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## Risk factors associated with the expression of cross-sucking and intersucking in buffaloes

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The incidence and the potential risk factors for cross-sucking in calves and intersucking in lactating cows were assessed in 73 buffalo farms located in the area of PDO 'Mozzarella di Bufala Campana'. Two trained assessors collected the data through a questionnaire including resource and management measures, which were directly taken and asked to the farmer, respectively. The incidences of cross-sucking, intersucking and mortality were also asked to the farmer. The farm was used as experimental unit. For each discrete risk factor, an ANOVA was performed to assess the association with outcome variables (i.e. the incidence of cross-sucking and intersucking), whereas for continuous risk factors the association was tested using Pearson correlation coefficients. Cross-sucking and intersucking were observed in 91% and 45% of the farms, respectively; their mean incidences were  $21.9 \pm 22.3$  and  $1.2 \pm 1.9$  (mean  $\pm$  SD), respectively. Cross-sucking was negatively correlated with the number of calves in the collective pen ( $n = 34$ ,  $r = -0.47$ ,  $p = .01$ ). Furthermore, cross-sucking tended to be positively correlated with calf mortality from 24 h to weaning ( $n = 34$ ,  $r = .27$ ,  $p = .11$ ). The incidence of intersucking was positively correlated with the number of buffalo cows on the farm ( $n = 73$ ,  $r = .26$ ,  $p = .03$ ), the number of lactating buffalo in the pen ( $n = 73$ ,  $r = 0.23$ ,  $p = .04$ ), the percentage of weaned calves cross-sucking ( $n = 73$ ,  $r = .29$ ,  $p = .01$ ), the percentage of heifers and pregnant heifers cross-sucking ( $n = 73$ ,  $r = .61$ ,  $p = .001$  and  $n = 73$ ,  $r = .52$ ,  $p = .001$ ; respectively). As expected the percentage of buffalo cows with nose-ring was highly correlated with the incidence of intersucking ( $n = 73$ ,  $r = 0.75$ ,  $p = .001$ ). Moreover, the expression of cross-sucking in the categories of weaned calves, heifers and pregnant heifers induced a higher incidence of intersucking ( $F_{1,69} = 5.5$ ,  $p = .02$ ,  $F_{1,70} = 28.8$ ,  $p = .0001$  and  $F_{1,71} = 15.5$ ,  $p = .0002$ ; respectively). We conclude that appropriate management strategies should be implemented in order to reduce the incidence of cross-sucking and intersucking. In addition, the onset of cross-sucking in replacement stock, such as calves and heifers, may favour the development of intersucking in their later lives.

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## How can we understand the emotional state of cattle for welfare evaluation purpose?

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This study explored whether the visible eye white and ear posture can reliably contribute to interpret the level of arousal in dairy cows. The research was conducted in five Italian farms. Lactating cows were loose housed in cubicles, except in farm 3 (tie stalls), and they were fed a TMR, except in farm 5 (ventilated hay and fresh grass). In farm 5, cows also had daily access to pasture. More than 500 photos of cows' heads were taken during feeding, resting, grazing and an avoidance distance test at the feeding rack (ADF). From these photos, for each animal we classified eye white as 'high arousal' (EWhigh = 1: eye white visible), and 'low arousal' (EWlow = 0: no visible white, including half-closed eye); ear posture was classified as 'high arousal' (EPhigh = 1: ear held upright or horizontally), and 'low arousal' (EPlow = 0: ear held backwards or hung down). Eye white and ear posture were significantly correlated (Spearman rank correlation test:  $r = 0.482$ ;  $p = .001$ ). The context significantly affected the level of arousal (Chi Square test:  $p = .001$ ): the highest percentage of EWhigh (95.5% of cows) and EPhigh (70.1%) were recorded during ADF test, as the most stressful situation, whereas the highest percentage of EWlow (77.3%) and EPlow (86.4%) were observed during grazing, when cows are usually relaxed. During feeding, the highest level of arousal was observed in farm 3 (EWlow = 28.6%), where cows were housed in tie stalls, and 2 (EWlow = 55.1%), that had the lowest ratio feeding places/cow (0.68), and the lowest level of arousal was recorded in farms 4 and 5 (EWlow = 81.8% and 81.3%, respectively; Chi-Square test:  $p = .028$ ). In farm 5, all cows with low arousal had half-closed eyes, which suggests that they were in a positive mood, probably due to the high number of feeding places/cow (1.72 feeding places/cow) and to the preference of cows for hay and grass, rather than for TMR distributed in all other farms. During testing, the level of arousal was significantly lower in farms 4 and 5 (Chi Square test:  $p = .001$ ), that had the higher proportion of cubicles per cow (1.15/cow and 1.12/cow, respectively): EWlow was 85.4% in farm 4 and 100.0% in farm 5 and EPlow was 72.1% in farm 4 and 92.6% in farm 5. Results suggest that eye white and ear posture are indicators of the level of arousal in dairy cows, which may depend on some

resource-based factors, and support their inclusion for welfare evaluation.

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### Behaviour assessment of different poultry genotypes organically reared with computerised video recording system

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The Regulation (EC) n. 889/2008 suggests that in organic production the breed choice should take into account the capacity to adaptation to local conditions. Unfortunately, the choice of genotypes is not compulsory and often the farmers base the choice only upon the productivity aspect.

The aim of the present study was to evaluate the behaviour of six different chicken genotypes organically reared using a computerised video recording system. The studied genotypes were: Ranger Classic (R1) Ranger Gold (R2), Rowan Ranger (R3), Campese (C), CYgen5 × JA87 (CY), M22 × JA87(M). The behaviour analysis was carried out through a Media Recorder for the video capture. The evaluation was done on the initial interest of bird and its activity from 5 to 10 m to the shelter. All the videos were elaborated using the Observer XT with a coding scheme that permits the identification of each behaviour. The main analysed behaviours were: resting, walking, roost, eating feed, eating grass, drinking and comfort. Data were statistically elaborated with a linear model and Bonferroni multiple *t*-test. The results showed a higher initial interest of the R3 and C genotypes, on the contrary, CY and R performed a low exploratory activity. Moreover, no subject, belonging to the M genotype, leave the shelter during the established time unit (5 minutes).

At 5 m from the shelter the genotypes did not show any difference on the walking behaviour. R3 had the lower frequency of rest and C the higher, CY exhibited intermediate frequencies. The roost behaviour showed a large variability between genotypes: R3 genotype exhibited the lower frequency; M and R1 were the genotypes that most expressed this behaviour while C, R2 and CY genotypes showed intermediate frequencies. The most frequent feed

behaviour was observed in R2 genotype, while the R3 showed higher grass consumption.

At 10 m from the shelter the M and CY genotypes were observed occasionally due to their low explorative and kinetic activity. Concerning walking and resting behaviours there was no significant difference between the genotypes. R3 showed a low roosting behaviour followed by C. At this distance, R3 exhibited higher feed consumption while C higher grass consumption.

In conclusion, the behavioural analysis suggests that CY and M genotypes showed less adaptability to the organic rearing systems, on the contrary, C and R3 seem more suitable due to the active behaviours and grass intake at both the distances from the shelter.

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## LIVESTOCK SYSTEMS – COMPANION ANIMALS II

## O170

### Interplay between salivary biomarkers and gut microbiome in dogs

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The study aimed at evaluating the role of a standardised polyphenol phytocomplex from grapes (GP) on the microbiome-gut-brain axis in dogs. For the trial, 24 dogs were recruited in a local shelter. The animals were housed in separated box and were adult, clinically healthy and of both sex and of mixed breed or different pure breeds. The dogs were assigned to 3 groups of 8 subject each and fed the same commercial kibble diets for 2 months before the beginning of the study. One group (CTR) received a placebo tablet without GP, the second group (D20) and the third group (D60) was supplemented with tablets to achieve 1 or 3 mg/kg live weight of GP, respectively. At day 0 (T0), after 14 (T14) and 28 (T28) days, faecal and saliva samples were collected from each dog. Furthermore, at T0 and T28 the hair was also collected. The DNA of faecal samples were extracted with commercial kit and sequenced in 300 bp pair ends for V3 and V4 regions of the 16S rRNA using an Illumina platform. Reads were annotated using the QIIME bioinformatic pipeline and operational taxonomic units used to build the taxonomic tree. Faecal samples were also analysed for short chain fatty acids (SCFA) and lactic acid (LA). The serotonin and cortisol concentrations of saliva and cortisol in hair were analysed with ELISA. The supplementation of GP to the canine diet caused a significant increase ( $p < .05$ ) of serotonin and serotonin/cortisol ratio in the D60 group in comparison to CTR and D20 groups, suggesting a positive interplay between