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**Επίδραση της προσθήκης χηλικών ιχνοστοιχείων στα σιτηρέσια και την αναπαραγωγική ικανότητα των χοιρομητέρων**

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## Effect of sow diets supplementation with chelated trace minerals on their reproductive performance

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## Επίδραση της προσθήκης χηλικών ιχνοστοιχείων στα σιτηρέσια και την αναπαραγωγική ικανότητα των χοιρομητέρων

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**ABSTRACT.** Trace minerals are constituents of proteins and enzymes that are involved in a variety of metabolic processes, having functional implications in growth, development, reproduction and health. Chelated minerals, are minerals bound to organic ligands, usually amino acids, providing higher levels of bioavailability compared to conventional inorganic mineral sources. In the present study, we investigated the effect of partial substitution of inorganic mineral sources with chelated minerals in sows' diets, on three important reproductive parameters, the number of liveborn and weaned piglets and the wean-to-first service interval, in three Greek farrow-to-finish herds. Before initiation of the study the sows were on diets supplemented with 15 mg/kg feed Cu (from CuSO<sub>4</sub>), 125 mg/kg Zn (from ZnO) and 40 mg/kg Mn (from MnO). After exiting the farrowing facilities they were offered diets in which 93.3%, 36.0% and 62.5% of the supplemented Cu, Zn and Mn, respectively, originated from commercially available chelated minerals. For the last farrowing on inorganic minerals diet and the subsequent one or two (for 35.0% and 65.0% of the studied sows, respectively) on chelated minerals, each sow's reproductive data (total number of parities, number of liveborn and weaned piglets and wean-to-first service interval) were recorded. The reproductive parameters, before and after the supplementation with chelated minerals, were compared in two mixed-effect linear regression models, for the number of liveborn and weaned piglets, and in a zero inflated negative binomial model for the weaning-to-first service interval. There was an improvement of the mean number of liveborn piglets after partial substitution of inorganic source of minerals with chelated minerals by almost half a piglet ( $P=0.015$ ), whereas there was no effect on the total number of weaned piglets ( $P=0.15$ ) and the wean-to-first service interval ( $P=0.65$ ). The increase in the number of liveborn piglets may be directly attributed to improved embryo survival due to increased bioavailability of the organic minerals or indirectly to the beneficial effect of chelated minerals on sows' hoof health, locomotor ability, feed intake and body condition.

**Keywords:** sows, chelated or organic minerals, reproductive performance

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**ΠΕΡΙΛΗΨΗ.** Τα ιχνοστοιχεία αποτελούν συστατικά πρωτεϊνών και ενζύμων που εμπλέκονται σε μια ποικιλία μεταβολικών διεργασιών, συμμετέχοντας λειτουργικά στην ανάπτυξη, την αναπαραγωγή και την υγεία του ζώου. Τα οργανικά ή χηλικά ιχνοστοιχεία είναι μέταλλα συνδεδεμένα με οργανικούς φορείς, συνήθως αμινοξέα, που σε σύγκριση με τα ανόργανα άλατα των μετάλλων έχουν μεγαλύτερη βιο-διαθεσιμότητα. Στην παρούσα μελέτη διερευνήσαμε την επίδραση της αντικατάστασης, στα σιτηρέσια χοιρομητέρων, μέρους των ανόργανων με χηλικά ιχνοστοιχεία σε τρεις από τους σημαντικότερους δείκτες αναπαραγωγικής απόδοσης, τον αριθμό των γεννηθέντων ζωντανών και απογαλακτισθέντων χοιριδίων και το μεσοδιάστημα απογαλακτισμού μέχρι την πρώτη σπερματέγχυση, σε τρεις Ελληνικές χοιροτροφικές εκμεταλλεύσεις. Πριν την έναρξη της μελέτης τα σιτηρέσια των χοιρομητέρων περιείχαν ανά κιλό τροφής 15mg Cu (από CuSO<sub>4</sub>), 125mg Zn (από ZnO) και 40mg Mn (από MnO). Μετά την έξοδο από τους θαλάμους τοκετού στα σιτηρέσια των χοιρομητέρων το 93,3%, 36,0% και 62,5% του προστιθέμενου Cu, Zn και Mn, αντίστοιχα, να προέρχεται από εμπορικά διαθέσιμες πηγές χηλικών ιχνοστοιχείων. Καταγράφηκαν τα αναπαραγωγικά δεδομένα που αφορούσαν το συνολικό αριθμό τοκετών, τον αριθμό των γεννηθέντων ζωντανών και των απογαλακτισθέντων χοιριδίων και το μεσοδιάστημα απογαλακτισμού μέχρι την πρώτη σπερματέγχυση σε κάθε μία από 446 χοιρομητέρες, τόσο κατά τον τελευταίο τοκετό με διατροφή που περιείχε ιχνοστοιχεία ανόργανης προέλευσης όσο και για τους επόμενους έναν ή δύο (αντίστοιχα στο 35,0% και 65,0% του συνόλου των χοιρομητέρων) τοκετούς μετά την προσθήκη των χηλικών ιχνοστοιχείων. Για τη σύγκριση του αριθμού των γεννηθέντων ζωντανών και απογαλακτισθέντων χοιριδίων, πριν και μετά την προσθήκη των χηλικών ιχνοστοιχείων, χρησιμοποιήσαμε δύο μικτά γραμμικά μοντέλα παλινδρόμησης, ενώ η διερεύνηση του αντίστοιχου συσχετισμού για το μεσοδιάστημα απογαλακτισμού μέχρι την πρώτη σπερματέγχυση έγινε με τη χρήση ενός αρνητικού διωνυμικού μοντέλου μηδενικού-πληθωρισμού. Βρήκαμε αύξηση ( $P=0.015$ ) του μέσου όρου των γεννηθέντων ζωντανών χοιριδίων, μετά την προσθήκη των χηλικών ιχνοστοιχείων κατά μισό περίπου χοιρίδιο ανά τοκετό, ενώ αντίθετα δεν υπήρξε καμία επίδραση στον αριθμό των απογαλακτισθέντων χοιριδίων ( $P=0.15$ ) καθώς και στο μεσοδιάστημα απογαλακτισμού μέχρι την πρώτη σπερματέγχυση ( $P=0.65$ ). Η μερική αντικατάσταση των ανόργανης προέλευσης με χηλικά ιχνοστοιχεία αυξάνει τον αριθμό των γεννηθέντων ζωντανών χοιριδίων, πιθανότατα μέσω της βελτίωσης της βιωσιμότητας των εμβρύων. Ωστόσο, είναι πιθανή και η έμμεση επίδραση των χηλικών ιχνοστοιχείων λόγω της βελτίωσης της υγείας των χηλών και της κινητικής ικανότητας των χοιρομητέρων καθώς και της επακόλουθης βελτίωσης της διατροφικής και σωματικής τους κατάστασης.

**Λέξεις κλειδιά:** χοιρομητέρες, χηλικά ιχνοστοιχεία, αναπαραγωγικές αποδόσεις

## INTRODUCTION

The minimum levels of trace minerals required to overcome deficiency syndromes and not necessarily to promote optimum productivity or enhance immunity are listed in the National Research Council (NRC) review (1998). In a survey of the allowances commonly provided in diets in several European countries (Whittemore et al., 2002) NRC minimum levels were usually exceeded by the industry in order to achieve optimal animal performance. For hyperprolific modern sows the consequences of an inadequate supply of dietary minerals have been reported on by Mahan and Newton (1995). They showed that, when supplied according to NRC standards, the body mineral reserves of third parity sows were lower in sows with high than low mean litter weight at 21-days. Furthermore, for either of the latter groups of sows the mineral reserves were lower, by as much as 20%, than those of unbred control animals of similar age. To provide the trace minerals, customarily, inorganic salts such as sulphates (e.g. copper sulphate) and oxides (e.g. zinc oxide) are added to the diet. In the digestive tract these salts are broken down to form free ions which are then absorbed by the animal only when they are not complexed with other dietary molecules such as phytate. Therefore, not only the availability of trace minerals to the animal may vary considerably but

also significant percentage of the unabsorbed minerals may be fecally excreted and cause environmental pollution (Close, 2003). To limit harm to the environment especially in pig-dense areas the Regulation (EC) No 1334/2003 proposed maximum inclusion levels of minerals which may not be sufficient to meet the needs of modern sows with high productivity potential. Bio-availability is normally defined as the degree to which an ingested nutrient in a particular source is absorbed in form that can be utilized by the animal. In the organic or chelated mineral sources, the minerals are bound to organic ligands, usually comprising a mixture of amino acids. Consequently mineral chelates can utilize amino acid uptake mechanisms in the intestine (Ashmead, 1993; Power and Horgan, 2000).

Minerals in sows are essential for growth and integrity of the skeleton and hooves (van Riet et al., 2013), for growth of fetuses and for mammary secretions (Mahan, 1990). Therefore, for EU countries where there are limits to maximum inclusion levels of minerals, increasing their bioavailability may improve the reproductive performance of the modern hyperprolific sows. Yet, studies documenting the possible beneficial effect on reproductive performance of substitution of inorganic with organic minerals in sow diets are sparse. Miranda et al. (1993) reported that 25.0% substitution of inorganic mineral sources with chelated mineral sources improved

overall sows' reproductive performance because more sows on the chelated diet became pregnant and had more live and less dead fetuses than the sows fed the inorganic diet. Similarly, in a study by Zhao et al. (2012) sows on diets with 50.0% substitution of inorganic sources of Cu, Zn and Mn with chelated sources had more total piglets born, more piglets born alive and more stillborn piglets than sows on diets with only inorganic minerals. However, in this study the former where on a different farm than the latter animals and the data analyses failed to control for the distribution of likely confounders such as sow age, genetics and other management factors.

Therefore the aim of this study, which was conducted on three Greek farrow-to-finish farms, was to evaluate the effect of partial supplementation of in-organic mineral sources with chelated mineral sources, on three of the most important parameters for sow reproduction performance, the number of liveborn and weaned piglets and the wean-to-first service interval.

## MATERIALS AND METHODS

The studied herds with farrow-to-finish with 350 (A), 190 (B) and 900 (C) sows, respectively, with different genetics coded as 1 (A, B) and 2 (C). The study population comprised 446 sows, 120 of which were in A, 112 in B, and 214 in C. During gestation they were loose housed in static groups of 8-12 on combinations of concrete and slatted flooring. All herds operated on weekly farrowing schedules. For participation in the study the only criterion was the owners' written consent. The authors declare that the study was conducted in farms which complied with the current laws concerning the protection of animals kept for farming in the European Union (European Council Directive 98/58/EC).

Before initiation of the study the sows were on diets supplemented with 15 mg/kg feed Cu (from  $\text{CuSO}_4$ ), 125 mg/kg Zn (from ZnO) and 40 mg/kg Mn (from MnO). After exiting the farrowing facilities they were offered diets in which 93.3%, 36% and 62.5% of the supplemented Cu, Zn and Mn, respectively, were from commercially available chelated mineral sources. During gestation the sows were automatically fed a total of 2.6-2.8 kg dry matter (DM) per sow daily of typical dry sow diets containing 12.7 MJ metabolizable energy (ME) per kg DM given either in one meal at 07:00 h (B and C) or split in half and offered in two meals at 07:00 and 16:00 h (A). Feed allowance was increased to 3.5 kg DM per sow daily after 90 days-in-pig until one week before the expected farrowing. One week before the expected farrowing, sows were transferred to the farrowing rooms. In the lactation facilities, they were restricted fed until 5 days after farrowing

and then were offered ad libitum typical lactation diets containing 13.5 MJ ME/kg DM. For the last farrowing on inorganic minerals and the subsequent two on chelated minerals we retrieved each sow's data, concerning the total number of parities (sow age), the number of liveborn and weaned piglets and the wean-to-first service interval from each farm's records. Therefore, for each sow the database included the relevant information twice, prior to and after the nutritional intervention. For almost one third of the sows, recordings were for the first farrowing after the nutritional intervention, because they were not on farm for the second farrowing, whereas for the remaining sows recordings concerned the second farrowing after supplementation with chelated minerals.

## STATISTICAL ANALYSIS

All statistical analyses were performed using Stata 13.1 (Stata Statistical Software. College Station, TX) and interpreted for significance at the 5% level.

In order to compare the number of liveborn and weaned piglets before and after supplementation with chelated minerals, two mixed-effect linear regression models were fitted. The distribution of the observed wean-to-first service interval was over-dispersed (Lord et al. 2007), because most sows were inseminated within five days after weaning. Therefore, data were initially recoded so that intervals up to five days, representing sows with a normal weaning-to-first service interval, were designated with zero. Intervals of more than five days were transformed into  $k-5$ , where  $k$  was the actual number of days from weaning-to-first service. Then, we fitted a zero inflated negative binomial (ZINB) model (Carrel et al., 2010) to investigate the possible association between the wean-to-first service interval and diet supplementation with chelated minerals. The ZINB model generates two separate models and then combines them; first a logit model comparing sows which were with those which were not in-heat within five days after weaning and second a negative binomial model comparing sows which exhibited estrus after more than five days from weaning (Long and Freese, 2006).

In each of the above models the reproductive parameter was the dependent variable whereas the dietary status (before or after supplementation with chelated minerals), the sow's parity and the farm of sow's origin were the independent variables. The latter two variables were forced in the models in order to account for their likely confounding effects. Furthermore, in the mixed-effect linear models a random-effect term for sow was included in order to account for repeated measurements on the same animal. In the ZINB model,



standard errors were adjusted to account for intra-sow correlation of repeated observations with the use of vce (cluster) option.

## RESULTS

Of the 446 studied sows, 292/446 (65.4%) were followed-up for two whereas the remaining 154/446 (34.5%) for one farrowing. The proportion of sows studied for one gestation was 38.4%, 31.8% and 33.3%, respectively for herds A, B and C. The mean number of liveborn and weaned piglets, the median weaning to first service interval and the sow parity before and after the partially supplemented with chelated minerals diets, by herd, are in Table 1. Since weaning was the time when farm managers decided whether a sow will be re-served or culled, the wean-to-first service interval was available for 310 sows (79, 69 and 162 sows in herds A, B and C, respectively).

There was an improvement of the mean number of liveborn piglets after partial substitution of inorganic source of minerals with chelated minerals (Table 2). Specifically, after sows were fed with chelated minerals, they farrowed 0.54 more ( $P=0.015$ ) liveborn piglets. For example, based on the fitted model, a third-parity sow in herd A is expected to farrow 15.2 (CI: 13.4, 16.4) liveborn piglets after chelated mineral nutrition, whereas the same sow was expected to farrow 14.3 (CI: 13.3, 15.4) liveborn piglets after inorganic only mineral nutrition. Chelated trace mineral nutrition affected neither the total number of weaned piglets ( $P=0.15$ ) nor the weaning-to-first service interval, both in the logit ( $P=0.07$ ) and the negative binomial ( $P=0.65$ ) part of the ZINB model.

## DISCUSSION

Trace minerals are constituents of proteins and enzymes that are involved in a variety of metabolic functions. They have functional implications in growth and development, reproduction and health. Sows' mineral reserves decline over several reproductive cycles and depletion is exacerbated for hyper-prolific sows which are able to support larger litter growth rates (Mahan and Newton, 1995). Mineral absorption increases during lactation in response to the high nutrient demand for milk production, but when dietary mineral intake is insufficient, the sow will mobilize her body mineral reserves, particularly from the liver to meet the demand (Mahan and Newton, 1995). An inadequate mineral intake may affect hormonal secretion, enzyme activity, muscle function, bone mineral content, and other body mineral functions (Peters and Mahan, 2008). Chelated minerals are more bio-available than inorganic minerals because they are protected from physiochemical factors or from negative interactions with dietary components, as phytate, which binds cations making them unavailable for absorption (Fairweather-Tait, 1996). Therefore, partial substitution of inorganic with chelated mineral sources in sow rations may improve mineral uptake of modern sows.

We found that nutrition with chelated Cu, Zn and Mn improved the total number of liveborn piglets. Peters and Mahan (2008) and Mirando et al. (1993) also reported that substitution of inorganic with organic mineral sources improved the number of total born piglets. Partial replacement of inorganic with organically-bound Cu, Mn, and Zn resulted in higher conception rates, more live fetuses and fewer dead embryos at 30days post-coitum (Mirando et al., 1993). In this study, the

**Table 1.** Mean number of liveborn and weaned piglets and median (range) of weaning-to-first service interval, by herd, before and after one or two farrowings on gestation and lactation diets partially supplemented with chelated Cu, Zn and Mn, of 446 sows in three Greek herds.

Reproductive parameters	Herd A (120 sows)		Herd B (112 sows)		Herd C (214 sows)	
	Before	After	Before	After	Before	After
Mean number of live-born piglets*	13.4 <sup>a</sup>	14.8 <sup>b</sup>	14.1 <sup>a</sup>	14.7 <sup>b</sup>	11 <sup>a</sup>	12.3 <sup>b</sup>
Mean number of weaned piglets*	12.4 <sup>a</sup>	12.9 <sup>a</sup>	11.4 <sup>a</sup>	11.9 <sup>a</sup>	10.2 <sup>a</sup>	10.5 <sup>a</sup>
Median (range) of wean-to-first service interval**	6 (5-47) <sup>a</sup>	6 (4-78) <sup>a</sup>	5(4-20) <sup>a</sup>	5(3-37) <sup>a</sup>	5(4-76) <sup>a</sup>	5(1-68) <sup>a</sup>

\*comparisons of both the mean numbers of liveborn and weaned piglets, before and after the partial supplementation of sows' diets with chelated minerals, were performed in mixed-effect linear regression models, fitted for each herd separately, adjusting for sows' age and repeated measurements on each sow.

\*\*comparison of the wean-to-first service interval, before and after the partial supplementation of sows' diet with chelated minerals, was performed in a zero-inflated negative binomial model, fitted for each herd separately, adjusting for sows' age. Data for this parameter were available for 79, 69 and 162 sows in herds A, B and C, respectively.

a,b different within-herd superscripts in the rows suggest statistically significant difference ( $P<0.05$ ) in the considered parameter before and after the partial supplementation of sows' diet with chelated minerals.

number of corpora lutea was similar between diets, but the number of live embryos was greater when organic trace minerals were fed, suggesting that organic minerals improved embryo survival. Additionally, gilts fed with Cu, Mn, and Zn proteinates had higher concentration levels of these minerals in the conceptus products than in the surrounding endometrial tissues and ovaries between 12 and 30 days post-coitum (Hostetler et al., 2000); indicating an increased uptake and/or improved utilization of trace minerals by the embryo and fetus during early pregnancy.

The fact that there was difference neither in the number of weaned piglets nor in the wean-to-rebreed interval between the two mineral source diets, could probably reflect the absence of any comparative advantage of the organic over the inorganic mineral source in the lactation performance (Mirando et al. 1993), the lactation feed intake and milk fat content (Peters and Mahan, 2008). Others also found that estrus-rebreeding intervals (Peters and Mahan, 2008) or pregnancy and ovulation rates (Hostetler and Mirando, 1998) were similar between different trace mineral source diets.

Chelated mineral nutrition has a beneficial effect on sows' locomotor system development (van Riet et al., 2013) and on reduction of hoof lesions (Anil 2010). Particularly Zn, Cu and Mn have been identified as instrumental minerals in the processes of hoof keratinization (Smart and Cymbaluk, 1997; Mülling et al., 1999; Mülling, 2000), through the activation of enzymes with catalytic, structural and regulatory role (Cousins, 1996; Van Riet et al., 2013). Previous studies reported the impact of hoof lesions on sows' longevity, lameness (Anil et al. 2007; Anil et al. 2009) and increased risk of culling (Engblom et al. 2008). Furthermore, heel lesions

and wall cracks have been associated with various sow reproductive performance parameters, such as decreased litter weight, increased pre-weaning piglet mortality and higher odds of stillborn piglets (Fitzgerald et al. 2012; Pluym et al. 2013). Hoof lesions occur more frequently and severely in older sows (Dewey et al. 1993; Pluym et al. 2011; Fitzgerald et al. 2012), suggesting a time-related pattern of occurrence and likely a cumulative effect over time on the animal's reproductive performance. Once a lesion is created the most probable outcome is its deterioration over time, thus justifying an overall negative impact on sows' reproductive performance and longevity (Fitzgerald et al. 2012; Pluym et al. 2013). In addition, hoof lesions may influence the postural behavior of the sow, with affected sows exhibiting higher relative frequency of lying and lower of standing posture (Enokida et al. 2011), spending accordingly less time feeding and drinking. Reduced feed consumption as a consequence of hoof lesions could be either or both the result of an existing inflammatory process or an impaired locomotor ability. Thus, improving hoof health with the use of chelated trace minerals could enhance sows' ability to move and feed properly, withstand the highly demanding period of lactation and manifest their genetic potential, leading to improved productivity and longevity.

## CONCLUDING REMARKS

In conclusion our results indicate that partial replacement of inorganic trace minerals with organic sources may improve the number of liveborn piglets but not the number of weaned piglets or the weaning-to-rebreeding interval. This effect may be ascribed to improved embryo survival due to increased uptake and/or enhanced utilization of chelated trace minerals during early pregnancy. However, the beneficial effect of organic trace minerals may also be partially attributed to an improvement of sows' hoof health and locomotor ability, providing optimal feed intake and body condition, which are decisive for the subsequent litter size.

## CONFLICT OF INTEREST STATEMENT

There are no conflicts of interests professionally or financially with this manuscript that the co-authors are aware of.

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**Table 2.** The mean difference in the number of liveborn piglets farrowed from sows which were on diets where inorganic sources of Cu, Zn and Mn were partially substituted with chelated sources compared to the same sows being on diets with inorganic only sources of those minerals (Diet), adjusted for the effect of herd of sow origin (Farm) and of sows' age (Parity) in a linear mixed-effect model with sow as random-effect term.

Number of liveborn piglets		
Covariates	Coefficient (CI)	P-value
Diet	0.54 (0.10, 0.98)	0.015
Parity	0.18 (0.06, 0.30)	0.003
Farm	-1.13 (-1.3, -0.95)	<0.001
Intercept	14.91 (14.39, 15.43)	<0.001

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