NON RUMINANT NUTRITION

The effect of hydroxychloride trace minerals on the growth performance and carcass quality of grower/finisher pigs: a meta-analysis

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Abstract

The current study compared the effect of hydroxychloride trace minerals (**HTM**) with the effect of inorganic trace minerals (**ITM**) on growth performance and carcass quality in grower-finisher pigs. The results of 6 studies conducted throughout Europe were combined into one meta-analysis. All included studies were performed using pigs from about 19 kg of body weight until slaughter. In all studies, 2 different mineral sources were compared, HTM and sulfates as ITM. Zn from either HTM or ITM was added at a level of 80 ppm to the diet, and Cu was added at a level of 15 ppm from the same source as Zn. In most studies, an additional treatment was included in which 20 ppm Zn was used from either source in combination with 15 ppm Cu from the same source. Diets were fed in 3 phases according to local commercial standards. The body weight, average daily gain, average daily feed intake, and gain:feed ratio were measured at the end of each phase. At the end of each study, the carcass yield, back fat thickness, and lean meat percentage were measured at commercial slaughterhouses. The meta-analysis was conducted using a MIXED model in SAS taking into account the within-study and between-study variation. The comparison was done only between HTM and ITM added at the same Zn level. No statistical differences were observed for growth performance or carcass characteristics between the mineral sources in pigs fed 20 ppm Zn. When 80 ppm Zn was used, a significant improvement in lean meat percentage was observed in pigs fed HTM compared with pigs fed ITM. In the overall study period, there was a tendency towards an increased gain:feed ratio in pigs fed 80 ppm Zn from HTM. In the last feeding phase, before slaughter gain:feed ratio and average daily gain were both significantly improved by 3.9%. In conclusion, HTM addition improved growth performance and lean meat percentage in grower-finisher pigs.

Key words: carcass characteristics, grow-finishing pigs, growth performance, hydroxychloride trace minerals, meta-analysis, sulfate trace minerals

Introduction

Trace minerals are essential in numerous biochemical processes in the body. Zinc is described to be important amongst other processes in many enzymes, collagen and keratin formation, and several immune functions (Richards et al., 2010). Copper is not only an enhancer of Zn functions, but is also involved via Cu dependent enzymes and crosslinking in collagen (Richards et al., 2010). Deficiency of either Zn or Cu may therefore have undesired consequences for the growth and health of animals. To prevent deficiencies, Zn and Cu are added to commercial diets of livestock animals at higher levels than the National Research Council (NRC) requirements (NRC, 2012). A higher dietary level of minerals may also result in a substantial

amount of excretion of minerals into the environment (Creech et al., 2004). The European Union took precautions by limiting the maximum allowed levels of Zn and Cu in animal diets. For example, in fattening pigs, maximum 120 ppm Zn and 25 ppm Cu are allowed in the complete feed (EFSA, 2014). When only these low amounts of minerals are allowed, it is utmost important to choose a good mineral source, meaning a lower potential reaction with vitamins, enzymes, and other nutrients, as well as a higher bioavailability. An example of these minerals is hydroxychloride trace minerals (**HTM**). These minerals are not soluble at a pH above 4 (Miles et al., 1998). As a result, the minerals will not react to other nutrients in the diets such as phytate in contrast to commonly used inorganic trace minerals, resulting in an increased activity of phytase (Lu et al., 2010). In addition, the same study (Lu et al., 2010) proved an increased stability of vitamin E in diets containing HTM compared with inorganic trace minerals (**ITM**). Both the minerals and other nutrients will remain available to the animals (Lu et al., 2010). The beneficial effects of HTM have already been proven in broiler chickens. Olukosi et al. (2018) describe increased growth performance of broiler chickens fed hydroxychloride Zn and Cu compared with birds fed Zn and Cu from sulfates.

It is known that in finishing pigs, addition of minerals and vitamins to the diets is essential for feed efficiency, weight gain, and feed intake (Edmonds and Arentson, 2001). An abstract published by Tsai et al. (2015) describes beneficial effects of hydroxychloride zinc, in presence of 166 ppm hydroxychloride Cu, on growth performance, and carcass quality. So far, the effects specifically for HTM on growth performance of grower-finisher pigs are not described elaborately in the literature. Therefore, a series of studies were performed to demonstrate the effects of HTM compared with ITM in the sulfate form. Due to regulatory limitations, the effects of different mineral sources are expected to be different in the European Union compared with other parts of the world. In Europe, only small amounts of minerals may be fed to grower-finisher pigs, and as a result only small differences may be expected. Taking this into account, a meta-analysis was performed, in which the results of several studies were combined to analyze the overall effect of HTM. The combination of several trials into a meta-analysis would increase the power of analysis and it was expected that this approach would better show beneficial effects of HTM on the growth performance and carcass quality in grower-finisher pigs.

Materials and Methods

All animal procedures in the different studies were approved by the local animal committees according to the standard procedures of the universities/institutes where the studies were performed.

Selection of the Trials

Selection criteria to include or exclude studies from the meta-analysis were defined. The inclusion criteria included: 1) the study must have been done in grower-finisher pigs starting from about 19-kg body weight until slaughter, the total length of the study may differ; 2) the treatments in the studies must compare HTM with inorganic trace minerals from sulfates at the same inclusion levels of the minerals; 3) the mineral levels tested must be 80 or 20 ppm Zn added for each source, in addition of 15 ppm Cu was added of the same source as Zn; 4) the raw data should be available for analysis; 5) the study must have been performed under European circumstances regarding mineral levels, diets, husbandry, and use of medication. Studies were excluded from the meta-analysis when 1) the inclusion criteria were not met; 2) if special circumstances occurred during the study resulting in a nonrepresentative result.

Study Design

All studies were done with grower/finisher pigs starting at about 9 to 10 wk of age, which was about 19 kg of body weight. In all studies, the pigs received commercial diets according to the local recommendations. The nutrient requirements met or exceeded those advised by NRC (NRC, 2012). The treatments were added to a premix without copper or zinc, and this premix was mixed well into the complete feed. All studies included 2 treatments in which 80 ppm Zn was added from either hydroxychloride Zn (IntelliBond, Trouw Nutrition, The Netherlands) or $ZnSO_4$ (various sources and origins, but all commercial, in the different studies). Besides, five of the studies included 2 additional treatments in which 20 ppm Zn was added from either hydroxychloride Zn or $ZnSO_4$. In all diets, 15 ppm Cu was added of the same source as the Zn. In all studies, the diets were fed in 3 different feeding phases. These phases differed slightly in length among the studies. All diets were analyzed for mineral contents and the final levels were close to the intended levels. The details of the studies that were included in the meta-analysis are summarized in Table 1.

Measurements

At the start of the study, and the end of each feeding phase, the body weight of the pigs was measured on pen level. The feed intake was measured at the end of each feeding phase. The body weight and feed intake were used to calculate the average daily gain, the average daily feed intake, and the feed efficiency. At the end of each study, all animals were slaughtered in local commercial slaughterhouses. The carcass yield, back fat thickness, and lean meat percentage were measured for individual pigs and averaged per pen.

Statistics

The raw data of 6 individual studies were integrated into one Excel file. The individual pens were regarded as the experimental units. The statistical analysis was performed by SAS software (SAS Institute Inc., Cary, NC). The body weight, average daily gain, average daily feed intake, and feed efficiency (gain:feed) for the overall study periods and the back fat thickness were analyzed using PROCMIXED. In our models, we included the within-study variation and between-study variation in the random and repeated statement, respectively, not assuming equal variances of treatments. For the analysis of the feeding phases, the same models were employed by only adding the feeding phase in the repeated statement via the group option. The carcass yield and lean meat percentage were analyzed via PROC GLIMMIX, using a beta distribution for continuous non-normal data. The data were only compared between different sources fed at the same Zn levels. Adjustments for multiple comparisons were made using the simulate option. Results were regarded as significant at $P < 0.05$.

Results and Discussion

Selection of Studies

In total, 6 studies were included in the meta-analysis based on the inclusion and exclusion criteria. One study was not

Table 1. Overview of studies included in the meta-analysis

Study number	Location	Country	Year	Duration	Replicates per treatment	Starting age and weight	Feeding phases	Breed
1	KU Leuven	Belgium	2016–2017	120 d	12	Age: 9.5 wk	Phase 1:	Topigs 20 sows x Pietrain
					(6 pigs/pen)	Weight: ~19 kg	days 0–36 Phase 2: days 36–71 Phase 3: days 71–120	
2	IRTA	Spain	2017	126 d	12	Age: 10 wk	Phase 1:	Pietrain x (LargeWhite x Landrace)
					(6 pigs/pen)	Weight: ~27 kg	days 0–42 Phase 2: days 42–84 Phase 3: days 84–126	
3	Universitat Autònoma de Barcelona (UAB)	Spain	2017	105 d	9	Age: 9 wk	Phase 1:	[Duroc x Landrace] x Pietrain
					(11 pigs/pen)	Weight: ~19 kg	days 0–21	
							Phase 2: days 21–63 Phase 3: days 63–105	
4	ILVO	Belgium	2017	121 d	12	Age: 10 wk	Phase 1:	Pietrain x RA-SE hybrid or Pietrain x Topigs20
					(6 pigs/pen)	Weight: ~26 kg	days 0–35 Phase 2: days 35–70 Phase 3: days 70–121	
5	University of Applied Sciences TH Bingen	Germany	2017	90 d	24	Age: 10 wk	Phase 1:	Topigs x Pietrain
					(2 pigs/pen)	Weight: ~28 kg	days 0–28 Phase 2: days 28–63 Phase 3: days 63–90	
6	University of Applied Sciences TH Bingen	Germany	2018	91 d	24	Age: 10 wk	Phase 1:	Topigs x Pietrain
					(2 pigs/pen)	Weight: ~25 kg	days 0–26 Phase 2: days 26–49 Phase 3: days 49–91	

considered in this study due to different Zn levels in the diets compared with the other studies.

Meta-Analysis of High (80 ppm) Zn Levels for the Overall Study Period

The results of the meta-analysis comparing the effects of 80 ppm Zn from HTM with ITM on growth performance for the overall study period and carcass characteristics are shown in Table 2. The gain:feed for the overall study period tended (P = 0.0925) to improve (1.3%) when 80 ppm Zn from HTM was fed to the pigs compared with 80 ppm Zn from ITM fed pigs.

In addition, the lean meat percentage significantly improved (P = 0.0009) by 1.0% in the pigs fed HTM compared with the pigs fed ITM. Although not significant, the back fat thickness showed a similar numerical difference, being lower in HTM fed pigs than in ITM fed pigs. The improved carcass characteristics are in line with Carpenter et al. (2016), who observed an improved hot carcass weight and numerically lower back fat thickness in pigs fed Zn from HTM compared ITM. The same study did not observe any additional benefits of HTM on growth performance. In contrast, comparisons of organic trace minerals (OTM) to ITM did not result in performance benefits, even if tested at different

Table 2. Results of the meta-analysis for high Zn levels[1]

	BW, kg	ADG, kg	ADF, kg	Gain:feed	Carcass yield[2], %	Back fat[2], Mm	Lean meat[3], %
ITM	107.81	0.789	1.981	0.399	74.42	11.60	61.31
HTM	107.96	0.797	1.980	0.404	74.44	11.25	61.91
Δ[4]	0.149	0.0079	−0.0014	**0.0053***	0.001	−0.350	**0.026****
95% LCL[5]	−2.059	−0.0078	−0.0463	**−0.0010***	−0.023	−0.838	**0.011****
95% UCL[6]	2.356	0.0236	0.0434	**0.0114***	0.025	0.139	**0.041****
% difference	0.14	1.0	−0.7	**1.3***	0.03	−3.0	**1.0****
P-value	0.8946	0.3180	0.9499	**0.0925***	0.9407	0.1568	**0.0009****

[1]ITM = inorganic trace minerals; HTM = hydroxychloride trace minerals; BW = body weight; ADG = average daily gain; ADF = average daily feed intake.
[2]Study number 1 was not included in the analysis of carcass yield or back fat thickness due to missing data.
[3]Study number 6 was not included in the analysis of lean meat percentage due to missing data.
[4]The difference in LS means. In case of carcass yield and lean meat percentage, this is the difference between the log-transformed LS means.
[5]LCL = lower confidence limit of the differences. In case of carcass yield and lean meat percentage, these are the 95% lower confidence limits of the difference between the log-transformed LS means.
[6]UCL = upper confidence limit of the differences. In case of carcass yield and lean meat percentage, these are the 95% upper confidence limits of the difference between the log-transformed LS means.
*Bold value indicates when the *P*-value was <0.1.
**Bold italic value indicates when *P*-value was <0.05.

Table 3. Results of the meta-analysis for high Zn levels in the different feeding phases[1]

		ADG, kg	ADF, kg	Gain:feed
Phase 1	Δ[2]	0.0020	0.0189	−0.0060
	95% LCL	−0.0232	−0.0240	−0.0196
	95% UCL	0.0272	0.0617	0.0077
	% difference	0.1	2.5	−0.3
	P-value	0.8761	0.3852	0.3859
Phase 2	Δ	−0.0063	−0.0141	0.0023
	95% LCL	−0.0268	−0.0779	−0.0086
	95% UCL	0.0136	0.0496	0.0132
	% Difference	−0.7	−0.7	0.6
	P-value	0.5148	0.6604	0.6739
Phase 3	Δ	**0.0290***	0.0121	**0.0134****
	95% LCL	**−0.0018***	−0.0670	**0.0047****
	95% UCL	**0.0598***	0.0912	**0.0221****
	% Difference	**3.9***	0.5	**3.9****
	P-value	**0.065***	0.7610	**0.0031****

[1]ADG = average daily gain; ADF = average daily feed intake; 95% LCL = 95% lower confidence limit; 95% UCL = 95% upper confidence limit.
[2]Δ is the difference between IntelliBond and sulfate.
*Bold value indicates when the *P*-value was <0.1.
**Bold italic value indicates when *P*-value was <0.05.

Zn levels (Wedekind et al., 1994; Creech et al., 2004; Burkett et al. 2009). This suggests that OTM do have a different mode of action in the animal body compared with HTM. As a result, all information known about the mechanisms of OTM cannot be used to describe the mechanisms of HTM, so more research is needed specifically by focusing on HTM.

Meta-Analysis of High (80 ppm) Zn Levels Per Feeding Phase

Table 3 shows the results of the meta-analysis comparing the effects of 80 ppm from HTM with ITM per feeding phase. During the first 2 feeding phases, no significant differences were observed. The main differences were observed during the last feeding phase before slaughter. In this last feeding phase, a significant improvement (*P* = 0.0031) of 3.9% in gain:feed was observed. Furthermore, the average daily gain also showed a tendency (*P* = 0.065) towards a similar improvement of 3.9%. From

the current results, it is unclear whether the same effects of HTM may be expected when only fed during the last feeding phase or when feeding HTM is already started in the nursery phase.

It is likely that the improved growth performance in the last feeding phase before slaughter is related to the increased lean meat percentage observed in the carcass characteristics of HTM fed pigs. In a pig's life, the body composition changes, where the protein percentage of the body increases until a pig reaches about 73 kg; thereafter, the protein percentage decreases while the fat percentage increases (Shields et al., 1983). In the current study, the average body weight at the end of feeding phase 2 was 78.4 kg. According to the study of Shields et al. (1983), this is the point where less protein and more fat are deposited. HTM seems to influence the ratio of protein to fat deposition, especially in this last phase before slaughter.

Another important aspect of HTM is the stability in feed. It has been proven that the vitamin E stability in feed is improved in diets included with Cu from HTM (Luo et al., 2005; Lu et al., 2010), and that similar effects may be expected from Zn from HTM. Without any vitamins or trace minerals added to the diet, mainly the vitamin E content of muscles decreases (Edmonds and Arentson, 2001), indicating that vitamin E is important in meat production. Although a clear relation between dietary vitamin E and performance or carcass yield was not yet demonstrated, vitamin E did have some effects on meat quality of pigs fed high fat diets (Guo et al., 2006). Vitamin E was described to protect pork meat from oxidation and to change the fatty acid profile in meat (Guo et al., 2006). The meat quality was not measured in the studies included in the present meta-analysis and should be studied in more detail in relation to feeding HTM. Especially when taking into account that Cu from HTM has also been shown to decrease oxidative stress in nursery pigs (Fry et al., 2012), HTM may have some direct or indirect, via vitamin E stability in feed, effects on oxidative stress and meat quality. This should be the focus of future research to elucidate the mode of action behind increased lean meat percentage when feeding HTM, and its relationship with the age of the pigs.

Meta-Analysis of Low (20 ppm) Zn Levels for the Overall Study Periods

The results of the comparison between 20 ppm Zn from HTM and from ITM are shown in Table 4. The results show that there were no significant differences between the mineral sources at these

Table 4. Results of the meta-analysis for low Zn levels[1]

	BW, kg	ADG, kg	ADF, kg	Gain:feed	Carcass yield[2], %	Back fat[2], mm	Lean meat[3], %
ITM	107.49	0.769	1.934	0.400	74.34	11.96	62.12
HTM	107.84	0.762	1.913	0.399	74.32	11.61	62.28
Δ[4]	0.346	−0.0063	−0.0215	−0.0010	−0.020[4]	−0.354	0.160[4]
95% LCL[5]	−2.530	−0.0234	−0.0666	−0.0074	−0.025	−0.823	−0.015
95% UCL[6]	3.222	0.0107	0.0236	0.0055	0.022	0.115	0.029
% Difference	0.3	−0.8	−1.1	−0.3	−0.03	−3.0	0.3
P-value	0.8118	0.4630	0.3445	0.7657	0.9170	0.1360	0.5538

[1]ITM = inorganic trace minerals; HTM = hydroxychloride trace minerals; BW = body weight; ADG = average daily gain; ADF = average daily feed intake; FCR = feed conversion ratio; FE = feed efficiency.
[2]Study number 1 was not included in the analysis of carcass yield or back fat thickness due to missing data
[3]Study number 6 was not included in the analysis of lean meat percentage due to missing data.
[4]The difference in LS means. In case of carcass yield and lean meat percentage, this is the difference between the log-transformed LS means.
[5]LCL = lower confidence limit of the differences. In case of carcass yield and lean meat percentage, these are the 95% lower confidence limits of the difference between the log-transformed LS means.
[6]UCL = upper confidence limit of the differences. In case of carcass yield and lean meat percentage, these are the 95% upper confidence limits of the difference between the log-transformed LS means.

lower Zn levels. No differences were observed for both the growth performance and the carcass characteristics. Because of the lack of any response for the low Zn levels, no separate analysis was done for the different feeding phases. The lack of response at low Zn levels in contrast to high Zn levels suggests that ITM may have negative effects at higher levels rather than HTM in relation to the bioavailability and function of Zn. At lower dietary Zn levels, the negative impact of ITM sources may not be as pronounced as at higher levels. At higher levels, more negative impact from ITM may be expected, whereas HTM would not cause negative effects. Consequently, more nutrients and energy could become available for growth performance and carcass development.

The results observed when feeding high Zn levels were not compared with the results when feeding low Zn levels, since this was beyond the scope of this study. Burkett et al. (2009) concluded that lower levels of OTM resulted in less Zn excretion without negatively affecting growth performance or carcass quality compared with commercial levels of ITM. Similar conclusions were drawn by Creech et al. (2004). The latter study also showed a benefit of reduced OTM compared with the reduced ITM. Although OTM may have different mechanisms from HTM, for environmental reasons lower Zn levels may be considered.

Conclusions

Combining 6 studies into one meta-analysis showed a significant improvement in lean meat percentage in grower-finisher pigs fed 80 ppm Zn from HTM compared with 80 ppm Zn from ITM. In addition, in the overall study period, a tendency towards an improved gain:feed was observed in pigs fed 80 ppm Zn from HTM. This improvement was mainly due to a 3.9% improvement in both ADG and gain:feed during the last phase before slaughter in HTM fed pigs. The effects of mineral source were less pronounced when 20 ppm Zn was fed. The current research clearly demonstrated the benefits of HTM on growth performance, mainly in the last phase, and carcass characteristics. Future research should focus on the mechanisms behind the growth performance and carcass yield improvements in pigs fed HTM, and the effect of age on the mode of action of HTM.

Acknowledgments

Conflict of Interest

The individual studies were performed by the researchers/ institutes mentioned in Table 1 and the acknowledgments. The researchers mentioned in the acknowledgments were not involved in the determination of the selection criteria, the selection of the studies nor the execution, interpretation, and writing of the meta-analysis. This was done by the authors of this publication. The authors declare no conflict of interest.

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