

Guilliermondii (PG) and the combination of both and the base gestation diet on some inflammatory biomarkers and growth performance of nursery piglets from birth until 30 days after weaning. During the last week of gestation a total 20 sows (Large White × Large White) homogeneous for parity, BCS and farrowing date were randomly assigned to one of the four dietary treatments: CTRL, basal gestation diet without any supplement; PRO, basal gestation diet supplemented with 90 mg of BS; PRE, basal gestation diet supplemented with 6 mg of PG and PRE/PRO, basal gestation diet supplemented with 90 mg of BS and 6 mg of PG. Starting from farrowing until weaning the piglets received the powder of different treatment diluted with water the same supplement of their dams by means of an oral-syringe in the follow amount CTRL water; PRO, 9 mg of BS; PRE, 300 mg PG; 9 mg of BS +300 mg of PG for group PRE/PRO. During lactation piglets' health status were monitored every day while body weights were recorded at birth and on +7, +14, +28 days after. At weaning, the piglets were blood sampled to investigate the serum levels of Interleukin 1B (IL-1B), Interleukin 6 (IL-6), Interleukin 10 (IL-10) and interferon gamma (IFN- γ). The piglets of PRE-PRO and PRO showed higher body weights at 21 and 28 days after birth compared to the other two groups. Serum levels of IL-1B and IL-6 were lower ($p < 0.01$) in group PRE/PRO compared to the other three groups. IL-10 and IFN- γ were higher ($p < 0.01$) in groups CTRL and PRE compared to PRO and PRE/PRO. Our findings evidenced that the group of piglets that received probiotics alone or in combination with prebiotics (PRO or PRE/PRO) in the diet decrease significantly pro-inflammatory serum cytokines levels. Moreover, higher anti-inflammatory IL-10 levels may be the result of a more evident inflammatory status of the CTRL and PRE compared to PRO or PRE/PRO-groups.

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Horse transportation by air: routes, practices and welfare implications

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Horse transportation by air is common, but information regarding its routes, practices, and welfare implications is scant. Our study aimed to describe the routes, management, and welfare status of horses travelling by air. Data were collected from 77/402 horses travelling on 23 journeys. Information was gained from before to 5 days after the journey. The horses were moved for sale (67%), equestrian competitions (17%), racing (9%), breeding (6%), or relocation (1%). Belgium was the most frequent departure country (15/23 flights, 65%; 38/77 horses, 49%), followed by The Netherlands (4/23 flights, 17%; 20/77 horses 26%), Germany (2/23

flights, 9%; 7/77 horses, 9%), UK (1/23 flights, 4%; 6/77 horses, 8%) and the USA (1/23 flights, 4%; 6/77 horses, 8%). The USA was the most frequent destination (10/23 flights, 43%; 19/77 horses, 25%), followed by Japan (7/23 flights, 30%; 30/77 horses, 39%), South Africa (3/23 flights, 13%; 14/77 horses, 18%), Australia (2/23 flights, 9%; 8/77 horses, 10%) and New Zealand (1/23 flights, 4%; 6/77 horses, 8%). Before departure, horses stayed in quarantine (15 ± 15 days), and on the last day, were considered fit for transport. At the departure airport, 17% of horses showed nasal discharge and two horses had minor injuries. The horses were loaded into 2-horse (17% of horses) or 3-horse type (83% of horses) jet stalls, located before (38%), in between (31%) and after the wings (31%). Inside the stalls, the horses were untied (23% of horses), long (possibility to low head to knee height; 52% of horses) or short tied (25% of horses) and shaving was the most common bedding. Grass hay (89% of horses) or haylage (11% of horses) was offered *ad libitum*, while water was given *ad libitum* (10% of horses), every two (31% of horses) or every 3 h (59% of horses); almost all horses ate (12 ± 7 kg/horse) and drank (17 ± 13 L/horse) in transit. At the check performed before landing, one horse was found with a swelling on the chest and 30% of horses showed nasal discharges. At the arrival quarantine, four horses showed health problems, namely two cases of superficial lesions (cut on the back limb and skin rubbed raw at head level), one case of diarrhoea, and one case of fever (40.7°C). All horses recovered after 5 days. Overall, this preliminary study has increased our knowledge of transportation by air and its implication for horse health and welfare.

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Effects of milk exosomes from heat-stressed dairy cows on BME-UV1 cells

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Heat stress (HS) represents one of the main problems in the livestock sector and in dairy cows, affecting productivity, health, and general well-being. Recent studies have observed and increased release of membranous extracellular vesicles, exosomes (EXO) in particular, in subjects exposed to HS conditions.

EXO are membrane microvesicles of 30–100nm in diameter that contain a load of substances deriving from the cells including proteins, lipids, miRNA and DNA able to transmit signals to other cells. The present study aimed at verifying the effects of EXO, isolated from milk of 4 Holstein Friesian (FR) and 4 Brown (BR) lactating cows, reared in commercial farms, under thermo-neutral (TN) or heat stress (HS) conditions, on cellular responses in bovine mammary epithelial cell line (BME-UV1).

Preliminary step for removing cell debris and fat globules was performed before the separation of EXO from milk. Milk samples were then centrifuged at 10,000g for 30 min at 4 °C. The supernatant was collected and diluted in sterile PBS and ultracentrifuged at 100,000g for 1h at 4 °C to obtain the EXO. The EXO pellet was collected and further purified through size exclusion chromatography. After isolation and characterization EXO were used for *in-vitro* test. The BME-UV1 were grown in flasks of 75 cm³ at 37 °C until they reached confluence. Subsequently, 2.5×10⁴ cells were plated in 0.3 cm³ wells using FBS medium without EXO and incubated at 37 °C. After 24 h, the medium was replaced with medium enriched with EXO FR-TN, FR-HS, BR-TN, BR-HS, and without EXO (control). Before the addition of EXO to cells, EXO were purified using the Toxin Eraser Endotoxin Removal Kit. Cell viability rate was assessed by XTT test, cellular apoptosis susceptibility by using the Apo-ONE[®] Homogeneous Caspase-3/7 Assay kit and mRNA expression of genes linked to apoptosis by PCR-Real Time.

The results showed a reduction of cell viability in FR-HS compared to FR-TN and an increase of apoptosis (21.2%) in FR-HS compared with FR-TN. These results were also confirmed by an over-expression of pro-apoptotic Casp-3 gene in FR-HS compared to FR-TN. In contrast, no statistical differences were observed between BR-TN and BR-HS. These findings provide insight into the ability of EXO isolated from HS animals to modulate the cellular response and gene expression of BME-UV1 *in vitro* and highlighted that FR-HS and BR-HS EXO were able to induce a breed-specific response in BME-UV1 cells.

We aimed to assess the effect of a hemp cake-based diet on the behaviour of organically reared Lohmann White hens. We used four sub-groups of 25 animals each. Two sub-groups received a standard diet based on corn flour and soya cakes (50 animals; Group C), two others received the same diet, integrated with 30% hemp cake (50 animals; Group H). The following behavioral categories were identified during two preliminary *ad libitum* observation sessions: feeding, drinking, locomotion, inactivity, laying, exploration, self-grooming, dust bathing. Eight observation sessions were conducted at 1-week intervals from 9.00 to 13.00 using the instantaneous scan sampling technique (3 min sampling intervals). The location (nest, indoor, outdoor) and posture (lying, standing) were also registered. The day of observation was used as experimental unit. Data were subjected to ANOVA using diet, hour of observation and their interaction as factors. Egg production (number and weight of eggs per 25-hen sub-group) was recorded over a period of 10 weeks and subjected to ANOVA using diet as factor. Egg production was higher in Group H than in Group C both in terms of number (17.29 ± 1.29 vs. 13.25 ± 0.58; *p* < 0.05) and total weight (1119.47 ± 68.54 vs. 811.61 ± 44.2 g; *p* < 0.05). Hens from Group H tended to be located more often in the nest (0.26 ± 0.02 vs. 0.20 ± 0.02; *p* < 0.10) and tended to be observed more often inactive (0.21 ± 0.02 vs. 0.18 ± 0.03; *p* < 0.10) and less often feeding (0.17 ± 0.02 vs. 0.22 ± 0.02; *p* < 0.10). In addition, animals from Group H were observed more often laying (0.17 ± 0.01 vs. 0.12 ± 0.01; *p* < 0.05). The results concerning production, location and behaviour all converged towards the same implications as the hens fed a hemp integrated diet, possibly due to a higher level of satiety, ate less, were less active, had a higher production level and as a consequence were more often located in the nest. We conclude that the inclusion of hemp cake at 30% in the hen diet may promote egg production with a reduction of the activity expressed by laying hens.

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Effect of hemp cake-based diet on laying hen behaviour

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Surgical castration: does a non-pharmacological approach improve piglet welfare?

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