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# Effect of dietary palygorskite on performance and blood parameters of lactating Holstein cows

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ABSTRACT

Sixteen lactating Holstein cows were used in an experiment to determine the effects of dietary palygorskite (Pal) supplementation on productivity and milk composition, and blood parameters. In the experiment, which started on week 12 postpartum, cows were allocated, after equal distribution relative to milk yield and lactation number (i.e., 2 or 3), into 2 treatments being Pal0 and Pal10 of 8 cows each. For a period of 12 weeks (i.e., weeks 12–24 postpartum), cows were fed one of two isonitrogenous (crude protein 178 g/kg, dry matter — DM basis) and isoenergetic (net energy for lactation 7.85 MJ/kg, DM basis) concentrates (12.3 kg DM/cow/day), alfalfa hay (5.4 kg DM/cow/day), corn silage (3.3 kg DM/cow/day) and wheat straw (1.8 kg DM/cow/day). The concentrate for treatment Pal0 (control) had no Pal, while that for treatment Pal10 contained 10 kg/t of Pal (as mixed basis), respectively. To examine the tolerance on excess of Pal feeding, 8 more lactating Holstein cows in an additional treatment group (Pal100, 100 kg Pal/t of concentrate, as mixed basis) were used for a period of 8 weeks (i.e., weeks 12–20 postpartum). The concentrate for treatment Pal100 was isonitrogenous and isoenergetic to treatments Pal0 and Pal10. Treatment Pal100 was solely used to determine the health status and behavior of cows and was not compared to the other treatments. All cows, including those in treatment Pal100, remained healthy until the end of the experiment without altering their behavior. In the 12-week experimental period, there were no differences between Pal0 and Pal10 treatments ($P > 0.05$) in milk fat (32.0 g/kg), protein (33.4 g/kg), lactose (48.9 g/kg) or ash (6.7 g/kg) contents. With increased Pal feeding, protein yield increased (0.82 vs. 0.86 kg/day; $P < 0.001$) and colony forming units (CFU) decreased (58.6 vs. 41.9 × 1000 CFU/ml; $P < 0.001$), but average milk yield (25.4 kg/day), other yields of components and somatic cell counts were not affected ($P > 0.05$). Moreover, some differences in blood parameters occurred ($P < 0.05$) between treatments. Dietary Pal supplementation, at levels up to 10 kg/t, in isonitrogenous and iso- (net energy) energetic diets for lactating cows improved cow milk yield and quality increasing protein yield and decreasing milk microorganisms. Lactating cows exhibit tolerance to excess of Pal feeding, at inclusion level 100 kg/t of concentrate, for up to 8 weeks.

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## 1. Introduction

Palygorskite (Pal) is among the world's most important and useful industrial clay minerals, and belongs to phyllosilicates (Brigatti et al., 2013; Galán, 1996; Murray, 2000). Pal is normally light tan or cream to brown in color, although it sometimes has a blue-green tint (Murray, 1991), and its chemical formula is $(Mg, Al, Fe^{3+})_5(Si, Al)_8O_{20}(OH)_2(OH_2)_4 \cdot 4H_2O$ (Carretero and Pozo, 2010). Pal consists of double silica tetrahedral chains linked together by octahedral oxygen and hydroxyl groups containing aluminum and magnesium ions in a chain-like structure (Murray, 1991). It is classed as a three-layer inverted structure and iron commonly substitutes for aluminum and magnesium in the octahedral layer, thus producing a moderately high charge on the structure (Murray, 1991). This layer charge and the high specific surface area give Pal an intermediate cation exchange capacity normally about 30 to 40 meq/100 g. The high specific surface area, the charge on the lattice, and the inverted structure, which leaves parallel channels through the lattice, give Pal a high absorption and adsorption capacity (Galán, 1996; Murray, 2000). These properties, along with Pal's elongated shape and high viscosity, make it useful in many industrial applications (Galán, 1996; Harvey and Lagaly, 2013; Murray, 2000).

Among other industrial uses, Pal can be used in the animal industry as aflatoxin adsorbent (Schell et al., 1993), small domestic animal bedding material (Konta, 1995), cat box absorbent and animal feed bondant (Galán, 1996; Murray, 2000), tannin adsorbent (Huang et al., 2008), excipient in pharmaceutical preparations (Carretero and Pozo, 2009),

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as well as antacid, gastrointestinal protector and antidiarrheic (Carretero and Pozo, 2010). Due to its absorption and adsorption properties, Pal has been tested on performance of weaned piglets (Zhang et al., 2013), growing pigs (Schell et al., 1993) and broiler chickens (Pappas et al., 2010). However, no evidence is available on the effect of dietary Pal supplementation in ruminants. Thus, the objective of this study was to evaluate Pal supplementation in diets of lactating Holstein cows, relative to performance and chemical composition of milk, as well as hematological values and serum biochemical constituents. Moreover, tolerance of cows on excess of Pal feeding was examined.

## 2. Materials and methods

### 2.1. Palygorskite

Pal was used in an experiment with lactating Holstein cows, at the Animal Research Institute, Hellenic Agricultural Organization — Demeter (Giannitsa, Greece; 40°44′ N, 22°27′ E). Pal was obtained from the Geohellas S.A. (Athens, Greece) as a little gray powder (AFG-60 mesh), and comprised of a minimum of 750 g/kg Pal and 250 g/kg bentonite–saponite. The main chemical components of the Pal supplement, which was mined in Greece, were: $SiO_2$ 560 g/kg, MgO 150.5 g/kg, $Fe_2O_3$ 106 g/kg, $Al_2O_3$ 49.7 g/kg, CaO 3.3 g/kg, $K_2O$ 3 g/kg, $Na_2O$ 0.6 g/kg, and Loss on Ignition (water content) 109.5 g/kg.

### 2.2. Experiment: Lactating cows

Sixteen lactating and clinically healthy Holstein cows were used to determine the effects of dietary Pal supplementation on productivity and milk composition, and blood parameters. All animals used in the experiment were cared for according to the applicable recommendations of the U.S. National Research Council (1996). Milk yield of the cows was recorded immediately before the commencement of the study. The mean milk yield at the beginning of the experiment was 28.03 ± 0.331 kg/day. Cows were allocated at the beginning of the experiment, after equal distribution relative to milk yield and lactation number (i.e., 2 or 3), into two treatment groups (Pal0 and Pal10) of 8 cows each and accommodated in individual tie stalls. All tie stalls were identical, with the same direction and orientation and the same covered area (3 $m^2$/cow), and all were equipped with similar troughs for providing grain concentrates, hay, silage and water. For a period of 12 weeks (i.e., weeks 12–24 postpartum), all cows were offered alfalfa hay (5.4 kg dry matter — DM/cow/day), corn silage (3.3 kg DM/cow/day) and wheat straw (1.8 kg DM/cow/day; Table 1), and a concentrate (12.3 kg DM/cow/day). The concentrate (Table 2) for treatment Pal0 (control) had no Pal, while that for treatment Pal10 contained 10 kg/t of Pal (as mixed basis), respectively. The inclusion level of Pal in treatment Pal10 was similar to that used in practice. Both concentrates were isonitrogenous and isoenergetic, according to the National Research Council (2001) nutrient composition values.

To examine tolerance on excess of Pal feeding, 8 more lactating and clinically healthy Holstein cows in an additional treatment group (Pal100) were housed, fed and cared similarly to treatments Pal0 and Pal10, for a period of 8 weeks (i.e., weeks 12–20 postpartum). The concentrate for treatment Pal100 contained 100 kg/t of Pal (as mixed basis), and was isonitrogenous and isoenergetic to treatments Pal0 and Pal10. The inclusion level of Pal in treatment Pal100 (tolerance study) was ten times higher compared to that in treatment Pal10, which is used in practice (efficacy study), as European Food Safety Authority requires (EFSA, 2011). Treatment Pal100 was solely used to determine the health status and behavior of cows and was not compared to the other treatments.

Feed intake was measured daily for each cow. Cows had free access to water and were machine milked twice daily at 07:00 and 18:00 h with an individual DeLaval (Thessaloniki, Greece) milking machine. During the experimental period, the health status and behavior of all cows were monitored on a daily basis. Milk yield was recorded weekly on a morning and afternoon milking. Milking was conducted at a vacuum level of 50 kPa, pulsation rate of 60 $min^{-1}$ and pulsation ratio of 50/50. Each week, during 13 morning and afternoon milkings for treatments Pal0 and Pal10 (i.e., 12th to 24th week postpartum) and during 9 morning and afternoon milkings for treatment Pal100 (i.e., 12th to 20th week postpartum), samples were collected from each cow, after cleaning and disinfecting the teats. The morning and afternoon milk samples of each cow, taken in proportion to the different yield, were finally pooled and kept refrigerated (+4 °C) until chemical analysis.

Blood samples were obtained from all 24 cows of treatments Pal0, Pal10 and Pal100 at weeks 12, 16 and 20 postpartum. The blood samples were obtained at 08:00 h from the coccygeal vein into vacuum tubes (10 ml) with citric acid as additive contained, and stored at +2 °C until hematological value analysis within 4 h according to Thrall and Glade Weiser (2002). In addition, blood samples were obtained from the coccygeal vein into vacuum tubes (10 ml) with no additive contained; then centrifuged at 1509 ×g for 15 min to obtain blood

**Table 1**
Chemical composition (g/kg) of alfalfa hay, corn silage and wheat straw (dry matter — DM basis).

| | Alfalfa hay | Corn silage | Wheat straw |
|---|---|---|---|
| Dry matter (as fed) | 900 | 351 | 921 |
| Crude protein | 189 | 88 | 49 |
| Crude fat | 33 | 32 | 15 |
| Neutral detergent fiber | 500 | 450 | 726 |
| Acid detergent fiber | 333 | 281 | 491 |
| Ash | 117 | 43 | 73 |

Values for alfalfa hay and wheat straw represent duplicate assays of two samples for each material.
Values for corn silage were obtained from the National Research Council (2001).

**Table 2**
Concentrate composition of lactating cow rations.

| | Treatment[a] | |
|---|---|---|
| | Pal0 | Pal10 |
| Ingredient composition (kg/t, as mixed) | | |
| Corn grain, ground | 400 | 400 |
| Barley grain, ground | 94 | 94 |
| Wheat grain, ground | 191 | 172 |
| Wheat bran | 100 | 100 |
| Soybean meal (435 g/kg CP) | 80 | 85 |
| Cotton seed meal (400 g/kg CP) | 100 | 100 |
| Vegetable fat | 0 | 4 |
| Palygorskite (Pal) | 0 | 10 |
| Limestone | 19 | 19 |
| Monocalcium phosphate | 6 | 6 |
| Salt | 4 | 4 |
| Vitamin–trace mineral premix[b] | 6 | 6 |
| Chemical composition[c] (g/kg dry matter-DM) | | |
| Dry matter (as fed) | 880 | 882 |
| Crude protein (CP) | 178 | 178 |
| Crude fat | 32 | 36 |
| Neutral detergent fiber | 169 | 168 |
| Acid detergent fiber | 72 | 71 |
| Ash | 32 | 32 |
| Calcium | 11.8 | 11.8 |
| Phosphorus | 6.9 | 6.9 |
| Sodium | 2.3 | 2.3 |
| Net energy for lactation (MJ/kg DM) | 7.85 | 7.85 |

[a] Pal0 = control treatment, Pal10 = treatment with 10 kg/t palygorskite.
[b] Premix contained 232 g/kg Ca and 48 g/kg P and supplied/kg of concentrate: 8500 IU vitamin A; 0.75 mg vitamin $B_1$; 1100 IU vitamin $D_3$; 21 mg vitamin E; 0.4 mg Co; 15 mg Cu; 1.32 mg I; 30 mg Fe; 57 mg Mn; 0.24 mg Se; 70 mg Zn.
[c] Concentrates were analyzed for dry matter, crude protein, crude fat and ash according to the Association of Official Analytical Chemists (1990), and for neutral detergent fiber and acid detergent fiber according to Van Soest et al. (1991). All other values were calculated from the National Research Council (2001) values.

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