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Methane emission and energy partitioning in Hanwoo Steers supplemented with seeds of *Pharbitis nil*

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Two *in vivo* experiments were conducted to evaluate the potential of *Pharbitis nil* seeds (PA) as an anti-methanogenic feed additive to ruminants. In experiment 1, six Hanwoo steers (411±57 kg) were fed either total mixed ration (TMR; Control) or TMR supplemented with PA at 5% dry matter intake (DMI; PA5) for two consecutive periods of 35 days in pairwise comparison. Faecal and urine output were measured in apparent digestibility trial. The methane (CH₄) and heat energy (HE) were measured using respiratory chambers equipped with gas analysers. Although no differences (P>0.05) in nutrients or gross energy intake between the periods, an increase (P<0.05) in apparent digestibility of DM (8%) and neutral detergent fibre (18%) was observed in PA5. A pronounced decrease (P<0.05) in CH₄ yield (16%) and urinary energy (30%) and nitrogen (N) excretion (25%) was noted in PA5 which led to an increased metabolisable energy intake by 16%. However, only numerical increase (P>0.05) in retained energy and average daily gain was noted due to increase in HE loss. In experiment 2, five rumen cannulated Holstein steers (744±35 kg) were assigned to PA5 diet for 40 days to study the rumen fermentation characteristics. Supplementing PA decreased (P<0.05) rumen ammonia concentration and pH, associated with an increased (P=0.091) rumen short-chain fatty acid concentrations at 3 h post feeding. A 29% increase (P<0.05) in propionate proportion clearly reflected a shift in the ruminal H₂ sink in PA5. A 40% reduction (P=0.067) in relative abundance of *Entodinium caudatum* supported by the *in silico* binding of secondary metabolites from PA to the cyclic GMP (cGMP)-dependent protein kinase (cGK) of *E. caudatum* was also noted. Overall, seeds of *P. nil* could be a potential alternative for ionophores in reducing CH₄ and N emission from livestock production.

Silages of agro-industrial by-products in lamb diets – effect on performance and methane emissions

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The use of agro-industrial by-products in animal feed is an opportunity to value these products and ensure low-cost feedstuffs. We aimed to produce silages based on by-products of carrot, sweet potato, potato, and tomato and integrate them in lamb diets replacing 50% of a conventional feed. The effect on animal performances and methane production was evaluated. Three silages were prepared with 35% tomato pomace + 20% wheat bran + 15% grass hay associated with 30% of potato (Psil) or 30% sweet potato (SPsil) or 30% of carrot (Csil). Thirty-two lambs were divided into four groups fed with: 85 concentrate/15% hay (control); 50% of concentrate and 50% of Psil or SPsil or Csil. After 6 weeks animals were slaughtered, and rumen content collected. The rumen fluid of each animal was incubated for 48 hours with 1 g DM of the lambs' diets in fermentation bottles with gas detectors (Ankom Tech.). The pressure and temperature in the bottles were recorded every 20 minutes during the incubation period and converted into ml of gas produced. Methane was quantified in gas by gas chromatography. No differences among diets were detected in dry matter (DM) and crude protein (CP) intake, averaging 1,046 g and 170 g respectively. Animals fed silage diets ingested more NDF (184 g/d) than those fed the control diet (312 g/d). The ADG was lower with silage diets than in control (316 vs 348 g/d, respectively). Among diets, no differences were found in DM conversion ratio (2.84). Silage diets did not affect the gas and methane production, averaging 204 ml and 46 ml/g DM, respectively. By-product silage can be a good strategy to replace part of the concentrate feed in lamb diets without increase methane production. This work is funded by project SubProMais (PDR2020-101-030988; PDR2020-101-030993), FCT – Foundation for Science and Technology projects UIDB/05183/2020 MED and UIDP/CVT/00276/2020 (CIISA), and the PhD grant awarded to LC (2020.05712.BD).

Difference and regression methods give similar energy values for dried brewers' spent grains in pigsL. Piquer¹, A. Cerisuelo¹, S. Calvet², C. Cano¹, D. Belloumi¹ and P. García-Rebollar³¹Instituto Valenciano de Investigaciones Agrarias, CITA, Polígono de la Esperanza 100, 12400 Segorbe, Spain, ²Universitat Politècnica de València, ICTA, Camí de Vera s/n, 46022 Valencia, Spain, ³Universidad Politécnica de Madrid, Dpto. Producción Agraria, ETSIAAB, Av. Puerta de Hierro, 2-4, 28040 Madrid, Spain; piquer.lai@gmail.com

The use of local fibrous by-products dehydrated by environmentally friendly drying procedures can contribute to increase sustainability of pig feed. The digestible energy (DE) content of brewers' spent grains (BSG) dried with two different low temperature drying processes, based on biomass or solar energy sources for air heating, were determined through the difference and the regression methods. Thirty growing pigs of 59.9±3.66 kg body weight were distributed in five experimental diets, allowing for two replicates in two periods and six replicates per diet. The experimental diets included a basal and four additional diets in which the energetic part of the basal diet was replaced by 150 or 300 g/kg of each BSG. Experimental periods consisted of 14 days of adaptation to diets and 4 days of total collection of faeces per animal. Feed intake and faeces production were quantified per animal and analysed for dry matter (DM) and gross energy content. The coefficient of total tract apparent digestibility of energy was then determined by diet. The final amount of DE in both BSG was calculated by difference (using 0 and 300 g/kg treatments; MIXED procedure in SAS) or regression (using all treatments; REG procedure in SAS) and compared. The DE values obtained using the difference procedure were considered not different from those obtained using linear regression if the values were within the 95% confidence interval (CLB statement in SAS) for the DE estimated using linear regression. The DE calculated using the difference method were 12.6 kJ/kg DM for biomass and 11.4 kJ/kg DM for solar dried BSG. The values obtained for DE using the regression method were 13.1 kJ/kg DM for biomass and 11.1 kJ/kg DM for solar dried BSG. The difference and regression procedures do not give different DE values for each type of BSG.

Agro-industrial by-products in silages for lactating ewesE. Jerónimo^{1,2}, L. Cachucho^{2,3}, D. Soldado^{2,3}, O. Guerreiro^{1,2}, H. Alves⁴, S. Gomes⁵, N. Alvarenga⁵, K. Paulos⁵, C. Costa⁵, J. Costa⁵, J. Santos-Silva⁵ and T. Dentinho⁵¹Mediterranean Institute for Agriculture, Environment and Development, MED, Beja, Portugal, ²Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, IPBeja, Beja, Portugal, ³Centro Investigação Interdisciplinar em Sanidade Animal, CHISA, Lisboa, Portugal, ⁴Sociedade Agropecuária Carlos e Helder Alves, Funcheira, Ourique, Portugal, ⁵Instituto Nacional de Investigação Agrária e Veterinária, INIAV, Santarém, Portugal; liliana.cachucho@cebal.pt

The use of by-products in animal feed can be a strategy to reduce external dependence on conventional raw materials and associated feeding costs. The objective of this work was to evaluate the effect of using silages containing by-products in lactating ewes diets on milk quality and lamb growth compared to a concentrate-feed based diet. Fifty-four Merino crossbred ewes at the end of gestation and during lactation were fed either concentrate or two silages supplemented with concentrate. Silages were composed by alfalfa hay, brewers grains and sweet potato or almond hull. All groups received hay *ad libitum*. Eighteen ewes from each diet and their respective lambs were randomly distributed among 3 pens. Lambs were weighed at birth and weekly throughout 8 weeks of trial. Milk samples were collected for analysis. The intake was monitored daily, and the feeding cost was determined. The use of silages with by-products increased ($P=0.017$) the milk fat content compared to concentrate (7.81 vs 7.12%). Protein, lactose, dry residue and fat-free dry residue content, pH and somatic cell count of milk were not affected by the dietary treatments. Lambs average daily gain was not affected by the diet fed to ewes ($P=0.231$, 0.271 g/day). Replacement of concentrate by silages reduced up to 37% the feeding costs of ewes during lactation. Results show that the use agro-industrial by-products silages are a promising approach for its use in lactating ewes' diets, with reduced feed costs without compromising the lamb growth. This work is funded by project SubProMais (PDR2020-101-030988, PDR2020-101-030993) through FEADER and by the projects UIDB/05183/2020 (MED) and UIDB/00276/2020 (CHISA), and the PhD grant awarded to LC (2020.05712.BD) funded by national funds through FCT – Foundation for Science and Technology.

Use of almond hull in lamb diets – effect on growth performance

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Almond production is growing in the Mediterranean region, particularly in southern Portugal, with increased availability of its by-products, such as almond hull (AH). Almond hull, which consists of the green outer covering of the almond, represents 52% of the total fruit fresh weight and contains high sugar content (18-30% dry matter (DM)). The main objective of this work was to evaluate the effect of partial replacement of cereals in the diet by increasing levels of AH on the growth performance of lambs. Twenty-four ram lambs were individually housed and randomly assigned to the 3 diets (8 lambs per diet), with feed offered *ad libitum*. All diets included 40% dehydrated lucerne, 6% soybean oil, soybean meal 44, sunflower meal 28, and cereals (maize, barley, and wheat), which were stepwise replaced by AH, reaching 0% (control), 9% (AH9) and 18% (AH18) of AH in diets. All diets contain 14% crude protein. The trial started after an adaptation period of 7 days, and Average daily gain (ADG) and feed intake were evaluated over 6 weeks of the experiment. Preliminary results showed that partial replacement of cereal in the diets by the AH up to levels of 18% DM did not affect the growth of lambs ($P=0.283$; $ADG=383$ g/day) and the dry matter intake ($P=0.391$; 1,745 g/day). Diets with 18% of AH increased the feed conversion ratio ($P=0.013$; 4.96) compared to the diets with 0 and 9% of AH (4.37). The use of AH in animal nutrition could be a promising strategy to replace conventional raw materials, with no relevant effect on growth performance. Funding: Project SubProMais (PDR2020-101-030988, PDR2020-101-030993)-FEADER; Projects UIDB/05183/2020 (MED) and UIDB/00276/2020 (CIISA), and PhD grant of LC (2020.05712.BD)-FCT.

Colour stability and a-tocopherol content of meat from lambs fed *Cistus ladanifer* plant and extract

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Cistus ladanifer L. (CL) is an abundant shrub in Mediterranean countries, containing high levels of condensed tannins (CT) and other antioxidant compounds, such as a-tocopherol. Incorporation of CL aerial part or its CT extract in the lamb diets can improve the oxidative stability of meat. This study aimed to evaluate the effect of the inclusion of aerial part and CT extract from CL in lamb diets on meat a-tocopherol content and colour stability. Six diets were formulated considering three levels of CL CT (0, 1.25 and 2.5% CT) and two ways of CT supply (CL aerial part vs CL CT extract). Basal diet was composed of dehydrated Lucerne supplemented with soybean oil (60 g/kg). Thirty-six lambs were housed individually (six lambs/diet). The trial lasted for 35 days. Content of a-tocopherol was analysed in Longissimus thoracis (LT) muscle samples, immediately collected after slaughter. Meat colour was analysed, using CIELAB system, in LT muscle collected seventy-two hours after slaughter and stored at 2 °C for 0, 3 and 7 days. Muscle a-tocopherol content was not affected by dietary treatments ($P>0.05$). Meat colour coordinates L* and b* were affected by dietary treatments, with higher L* values in diets containing 2.5% CT than in diets with the lower CT levels ($P=0.018$). *C. ladanifer* CT extract resulted in meats with lower values of b* than CL aerial part ($P=0.028$). During storage time, colour parameters L*, b*, C* and H* increased, while a* decreased ($P<0.001$). In this work, the inclusion of CL aerial part and CT extract in lamb diets did not affect the a-tocopherol content or improve the colour stability of meat. Funding: Project CistusRumen (ALT20-03-0145-FEDER-000023) – FEDER; Projects UIDB/05183/2020 (MED) and UIDP/00276/2020 (CIISA), and PhD grant awarded to DS (SFRH/BD/145814/2019) – FCT.

Effects of guanidinoacetic acid on rumen microbial fermentation in continuous culture systemR. Temmar¹, M.E. Rodríguez-Prado¹, A. Kihal¹, V.K. Inhuber² and S. Calsamiglia¹¹Universidad Autónoma de Barcelona (UAB), Animal Nutrition and Welfare Service (SNiBA), Edifici V, Travessera dels Turons, Cerdanyola del Vallès, 08193 Barcelona, Spain, ²Alchem Trostberg GmbH, Dr.-Albert-Frank-Straße 32, 83308 Trostberg, Germany; temmarrokia@gmail.com

The objective of the study was to evaluate the effect of guanidinoacetic acid (GAA) on rumen microbial fermentation and nutrient digestion. The study was a randomized block design with 8 dual flow continuous culture fermenters and 2 periods. Treatments (n=4) arranged in a 2×2 factorial, with factors being the type of fermentation environment: dairy (pH between 5.8 and 6.8; diet 50:50 forage:concentrate, 17.1% CP and 30.0% NDF) or beef (pH between 5.5 and 6.5; diet 10:90 forage:concentrate, 16.3% CP and 17.6% NDF); and GAA: 0 vs 2 g/d. Temperature (38.5 °C), liquid (0.10/h) and solid (0.05/h) dilution rates were kept constant. Diets (90 g/d DM) were fed 3 portions/d. Effluent samples were collected from a composite of the 3 sampling days, and bacteria were isolated on the last day of each period from fermenters. Fermenter samples were taken 3 h after the morning feeding for microbiome analysis. Fermentation data were analysed with the PROC MIXED of SAS and the microbiome diversity and composition with R-Studio. Significance was set at P<0.05. No differences were observed on true OM (59.4±7.94%) degradability. Degradability of NDF (49.9 vs 31.2±3.79%), the proportions (mol/100 mol) of acetate (51 vs 44±1.7) and butyrate (19 vs 11±1.5), the acetate to propionate ratio (2.02 vs 1.12±0.091), NH₃-N concentration (8.18 vs 2.92±0.662 mg/100 ml), the flow (g/d) of total (2.83 vs 2.67±0.023) and ammonia (0.27 vs 0.09±0.021) N, the efficiency of microbial protein synthesis (32 vs 22±2.3 g N/kg OM truly digested) and the relative abundance and diversity of microbial population were higher in dairy than in beef. Total VFA (113 vs 100±1.9 mM) and the propionate proportion (41 vs 26±1.8 mol/100 mol) were higher in beef than in dairy. The GAA increased NH₃-N concentration (7.31 vs 3.78±0.662 mg/100 ml) and the flow (g/d) of total (2.97 vs 2.71±0.023) and ammonia (0.24 vs 0.12±0.021) N. The degradation of GAA was higher in dairy (69.8%) than in beef (6.3%). The use of GAA in dairy cow, but not in beef, may require protection.

Silages of agro-industrial by-products in lamb diets – effect on carcass and meat qualityK. Paulos¹, C. Costa¹, J. Costa¹, L. Cachucho^{2,3}, P.V. Portugal¹, J. Santos-Silva^{1,3}, J.M. Almeida^{1,3}, E. Jerónimo^{2,4} and M.T.P. Dentinho^{1,3}¹INIAV, Fonte Boa, Santarém, Portugal, ²CEBAL, Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja, Portugal, ³CIISA, Avenida Universidade Técnica, Lisboa, Portugal, ⁴MED, Mediterranean Institute for Agriculture, Environment and Development, Évora, Portugal; katia.paulos@iniav.pt

In Portugal large amounts of agro-industrial by-products are generated which can ensure low-cost feedstuffs. We aimed to evaluate the effect of silages produced with by-products of potato, sweet potato, tomato pomace and carrot on lamb growth performance, carcass composition and meat quality. Three silages were performed using 35% tomato pomace + 20% wheat bran + 15% of grass hay + 30% potato (Psil) or 30% sweet potato (SPsil) or 30% carrot (Csil). Good quality silages were obtained, with pH 4.16 (P), 3.97 (SP), and 4.10 (C). Thirty-two lambs were housed individually in parks and divided into four groups (8 animals/group) each group with the following diet: Control – 85% commercial concentrate and 15% oat/vetch hay; P- 50% concentrate and 50% Psil in DM; SP – 50% concentrate and 50% SPsil in DM; C – 50% concentrate and 50% Csil in DM. After 6 weeks of trial, the animals were slaughtered and the carcass and the meat were analysed for chemical, physical and sensorial characteristics. Colour and lipid stability over 7 days of storage at 4 °C was also evaluated. Carcass traits and shoulder tissues composition were similar among diets. With silage diets the L* of dorsal subcutaneous fat was higher than control, averaging 78.2 and 75.3 respectively. The Longissimus lumborum colour was affected by diets with a* and C* higher with C diet. No differences among diets were found on shear force, cooking losses, oxidative stability after 7 days of storage and sensorial characteristics. We can conclude that the partial replacement of concentrate-feed by by-product silages in lamb diets is a good option not compromising the quality of the lambs' carcasses and meat. This work is funded by project SubProMais (PDR2020-101-030988; PDR2020-101-030993) and by National Funds through Foundation for Science and Technology projects UIDB/05183/2020 (MED) and UIDP/CVT/00276/2020 (CIISA), and the PhD grant awarded to LC (2020.05712.BD).