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 10,000*g* for 30 min at 4 °C. The supernatant was collected and diluted in sterile PBS and ultracentrifuged at 100,000*g* 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 cm^3 at 37 °C until they reached confluence. Subsequently, 2.5×10^4 cells were plated in 0.3 cm^3 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.

P158

Effect of hemp cake-based diet on laying hen behaviour

Ada Braghieri[a], Claudia Lambiase[b], Francesco Serrapica[b], Roberto Di Matteo[b], Amelia Maria Riviezzi[a], Fabio Napolitano[a] and Giuseppe De Rosa[c]

[a]*Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy*
[b]*Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici, Italy*
[c]*Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici (Napoli), Italy*

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 subgroup) 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.

Acknowledgements
This research was funded by MIPAAF within the project 'PROteine per la FILiera Avicola – PROFILA'.

P224

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

Gaia Pesenti Rossi[a], Sara Barbieri[a], Ambra Motta[b], Alessandro Gastaldo[c], Alessandro Pecile[a], Elisabetta Canali[a], Michela Minero[a], Laura Martinelli[a], Giacomo Cavallaro[d] and Emanuela Dalla Costa[a]

[a]*Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy*
[b]*Centro Ricerche Produzioni Animali – CRPA Soc.Cons.p.A., Reggio Emila, Italy*