377 Essential oils in feed: Development of a quantification method. D. Bellenot[1], V. Hocde[6], J.-Y. Anizon[2], Y. Riou[3], C. Ionescu[9], C. Genouel[5], C. Langella[4], T. Banchereau[8], S. Oguey[13], V. Guitton[11], A. Guyonvarch[12], P. Metra[7], F. Recoquillay[10], S. Kerros[10], P. Schupfer*[14], [1]*ITEIPMAI*, *Chemillé, France*, [2]*ARCHIMEX*, *Vannes, France*, [3]*TECALIMAN*, *Nantes, France*, [4]*DGCCRF-Marseille*, *Marseille, France*, [5]*DGCCRF-Rennes*, *Rennes, France*, [6]*CCPA DELTAVIT*, *Janzé, France*, [7]*LAREAL*, *Saint-Nolff, France*, [8]*TECHNA*, *Coueron, France*, [9]*AXISS FRANCE S.A.S*, *Bellegarde-sur-Valserine, France*, [10]*PHYTOSYNTHESE*, *Saint Bonnet de Rochefort, France*, [11]*INZO*, *Paris, France*, [12]*EVIALIS*, *Vannes, France*, [13]*PANCOSMA*, *Genève, Switzerland*, [14]*INTERVET-CRINA*, *Gland, Switzerland*.

A working group was created in France in order to develop an analytical method ensuring a complete traceability of feed additives based on essential oils (eo) during the whole process of production.

For the development of the method, three kinds of feed additives (encapsulated, mineral, vegetable) containing five eo markers (1,8-cineole, thymol, carvacrol, cinnamaldehyde, eugenol) were incorporated through premixes into three commercial-type final feeds (turkey, piglet, rabbit), so that the theoretical concentration of each analyte was 10 ppm (parts-per-million). Pelleting of the resulting meals led to the feed matrices used for developing the method.

Several chromatographic methods (GC/FID, GC/MS, HPLC/UV) and extraction techniques (steam-distillation, Soxhlet, ASE, Lickens-Nickerson, etc) were considered, as well as different extraction solvents (acetone, pentane, dichloromethane, etc). After the choice and the optimization of the entire analytical process, a first ring test was carried out.

The method adopted was as follows, first Soxhlet extraction of ground feed (20 g) in n-pentane (200 ml) for 20 cycles, followed by addition of azulene (0.1 mg) used as an internal standard (IS) prior to GC injection. For the ring test, participants (n=8) received an IS solution, four calibrating solutions and three feed samples (turkey, piglet, rabbit). The results achieved were promising for phenols (4.4-5.5 ppm found with <20% of relative standard deviation, RSD), but unsatisfactory for cineole and cinnamaldehyde (1.5-2.5 ppm; >30%RSD).

Further developments and ring tests are underway.

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Key Words: Essential Oil, Analytical Method, Feed

378 Orange pulp reduces growth of E. coliO157:H7 and *Salmonella* Typhimurium in pure culture and in vitro mixed ruminal microorganism fermentation. T. Callaway*[1], J. Carroll[2], J. Arthington[3], R. Anderson[1], T. Edrington[1], K. Genovese[1], and D. Nisbet[1], [1]*ARS/USDA, Food and Feed Safety Research Unit*, *College Station, TX*, [2]*ARS/USDA, Livestock Issues Research Unit*, *Lubbock, TX*, [3]*Range Cattle Research and Education Center, Univ. Florida*, *Ona, FL*.

Orange peel and orange pulp are by-products that are included in cattle rations in regions of the U.S. where citrus fruits are grown and processed. They are included in feedlot and dairy cattle rations due to their low cost, nutritional qualities, and palatability. The antimicrobial activity of citrus oil and other citrus-derived products have been previously reported. *Escherichia coli* O157:H7 and *Salmonella* spp. are human food borne pathogenic bacteria that can be carried in the gastrointestinal tract of cattle. Therefore, the present study was carried out to determine if these citrus by-products in a cattle ration exert antimicrobial effects on *E. coli* O157:H7 and *Salmonella* typhimurium populations. The specific growth rate of pure cultures (n = 3) of *E. coli* O157:H7 and *Salmonella* typhimurium were reduced ($P < 0.05$) by addition of 2% (w/v) orange pulp and orange peel. Ruminal fluid was collected from cattle (n = 2), diluted with growth medium containing 1 g/L soluble starch, and *E. coli* O157:H7 or *Salmonella* typhimurium were added to the ruminal fluid. The addition of orange pulp and peel to in vitro mixed ruminal microorganism fermentations (n = 2) demonstrated that both orange pulp and peel reduced *E. coli* O157:H7 and *Salmonella* typhimurium populations at least 2 log10 in mixed ruminal fluid fermentations. Other in vitro ruminal fermentations (n = 3) contained *E. coli* O157:H7 or *Salmonella* typhimurium and contained additions of: 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 % (w/v) of feed-grade orange pulp. Addition of orange pulp reduced ($P < 0.05$) *E. coli* O157:H7 populations from 10^5 to 10^2 CFU/ml and *Salmonella* typhimurium populations ($P < 0.05$) from 10^4 to 10^2 CFU/ml. These results indicate that orange pulp and/or peel included in ruminant rations could decrease ruminal populations of food-borne pathogenic bacteria. Further research is needed to determine if the antimicrobial activity of orange products against *E. coli* O157:H7 or *Salmonella* typhimurium continues in the lower gastrointestinal tract.

Key Words: Pathogen, Intervention, Food Safety

379 Effects of an experimental vaccine on *Escherichia coli* O157:H7 prevalence in the feces and colonized at the terminal rectum in beef feedlot cattle. R. Peterson*, D. Smith, R. Moxley, T. Klopfenstein, G. Erickson, and S. Hinkley, *Univeristy of Nebraska*, *Lincoln*.

A clinical trial was conducted to test the effect of vaccination against EHEC type III secreted proteins on the probability for feedlot steers to shed *E. coli* O157:H7 in the feces, and for animals to be colonized by this organism in the terminal rectum. Medium-weight steers (N=288) were assigned randomly to 36 pens (8 head/pen) and to vaccination treatment. Treatments included vaccination (3 doses at three-week intervals) or no vaccination. Steers assigned to the no-vaccination treatment received a dose of adjuvant at the same time as vaccinated steers. Fecal samples were collected (n=1,416) from each steer on d 0 (pre-treatment), and d 14, 28, 42, and 56 post-treatment by rectal palpation. Rectoanal mucosal samples were collected at slaughter (d 57 post-treatment) by scraping the mucosa of the terminal rectum 3-5 cm proximal to the rectoanal juncture. *E. coli* O157:H7 was isolated and identified from both types of samples using standard methods involving selective enrichment, immunomagnetic separation, agar plating, biochemical and immunological testing, and PCR confirmation. The outcome variables were recovery of *E. coli* O157:H7 from the feces or rectoanal mucosa. The outcome was analyzed using the GENMOD procedure of SAS accounting correlation of observations within pens and by repeated measures. Pre-treatment prevalence of *E. coli* O157:H7 differed ($P<0.10$) between treatments and averaged 6.4 and 1.4% in vaccinated and non-vaccinated pens, respectively. The probability for vaccinated or non-vaccinated steers to shed *E. coli* O157:H7 in the feces was not significantly different (OR=0.87, $P>0.10$). However, the probability for steers to be colonized by *E. coli* O157:H7 in the terminal rectum was greatly reduced (OR=0.02, $P<0.001$) for vaccinated (0.7%) compared with non-vaccinated (27.0%) cattle.

Key Words: Cattle, *Escherichia coli*, Vaccination

380 A novel concept for simultaneous deactivation of various mycotoxins in piglets. G. Schatzmayr*[1], D. Schatzmayr[1], V. Starkl[1], S. Nitsch[1], M. Forat[2], and E. Binder[3], [1]*Biomin GmbH*, *Herzogenburg, Austria*, [2]*Instituto Internacional de Investigacion Animal*, *Queretaro, Mexico*, [3]*Erber AG*, *Herzogenburg, Austria*.

Mycotoxins are secondary metabolites produced by many fungi under various conditions. In animal husbandry mycotoxins decrease productivity and increase disease incidence due to their immune-suppressive effects. In spite of all efforts to prevent formation of mycotoxins in feeds, significant contaminations still occur. Therefore strategies for detoxification have become very important. It has been known for years that clay minerals can be used for detoxification of

aflatoxins but also that these enterosorbents do not work on other mycotoxins. It has been reported that an anaerobic rumen bacterium (*Eubacterium BBSH 797*) is able to deactivate trichothecenes by biotransformation of the epoxide ring. Further a novel yeast strain (*T. mycotoxinivorans*) with the capability to deactivate ochratoxin A (OTA) and zearalenone (ZON) was recently isolated and characterized.

The objective of the presented study was to test the combination of minerals, *Eubacterium BBSH 797* and *T. mycotoxinivorans* (MTV) in a feeding trial with weaning piglets for detoxification of 500 µg/kg OTA and 200 µg/kg ZON. 96 weaning piglets (age 21 days, 50% male, 50% female) were assigned to 4 groups: A negative control group (A) neither receiving mycotoxins nor the deactivator, a toxin group (B) receiving the mycotoxins and 2 test groups (C, D) receiving mycotoxins and the deactivator at 2 different concentrations (0.5 kg/to feed; 1kg/to feed). After 42 days the piglets in group B were lighter than in group A (21.72 vs. 24.00 kg). Piglets in group C (0.5 kg of additive) weighed 23.08 kg and those in group B (1 kg of additive) weighed 24.39 kg. The average daily weight gain data showed a significant improvement in the test groups in comparison to the toxin group (A = 437 g, B = 380 g, C = 412 g and D = 443 g). Performance data were confirmed by clinical results (reduction of swollen vulvas, rectum prolapses and diarrhea in test groups) as well as by histopathological findings.

This trial revealed that a combination of minerals, *Eubacterium BBSH 797* and *T. mycotoxinovorans* is able to abolish negative effects of the mycotoxins ochratoxin A and zearalenone.

Key Words: Mycotoxins, Detoxification, Biotransformation

381 Assessing the relationship between ruminal perchlorate infusion in dairy cows and its concentration in milk. A. V. Capuco*, R. L. Baldwin, C. P. Rice, W. Hare, M. J. Paape, D. D. Bannerman, A. Kauf, G. W. McCarty, A. M. Sadeghi, J. L. Starr, L. L. McConnell, C. J. Hapeman, and C. P. Van Tassell, *USDA-ARS*, *Beltsville, MD*.

Perchlorate is a goitrogenic anion that is a competitive inhibitor of the sodium-iodide symporter. At sufficient concentration, perchlorate can reduce thyroid uptake of iodine and ultimately reduce the secretion of thyroxine and triiodothyronine. Perchlorate has also been perceived as an environmental contaminant of concern in regions of the country where it has been released during its manufacture or distribution for use in rocket fuel and other oxidative products. Recent studies have shown the presence of perchlorate in dairy cow feed components, such as alfalfa, and in milk samples collected from cows throughout the U.S. A comprehensive study was performed to determine the relationship between dietary perchlorate and concentrations of perchlorate in milk, as well as its effect on thyroid hormone secretion and general animal health. Sixteen Holstein cows were randomly assigned to receive 0, 0.4, 4 or 40 mg of perchlorate daily as a 22-h continuous infusion. The total mixed ration contained an average of 21 ppb perchlorate and 1.6 ppm of iodine. Concentrations of perchlorate in milk averaged 4.6, 7.7, 20.0 and 91.4 ng/ml for the 0, 0.4, 4.0 and 40 mg/d doses, respectively. The Pearson correlation coefficient of milk perchlorate vs. perchlorate intake was 0.99. The daily total output of perchlorate in milk, urine and feces was significantly less than that infused, suggesting that a large portion of the infused perchlorate was metabolized. Concentrations of thyroid hormones in the circulation were not influenced by perchlorate treatment during the 5-wk infusion period. Upon termination of perchlorate infusion, concentrations in milk, blood and urine returned to control values within 72 h. Overall, our data indicate that milk perchlorate is highly correlated with perchlorate intake and, within the confines of a 5-wk treatment, no demonstrable health effects on the cow were observed.

Key Words: Mammary Gland, Perchlorate, Thyroid Hormones

382 The effect of dried yeast culture on the carry over of aflatoxin in sheep milk. G. Battacone*[1], A. Nudda[1], M. Palomba[1], M. Pascale[2], A. Mazzette[1], P. Nicolussi[3], and G. Pulina[1], [1]*University of Sassari*, *Sassari, Italy*, [2]*CNR Istituto di Scienze delle Produzioni Alimentari*, *Bari, Italy*, [3]*Istituto Zooprofilattico Sperimentale della Sardegna*, *Sassari, Italy*.

A study was conducted to evaluate whether dried yeast culture (DYC) of *Kluyveromyces lactis*, a source of mannan oligosaccharides, reduced the carry over on aflatoxin M1 (AFM1) in sheep milk. Eighteen Sarda dairy ewes were divided in three groups fed 1.4 kg/d of TMR with different percentage of wheat meal (WM) naturally contaminated with aflatoxin B1 (AFB1, 11 µg/kg): Group 1 (G1) 18%, Group 2 (G2) 36%, and Group 3 (G3) 54%. The trial lasted 14 days. Between days 8 and 14 the three rations were supplemented with DYC (12 g/d). Individual milk yield was recorded and milk samples were collected at each milking. Blood was sampled on day 5 and 12 and analyzed for haematological and serum parameters, in order to assess the haematotoxicity. The AFM1 concentration in milk was determined using an immunoaffinity column-HPLC method. Milk production (1.17 ± 0.015 kg/d) was not affected by treatments. All hematological values were considered to be within the acceptable physiological range. The AFM1 concentration in milk increased as the AFB1 intake increased (30.3, 40.9, 70.2 ng/L; $P \leq 0.01$). The AFM1 concentration in milk tended to be higher in all experimental groups when the rations were supplemented with DYC ($P = 0.059$). Our preliminary results showed that the inclusion of DYC in the diets did not reduce the transfer of the AFB1 metabolite into milk.

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Key Words: Aflatoxin, Sheep Milk, Dried Yeast

383 Detection of feed-ingested plant DNA fragments in salt-cured pork product. T. Reuter*[1,2], K. Aulrich[2], W. Schnäckel[3], and T. McAllister[1], [1]*Agriculture and Agri-Food Canada Research Centre*, *Lethbridge, AB, Canada*, [2]*Federal Agriculture Research Centre*, *Westerau, Germany*, [3]*Hochschule Anhalt*, *Bernburg, Germany*.

The use of genetically modified (GM) plants in animal nutrition is increasing, and feed related DNA-fragments are constantly exposed to the gastrointestinal cell wall and are able to enter the tissues of humans and animals. This leads to the possibility that, foreign DNA in food animals may survive food processing and be consumed. We used PCR techniques to track feed-ingested DNA fragments in a minimally processed salt-cured pork product. The presence of plant-specific DNA (a 140-bp fragment of chloroplast *rbcL*, encoding Rubisco) was confirmed in 53 of 144 muscle tissue samples collected from 48 pigs fed to slaughter on diets containing 70% parental or GM maize ($n = 12$; $n = 36$). Gammon was produced from 12 frozen vacuum-packed *rbcL*-positive *M. gluteus maximus* samples. Samples (800 g) were thawed at 6°C, then dry-salted, cured (14 d; 6°C), rinsed (2 h), dried (5 h), and smoked (7 d) at <20°C. Total DNA was extracted from 25 mg subsamples and PCR was conducted using the primer pair Rub01/Rub02 to amplify the *rbcL* 140-bp fragment with consideration for the appropriate positive and negative controls. Feasibility of the PCR was confirmed and the limit of detection was established at 0.8 pg/µL. Gel electrophoresis revealed substantial degradation of DNA during gammon production. However, the 140-bp fragment was detected in 6 of the 12 samples. To date, only native (i.e., non-GM) plant DNA has been detected in animal tissues or food products. Research suggests that foreign DNA in GM crops behaves similarly to endogenous DNA, thus rates of uptake and/or survival of foreign and endogenous DNA would presumably also be similar. The likelihood of uptake of a transgenic fragment will increase with the prevalence of GM feedstuffs, but low copy number of introduced genes in GM crops may continue to hinder their detection.

Key Words: GMO, Food, Pig