (p=0.00003) versus the control group. While organic copper increased by 27.47 g/day (p<0.00001). In the growth-fattening stage, organic copper increased 33.91 g/day (p=0.00002) compared to the control group. Meanwhile, supplemented inorganic copper increased 14.99 g/day (p=0.02973) versus the control group.

In the case of FC, its evident decrease showed that the inclusion of inorganic copper in the pig-general stage was significantly favorable (p=0.01627), therefore, this group required consuming 113.1 g less feed to produce 1 kg of weight compared to pigs fed diets that did not include copper. In the piglet-rearing stage, they needed to consume 86.1 g (p=0.00108) less organic copper feed to produce 1 kg of weight in relation to the control group. In growth-fattening they needed to consume 74.6 g (p<0.00001) less organic copper compared to the control group. The ring structure formed by the chelating reaction in organic minerals protects organic Cu against chemical reactions at low pH and makes it more stable in the gastrointestinal tract of animals (Elliott *et al.*, 2017, Sandström, 2001). Organic sources of copper have shown benefit on the intestinal microbiota and reduce copper excretion into the environment (Wen *et al.*, 2022).

Table 10. MA of organic and inorganic copper sources in dietary inclusion on ADG, ADFI, FC and FE in pigs.

Productive parameter	Meta-analysis		MD	CI		p
ADG (g/day)	Pigs (general)	Inorganic	34.9853	22.735	47.235	<0.00001
		Organic	26.5491	19.357	33.741	<0.00001
	Piglets - Rearing	Inorganic	48.0923	25.322	70.861	0.00003
		Organic	27.4660	23.012	31.920	<0.00001
	Growth-Fattening	Inorganic	14.9943	1.4744	28.514	0.02973
		Organic	33.9070	18.379	49.434	0.00002
ADFI (g/day)	Pigs (general)	Inorganic	13.5409	-1.7637	28.845	0.0829
		Organic	31.9839	23.044	40.923	<0.00001
	Piglets - Rearing	Inorganic	38.0983	23.807	52.388	<0.00001
		Organic	28.0980	22.586	33.536	<0.00001
	Growth-Fattening	Inorganic	-0.5895	-29.403	28.224	0.96801
		Organic	32.1271	12.672	51.582	0.00121
FC (kg/kg)	Pigs (general)	Inorganic	-0.1131	-0.2054	-0.0208	0.01627
		Organic	-0.084	-0.1245	-0.0434	0.00005
	Piglets - Rearing	Inorganic	-0.0686	-0.1081	-0.0291	0.00066
		Organic	-0.0861	-0.1378	-0.0345	0.00108
	Growth-Fattening	Inorganic	-0.2014	-0.4774	0.0745	0.15252
		Organic	-0.0746	-0.1051	-0.0441	<0.00001
FE (kg/kg)	Pigs (general)	Inorganic	0.00593	0.00074	0.01113	0.02515
		Organic	0.00908	-0.00005	0.01821	0.05117
	Piglets - Rearing	Inorganic	0.03329	0.01235	0.05423	0.00183
		Organic	0.00908	-0.00005	0.01821	0.05117
	Growth-Fattening	Inorganic	0.00216	-0.00142	0.00574	0.2367
		Organic	All studies tested inorganic source			

MD: mean difference; CI: confidence interval; p: probability value.

In FC, general pigs gained 5 g (p=0.02515) of additional weight from each kg of feed with inorganic copper consumed as opposed to feed that did not include copper. In the piglet-rearing stage they gained 33 g (p=0.00183) of weight from each kg of feed added with inorganic copper versus control diets. In the growth-fattening stage it was observed that, from each kg of feed with inorganic copper, they gained 2 g (p=0.2367). Organic Cu has the advantage of minimizing the formation of bonds between minerals and phytic acid, thus favoring its absorption in the small intestine. It has also been suggested that organic Cu may increase phosphorus (P) absorption by decreasing complex formation between P and other minerals in a plant-based diet (Liu *et al.*, 2014). In a recent meta-analysis it was found that copper levels between 81 to 200 mg/kg favor productive performance, however no benefit was found above these levels. Furthermore, it was found that these effects were independent of the copper source (Galiotto Miranda *et al*., 2024).

In light of the environmental trade-off, our results demonstrate that copper supplementation increased ADG by 26.55 g/day. However, to mitigate risks such as bacterial resistance and toxicity, optimizing retention efficiency is essential. The use of organic copper sources emerges as a key strategy for sustainability; due to their higher bioavailability, these sources could allow for lower inclusion doses without compromising the +26.55 g/day performance benefit identified in this meta-analysis, thereby reducing mineral excretion into the environment

CONCLUSIONS

Copper supplementation favors productive performance (ADG, ADFI, FC and FE) in pigs. The benefit is greater in rearing piglets versus grower-fattening pigs. The effect of moderators (nutrients) should be considered for the development of balanced diets or for future experiments.

Funding. the authors did not receive funding.

Conflict of interest statement. The autors declare that there is not conflict of interest.

Compliance with ethical standards. Do not apply.

Data availability. Data is available upon reasonable request with the corresponding author (jrquisirumbay@uce.edu.ec).

Author contribution statement (CRediT). V.D. Tufiño-Loza – Investigation and writing-original draft. **R.P. Mena-Pérez**– Conceptualization, Supervision and writing -review & editing. **D.F. Rodriguez-Saldaña**–Methodology, Supervision and writing -review & editing. **J.R. Quisirumbay-Gaibor**–Conceptualization, Project administration, Supervision and Writing -review & editing.

REFERENCES

Aaron, D.K. and Hays, V.W., 2004. How many pigs? Statistical power considerations in swine nutrition experiments. *Journal of Animal Science*, 82(13_suppl), pp. E245–E254. https://doi.org/10.2527/2004.8213_supplE245x

Ayuso, M., Óvilo, C., Fernández, A., Nuñez, Y., Isabel, B., Daza, A., López-Bote, C.J. and Rey, A.I., 2015. Effects of dietary vitamin A supplementation or restriction and its timing on retinol and α-tocopherol accumulation and gene expression in heavy pigs. *Animal Feed Science and Technology*, 202, pp. 62–74. https://doi.org/10.1016/j.anifeedsci.2015.01.014

Bax, L., 2016. *MIX 2.0 - Professional software for meta-analysis in Excel*. [Software].

Blavi, L., Solà, D., Monteiro, A., Pérez, J.F. and Stein, H.H., 2021. Inclusion of dicopper oxide instead of copper sulfate in diets for growing–finishing pigs results in greater final body weight and bone mineralization, but reduced accumulation of copper in the liver. *Journal of Animal Science*, 99(6), skab127. https://doi.org/10.1093/jas/skab127

Burrough, E.R., De Mille, C. and Gabler, N.K., 2019. Zinc overload in weaned pigs: tissue accumulation, pathology, and growth impacts. *Journal of Veterinary Diagnostic Investigation*, 31(4), pp. 537–545. https://doi.org/10.1177/1040638719852144

Canibe, N., Højberg, O., Kongsted, H., Vodolazska, D., Lauridsen, C., Nielsen, T.S. and Schönherz, A.A., 2022. Review on Preventive Measures to Reduce Post-Weaning Diarrhoea in Piglets. *Animals*, 12(19), 2585. https://doi.org/10.3390/ani12192585

Cao, S., Hou, L., Sun, L., Gao, J., Gao, K., Yang, X., Jiang, Z. and Wang, L., 2022. Intestinal morphology and immune profiles are altered in piglets by early-weaning. *International Immunopharmacology*, 105, 108520. https://doi.org/10.1016/j.intimp.2022.108520

Coble, K.F., Burnett, D.D., DeRouchey, J.M., Tokach, M.D., Gonzalez, J.M., Wu, F., Dritz, S.S., Goodband, R.D., Woodworth, J.C. and Pluske, J.R., 2018. Effect of diet type and added copper on growth performance, carcass characteristics, energy digestibility, gut morphology, and mucosal mRNA expression of finishing pigs. *Journal of Animal Science*, 96(8), pp. 3288–3301. https://doi.org/10.1093/jas/sky196

Di Giancamillo, A., Rossi, R., Martino, P.A., Aidos, L., Maghin, F., Domeneghini, C. and Corino, C., 2018. Copper sulphate forms in piglet diets: Microbiota, intestinal morphology and enteric nervous system glial cells. *Animal Science Journal*, 89(4), pp. 616–624. https://doi.org/10.1111/asj.12948

Diao, H., Yan, J., Li, S., Kuang, S., Wei, X., Zhou, M., Zhang, J., Huang, C., He, P. and Tang, W., 2021. Effects of Dietary Zinc Sources on Growth Performance and Gut Health of Weaned Piglets. *Frontiers in Microbiology*, 12, 771617. https://doi.org/10.3389/fmicb.2021.771617

Dove, C.R., 1995. The effect of copper level on nutrient utilization of weanling pigs. *Journal of Animal Science*, 73(1), pp. 166–171. https://doi.org/10.2527/1995.731166x

Egger, M., Smith, G.D., Schneider, M. and Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315(7109), p. 629. https://doi.org/10.1136/bmj.315.7109.629

Elliott, S., Frio, A. and Jarman, T., 2017. Heavy metal contamination of animal feedstuffs – a new survey. *Journal of Applied Animal Nutrition*, 5, e8. https://doi.org&10.1017/jan.2017.7

Espinosa, C.D., Fry, R.S., Kocher, M.E. and Stein, H.H., 2019. Effects of copper hydroxychloride and distillers dried grains with solubles on intestinal microbial concentration and apparent ileal and total tract digestibility of energy and nutrients by growing pigs. *Journal of Animal Science*, 97(12), pp. 4904–4911. https://doi.org/10.1093/jas/skz340

Espinosa, C.D., Fry, R.S., Usry, J.L. and Stein, H.H., 2017. Copper hydroxychloride improves growth performance and reduces diarrhea frequency of weanling pigs fed a corn–soybean meal diet but does not change apparent total tract digestibility of energy and acid hydrolyzed ether extract. *Journal of Animal Science*, 95(12), pp. 5447–5454. https://doi.org/10.2527/jas2017.1702

Espinosa, C.D. and Stein, H.H., 2021. Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: a review. *Journal of Animal Science and Biotechnology*, 12(1), 13. https://doi.org/10.1186/s40104-020-00533-3

Feng, J., Ma, W.Q., Gu, Z.L., Wang, Y.Z. and Liu, J.X., 2007. Effects of Dietary Copper (II. Sulfate and Copper Proteinate on Performance and Blood Indexes of Copper Status in Growing Pigs. *Biological Trace Element Research*, 120(1-3), pp. 171–178. https://doi.org/10.1007/s12011-007-8001-y

Fry, R.S., Ashwell, M.S., Lloyd, K.E., O'Nan, A.T., Flowers, W.L., Stewart, K.R. and Spears, J.W., 2012. Amount and source of dietary copper affects small intestine morphology, duodenal lipid peroxidation, hepatic oxidative stress, and mRNA expression of hepatic copper regulatory proteins in weanling pigs. *Journal of Animal Science*, 90(9), pp. 3112–3119. https://doi.org/10.2527/jas.2011-4403

Galiotto Miranda, P.A., Remus, A., Dalto, D.B., Hilgemberg, R., Beber Jasluk, G., Rosário Silva, B.C. and Lehnen, C.R., 2024. A Systematic Review and Meta-Analysis of the Effects of Various Sources and Amounts of Copper on Nursery Piglets. *Veterinary Sciences*, 11(2), 68. https://doi.org/10.3390/vetsci11020068

Higgins, J.P.T. and Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine*, 21(11), pp. 1539–1558. https://doi.org/10.1002/sim.1186

Hill, G., ed.. 2023. *Sustainable Swine Nutrition*. 2nd edn. John Wiley & Sons Ltd.

Holman, D.B. and Chénier, M.R., 2015. Antimicrobial use in swine production and its effect on the swine gut microbiota and antimicrobial resistance. *Canadian Journal of Microbiology*, 61(11), pp. 785–798. https://doi.org/10.1139/cjm-2015-0239

Huting, A.M.S., Middelkoop, A., Guan, X. and Molist, F., 2021. Using Nutritional Strategies to Shape the Gastro-Intestinal Tracts of Suckling and Weaned Piglets. *Animals*, 11(2), 402. https://doi.org/10.3390/ani11020402

Ketata, M.A., Létourneau-Montminy, M.-P. and Guay, F., 2023. Estimation of digestible zinc and copper in pigs: a meta-analysis approach. *Canadian Journal of Animal Science*, 103(4), pp. 418-433. https://doi.org/10.1139/cjas-2023-0064

Lauridsen, C., Matte, J.J., Lessard, M., Celi, P. and Litta, G., 2021. Role of vitamins for gastro-intestinal functionality and health of pigs. *Animal Feed Science and Technology*, 273, 114823. https://doi.org/10.1016/j.anifeedsci.2021.114823

Lee, S., Hosseindoust, A., Goel, A., Choi, Y., Kwon, I.K., and Chae, B., 2016. Effects of dietary supplementation of bacteriophage with or without zinc oxide on the performance and gut development of weanling pigs. *Italian Journal of Animal Science*, 15, 412–418. https://doi.org/10.1080/1828051X.2016.1188676

Lewis, E.D., Meydani, S.N. and Wu, D., 2019. Regulatory role of vitamin E in the immune system and inflammation. *IUBMB Life*, 71(4), pp. 487–494. https://doi.org/10.1002/iub.1976

Li, J., Yan, L., Zheng, X., Liu, G., Zhang, N. and Wang, Z., 2008. Effect of high dietary copper on weight gain and neuropeptide Y level in the hypothalamus of pigs. *Journal of Trace Elements in Medicine and Biology*, 22(1), pp. 33–38. https://doi.org/10.1016/j.jtemb.2007.10.003

Liu, Y., Ma, Y.L., Zhao, J.M., Vazquez-Añón, M. and Stein, H.H., 2014. Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. *Journal of Animal Science*, 92(8), pp. 3407–3415. https://doi.org/10.2527/jas.2013-7080

Manto, M., 2014. Abnormal Copper Homeostasis: Mechanisms and Roles in Neurodegeneration. *Toxics*, 2(2), pp. 327–345. https://doi.org/10.3390/toxics2020327

Moreira, R.H.R., Pérez Palencia, J.Y., Moita, V.H.C., Caputo, L.S.S., Saraiva, A., Andretta, I., Ferreira, R.A. and de Abreu, M.L.T., 2020. Variability of piglet birth weights: A systematic review and meta-analysis. *Journal of Animal Physiology and Animal Nutrition*, 104(2), pp. 657–666. doi.org. https://doi.org/10.1111/jpn.13264

National Research Council, 2012. *Nutrient requirements of swine*. 11th edn. Washington: National Academies Press.

Palencia, J.Y.P., Lemes, M.A.G., Garbossa, C.A.P., Abreu, M.L.T., Pereira, L.J. and Zangeronimo, M.G., 2018. Arginine for gestating sows and foetal development: A systematic review. *Journal of Animal Physiology and Animal Nutrition*, 102(1), pp. 204–213. https://doi.org/10.1111/jpn.12679

Pluske, J.R., Turpin, D.L. and Kim, J.-C., 2018. Gastrointestinal tract (gut. health in the young pig. *Animal Nutrition*, 4(2), pp. 187–196. https://doi.org/10.1016/j.aninu.2017.12.004

Sampath, V., Sureshkumar, S., Seok, W.J. and Kim, I.H., 2023. Role and functions of micro and macro-minerals in swine nutrition: a short review. *Journal of Animal Science and Technology*, 65(3), pp. 479–489. https://doi.org/10.5187/jast.2023.e9

Sandström, B., 2001. Micronutrient interactions: effects on absorption and bioavailability. *British Journal of Nutrition*, 85(S2), pp. S181-S185. https://doi.org/10.1049/BJN2000312

Smith II, J.W., Tokach, M.D., Goodband, R.D., Nelssen, J.L. and Richert, B.T., 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs2. *Journal of Animal Science*, 75, pp.1861–1866. https://doi.org/10.2527/1997.7571861x

Tang, Q., Lan, T., Zhou, C., Gao, J., Wu, L., Wei, H., Li, W., Tang, Z., Tang, W., Diao, H., Xu, Y., Peng, X., Pang, J., Zhao, X., Sun, Z., 2024. Nutrition strategies to control post-weaning diarrhea of piglets: From the perspective of feeds. *Animal Nutrition*, 17, pp.297-311. https://doi.org/10.1016/j.aninu.2024.03.006

Tang, W., Qian, Y., Yu, B., Zhang, T., Gao, J., He, J., Huang, Z., Zheng, P., Mao, X., Luo, J., Yu, J., Chen, D., 2019. Effects of Bacillus subtilis DSM32315 supplementation and dietary crude protein level on performance, gut barrier function and microbiota profile in weaned piglets1. *Journal Animal Science*, 97, 2125–2138. https://doi.org/10.1093/jas/skz090

Veum, T.L., Carlson, M.S., Wu, C.W., Bollinger, D.W., Ellersieck, M.R., 2004. Copper proteinate in weanling pig diets for enhancing growth performance and reducing fecal copper excretion compared with copper sulfate1. *Journal Animal Science*, 82, pp.1062–1070. https://doi.org/10.1093/ansci/82.4.1062

Wang, J., Zhu, X., Guo, Y., Wang, Z., Zhao, B., Yin, Y., Liu, G., 2016. Influence of Dietary Copper on Serum Growth-Related Hormone Levels and Growth Performance of Weanling Pigs. *Biological Trace Element Research*, 172, pp.134–139. https://doi.org/10.1007/s12011-015-0574-2

Wang, M., Wang, Lixia, Tan, X., Wang, Lei, Xiong, X., Wang, Y., Wang, Q., Yang, H., Yin, Y., 2022. The developmental changes in intestinal epithelial cell proliferation, differentiation, and shedding in weaning piglets. *Animal Nutrition*, 9, pp.214–222. https://doi.org/10.1016/j.aninu.2021.11.006

Wang, M.-Q., Du, Y.-J., Wang, C., Tao, W.-J., He, Y.-D., Li, H., 2012. Effects of Copper-Loaded Chitosan Nanoparticles on Intestinal Microflora and Morphology in Weaned Piglets. *Biological Trace Element Research*, 149, pp.184–189. https://doi.org/10.1007/s12011-012-9410-0

Wang, Z., Li, J., Wang, Y., Wang, L., Yin, Yuebang, Yin, L., Yang, H., Yin, Yulong, 2020. Dietary vitamin A affects growth performance, intestinal development, and functions in weaned piglets by affecting intestinal stem cells. *Journal Animal Science*, 98, pp.skaa020. https://doi.org/10.1093/jas/skaa020

Wen, Y., Li, R., Piao, X., Lin, G., He, P., 2022. Different copper sources and levels affect growth performance, copper content, carcass characteristics, intestinal microorganism and metabolism of finishing pigs. *Animal Nutrition*, 8, 321–330. https://doi.org/10.1016/j.aninu.2021.10.007

Xia, J., Fan, H., Yang, J., Song, T., Pang, L., Deng, H., Ren, Z., Deng, J., 2022. Research progress on diarrhoea and its mechanism in weaned piglets fed a high-protein diet. *Journal Animal Physiology Animal Nutrition (Berl)*, 106, pp.1277–1287. https://doi.org/10.1111/jpn.13654

Zhou, W., Kornegay, E.T., van Laar, H., Swinkels, J.W.G.M., Wong, E.A., Lindemann, M.D., 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. *Journal of Animal Science*, 72, pp.2385–2394. https://doi.org/10.2527/1994.7292385x