Optimal dietary copper levels for pig growth

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SUMMARY

Pharmacological levels of 250 mg copper/kg diet were found to increase the growth rate of pigs. However, responses were not consistent among studies, and the calculation of an optimal dietary copper level to obtain maximum performance was hindered by a method to effectively account for differences in experimental protocols. In the current evaluation the random effects regression method, was used to compute relationships between total dietary copper levels or intakes as explanatory variables, and average daily gain, average daily feed intake, or feed conversion ratio as response variables. Based on several selection criteria, such as copper sulphate as source of copper supplementation, and treatment means from a minimum of five independent experiments, results from experiments reported in 55 studies could be divided into categories of different phases (postweaning, growth, finisher, and combinations thereof) and countries (UK, USA). Experiments within countries concurred in characteristics such as the frequency of zinc and iron supplementation, feeding method, and main feed ingredients. Based on initial visual inspection, linear and quadratic relationships could be used to explore the relationships between fixed explanatory and response variables, with experiment included as a random effect. Quadratic relationships indicated that total dietary Cu contents of 253 and 230 mg copper/kg diet would result in maximum average daily gain of pigs during the postweaning and growth-finishing phases, respectively, when antibiotics were not added to USA diets. Relationships, when detected, were predominantly linear with results from UK experiments, ascribed to an upper supplementation level of 250 mg copper/kg diet applied. It can be concluded from this study that any decrease in total dietary copper to levels lower than 200 mg copper/kg diet will deteriorate growth performance of pigs during the respective life phases from postweaning to finishing.

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INTRODUCTION

The requirement for copper (Cu), an essential trace element needed by pigs to prevent microcytic hypochromic anaemia, dermatosis and bone marrow problems, maintain the integrity of the cardiovascular system, and perform metabolic functions as a constituent of several enzymes systems in the body (Omole, 1980), is probably not greater than a level of 5 to 6 mg Cu/kg of the pig's diet (NRC, 1998). However, after pioneering work by Braude in 1945, with numerous follow-up studies, it was recommended (Braude, 1967) that supplementation of pig diets with 250 mg Cu/kg diet would result in improved average daily gain (ADG) and feed conversion ratio (FCR). In recent years, however, these high Cu levels have become an environmental hazard. With most of the dietary Cu supply being excreted, pig manure application to arable lands increases Cu soil contents, with subsequent negative environmental effects. As a preventive measure, considerable lower levels of 170 mg Cu/kg diet for pigs up to 12 weeks and 25 mg Cu/kg diet for all other types have been introduced in the European Union (European Commission, 2003a).

Although the modes of action of Cu are not fully explained, an original unresolved hypothesis ascribed the growth-stimulating effects of Cu to its antimicrobial actions (Fuller et al., 1960). More recently, both intestinal (Radecki et al., 1992; Zhao et al., 2007) and systemic (Zhou et al., 1994a; b) effects have been suggested as possible mechanisms for the action of Cu on the growth of pigs.

Throughout the scientific literature, reports of pig responses on Cu supplementation are inconsistent concerning the optimum level of supplementation and phase when it has to be supplemented. Previous attempts (Wilson et al., 1979; European Commission, 2003b; Jongbloed et al., 2011) to find the most effective level of dietary Cu across studies were restrained by the absence of a proper method to incorporate the variability caused by different methodologies among studies. Ignorance of this variability could result in considerable bias in the estimates of a regression equation. However, with the random effects regression method, variance among studies not accounted for by variables other than the fixed effects under evaluation, could be represented by inclusion of experiment as a random effect (St-Pierre, 2001). Therefore this method was applied in the current evaluation to quantify the effects of dietary Cu on performance of pigs from postweaning to finishing, which would give an indication of an optimal level of Cu needed in the diet.

Material and Methods

Selection of studies

Peer-reviewed studies that have evaluated the effects of pharmacological Cu additions to pig diets on animal performance were identified by internet search engines (Google Scholar, ISI Web of Science, PubMed and ScienceDirect), and reviews of citations of retrieved articles. Performance was identified by ADG (g), average daily feed intake (ADFI; g) and FCR (kg feed/kg live weight increase). Supplementation should have been applied during the different phases from postweaning to finishing. Copper carbonate, Cu chloride, Cu-lysine complex, and organic and inorganic chelated forms of Cu have been reported to be as effective as Cu sulphate, with the sulphide and oxide Cu salts found to be as poorly available to pigs (Cromwell et al., 1998). However, possible differences in absorption rates from these formulations could change the transfer of the amounts of Cu from the intestine into the organism, which would change the threshold Cu value for maximum animal performance (Windisch et al., 2001). Consequently, Cu sulphate as source of supplementation was included as a main selection criterion of studies because of its extensive frequency of use. Other criteria included: (1) the presence of a control treatment without Cu addition other than in the basal dietary mineral mixture; (2) no confounding of Cu supplementation with other feed additives; and (3) written in English. These criteria identified 77 studies with suitable results for analyses.

Table 1. Distribution of studies according to phase, country and the presence or absence of antibiotic in all treatments.

Phase	Country	Antibiotic added	Live weight range (kg)	Studies	Exp[a]	*n*[b]
Postweaning	Canada	Yes	13.5–23.0	2 (0)[c]	2 (0)	4 (0)
		No	5.5–22.8	2 (2)	3 (3)	6 (6)
	China	No	7.5–25.6	2 (2)	2 (2)	6 (6)
	Germany	No	7.6–31.3	1 (1)	3 (3)	9 (9)
	UK	Yes	3.6–20.5	1 (1)	1 (1)	2 (2)
		No	3.6–20.9	1 (1)	1 (1)	2 (2)
	USA	Yes	4.5–25.5	12 (12)	21 (21)	71 (71)
		No	4.3–32.7	19 (17)	33 (29)	91 (83)
Growth	Canada	Yes	19.1–71.2	2 (1)	2 (1)	6 (2)
		No	11.3–72.9	3 (3)	4 (4)	24 (24)
	China	No	21.2–41.0	1 (1)	1 (1)	2 (2)
	Denmark	No	20.0–50.0	1 (0)	1 (0)	4 (0)
	Korea	No	21.5–55.8	1 (1)	1 (1)	3 (3)
	UK	No	18.2–67.9	8 (8)	10 (10)	31 (31)

	USA	Yes	17.4–61.0	3 (3)	4 (4)	15 (15)
		No	13.8–67.6	9 (8)	16 (13)	39 (33)
Postweaning-Growth	Canada	Yes	13.5–69.0	2 (0)	2 (0)	4 (0)
		No	N[d]–68.3	1 (1)	1 (1)	4 (4)
	China	No	12.5–45.8	1 (1)	1 (1)	3 (3)
	USA	Yes	10.8–77.0	2 (2)	4 (4)	16 (16)
		No	6.4–56.8	2 (2)	2 (2)	6 (6)
Finishing	Canada	No	47.0–90.0	2 (2)	4 (4)	22 (22)
	Denmark	No	50.0–90.0	1 (0)	1 (0)	4 (0)
	UK	No	45.5–93.2	7 (7)	10 (10)	29 (29)
	USA	Yes	52.8–111.7	2 (2)	2 (2)	5 (5)
		No	47.6–108.0	4 (3)	6 (3)	14 (8)
Growth-Finishing	Canada	No	11.3–123.0	2 (2)	4 (4)	24 (24)
	Ireland	No	23.8–81.5	1 (1)	1 (1)	4 (4)
	Germany	No	27.0–93.0	1 (1)	1 (1)	2 (2)
	Denmark	No	20.0–90.0	1 (0)	1 (0)	4 (0)
	UK	Yes	23.3–95.1	1 (1)	1 (1)	2 (2)
		No	14.2–96.0	14 (14)	29 (29)	74 (74)
	USA	Yes	17.4–111.7	4 (4)	5 (5)	15 (15)
		No	13.8–96.9	11 (11)	21 (21)	60 (60)
Postweaning-Finishing	Canada	Yes	13.5–92.0	2 (2)	4 (2)	13 (9)
		No	5.5–91.7	2 (2)	2 (2)	4 (4)
	USA	No	6.4–108.0	6 (5)	8 (7)	20 (14)

[a]Number of experiments.
[b]Number of treatment means.
[c]Values in parentheses indicated numbers obtained for variables in which feed intake was accounted for.
[d]No information available.

Classification according to phase, country and the presence or absence of antibiotic in all treatments (including the control), resulted in a distribution of studies as presented in Table 1. Phases were as indicated in the respective experiments, or when unspecified, identified according to the live weight range. When Cu sulphate supplementation was reported at different crude protein, fat, calcium or ingredient dietary contents, with different protein or fat sources, and for various feeding or housing systems, means from each treatment within studies were tabulated. Some studies presented treatment means across experiments or experimental stations, and were used as such. An average value was calculated when means were separate reported for males and females, and a weighted average when indicated for specific periods within a phase.

To prevent bias, a minimum of five experiments was arbitrarily set as requirement for any category to be evaluated. These additional criteria eliminated 22 studies (Appendix A of Electronic Supplementary Data) from the database. Finally, treatment means from 55 studies (Appendix B of Electronic Supplementary Data) could be distributed across phases and countries, as characterized in Table 2.

Table 2. Characteristics of final data sets.

Phase	Country	Antibiotic added	≤ 1975[a]	Number of treatment means						Cu added
				Zinc[c]	Iron[d]	Zinc and iron	*Ad libitum* feeding	Maize, soy[e]	Barley, fishmeal[e]	(mg Cu/kg diet)[b]
Postweaning	USA	Yes	12 (12)[f]	71 (71)	71 (71)	71 (71)	71 (71)	71 (71)	0 (0)	250
		No	4 (3)	91 (83)	91 (83)	91 (83)	85 (79)	89 (81)	0 (0)	550
Growth	UK	No	8 (8)	13 (13)	11 (11)	11 (11)	6 (6)	4 (4)	21 (21)	250
	USA	No	3 (2)	39 (33)	39 (33)	39 (33)	37 (31)	37 (31)	0 (0)	375
Finishing	UK	No	7 (7)	13 (13)	11 (11)	11 (11)	2 (2)	4 (4)	14 (14)	250
	USA	No	3 (2)	14 (8)	14 (8)	14 (8)	14 (8)	14 (8)	0 (0)	250
Growth-Finishing	UK	No	14 (14)	10 (10)	8 (8)	8 (8)	4 (4)	4 (4)	54 (54)	250
	USA	Yes	1 (1)	15 (15)	15 (15)	15 (15)	15 (15)	15 (15)	0 (0)	250
		No	7 (7)	56 (56)	56 (56)	56 (56)	60 (60)	60 (60)	0 (0)	750
Postweaning-Finishing	USA	No	3 (3)	18 (12)	20 (14)	18 (12)	20 (14)	20 (14)	0 (0)	250

[a]Number of studies published before or in 1975.
[b]Cu, copper; maximum copper added from copper sulphate.
[c]Zinc added (40 – 200 mg zinc/kg diet) from the basal mineral mixture.
[d]Iron added (10 – 200 mg iron/kg diet) from the basal mineral mixture.
[e]Main energy and protein sources used in diets.
[f]Values in parentheses indicated numbers of studies or treatment means used for variables in which feed intake was accounted for.

Description of database

Data sets showed a high degree of agreement within country for the characteristics indicated in Table 2. Most studies were reported before or in 1975. However, several USA studies were published after 1975 for the postweaning (without antibiotics added), growth and growth-finishing phases. Zinc and iron were predominantly supplemented in basal mineral mixtures in USA treatments, but not in UK data sets. Whereas almost all treatments in the US data sets applied *ad libitum* feeding regimes, with maize and soybean as the main dietary energy and protein sources, respectively, restricted or semi *ad libitum* feeding (unlimited feed supply for a specific time period per day), with barley and fishmeal diets, were dominant in the UK treatments. Phases

where Cu were supplemented at levels of higher than 250 mg Cu/kg diet were postweaning, growth and growth-finishing, all without antibiotics added to diets, and conducted in the USA. In all treatments, calcium was supplemented as some source (calcium carbonate, calcium phosphate) in the basal diets. Durations of treatments varied from 10 to 56, and 61 to 116 days, for the postweaning and growth-finishing phases, respectively. For other phases treatment periods were seldom reported.

Ranges of means for Cu treatments (total dietary contents and intakes) and response variables are presented in Table 3. When not presented by studies, total dietary Cu content was calculated from the sum of the amounts provided by dietary ingredients using values reported by NRC (1998), Cu added from the trace mineral premix, and any additional Cu sulfate supplementation. Tabulated NRC (1998) values and diet compositions in studies were expressed on an as-fed basis. Total dietary Cu intakes were computed as total dietary Cu content multiply by ADFI. Feed conversion ratio, when absent in experiments, was calculated from ADG and ADFI. Similarly, ADFI was derived from FCR. Dietary nutrient composition was rarely reported in experiments. Where indicated, dietary crude protein (the dietary nutrient most frequently stated) varied from 155 to 229, 145 to 181, and 119 to 181 g/kg for the postweaning, growth, and finishing phases, respectively.

Table 3. Ranges of treatment means for explanatory and response variables in the respective data sets.

Phase	Country	Antibiotic added	Total dietary Cu (mg Cu/kg diet)[a]	Total dietary Cu intake (mg Cu/day)	Average daily gain (g)	Average daily feed intake (g)	Feed conversion ratio	Reference[b]
Postweaning	USA	Yes	12–289	5–216	107–593	246–939	1.35–2.38	3, 4, 20, 22, 24, 27, 30, 34, 48, 49, 51, 55
		No	4–580	2–342	127–670	350–1230	1.41–3.01	2, 4, 16, 17, 19, 20, 22, 23, 28, 29, 30, 32, 46, 47, 49, 50, 53, 54, 55
Growth	UK	No	6–285	9–561	409–736	1228–2173	2.45–4.00	1, 7, 10, 12, 14, 35, 39, 40
	USA	No	8–386	17–749	559–830	1506–2882	2.50–3.72	21, 25, 32, 33, 43, 44, 45, 50, 53
Finishing	UK	No	6–278	15–763	571–798	2291–2955	3.24–4.80	7, 10, 12, 14, 35, 39, 40
	USA	No	6–264	21–839	650–920	1957–3173	2.94–4.18	18, 32, 43, 53
Growth-Finishing	UK	No	6–279	12–645	494–820	1819–2495	2.02–4.40	1, 5, 6, 7, 8, 9, 10, 11, 14, 15, 35, 39, 40, 41

	USA	Yes	11–262	25–665	726–950	2055–2945	2.70–3.26	4, 25, 42, 52
		No	7–780	14–1527	377–860	1514–3110	2.69–4.14	19, 21, 25, 26, 36, 37, 38, 43, 44, 45, 52
Postweaning-Finishing	USA	No	7–264	15–592	550–760	1875–2370	2.86–3.41	13, 18, 31, 32, 33, 53

[a] Cu, copper.

[a] From Electronic Supplementary Data Appendix B.

Data analysis

Visual evaluation of data, as suggested by Sauvant et al. (2008), presented evidence of linear and curvilinear intra- and interexperiment relationships between explanatory and response variables. Attempts to relate total dietary Cu contents or intakes (x) to the respective response variables (y) by two-slope broken-line models (Robbins et al., 2006) with the NLMIXED procedures of SAS Inc., (2008), failed. In these models experiment was included as a random effect to represent the variance among experiments not accounted for by fixed explanatory variables in the models. Although models converged to a solution for most data sets, a final Hessian that was not positive definite, and elements of the projected gradient greater than 0.001, were found. These indicated that the variance of some parameter estimates was zero or linearly related, or that the models may not have fully converged, which resulted in unrealistic and unreliable estimates. Consequently, linear ($y = a + bx$) and quadratic ($y = a + bx + cx^2$) relationships were calculated with the MIXED procedures of SAS Inc. (2008) according to methodology described by St-Pierre (2001). Total dietary Cu content or intake was included as a fixed effect, and the effect of experiment as random. An unstructured variance-covariance matrix was added to the random part of the model. The absence of a measurement of variation in numerous experiments, and the need to calculate ADFI or FCR in several treatments, prevented the weighing of treatment means to ensure homogeneity of variance for models. Response variables were considered significant at $P<0.05$, with a tendency for significance at $P<0.10$. The quadratic relationship was accepted if the quadratic term was significant, regardless of the significance of the linear term. For all models residual plots (residual *versus* predicted values) were visually evaluated to assess whether any patterns existed (Draper and Smith, 1981).

Root mean square errors were calculated after y-values were adjusted for the random effect of experiment (St-Pierre, 2001) to indicate the magnitude of variation. Contrary to fixed effects models, R^2-values, as indication of the fraction of variability that can be explained by the relationship, may actually decrease when explanatory variables are added to the fixed effects part of a

random effects model (Zanton and Heinrichs, 2005). Therefore, no R^2-values were reported in this study. With quadratic regression equations, a minimum (when c>0) or maximum (when c<0) *x*-value, where the function turns direction (vertex), was calculated by -b/(2a). The asymptotic standard error of the vertex was calculated according to the delta method, as described by Kuha and Temple (2003):

$$\sqrt{\frac{b^2}{4c^2}\left[\frac{\sigma_b}{b^2}+\frac{\sigma_c}{c^2}-\frac{2\sigma_{bc}}{bc}\right]}$$

where σ_b is the variance of b, σ_c is the variance of c, and σ_{bc} is the covariance between b and c. Variance and covariance estimates of the fixed effects were obtained with the COVB option in the MODEL statement of the MIXED procedures of SAS Inc. (2008).

RESULTS

Quadratic and linear relationships obtained between total dietary Cu contents or intakes (*x*) and response variables (*y*), which presented coefficients with a significance of <0.10, are indicated in Tables 4 and 5, respectively. With all models, the estimated generalised matrix was not positive definite when an unstructured variance-covariance matrix was included in the random part. Because this implied that one or more variance components on the RANDOM statement were estimated to be zero, the variance-covariance matrix was removed from models.

Table 4. Relationships ($y = a + bx + cx^2$) between total dietary Cu content or intake (*x*) and response variables (*y*) with significant (P<0.05, except stated otherwise) quadratic regression coefficients (± standard errors).

Phase	Country	Antibiotic added	*y*[a]	a	b	c	RMSE[b]	Vertex[c]
Total dietary Cu content (mg Cu/kg diet)								
Postweaning	USA	No	ADG	325 ± 19.2	0.384 ± 0.0499	-0.00076 ± 0.000124	20.0	253 ± 17.9
		Yes	ADFI	530 ± 30.3	0.958 ± 0.313	-0.00259 ± 0.00115	33.1	185 ± 21.1
		No	ADFI	594 ± 37.3	0.555 ± 0.080	-0.00113 ± 0.000194	29.4	245 ± 33.6
		No	FCR	2.02 ± 0.061	-0.00114 ± 0.000335	2.36E-06 ± 8.1E-07	0.126	241 ± 70.1
Growth	USA	No	ADFI	1850 ± 96.3	1.10 ± 0.436	-0.00266 ± 0.00143[d]	53.0	208 ± 40.2
Growth-Finishing	USA	No	ADG	697 ± 12.6	0.392 ± 0.0911	-0.00085 ± 0.00014	43.6	230 ± 34.8
		No	ADFI	2327 ± 55.2	0.698 ± 0.306	-0.00165 ± 0.000484	132	211 ± 40.0

		No	FCR	3.33 ± 0.0529	-0.00083 ± 0.000267	1.729E-06 ± 4.237E-07	0.113	242 ± 77.2
Postweaning-Finishing	USA	No	ADG	698 ± 22.9	-0.544 ± 0.306	0.00221 ± 0.00118[d]	15.6	123[e]
Total dietary Cu intake (mg Cu/day)								
Postweaning	USA	No	ADG	298 ± 14.8	0.696 ± 0.0811	-0.00212 ± 0.000329	16.6	164 ± 16.8
		No	ADFI	595 ± 37.4	0.754 ± 0.155	-0.002 ± 0.000629	31.5	188 ± 27.8
		No	FCR	2.01 ±0.058	-0.00225 ± 0.000609	8.84E-06 ± 2.44E-06	0.129	127 ± 13.2
Growth	UK	No	ADFI	1551 ± 1394	-0.295 ± 0.156	0.000904 ± 0.000336	30.2	163 ± 33.5
	USA	No	ADG	638 ± 20.5	0.197 ± 0.0653	-0.0002 ± 0.000105[d]	18.8	491 ± 113
Finishing	UK	No	ADFI	2591 ± 52.5	-0.311 ± 0.171	0.000451 ± 0.000242[d]	25.3	345 ± 47.7
Growth-Finishing	UK	No	ADG	639 ± 11.6	-0.180 ± 0.0929	0.000448 ± 0.000157	17.2	201 ± 75.2
		No	ADFI	2151 ± 26.2	-0.658 ± 0.204	0.00130 ± 0.000343	37.7	254 ± 10.7
	USA	No	ADG	699 ± 15.0	0.178 ± 0.0521	-0.00019 ± 0.000043	46.9	479 ± 54.4
		No	ADFI	2331 ± 58.9	0.281 ± 0.172	-0.00032 ± 0.000144	146	438 ± 159
		No	FCR	3.33 ± 0.0542	-0.00042 ± 0.000141	4.44E-07 ± 1.187E-07	0.117	482 ± 157
Postweaning-Finishing	USA	No	ADG	717 ± 17.3	-0.360 ± 0.189	0.00066 ± 0.000324[d]	14.1	273 ± 30.5

[a] ADFI, average daily feed intake (kg); ADG, average daily gain (kg); FCR, feed conversion ratio.
[b] Root mean square error, adjusted for the random effect of experiment.
[c] Mean calculated as (b/(-2c)), calculation of the standard error presented in section on data analysis.
[d] 0.05<P<0.10.
[e] Presented an unexplained negative variance.

During the postweaning phase, total dietary Cu contents or intakes were quadratically related to all response variables when antibiotics were excluded from diets in USA experiments, with a maximum ADG obtained at a total dietary Cu intake of 164 mg Cu/day (Table 4). In contrast, when antibiotics were added to diets, relationships were found to be linear (Table 5), except for ADFI. However, ADFI can be ascribed as a secondary response variable compared to ADG and FCR.

Average daily gain and FCR responded in a linear mode to increasing total dietary Cu contents or intakes during the growth phase in the UK data set (Table 5). The quadratic relationship between total dietary Cu intake and ADFI found for this data set (Table 4), presented c>0 with a minimum ADG value at the turning point, whereas c<0 with a maximum ADFI was obtained in other relationships. Although visual inspection of residuals did not reveal evidence of any irregularities for this relationship, it could be regarded as an artifact.

Table 5. Relationships (y = a + bx) between total dietary Cu content or intake (x) and response variables (y) with significant (P<0.05, except stated otherwise) linear regression coefficients (± standard errors).

Phase	Country	Antibiotic added	y[a]	a	b	RMSE[b]
Total dietary Cu content (mg Cu/kg diet)						
Postweaning	USA	Yes	ADG	311 ± 20.1	0.203 ± 0.0332	25.0
		Yes	FCR	1.81 ± 0.050	-0.000363 ± 0.000137	0.105
Growth	UK	No	ADG	495 ± 19.8	0.189 ± 0.0348	18.7
		No	FCR	3.13 ± 0.087	-0.000819 ± 0.000217	0.118
	USA	No	ADG	670 ± 20.7	0.0984 ± 0.0451	25.2
Growth-Finishing	UK	No	ADG	634 ± 12.4	0.177 ± 0.0231	19.0
		No	ADFI	2136 ± 28.8	0.217 ± 0.0513	42.2
		No	FCR	3.39 ± 0.045	-0.000557 ± 0.00009	0.075
Total dietary Cu intake (mg Cu/day)						
Postweaning	USA	Yes	ADG	311 ± 18.9	0.339 ± 0.0517	24.6
		Yes	ADFI	544 ± 28.4	0.453 ± 0.0692	32.9
		Yes	FCR	1.80 ± 0.049	-0.000495 ± 0.000221	0.108
Growth	UK	No	ADG	494 ± 18.7	0.123 ± 0.0206	17.9
		No	FCR	3.12 ± 0.089	-0.000497 ± 0.000135	0.118
Growth-Finishing	UK	No	FCR	3.39 ± 0.045	-0.000258 ± 0.000041	0.074

[a] ADFI, average daily feed intake (kg); ADG, average daily gain (kg); FCR, feed conversion ratio.
[b] Root mean square error, adjusted for the random effect of experiment.

Significant relationships were absent in results from USA experiments during the growth phase, although quadratic trends were identified between total dietary Cu content and ADFI *(*P=0.080), and total dietary Cu intake and ADG (P=0.068). No relationships were detected with ADG and FCR as response variables during the finishing phase with UK results, and ADFI was quadratically related to total dietary Cu intakes with c>0 (Table 4). Too few USA experiments were available during the finishing phase (Table 1) for analysis of results.

In categories where successive phases were combined, no relationships were obtained when antibiotics were included in USA diets during the growth-finishing phase. However, when diets were supplemented with antibiotics, all response variables were quadratically related to total dietary Cu contents or intakes (Table 4). Variability in FCR was more profound than in ADG, as illustrated in the standard errors of the vertex. Performance characteristics responded linearly to increasing total Cu contents in growth-finishing results

from UK experiments, whereas responses on total dietary Cu intakes were quadratic for ADG and ADFI with c>0 (Table 5), and linear for FCR (Table 4). Quadratic trends during the postweaning-finishing phase in the USA data set between total dietary Cu content and ADG (P=0.090), and total dietary Cu intake and ADG (P=0.098), presented quadratic coefficients of greater than 0.

DISCUSSION

Evidence is presented in this study that the total dietary Cu contents to achieve maximum ADG in pigs should be above 200 mg Cu/kg diet. These values were calculated as the maximum vertex of quadratic relationships determined with treatment means from empirical studies conducted in the USA in which antibiotics were not added to diets during the postweaning and growing-finishing phases, with variability caused by differences in dietary nutrient contents other than Cu, housing systems, and other methodologies among experiments, included as a random effect in models. This is in agreement to an optimal level of 224 mg Cu/kg diet calculated by Wilson et al. (1979) from quadratic regression equations obtained with 1343 results from 129 studies with pigs in the live weight range 4-100 kg. In contrast, Jongbloed et al. (2011) found a considerable lower maximum value for ADG at 146 mg Cu/kg diet calculated from a quadratic regression containing 252 observations from pigs varying in live weight from 5 to 25 kg, with a maximum ADFI at 150 mg Cu/kg diet. In other weight classes (5-45 kg; 20-45 kg; 45-100 kg), Jongbloed et al. (2011) failed to demonstrate significant linear or quadratic effects of supplementary Cu on ADG, ADFI or FCR in most cases. In an empirical study, Cromwell et al. (1989) have determined a quadratic maximum ADG at 242 mg Cu/kg diet, supplemented as copper sulphate to an upper limit of 500 mg Cu/kg diet, for pigs during growth from 6.7 to 18.0 kg. Previous studies, however, have only considered the supplemented Cu levels, whereas the current study accounted for the contribution of Cu from feed ingredients and basal mineral mixes. When Cu contents are related to ADG, ADFI or FCR, any variability in the level of feed intake could affect the content of Cu needed in the diet. Although optimal total dietary Cu contents presented in the current evaluation were from experiments that have used *ad libitum* feeding systems, which would result in optimal feed intakes, optimal values were also presented as the daily intake of total dietary Cu.

Response variables during the growth and finishing phases across UK or USA studies were either not related, or mostly linearly related to total dietary Cu contents or intakes. This tendency was also illustrated during the growth-finishing phase across UK studies. The aforementioned is not surprising, seen that the maximum supplementation in these studies, with the exception of

studies conducted in the USA during the growth phase, was restricted to 250 mg Cu/kg diet. This value was determined to be near to the turning point for responses when limits were extended beyond 250 mg Cu/kg diet. Furthermore, it is generally accepted that the regulatory capacity of Cu metabolism in pigs is overcharged when levels of above 250 mg Cu/kg diet are fed for extended periods. As a result, excessive accumulation of Cu in tissues might cause Cu toxicity (NRC, 1998), with a consequently decrease in animal performance. Linear responses on Cu supplementation (up to 280 mg Cu/kg diet additional to that in feed ingredients and the basal mineral mixture) in the fraction of improvement in ADG were also observed with results across studies by the European Commission (2003b) for pigs from weaning to approximately 25 kg live weight, with no trends found during the growth or growth-finishing phases.

Although this study was not designed to evaluate the interactions of dietary Cu with antimicrobial agents, which was banned in animal feeds throughout the European Union in 2006 after much controversies (Zhao et al., 2007), an indication of the effects of these substances was prevented by the upper limit of 250 mg Cu/kg diet in treatments in which they were applied. This limitation also restricted information on the possible interactions of Cu supplementation with diet composition, supplementation of zinc and iron, and method of feeding, which could be illustrated by categorising studies according to the country in which studies were conducted. In pigs, Cu has been proved to be antagonistic to elements such as cadmium, iron, molybdenum and zinc (see Omole, 1980; Shuttle, 2010). Other factors which might have an influence on the growth-promoting effects of Cu in pig diets included type and level of protein (Braude, 1967; Omole, 1980), fat source (Dove, 1995), method of feeding, and different environmental conditions (Braude, 1967).

Quadratic responses in ADF on increased total Cu contents or intakes during the postweaning (without antibiotics added to diets) and growth-finishing phases in results from USA studies were reflected in ADFI and FCR responses. Optimal values obtained with the different response variables were in close approximation of each within phases, although ADG presented the least variability. Similarly, ADG, ADFI and FCR during the postweaning phase when antibiotics were added to USA diets, and in the growth-finishing phase across studies performed in the UK, responded all linearly to total Cu intakes and contents, respectively. These might indicated that an increased ADFI was not the only factor responsible for increased growth. An increase in feed intake upon stimulation of the secretion of neuropeptide Y secretion by Cu was suggested by Zhou et al. (1994b) to increase growth, and verified in pigs by Li et al. (2008). However, intravenously injected Cu, which bypassed the gastrointestinal tract, was found to stimulate pig growth (Zhou et al., 1994a).

This might have resulted from a direct action of Cu on the growth regulatory system (Zhou et al., 1994b).

Conclusions

A Cu content of above 200 mg Cu/kg diet on an as-fed basis is needed to obtain maximum growth in pigs. It appears that this level should be applied during all phases of the life cycle from postweaning to finishing. This study presented viable models that could be applied within the conditions under which they were established to calculate the reduction in growth and economic returns when dietary Cu levels are decreased. If the aim would be to sustain maximum growth, any lower dietary Cu levels because of the potential environmental hazard of Cu will have to be counteracted by alternative dietary components or management techniques.

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ELECTRONIC SUPPLEMENTARY DATA

Appendix A

Studies eliminated when classification was done according to stage of growth, country and the presence or absence of antibiotic in all treatments, and the additional criterion of five experiments per data set to prevent bias was introduced.

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Appendix B

Studies from which treatment means were used in final data sets.

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