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### Can haylage-based diets be a sustainable alternative to concentrate-based diets for finishing yeals?

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High quality forage-based diets for finishing cattle will reduce competition for human edible food resources, Portuguese dependence on cereal imports and protein rich commodities and potentially the carbon footprint of the operation. Sixteen crossbred Limousine × Alentejana 7 months weaned veals, were individually housed and randomly assigned to two diets during 64 days of finishing. The control diet (C) was based on a commercial concentrate and the Total Mixed Ration diet (TMR) contained 54% DM (dry matter) haylage, 36% DM concentrate and 10% DM sunflower seed. The haylage for TMR used was from a biodiverse mixture of annual species. The diets were formulated to have 16% DM of crude protein and were offered ad libitum. Intake was controlled daily and live weight fortnightly. Methane production was individually measured, using a GreenFeed unit (C-Lock, Rapid City, SD, USA). A Life Cycle Analysis was conducted to estimate the carbon footprint of the feeding systems. DM intake was 7.6% higher with TMR, corresponding to higher fat and fibre and lower starch intakes. Daily weight gain was not affected by the diets, averaging 1,598±g/day. Dry matter conversion ratio was 43% higher for TMR but feeding cost was not affected by the diet when normalized by live weight gain (2.37 E/kg). Concentrate consumption was 46% lower with TMR. Although methane production was 75% higher, the total carbon footprint of the TMR diet was slightly lower when compared to the Control. So, haylage-based diets did not compromise growth performance or meat quality of crossbred yeals and had small impact on the carbon footprint of yeals finishing phase when compared to conventional concentrate-base diets. Work funded by PDR2020 program through the FEADER under the project LegForBov (PDR2020-101-031179).

Session 38 Poster 15

# Improving sustainability of dairy cattle farms through the use of Limousin and Charolais beef sires

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The elimination of milk quotas at EU level resulted in restructuring of the dairy sector, intense cross-border milk commerce, increased volatility in production and prices, including a reorientation of some farmers towards beef production. Moreover, male veal-calves from Holstein-Friesian dairy breeds are regarded as uneconomical for fattening, given their slow growth rates and lower carcass attributes. The aim of the current research was to evaluate the use of Limousin and Charolais French beef sire breeds for crossbreeding with the Romanian Black and White Spotted HF dairy breed (n=20), with the ultimate purpose as to test their F<sub>1</sub> progeny performances for meat production and reproduction efficiency. At the age of 14 months the F<sub>1</sub> Limousin × Romanian Black and White Spotted heifers had on average 376.6±23.73 kg, while the F<sub>1</sub> Charolais × Romanian Black and White Spotted heifers had on average 459.67±21.63 kg, with differences between the two genotypes being significant (P≤0.01). Age at first conception was on average of 16.7±1.05 months for the Limousin sired heifers and of 20.3±2.63 months for the Charolais crossbreeds, differences between genotypes being significant ( $P \le 0.05$ ). Based on current preliminary results, when crossbreeding dairy breeds, the Limousin sires are recommended for farms which desire to transition to beef production, throughout the use of maternal composites, given the better performance of the Limousin crosses under semi-intensive systems when reproduction efficiency of heifers is concerned. Furthermore, in order to develop a beef composite breed, the F<sub>1</sub> Limousin and Charolais sired heifers were crossed Piemontese breed, to produce a three-way cross, in order to obtain animals with high genetic potential for growth rates and prime quality meat. The use of French beef sires for crossbreeding in Romanian dairy enterprises could represent a feasible alternative for cattle farmers to diversify their production, to produce calves with higher growth rates and improve the overall farm returns, while taking advantage of the heterosis effects and breeds complementarity.

Session 38 Poster 22

### Evaluation of carcass electrical stimulation on meat quality traits of F1 Angus-Nellore cattle

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Carcass electrical stimulation (ES) of medium voltage (120 V, 60 Hz) was evaluated in carcass of F1 Angus-Nellore bulls and heifers (30 per group) feedlot finished. At slaughterhouse, the carcasses were properly identified, washed and, before cooling, the left half carcass of each animal was submitted to ES for 10 seconds. The right half carcass of the same animal did not receive ES (control). Before deboning, the carcasses of all animals were evaluated for carcass weight, ossification, ribeve area, fatness, marbling score and final pH (48 h). Subsequently, between the 12th and 13th ribs (Longissimus thoracis - LT) meat samples were collected for physical-chemical analyses at four aging times (7, 14, 21 and 28 days). Other LT samples were used in sensory quality tests with the consumer, aged for 14 and 21 days. The ES did not influence (P>0.05) meat quality traits such as purge loss, cooking loss or meat colour (L, a\*, b\*, Chroma and Hue). On the other hand, shear force (SF) tended (P<0.10) to change in response to ES, with lower SF values (more tender meat) being observed more frequently in the ES treatment when compared to the control. Additionally, it was found that consumers tended (P<0.10) to attribute higher tenderness scores to meats from ES treatment. However, the same trend was not observed for flavour in meats aged for 21 days. The juiciness and overall satisfaction variables were not influenced by treatments (P>0.05) in the two aging times evaluated (14 and 21 days). Significant (P<0.01) and positive correlations were found between sensory tenderness, general satisfaction and flavour (r>0.70). In general, correlations between objective and sensorial variables of meat quality traits were of low magnitude or not significant (P>0.05). In this study, ES in half carcass did not result in consistent change in the meat quality traits of crossbred cattle (bulls or heifers) feedlot finished.

Session 38 Poster 23

## Biodiverse haylages in beef fattening diets - Effect on lipid oxidation of cooked meat

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In Portugal, bulls and heifers are fattened with concentrate-based diets mainly composed of cereals and oilseed derivatives, raw materials in which country is strongly dependent on the external market. Currently is possible produce high-quality havlages, even when the climate conditions in Spring are unfavourable. Under the project 'LegForBov - Alternative feeds in beef production', haylages were used to replace part of conventional raw materials and, thus, reduce external dependence and the environmental impact of beef production systems. In 3 experiments, haylage produced from a biodiverse mixture (Speedmix - Fertiprado, Portugal) was included at different levels (50, 54, 60 and, 67.5% DM) in bull and heifer diets (64 animals). The diets with 54 and 67.5% DM haylage contained sunflower seed (10% DM). The effect of haylage on meat lipid oxidation was compared with a conventional concentrate-based diet. Lipid oxidation (TBARS) was evaluated in muscle samples stored under vacuum at 2 °C from 3 to 14 days after slaughter and maintained at 4 °C during 0 and 3 days after cooking. On cooking day, the meat lipid oxidation was lower in haylage diets (P<0.001; 0.12 mg malonaldehyde (MDA)/kg muscle) than in concentrate-based diet (0.18 mg MDA/kg muscle). In cooked meat preserved for 3 days, lipid oxidation was not affected by the diet, averaging 3.09 mg MDA/kg muscle. The higher contents of antioxidant compounds, such as phenolic compounds in haylage diets than in concentrate diet (8.93 vs 1.84 mg gallic acid equivalents/g DM, respectively), may have contributed to the meat lipid protection. However, in more severe oxidative conditions (cooking and storage) the antioxidant effect of haylage was not enough to limit the lipid oxidation of meat. Funding: Project LegForBov (PDR2020-101-031179; PDR2020-101-031184) - FEADER; Projects UIDB/05183/2020 (MED) and UIDB/00276/2020 (CIISA), and PhD grants awarded to LC (2020.05712.BD) and LF (2020.04456.BD) - FCT.

Session 50 Poster 20

Are all the rumen ciliate protozoa equally related with the enteric methane emissions of ruminants?

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Due to their high fermentative activity, ciliate protozoa (PTZ) are the main rumen producers of hydrogen, that is then used by the methanogenic archaea to produce methane (CH<sub>4</sub>). The CH<sub>4</sub> has an impact as greenhouse gas 25 times higher than  $CO_2$ , and as ruminant digestive emissions contribute relevantly to the total anthropogenic  $CH_4$  emissions, strategies for their mitigation are required. The elimination of PTZ from rumen has been proposed to reduce the CH<sub>4</sub> emissions from ruminants. However, there is still a strong lack of knowledge on the links between PTZ taxa and rumen methanogenesis. This study evaluated the relationships between the PTZ genera present in the rumen fluid of 35 young crossbred beef bulls, used in two experiments and fed 6 different diets and the correspondent quantity of gas and CH<sub>4</sub> produced. Rumen fluid was collected at slaughter and PTZ enumerated and identified at genus level by optical microscopy. The total gas and CH<sub>4</sub> production were evaluated in vitro in glass bottles with gas detectors (Ankom system), incubating for 48 h the individual rumen fluid samples with the diet provided to the donor during the feeding experiment. PTZ data were subjected to a regression analysis, removing the variation due to the diet and considering the experiment as a random effect. Total abundance of PTZ (log10cells/ml rumen fluid) was not related with total gas or CH<sub>4</sub>. However, total gas production increased linearly with Entodinium (P=0.017) and decreased with Epidinium (P<0.001) counts. The CH<sub>4</sub> production decreased linearly with Epidinium counts (P<0.001) and the CH<sub>4</sub> proportion (% of total gas) decreased linearly with *Isotricha* counts (P<0.001). Ruminal total gas and  $CH_A$  production seems to depend on the PTZ genera present in the rumen fluid, being Epidinium and Isotricha related with lower CH<sub>4</sub> emissions. Work funded by the PDR2020 program through FEADER (LegForBoy project- PDR2020-101-031179) and by the Fundação para a Ciência e a Tecnologia (FCT) (PtzR Methane project - EXPL/CAL-ZOO/0144/2021).

Session 50 Poster 21

### Zinc-methionine alters the oxidative and inflammatory status of dairy cows under heat stress

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Modern dairy cows undergo thermal stress starting at an average temperature-humidity index (THI) of 68, whereas at THI>72 markedly hinders animal's productivity. Heat stress exacerbates oxidative stress and reactive oxygen species production in dairy cows. Alteration of oxidative metabolites in cows would influence the cytokine production during heat stress. Present work aimed to observe impact of a commercially available rumen-protected Zinc-Methionine complex (RPZM; Loprotin, Kaesler Nutrition GmbH, Cuxhaven, Germany) supplementation in early high producing Holstein cows during environmental heat stress. Sixty-two multiparous lactating Holstein cows [balanced by days in milk (mean ± SD)=28±7 d; lactation number = 2.9±0.6] were randomly assigned to one of two dietary treatments [total mixed ration with RPZM (LP group) or without the RPZM inclusion (CON group)]. RPZM was included as 0.131% diet DM for a total period of 6 weeks. Blood sampling was conducted bi-weekly via puncture of the coccygeal vessels and obtained serum was subjected to analyses for non-esterified fatty acids (NEFA), calcium, albumin, haptoglobin, total antioxidant status (TAS), malondialdehyde (MDA) and interleukin-1 beta (IL1-B). Data was statistically analysed using the Proc Mixed procedure of SAS for a completely randomized design with repeated measures. Circulating NEFA concentrations were not significantly different between the experimental groups (P>0.05). Blood calcium concentration in the LP group was clearly higher in comparison with the control (P<0.01). RPZM supplemented cows tended to have higher albumin concentration than control (P=0.08). Blood serum haptoglobin was evidently lower in cows in the LP group (P=0.001). Dairy cattle in the LP group had significant (P=0.017) higher blood TAS along with lower MDA concentration (P=0.08). Circulating IL1-B concentration in animals supplemented with RPZM was markedly lower in comparison with the CON (P=0.001). Current work underlines improved anti-oxidative capacity and lower systematic inflammation in dairy cattle fed the RPZM under environmental heat stress.