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## Effects of palygorskite dietary supplementation on back fat mobilization, leptin levels and oxidative stress parameters in sows

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### ABSTRACT

The effects of dietary supplementation of palygorskite on the metabolic and oxidative parameters during the peripartal period and performance of sows were assessed. Upon insemination sows were allocated to treatments: a) CON ( $n = 23$ ): sows were fed a basal diet; b) PAL ( $n = 21$ ): sows were fed the basal diet supplemented with 7 g/kg feed palygorskite; and c) PAL+ ( $n = 23$ ): sows were fed the basal diet supplemented with 8 g/kg feed of a palygorskite compound product. Sow plasma samples ( $n = 10$ /treatment) were obtained at days 108 of gestation, 1 and 14 postpartum. Leptin levels were higher in PAL and PAL+ groups ( $P < 0.001$ ). The levels of thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP) were higher in the CON group ( $P = 0.001$  and  $P = 0.003$ , respectively). PAL sows exhibited higher back fat (BF) loss from late gestation to weaning ( $P = 0.015$ ). Litter weight at weaning and litter weight gain during lactation were higher in CON sows ( $P < 0.001$ ). Overall, palygorskite supplementation affected peripartal energy balance of sows, which was characterized by the increased leptin levels and BF mobilization and accompanied by a decreased oxidative stress.

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### 1. Introduction

The use of clay minerals in animal nutrition has been strongly supported over the last years by the research community. Palygorskite (or attapulgitite, the term widely used in end users) is a clay mineral characterized of an elongated shape (Murray, 2000), in an arrangement of blocks separated by parallel channels composed by two layers of SiO<sub>2</sub> tetrahedral enclosing octahedral sheet containing mainly Mg and Al (Alvarez et al., 2011). Due to its absorptive, rheological and catalytic properties, palygorskite has recently received considerable attention highlighted by its insertion in the catalogue of feed materials (Commission regulation 575/2011). Published work in production animals has shown positive effects of its dietary inclusion in piglets (Zhang et al., 2013; Lv et al., 2015), lactating cows (Bampidis et al., 2014) and laying hens (Chalvatzi et al., 2014). However, in vivo studies on the use of palygorskite in the diets of sows are scarce.

In modern highly prolific sows, adequate nutrient and energy supply during gestation and lactation are essential (Kemp and Soede, 2012; Campos et al., 2012). In practice, gestating sows are fed on restricted levels, while the metabolic demands of the sow increase towards

farrowing (Close and Cole, 2003). Feeding management during the transition from gestation to lactation is important for performance and peripartal health and could affect adipocyte related factors such as non-esterified fatty acids (NEFAs) and plasma leptin (Papadopoulos et al., 2009, 2010; Mosnier et al., 2010; Hansen et al., 2012). Under restricted feeding conditions in sows, an alternative option is the inclusion of feeding additives such as clay minerals, with the potential to promote nutrient utilization and absorption (Slamova et al., 2011).

Based on the properties of palygorskite, it could be hypothesized that the gradual release of nutrients in the intestine of sows could affect their performance and energy balance. Hence, the objectives of the present study were to assess the effects of dietary supplementation of palygorskite, either as pure supplement or blended with other additives, on the metabolic and oxidative status of peripartal sows and its potential impact on their performance.

### 2. Materials and methods

All experimental procedures were approved by the Regional State Veterinary Authority of Pieria Prefecture and by the Ethical Committee branch of the Research Committee of Aristotle University of Thessaloniki (decision number 67555; project number 85152). The specifications of the trial complied with all welfare requirements of

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sows with regard to feed, water, space, and treatments according to Good Farming Practice Guidelines (Directive 2010/63/EC; Commission recommendation 2007/526/EC).

### 2.1. Trial farm description

The study was conducted at a commercial farm, situated in the Pieria prefecture in Greece, which had a capacity of 880 sows (TOPIGS40, Topigs Norsvin, 5263 LT Vught, The Netherlands). The farm operated a farrow to finish production system and implemented “all-in, all out” system. The farm has fully implemented sow group housing during gestation. Sows were housed during gestation in groups of six in pens with solid concrete floor (4.5 by 3.5 m) without straw bedding. The gestation pens included also an outdoor space (2 × 3.5 m) to which the sows had access via doors of double movement made by polyethylene leaves. Feeders in gestations pens were subdivided into smaller feeder spaces, resulting in at least six separate feeding positions. Approximately at seven days before the calculated farrowing date the sows were transferred to farrowing rooms (day 108 of gestation), where they were housed individually in farrowing pens (2.40 × 1.80 m) until weaning. Each pen consisted of fully slatted floor for the sow crate area, while the piglet creep area had a concrete floor. An infrared heat lamp was placed in each piglet creep area and was kept open from one day before farrowing till approximately two weeks of age of the piglets. Cross-fostering of piglets took place within 24 h after parturition and occurred only among sows of the same treatment. Overall target during cross-fostering was to standardize at 12 piglets per litter.

### 2.2. Palygorskite

The palygorskite used in the present study (AFG-60 mesh) was provided by Geohellas S.A., Greece. It was mined from deposits, located in Ventzia basin of Grevena, western Macedonia, Greece. The mineralogical characterization, its chemical composition and properties are provided in detail in a recent publication (Chalvatzi et al., 2014). Apart from the pure product consisted of 75% palygorskite and 25% bentonite-saponite (Optify®, Geohellas S.A., Athens, Greece), the blend of palygorskite with other additives is further named as “palygorskite +” (PAL+). This product (Ultrafed®, Geohellas S.A., Athens, Greece) contained in wt. %: the pure palygorskite product (Optify®) 87.5%, *Saccharomyces cerevisiae* (Actisaf® Sc47, strain CNCM I-4407, Lesaffre, Marcq-en-Baroeul Cedex, France) 1.56%, MgO 1.25%, lysine 1.25%, methionine 1.25%, palm oil 0.36%, NaCl 0.27%, vitamin and trace element supplements 1.00%, dextrose 3.60%, and enzymes (endo-1,4 β-glucanase & endo-1,4 β-xylanase) 1.66%.

### 2.3. Treatments

The treatment groups were: a) control group (CON): the sows were fed a basal diet according to their reproductive stage (gestation-lactation); b) palygorskite group (PAL): the sows were fed the basal diet according to their reproductive stage, which was supplemented with palygorskite at 7 g/kg feed; and c) palygorskite + group (PAL+): the sows were fed the basal diet according to their reproductive stage, which was supplemented with the palygorskite compound product at a 8 g/kg feed. This treatment group was included to check if palygorskite would still add benefits on top of more established additives such as probiotics and cell wall degrading enzymes. During gestation the individual sow was the smallest unit used and, therefore, it represented the experimental unit. Similarly, in the lactation period the sow and her litter were the experimental units.

### 2.4. Study population

The initial target was to include at least 20 sows in the experiment that would complete the gestation and lactation phase, constituting a

complete cycle within the same treatment. The farm was practicing on average weekly batches of minimum 30 sows that were weaned and inseminated afterwards. Sows from the same weekly batch after insemination were assigned to the same treatment. The sequence of allocation of sows to treatment groups was firstly the CON, secondly the PAL and thirdly the PAL+. The initial number of sows allocated to each treatment was 37 for CON, 32 for PAL and 33 for PAL+. Due to various reproductive and husbandry factors (culling, abortions, return into service) and experimental criteria (exclusion due to parity: <2, and higher than 7) the numbers of sows that were included in the final dataset and completed the gestation and lactation were 23 for CON, 21 for PAL and 23 for PAL+.

### 2.5. Experimental design for blood parameters

The sows were sampled at three time points. The sampling time points were: day 108 of gestation, day one (1) postpartum (day 1 of lactation) and day 14 postpartum (day 14 of lactation). The sows sampled at the first sampling day were randomly chosen from each treatment sow population. The same sows were sampled at the subsequent time points. Postprandial blood samples (10 ml) were taken at approximately 60 min after morning feeding from the jugular vein after restraining the sows with a snare. Time elapsed (60 min) was calculated from the moment of completed consumption of the morning feed portion. The morning feed portion was standardized to 1.5 kg. Due to the required precision of the blood sampling protocol, it was not possible to sample all available sows and therefore blood sampling was limited to 10 sows per treatment group. Blood samples were collected into heparinized tubes (10 ml BD Vacutainer, containing 170 IU spray dried lithium heparin, Becton, Dickinson and Company, Franklin Lakes, New Jersey, US) and till centrifugation were preserved into iced water. Within approximately 30 min after collection, blood samples were centrifuged for 15 min at 3000 × g at 4 °C. Afterwards, plasma samples collected were transferred in Eppendorf safe-lock tubes, labelled and stored at –20 °C until they were assayed.

### 2.6. Animals and feeding

The gestation feed offered to sows contained as main ingredients (as fed basis) corn 57.3%, wheat bean 25.0%, soybean meal (44% CP) 13.5%, soybean oil 0.5%, limestone 1.6%, monocalcium phosphate 0.6%, sodium chloride 0.5% and vitamin and mineral premix 1.0%. Its nutrient analysis was DM 85.5%, ash 6.3%, ME 12.7 MJ/kg, crude fat 4.0%, crude protein 14.3% and crude fiber 4.4% (calculated values based on table of feeding value of animal feed ingredients by Centraal Veevoeder Bureau CVB, Lelystad, The Netherlands, 2007). Daily feed allowance differed according to the stage of gestation: days 0 to 30 = 3 kg/day; days 31 to 85 = 2.5 kg/day; and days 86 to day of transfer to farrowing room = 3 kg/day. Gestation feed was offered twice daily (08.00 and 14.00 h). From the day of transfer to the farrowing room and till farrowing, sows received 3.5 kg of the lactation diet. The lactation feed contained as main ingredients (as fed basis) corn 51.8%, wheat bean 20.0%, soybean meal (44% CP) 18.0%, soybean oil 4.0%, fish meal (70%CP) 2.5%, limestone 1.6%, monocalcium phosphate 0.6%, sodium chloride 0.5% and vitamin and mineral premix 1.0%. Its nutrient analysis was DM 86.0%, ash 6.6%, ME 13.7 MJ/kg, crude fat 7.4%, crude protein 16.8% and crude fiber 4.1%. Post farrowing, sows received the lactation diet starting at a level of 2 kg at farrowing that was onwards further supplemented by a quantity of 400 g per piglet. Maximum feed allowance during lactation was reached within the fifth day post-farrowing. Lactation feed was divided into three equal daily portions (08.00, 12.00 and 16.00 h). Feeding procedure was done manually by the same person throughout the trial.

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