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## Effects of attapulgitte dietary supplementation on sow performance in two commercial farms in Greece

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## Μελέτη της επίδρασης της προσθήκης ατταπουλγίτη στα σιτηρέσια χοιρομητέρων, στις αποδόσεις τους, σε δυο εμπορικές εκτροφές στην Ελλάδα

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**ABSTRACT.** The present study investigated the effects of attapulgitte supplementation in sow diets during gestation and lactation on sow performance. The study comprised two reproductive phases (cycles) in two commercial farrow to finish farms: Farm A (capacity: 550 sows) and Farm B (capacity: 220 sows). The treatment groups were: a) control group (CN): the sows were fed a common gestation or lactation diet; b) attapulgitte group (AT): the sows were fed the CN diet supplemented with attapulgitte at 0,7% level; c) attapulgitte plus group (AT+): the sows were fed the CN diet supplemented with attapulgitte (0.7%) and a mix of enzymes, live yeast and amino acids (0.1%), at a total of 0.8% level. Within each cycle the sows included per treatment were: 24 for Farm A; 12 for Farm B. Initially data were analyzed per cycle and per each farm. Data from sows that completed both cycles within each farm, were analyzed by repeated

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measures analysis. Regarding sow parameters, sow body weight loss during lactation tended to be greater in AT sows compared to CN sows during cycle 1 in Farm B and was greater in AT and AT+ than CN sows in Farm A that completed both cycles ( $P=0.063$  and  $P=0.023$ , respectively). A greater litter size 24h postpartum was observed in favour of AT compared to CN group during cycle 1 in Farm A and in sows that completed both cycles in Farm A ( $P=0.001$  and  $P=0.011$ , respectively). Litter size at weaning was greater in sows from the AT group than CN during cycle 1 and 2 in Farm A, in cycle 1 in Farm B and in sows that completed both cycles in Farm A ( $P=0.004$ ,  $P=0.037$ ,  $P=0.037$ , and  $P=0.022$ , respectively). Piglet weight at weaning and average daily gain during lactation were greater in AT group than CN and AT+ in sows that completed both cycles in Farm A ( $P=0.049$  and  $P=0.040$  respectively). Notable similar effects, although not statistically significant, were also observed in Farm B. This field study suggests that attapulgite supplementation in sow diets can improve performance indexes. Further research should investigate the underlying mechanisms involved.

**Keywords:** Sows, Attapulgite (Palygorskite), Reproduction Performance

**ΠΕΡΙΛΗΨΗ.** Στόχος της παρούσας μελέτης ήταν η διερεύνηση της επίδρασης της προσθήκης ατταπουλγίτη στα σιτηρέσια των χοιρομητέρων, στις αποδόσεις τους κατά τη διάρκεια της κνοφορίας και της γαλουχίας. Η μελέτη συμπεριέλαβε δύο συνεχείς αναπαραγωγικούς κύκλους σε δύο εμπορικές εκτροφές: Εκτροφή Α (δυναμικότητας 550 χοιρομητέρων), Εκτροφή Β (δυναμικότητας 220 χοιρομητέρων). Οι πειραματικές ομάδες ήταν: α) ομάδα μάρτυρας (CN): οι χοιρομητέρες κατανάλωναν το σύνθηες σιτηρέσιο κνοφορίας και γαλουχίας, β) ομάδα ατταπουλγίτη (AT): οι χοιρομητέρες κατανάλωναν το σιτηρέσιο της ομάδας CN με επιπλέον 0,7% προϊόντος ατταπουλγίτη και γ) ομάδα σύνθητου ατταπουλγίτη (AT+): οι χοιρομητέρες κατανάλωναν το σιτηρέσιο της ομάδας CN με επιπλέον 0,7% ατταπουλγίτη και ενός μίγματος αποτελούμενου από ένζυμα, ζύμες και αμινοξέα (0,1%), σε σύνολο προσθήκης το 0,8%. Ο αριθμός των χοιρομητέρων σε κάθε πειραματική ομάδα και ανά κύκλο ήταν: 24 για την Εκτροφή Α, 12 για την Εκτροφή Β. Αρχικά, τα πειραματικά δεδομένα επεξεργάστηκαν στατιστικά για κάθε κύκλο και για κάθε εκτροφή χωριστά. Στη συνέχεια, η ανάλυση δεδομένων των χοιρομητέρων που ολοκλήρωσαν και τους 2 κύκλους στις 2 εκτροφές έγινε με ανάλυση διακύμανσης με επαναλαμβανόμενες μετρήσεις. Η απώλεια σωματικού βάρους των χοιρομητέρων κατά τη διάρκεια της γαλουχίας, ήταν οριακά υψηλότερη στην ομάδα AT σε σχέση με την ομάδα CN κατά τη διάρκεια του πρώτου αναπαραγωγικού κύκλου στην Εκτροφή Β και υψηλότερη στις ομάδες AT και AT+ σε σχέση με την ομάδα CN στις χοιρομητέρες που ολοκλήρωσαν και τους 2 αναπαραγωγικούς κύκλους στην Εκτροφή Α ( $P=0.063$  και  $P=0.023$ , αντίστοιχα). Το μέγεθος της τοκετοομάδας 24 ώρες μετά τον τοκετό ήταν υψηλότερο στην ομάδα AT σε σχέση με την ομάδα CN κατά τον πρώτο κύκλο στην Εκτροφή Α καθώς και στις χοιρομητέρες που ολοκλήρωσαν τους 2 κύκλους στην Εκτροφή Α ( $P=0.001$  και  $P=0.011$ , αντίστοιχα). Το μέγεθος της τοκετοομάδας στον απογαλακτισμό ήταν υψηλότερο στην ομάδα AT σε σχέση με την ομάδα CN στον πρώτο και δεύτερο κύκλο στην Εκτροφή Α, στον πρώτο κύκλο στην Εκτροφή Β και στις χοιρομητέρες που ολοκλήρωσαν τους 2 αναπαραγωγικούς κύκλους στην Εκτροφή Α ( $P=0.004$ ,  $P=0.037$ ,  $P=0.037$  και  $P=0.022$ , αντίστοιχα). Το βάρος των χοιριδίων στον απογαλακτισμό και η μέση ημερήσια αύξηση των χοιριδίων κατά τη διάρκεια της γαλουχίας ήταν υψηλότερα στην ομάδα AT σε σχέση με τις ομάδες CN και AT+ καθώς και στις χοιρομητέρες που ολοκλήρωσαν τους 2 κύκλους στην Εκτροφή Α ( $P=0.049$  και  $P=0.040$ , αντίστοιχα). Αξίζει να σημειωθεί ότι παρόμοια αποτελέσματα παρατηρήθηκαν και για την Εκτροφή Β, χωρίς όμως αυτά να είναι στατιστικά σημαντικά. Η παρούσα μελέτη, που διενεργήθηκε σε επίπεδο εκτροφής, έδειξε πως η χορήγηση του ατταπουλγίτη στα σιτηρέσια χοιρομητέρων είναι δυνατό να βελτιώσει τους αναπαραγωγικούς δείκτες. Ωστόσο, κρίνεται αναγκαία περαιτέρω έρευνα για τη διερεύνηση των μηχανισμών που σχετίζονται με τις ευεργετικές επιδράσεις του ατταπουλγίτη στις χοιρομητέρες.

**Λέξεις ευρητηρίας:** Χοιρομητέρες, Ατταπουλγίτης (Παλυγορσκήτης), Αναπαραγωγική Ικανότητα

## INTRODUCTION

It is well established that sows during lactation enter a catabolic state, in which feed intake is often not sufficient to fulfill the energy demands for maintenance and milk production (Eissen et al., 2003). To achieve sufficient milk production and support the growth of the progeny, sows catabolize their body reserves, as an energy source for milk (Noblet et al., 1998). However, extreme body weight losses during lactation, can negatively influence farrowing rate and litter size in subsequent parity (Prunier et al., 2003; Schenkel et al., 2010). Thus, it is essential to improve nutrient utilization in sows during gestation and lactation by nutritional means.

Clays represent a category of supplements that have been applied in pig nutrition. Clays are naturally occurring materials, having as basic constituent fine-grained minerals with specific structures of porous aluminosilicate-layers (Guggenheim and Martin, 1995; Papaioannou et al., 2005; Williams et al., 2009). Due to their absorptive capacity, supplementation of clays (e.g. smectite, kaolinite, clinoptilolite) in weaned pig diets ameliorated diarrhea incidence after experimental challenge with enterotoxigenic *E. coli* (Song et al., 2012). In particular, attapulgite (also referred as palygorskite) is a clay mineral that belongs to the group of hormites and is characterized by its elongated shape (Murray, 2000), in an arrangement of blocks separated by parallel channels composed by two layers of SiO<sub>2</sub> tetrahedral enclosing a layer of MgO octahedral (Alvarez et al., 2011). This shape is responsible for its high absorption capacity (Murray, 2000). A thermal or acid treatment enhances its sorptive properties and surface area (Alvarez et al., 2011). Dietary supplementation with 2000 mg/kg palygorskite improved growth performance and reduced the incidence of diarrhea in weaned piglets (Zhang et al., 2013). In lactating cows, palygorskite supplementation at levels up to 10 kg/t, improved cow milk yield, increased milk protein yield and decreased milk colony forming units (Bampidis et al., 2014). The latter authors attributed partly the positive effects of attapulgite in cows to the toxin binding effect of the material. However, the effects of attapulgite on a lactating animal could not only be attributed to the toxin binding effect, but also to plausible improvement of nutrient utilization. For example, a sparing effect of energy and nutrients for a better farrowing and lactation performance was

proposed as a plausible mechanism after clinoptilolite supplementation in sows (Kyriakis et al., 2002). It should be noted that the information of the dietary inclusion of attapulgite in sows is scarce. Hence, the objective of the present study was to evaluate the effects of attapulgite dietary supplementation on sow performance during gestation and lactation periods.

## MATERIALS AND METHODS

The experimental procedures in this study received approval from the Research Committee of the Aristotle University of Thessaloniki, Greece (Protocol No 67555/ Code 85152) and followed the guidelines of Directive 2010/63/EU for animal experiments.

### Study Farms

The present study was performed under field conditions. Two farms were selected, based on the following criteria: number of sows under production and geographical distribution. The farms were situated in different geographical parts of Greece: Farm A in Fillipiada of Preveza prefecture (capacity of 550 sows) and Farm B in Megalochori of Trikala prefecture (capacity of 220 sows). The study started during the same period in both farms. Both farms implemented vaccination protocols in the reproductive population against Aujersky's virus, Porcine Respiratory Reproductive Syndrome virus, Parvo virus, *Esypelothrix Rhusiopathiae* and *E. coli*.

### Treatments

The treatment groups were: a) control group (CN): The sows were fed a common diet according to their reproductive stage (gestation-lactation); b) attapulgite group (AT): The sows were fed the common diet according to their reproductive stage, which was supplemented with attapulgite at 0.7% level, which was the recommended level by the producing company (Optify<sup>®</sup>, Geohellas S.A., Athens, Greece) and c) attapulgite plus group (AT+): The sows were fed the common diet according to their reproductive stage, which was supplemented with attapulgite (0.7%) and a mix of vitamins, trace elements, enzymes, live yeast and amino acids (0.1%), at a total of 0.8% level (Ultrafed<sup>®</sup>, Geohellas S.A., Athens, Greece).

A detailed mineralogical characterization, chemical composition and other properties of the material used in here is described in a previous publication of our research group (Chalvatzi et al., 2014).

### Experimental Design

The aim of the trial was to implement a sufficient number of clinically healthy sows and to study the effects of supplementation of attapulgit on sow diets for 2 consecutive reproductive phases (cycles). To achieve the desired number of sows, a higher number of sows was allocated to the treatments at the onset of each reproductive cycle, in order to cover the unpredicted factors that could cause the exclusion of experimental animals, e.g. return to oestrus after insemination, culling due to lameness etc. Thus, a total of 24 sows in Farm A and 12 sows in Farm B were included per treatment for both cycles. It should be noted that the sows that completed both experimental phases (cycles) were in Farm A, 15 sows per treatment, while in Farm B, 8 sows per treatment. The detailed description of the experimental design is given on Table 1.

### Animals and Feeding

In both farms sows were group housed during gestation. Artificial insemination was used and estrus detection occurred with the presence of a teaser boar. In Farm A, sows were transferred to the farrowing

rooms approximately 4 days before the expected farrowing date. Transfer of sows to the farrowing crates in farm B was performed 3 days before the expected farrowing date. Farrowing induction was not regularly practiced in any of the two farms, unless a sow has exceeded by one day of the expected farrowing day. Cross fostering of piglets took place within 24h postpartum and was allowed only within the same treatment in both farms. Regular piglet husbandry procedures (teeth clipping, iron injection, tail docking and castration of male piglets) took place between the second and fourth day postpartum in both farms. Weaning took place at 21 days postpartum in Farm A and in 28 days postpartum in Farm B. Restricted feeding of sows was followed as a regular practice in both farms. Specifically, in Farm A during gestation the daily feed allowance was adjusted from after insemination to 90<sup>th</sup> day of gestation at 2.5 kg/day, and from 90<sup>th</sup> day of gestation to the day of transfer to farrowing room at 2.8 kg/day. In Farm B the daily feed allowance was adjusted from after insemination to 30<sup>th</sup> day of gestation at 3.0 kg/day, from the 31<sup>st</sup> day to 85<sup>th</sup> day of gestation at 2.3 kg/day, and from 86<sup>th</sup> day of gestation to the day of transfer to farrowing room at 2.6 kg/day. At placement in the farrowing crate, the diet of sows shifted to the lactation diet. In Farm A, during lactation sows were fed at a level of 2kg at farrowing plus 400g/ piglet, divided over two meals per day. The sows were fed on an

**Table 1.** Number of sows allocated per treatment at the different stages of the experiment and final number of sows that were included in the first and second reproductive cycle in both study farms

| Parameter                             | Farm A | Farm B   |
|---------------------------------------|--------|--|
| Sows Capacity                         | 550    | 220  |
| Breed                                 |        | Topigs 40  |
| Treatments                            |        | CN <sup>1</sup><br>AT <sup>2</sup><br>AT <sup>+</sup> <sup>3</sup> |
| Initial Allocation of sows/ cycle     | 120    | 45   |
| Sows by experimental criteria/ cycle  | 72     | 36   |
| Sows per treatment/ cycle             | 24     | 12   |
| Sows completed both cycles/ treatment | 15     | 8  |

1 control group: standard gestation and lactation diet

2 attapulgit group: standard gestation and lactation diets were supplemented with 7 g/kg of feed of attapulgit

3 attapulgit+ group: standard gestation and lactation diets were supplemented with 8 g/kg of feed of an attapulgit blended product

ascending scale until the 7<sup>th</sup> day of lactation after which maximum allowance was reached. In Farm B, during lactation sows were fed at a level of 2.5kg at farrowing plus 400g/ piglet, divided over two meals per day and the sows reached maximum feed allowance till the 6<sup>th</sup> day of lactation. Main ingredients and

basic nutrient composition (calculated with EvaPig® software) of the gestation and lactation diets is given in Table 2. Water was available *ad libitum*. In both farms, piglets were given free access to a commercial creep feed from the 7<sup>th</sup> day after farrowing until weaning.

**Table 2.** Main ingredients and nutrient composition of the gestation and lactation diet in Farm A and Farm B

| <i>Farm A</i>                  |                |                |
|--------------------------------|----------------|----------------|
|                                | Gestation Diet | Lactation Diet |
| Ingredients (kg/100kg of feed) |                |                |
| Maize                          | 7.5            | -              |
| Wheat soft                     | 40.0           | 46.0           |
| Wheat bran                     | 21.0           | 10.0           |
| Barley                         | 20.1           | 22.35          |
| Soybean 44% CP                 | 8.5            | 13.5           |
| Soybean oil                    | -              | 1.75           |
| Fish meal 70% CP               | -              | 3.5            |
| Calcium carbonate              | 1.65           | 1.5            |
| L-Lysine HCl                   | -              | 0.15           |
| Mineral and vitamin premix     | 1.25           | 1.25           |
| Nutrients                      |                |                |
| Crude Protein (%)              | 13.63          | 17.01          |
| Crude Fat (%)                  | 2.11           | 3.75           |
| Crude Fibre (%)                | 4.41           | 3.78           |
| Metabolizable Energy (MJ/kg)   | 12.27          | 13.08          |
| <i>Farm B</i>                  |                |                |
|                                | Gestation Diet | Lactation Diet |
| Ingredients (kg/100kg of feed) |                |                |
| Maize                          | 19.0           | 19.0           |
| Wheat soft                     | 17.4           | 20.5           |
| Wheat bran                     | 30.0           | 18.0           |
| Barley                         | 17.0           | 18.0           |
| Soybean 44% CP                 | 10.0           | 17.0           |
| Soybean oil                    | 1.5            | 2.0            |
| Fish meal 70% CP               | 1.0            | 1.0            |
| Yeast                          | -              | 0.5            |
| Mineral and vitamin premix     | 4.1            | 4.0            |
| Nutrients                      |                |                |
| Crude Protein (%)              | 14.55          | 16.47          |
| Crude Fat (%)                  | 4.08           | 4.36           |
| Crude Fibre (%)                | 4.93           | 4.38           |
| Metabolizable Energy (MJ/kg)   | 12.18          | 12.78          |

### Measured Parameters

In both farms sows' body weight (Sow BW) was measured at insemination (Sow BW<sub>i</sub>), at the transfer to the farrowing room (Sow BW<sub>f</sub>), and at weaning (Sow BW<sub>w</sub>). The body weight of the sows was measured by a digital scale. Sow body weight loss from late gestation to weaning was calculated (Sow

BW loss). In addition, the relative sow body weight loss (%) during lactation was calculated by the equation:  $\text{SowBWloss\%} = \frac{(\text{SowBW}_f - \text{SowBW}_w)}{\text{SowBW}_f} \times 100$ . Regarding piglet data, the litter size 24h postpartum (after cross-fostering) and at weaning was measured in both farms. The number of still-born piglets was also recorded. Piglets were weighed

**Table 3.** Sow and piglet performance in Farm A during cycles 1 and 2

| Parameters                               | Treatment         |                   |                   | SEM   | P-value |
|--|-------------------|-------------------|-------------------|-------|---------|
|  | CN<br>(n=24)      | AT<br>(n=24)      | AT+<br>(n=24)     |       |         |
| <i>Cycle 1</i>                           |                   |                   |                   |       |         |
| Parity                                   | 4.6               | 4.7               | 3.8               | 0.59  | 0.217   |
| Sow BW <sub>i</sub> <sup>1</sup> , kg    | 217.9             | 224.0             | 222.0             | 10.50 | 0.716   |
| Sow BW <sub>f</sub> <sup>1</sup> , kg    | 269.4             | 273.1             | 277.6             | 11.32 | 0.698   |
| Sow BW <sub>w</sub> , kg                 | 226.8             | 230.3             | 233.1             | 9.98  | 0.749   |
| Sow BW <sub>loss</sub> , kg              | 42.7              | 43.1              | 44.2              | 4.34  | 0.942   |
| Sow BW <sub>loss</sub> %                 | 15.6              | 15.6              | 16.1              | 0.95  | 0.912   |
| Litter size 24h postpartum <sup>1</sup>  | 10.7 <sup>a</sup> | 12.5 <sup>b</sup> | 12.1 <sup>b</sup> | 0.50  | 0.001   |
| Stillborn                                | 1.2               | 0.9               | 1.2               | 0.39  | 0.655   |
| Litter size weaning <sup>1</sup>         | 10.3 <sup>a</sup> | 11.3 <sup>b</sup> | 10.3 <sup>a</sup> | 0.35  | 0.004   |
| Piglet BW <sub>f</sub> , kg              | 1.6               | 1.5               | 1.6               | 0.06  | 0.124   |
| Piglet BW <sub>w</sub> <sup>1</sup> , kg | 6.5               | 6.6               | 6.6               | 0.24  | 0.846   |
| ADG <sub>1</sub> , g/day                 | 234.8             | 245.9             | 240.2             | 11.70 | 0.612   |
| <i>Cycle 2</i>                           |                   |                   |                   |       |         |
| Parity                                   | 5.1               | 4.8               | 4.0               | 0.58  | 0.121   |
| Sow BW <sub>i</sub> <sup>1</sup> , kg    | 213.3             | 219.0             | 220.9             | 10.40 | 0.528   |
| Sow BW <sub>f</sub> <sup>1</sup> , kg    | 275.1             | 275.9             | 272.0             | 8.13  | 0.883   |
| Sow BW <sub>w</sub> <sup>1</sup> , kg    | 228.8             | 235.2             | 228.0             | 7.10  | 0.548   |
| Sow BW <sub>loss</sub> , kg              | 46.3              | 40.8              | 43.8              | 4.75  | 0.501   |
| Sow BW <sub>loss</sub> %                 | 16.3              | 14.8              | 16.5              | 1.07  | 0.457   |
| Litter size 24h postpartum <sup>1</sup>  | 12.1              | 12.6              | 12.1              | 0.59  | 0.565   |
| Stillborn                                | 0.9               | 0.8               | 0.8               | 0.24  | 0.862   |
| Litter size weaning <sup>2</sup>         | 9.7 <sup>a</sup>  | 10.4 <sup>b</sup> | 10.6 <sup>b</sup> | 0.34  | 0.037   |
| Piglet BW <sub>f</sub> , kg              | 1.6               | 1.6               | 1.5               | 0.07  | 0.630   |
| Piglet BW <sub>w</sub> , kg              | 7.2               | 7.6               | 7.2               | 0.30  | 0.279   |
| ADG, g/day                               | 265.7             | 288.0             | 271.0             | 14.22 | 0.270   |

<sup>a,b</sup> Means with different superscripts within the same row differ significantly between them (P<0.05)

<sup>1</sup>Parity was used as covariate, mean values within the same row represent adjusted means

<sup>2</sup>Litter size at 24h postpartum was used as covariate, mean values within the same row represent adjusted means

Sow BW<sub>i</sub>= sow body weight at insemination; Sow BW<sub>f</sub>=sow body weight at arrival at farrowing room; Sow BW<sub>w</sub>= sow body weight at weaning; Sow BW<sub>loss</sub>= sow body weight loss from farrowing to weaning; Sow BW<sub>loss</sub> %= sow body weight loss from farrowing to weaning expressed in % related to sow body at farrowing; Piglet BW<sub>f</sub>= piglet body weight at 24h postpartum; Piglet BW<sub>w</sub>= piglet body weight at weaning; ADG= average daily gain of piglets during lactation

24h postpartum (Piglet BWf) and at weaning (Piglet BWw). Average daily gain (ADG) was calculated dividing the difference between Piglet BWw and Piglet BWf by the number of lactation days for each farm. Parity was also recorded.

### Statistical Analysis

All statistical procedures were performed using SPSS

(SPSS 22.0 Version, Chicago, IL, USA). Statistical significance was considered at  $P<0.05$ . Data were analyzed with analysis of variance (ANOVA) of GLM procedures. Data was checked for normality of distribution with Kruskal-Wallis test and equality of differences with Levene's test. Treatment was included as fixed factor.

At a first step, each cycle and for each farm was

**Table 4.** Sow and piglet performance in Farm B during cycles 1 and 2

| Parameters                              | Treatment          |                    |                     | SEM   | P-value |
|---|--------------------|--------------------|---------------------|-------|---------|
|   | CN<br>(n=12)       | AT<br>(n=12)       | AT+<br>(n=12)       |       |         |
| <i>Cycle 1</i>                          |                    |                    |                     |       |         |
| Parity                                  | 4.2                | 4.5                | 4.9                 | 0.64  | 0.761   |
| Sow BWi <sup>1</sup> , kg               | 215.3 <sup>x</sup> | 202.4 <sup>y</sup> | 201.1 <sup>y</sup>  | 10.66 | 0.092   |
| Sow BWf <sup>1</sup> , kg               | 259.5              | 257.9              | 262.7               | 6.35  | 0.747   |
| Sow BWw <sup>1</sup> , kg               | 219.6              | 214.9              | 215.0               | 8.16  | 0.809   |
| Sow BW loss, kg                         | 31.4 <sup>x</sup>  | 45.3 <sup>y</sup>  | 53.7 <sup>y</sup>   | 8.02  | 0.063   |
| SowBWloss%                              | 15.4               | 16.6               | 18.0                | 1.84  | 0.611   |
| Litter size 24h postpartum <sup>1</sup> | 10.6               | 11.1               | 10.2                | 0.59  | 0.252   |
| Stillborn                               | 0.75               | 0.5                | 1.1                 | 0.38  | 0.303   |
| Litter size weaning                     | 9.9 <sup>ab</sup>  | 10.2 <sup>a</sup>  | 8.9 <sup>b</sup>    | 0.51  | 0.037   |
| Piglet BWf, kg                          | 1.4                | 1.5                | 1.6                 | 0.09  | 0.412   |
| Piglet BWw <sup>2</sup> , kg            | 8.6                | 9.1                | 8.2                 | 0.58  | 0.174   |
| ADG, g/day                              | 214.7              | 245.5              | 221.2               | 14.89 | 0.109   |
| <i>Cycle 2</i>                          |                    |                    |                     |       |         |
| Parity                                  | 4.8                | 4.7                | 5.0                 | 0.89  | 0.960   |
| Sow BWi <sup>1</sup> , kg               | 220.5              | 209.5              | 216.3               | 8.07  | 0.362   |
| Sow BWf, kg                             | 262.3              | 250.8              | 259.9               | 7.31  | 0.276   |
| Sow BWw <sup>1</sup> , kg               | 239.8 <sup>x</sup> | 224.9 <sup>y</sup> | 228.5 <sup>xy</sup> | 7.47  | 0.080   |
| Sow BW loss, kg                         | 22.5               | 26.7               | 29.2                | 4.63  | 0.277   |
| SowBWloss%                              | 8.6                | 10.6               | 11.8                | 1.22  | 0.192   |
| Litter size 24h postpartum <sup>1</sup> | 10.2               | 10.6               | 11.0                | 0.96  | 0.685   |
| Stillborn                               | 0.5                | 0.5                | 0.4                 | 0.23  | 0.777   |
| Litter size weaning                     | 8.6                | 8.3                | 9.8                 | 0.94  | 0.248   |
| Piglet BWf, kg                          | 1.5                | 1.4                | 1.5                 | 0.09  | 0.250   |
| Piglet BWw <sup>2</sup> , kg            | 8.3 <sup>a</sup>   | 8.5 <sup>a</sup>   | 9.8 <sup>b</sup>    | 0.33  | <0.001  |
| ADG, g/day                              | 218.8              | 227.9              | 248.7               | 15.15 | 0.136   |

<sup>a,b</sup> Means with different superscripts within the same row differ significantly between them ( $P<0.05$ )

<sup>x,y</sup> Means with different superscripts within the same row tend to differ significantly between them ( $0.05<P<0.1$ )

<sup>1</sup>Parity was used as covariate, mean values within the same row represent adjusted means

<sup>2</sup>Litter size at 24h postpartum was used as covariate, mean values within the same row represent adjusted means

Sow BWi= sow body weight at insemination; Sow BWf=sow body weight at arrival at farrowing room; Sow BWw= sow body weight at weaning; Sow BW loss= sow body weight loss from farrowing to weaning; Sow BWloss %= sow body weight loss from farrowing to weaning expressed in % related to sow body at farrowing; Piglet BWf= piglet body weight at 24h postpartum; Piglet BWw= piglet body weight at weaning; ADG= average daily gain of piglets during lactation

analyzed separately. Parity, sow BW<sub>i</sub>s were tested as covariates for SowBW<sub>f</sub>. The SowBW<sub>f</sub> was used as a covariate for SowBW<sub>w</sub>. Parity was tested as a covariate for all the rest recorded parameters. Litter size 24h postpartum was tested as a covariate for piglet BW<sub>f</sub>, piglet BW<sub>w</sub> and ADG. At a second step, in order to evaluate the treatment effects in sows that completed both cycles in each farm separately, the GLM repeated measures analysis was used. Treatment was included as between subject factor, and cycle 1 and 2 were included as within subject factors. Results were reported as least square means  $\pm$  SEM. In case of significant relationship of a covariate, results were reported as estimated marginal means.

## RESULTS

### Results from each farm and per cycle

The results of sow and piglet performance parameters during both phases (cycles 1 and 2) from Farm A are presented in Table 3. No significant differences were detected for sow related characteristics either for cycle 1 or for cycle 2. Regarding piglet data,

during cycle 1 significant differences between treatments were noted for litter size 24h postpartum and at weaning. Specifically, litter size 24h postpartum was greater for AT and AT+ groups compared to CN ( $P=0.001$ ). Moreover, litter size at weaning was greater in AT than the other two groups ( $P=0.004$ ). During cycle 2, the only significant effect was detected for litter size at weaning, which was in favour of AT and AT+ compared to CN group ( $P=0.037$ ). One of the most important parameters to evaluate sow and litter performance during the lactation period is piglets' growth. Although not statistically significant, numerical differences were recorded in the ADG of piglets in favour of the attapulgitic supplemented groups.

The results of sow and piglet performance parameters during both phases (cycles 1 and 2) from Farm B are presented in Table 4. During cycle 1, sow BW at insemination tended to be higher in CN compared to the other two groups ( $P=0.092$ ). The sow BW loss from late gestation to weaning, tended to be higher in AT and AT+ than CN group ( $P=0.063$ ). During cycle 2, sow body weight at weaning tended to be higher in the CN than AT sows ( $P=0.080$ ). Regarding

**Table 5.** Treatment effects in Farm A after repeated measured analysis in sows that completed both reproductive cycles

| Parameters                  | Treatment          |                    |                    | SEM  | P-value |
|-----------------------------|--------------------|--------------------|--------------------|------|---------|
|                             | CN<br>(n=15)       | AT<br>(n=15)       | AT+<br>(n=15)      |      |         |
| Sow BW <sub>i</sub> , kg    | 214.4              | 219.1              | 228.2              | 9.19 | 0.561   |
| Sow BW <sub>f</sub> , kg    | 265.9              | 275.4              | 282.2              | 9.67 | 0.497   |
| Sow BW <sub>w</sub> , kg    | 227.5              | 227.8              | 236.1              | 8.96 | 0.744   |
| Sow BW loss, kg             | 38.4 <sup>a</sup>  | 47.5 <sup>b</sup>  | 46.0 <sup>b</sup>  | 2.40 | 0.023   |
| Sow BW loss%                | 14.5 <sup>x</sup>  | 17.4 <sup>y</sup>  | 16.2 <sup>y</sup>  | 0.82 | 0.052   |
| Litter size 24h postpartum  | 11.5 <sup>a</sup>  | 12.8 <sup>b</sup>  | 12.1 <sup>ab</sup> | 0.31 | 0.011   |
| Stillborn                   | 1.0                | 0.9                | 0.6                | 0.19 | 0.451   |
| Litter size weaning         | 10.2 <sup>a</sup>  | 11.2 <sup>b</sup>  | 10.3 <sup>a</sup>  | 0.25 | 0.022   |
| Piglet BW <sub>f</sub> , kg | 1.6                | 1.6                | 1.6                | 0.04 | 0.662   |
| Piglet BW <sub>w</sub> , kg | 6.7 <sup>a</sup>   | 7.3 <sup>b</sup>   | 6.8 <sup>a</sup>   | 0.18 | 0.049   |
| ADG, g/day                  | 183.7 <sup>a</sup> | 206.1 <sup>b</sup> | 185.9 <sup>a</sup> | 9.35 | 0.040   |

a,b Means with different superscripts within the same row differ significantly between them ( $P<0.05$ )

x,y Means with different superscripts within the same row tend to differ significantly between them ( $0.05<P<0.1$ )

Sow BW<sub>i</sub>= sow body weight at insemination; Sow BW<sub>f</sub>=sow body weight at arrival at farrowing room; Sow BW<sub>w</sub>= sow body weight at weaning; Sow BW loss= sow body weight loss from farrowing to weaning; Sow BW loss %= sow body weight loss from farrowing to weaning expressed in % related to sow body at farrowing; Piglet BW<sub>f</sub>= piglet body weight at 24h postpartum; Piglet BW<sub>w</sub>= piglet body weight at weaning; ADG= average daily gain of piglets during lactation

piglet data, during cycle 1 litter size at weaning was higher in group AT than AT+, with the CN group being indifferent from the other 2 groups ( $P=0.037$ ). During cycle 2, piglet weight at weaning was higher in AT+ than the other 2 groups ( $P<0.001$ ). The ADG of piglets in both cycles was again not statistically significant, but also numerically greater in the attapulgitic supplemented groups compared to the control one.

### Repeated Measures Analysis

The results of treatment effects after repeated measures analysis of data from sows that completed both cycles from Farm A and Farm B are presented in Tables 5 and 6, respectively.

In Farm A (Table 5), the calculated sow BW loss was greater for AT and AT+ groups compared to CN ( $P=0.023$ ). Likewise, the % of sow BW loss during lactation tended to be greater for AT and AT+ groups compared to CN ( $P=0.052$ ). Litter size 24h postpartum, litter size at weaning and piglet BW at weaning were higher in AT than CN and AT+ groups ( $P=0.011$ ,  $P=0.022$  and  $P=0.049$ , respectively). Finally, the ADG of piglets during lactation was higher in AT compared to CN and AT+ groups ( $P=0.040$ ).

In Farm B no significant differences between treatments were detected regarding sow and litter performance parameters. However, a clear trend regarding sow BW loss, sow BW loss (%) and ADG of piglets was observed for the attapulgitic supplemented groups, where values were greater compared to control group. Furthermore, litter size 24h postpartum and at weaning, was numerically higher in the attapulgitic supplemented groups compared to control group.

### DISCUSSION

The present study investigated the effects of dietary attapulgitic supplementation during gestation and lactation on sows' performance. The study protocol implemented the follow up of two consecutive reproductive phases (cycles) in two commercial farms. Thus, it was possible to investigate the repetition of treatment effects over a two cycle period. Moreover, as both farms differed in terms of size (number of sows), location and feeding practices the effects of attapulgitic supplementation under different husbandry conditions were also evaluated.

In the present study, litter size 24h postpartum was

**Table 6.** Treatment effects in Farm B after repeated measured analysis in sows that completed both reproductive cycles

| Parameters                  | Treatment   |             |              | SEM  | P-value |
|-----------------------------|-------------|-------------|--------------|------|---------|
|                             | CN<br>(n=8) | AT<br>(n=8) | AT+<br>(n=8) |      |         |
| Sow BW <sub>i</sub> , kg    | 210.3       | 200.5       | 204.5        | 6.82 | 0.603   |
| Sow BW <sub>f</sub> , kg    | 254.5       | 250.4       | 260.4        | 6.14 | 0.522   |
| Sow BW <sub>w</sub> , kg    | 224.4       | 213.7       | 221.0        | 6.35 | 0.489   |
| Sow BW loss, kg             | 30.1        | 37.3        | 39.4         | 3.28 | 0.133   |
| SowBWloss%                  | 11.8        | 14.6        | 15.2         | 1.32 | 0.176   |
| Litter size 24h postpartum  | 10.4        | 11.4        | 11.0         | 0.51 | 0.352   |
| Stillborn                   | 0.6         | 0.6         | 0.8          | 0.19 | 0.860   |
| Litter size weaning         | 9.1         | 9.4         | 9.6          | 0.50 | 0.768   |
| Piglet BW <sub>f</sub> , kg | 1.4         | 1.4         | 1.5          | 0.05 | 0.154   |
| Piglet BW <sub>w</sub> , kg | 8.7         | 8.8         | 8.8          | 0.25 | 0.958   |
| ADG, g/day                  | 216.9       | 240.8       | 234.4        | 8.26 | 0.132   |

Sow BW<sub>i</sub>= sow body weight at insemination; Sow BW<sub>f</sub>=sow body weight at arrival at farrowing room; Sow BW<sub>w</sub>= sow body weight at weaning; Sow BW loss= sow body weight loss from farrowing to weaning; Sow BWloss %= sow body weight loss from farrowing to weaning expressed in % related to sow body at farrowing; Piglet BW<sub>f</sub>= piglet body weight at 24h postpartum; Piglet BW<sub>w</sub>= piglet body weight at weaning; ADG= average daily gain of piglets during lactation

affected by attapulgitic supplementation in sows. This effect was more consistent in Farm A sows, and especially in those that completed both cycles. The same effect was also evident at litter size at weaning. Although litter size as a single factor for the evaluation of the reproductive performance of sows alone has a limited value, as it can be largely affected by numerous factors, the persistency of this effect in sows that completed both cycles, suggests that attapulgitic requires continuous supplementation in sow feeding in order to affect litter size parameters. The output of a pig farm is estimated upon the number of piglets weaned per sow per cycle or per year basis, and therefore influencing positively litter size at weaning is a demand in the field. Successful breeding programs have resulted in a significant increase in litter size in the modern hyper-prolific sow (Kemp and Soede, 2012). Litter size at farrowing can be influenced mainly during the early stages of gestation in sows. An increased feeding level during the first 4 weeks of gestation improved embryonic and placental development through an increased availability of specific micronutrients, such as folic acid and arginine (Matte et al., 1996). Thus, an increased feeding level might stimulate embryonic and placental development, and thereby embryo survival, by its influence on IGF-1 (Hoving et al., 2011). Similar results were previously reported after dietary inclusion of another clay (clinoptilolite) in sow diets, where an increased litter size both at birth and at weaning accompanied by increased piglet body weight at birth and at weaning (Papaioannou et al., 2002). The latter study attributed the improvements of piglet characteristics to an improvement in nutritional efficiency of the sow during late gestation and also to a sparing effect of nutrients and energy for a better lactation performance. In the present study sows from both farms were fed restrictively during gestation. Therefore, possible differences in litter size at farrowing could not be attributed to an increased feeding allowance during the first stages of gestation but to the effect of attapulgitic. The fact that attapulgitic supplementation in sows did not cause differences on piglet weight 24h postpartum, suggests that supply of nutrients *in utero* did not affect fetus growth and weight. Elsewhere, dietary supplementation of sows with clinoptilolite-rich tuff clay, resulted both

in improved litter size and piglet weight at farrowing (Kyriakis et al., 2002).

The finding of an increased sow BW loss in groups supplemented with attapulgitic, supports the finding of the improved piglet performance in the specific groups. The consistency of this finding was further validated by repeated measures analysis of sows that participated in both phases. Specifically, the repeated measures analysis of sow BW loss % in both farms, revealed that attapulgitic supplemented sows mobilized proportionally more body weight during lactation than the control ones. Litter size is the driving force of milk production in the sow and a higher litter size induces higher milk production (Auld et al., 1998). The sow during lactation mobilizes body reserves in order to support the growth of the nursing piglets, as feed intake is suboptimal (Eissen et al., 2003). The underlying mechanism under this metabolic effect of attapulgitic on increased sow body weight mobilization during lactation remains to be investigated. Attapulgitic, due to its high water binding capacity, has been used in human medicine as a product to reduce diarrhea (Carretero and Pozo, 2010). It could be hypothesized that in sows attapulgitic caused a decreased gut transition rate. This may have resulted in a gradual release of nutrients, inducing in this way a greater mobilization of body reserves to cover the needs for piglet growth. Consequently, the mobilization of body reserves by the attapulgitic supplemented sows reflected in improved weight gain of piglets during lactation. Such information on body weight changes of sows during lactation after clay supplementation is absent in previous studies that demonstrated improved piglet weight gain (Kyriakis et al., 2002; Papaioannou et al., 2002). These effects became significant in Farm A, whereas in Farm B numerical differences were detected in favour of attapulgitic groups; this should be attributed to the number of replicates, either in the single phases (cycle) or in the repeated measurements analysis of data in Farm B.

Despite the fact that the compound form (AT+) contained the same amount of attapulgitic as of the pure product (AT), the obtained results were different between farms. The latter could be attributed to the feeding regime in the two farms. As the total cereal amount containing Non-Starch Polysaccharides

(NSPs, e.g. barley and wheat) added was higher in Farm A, it is plausible that the additives such as yeasts or enzymes may not act synergistically with attapulgit. It is known that NSP enzymes reduce digesta viscosity and thus promote intestinal transit time (Diebold et al., 2004). In the contrary, a clear effect was evident in Farm B, where the total NSP cereals were lower (38.5% vs 68.4% for the lactation diets).

### CONCLUSIONS

Supplementation of attapulgit in sow diets during gestation and lactation in two commercial farms in Greece resulted in the improvement of performance indexes such as sow body reserves mobilization, litter size characteristics and piglet growth. However, results should be cautiously extrapolated to farms differing in the feeding regime, in their size and in their productivity. Further research is needed to eluci-

date the underlying mechanisms involved, especially regarding to sow metabolism.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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