# ADDING AN ANTIOXYDANT COCKTAIL TO PIG FEED REDUCED LUMINAL OXIDATION IN RATS FED A COOKED HAM DIET FROM SUPPLEMENTED REARING

A. Promeyrat[1*], M. Arturo-Schaan[2], A. Hamard[2], M. Carlier[1], J.L. Martin[1], B. Duchêne[1], E. Fouche[3], F. Gueraud[3], N. Naud[3], F.H.P. Pierre[3]

[1] IFIP – Institut du Porc, 41 Le Bois Hamon - Route de Miniac sous Bécherel, 35850 Romillé, France.

[2] CCPA groupe, ZA du Bois de Teillay, 35150 Janzé, France.

[3] ToxAlim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France.

*Corresponding author email: aurelie.promeyrat@ifip.asso.fr

## I. INTRODUCTION

Excessive consumption of cured meats correlates with an increase in the risk of developing colorectal cancer [1]. Different hypotheses have been explored to better understand the substances that are responsible for an increase in risk. Research has identified *N*-nitrosocompounds, such as carcinogenic *N*-nitrosamines [*i.e. N*-nitrosodimethylamine or *N*-nitrosodiethylamine], and the ability of heme iron to catalyse lipid oxidation and nitrosylation with nitrosylated heme iron. Lipid oxidation induces the formation of geno- and cytotoxic terminal aldehydes [malondialdehyde (MDA) and 4-hydroxy-2-nonenal]. To limit cancer risk, some studies have found that adding antioxidants or polyphenols when preparing processed meats inhibited these reactions [2]. Dietary vitamin E supplementation of pig feed may also be of interest to protect cured meat from the formation of nitrosamines and aldehydes in dry sausages [3]. In animal models, vitamin E supplementation can reduce the risk of preneoplasic lesions in rats fed with cooked sausages [4]. In the present study, we assessed the effect of adding a mix with of vitamin E and plant extracts in pig feed on the formation of faecal nitrosylation, nitrosation and peroxidation biomarkers associated with risk of colon cancer in rats fed a diet based on cooked ham from supplemented animals compared to rats fed a diet based on cooked ham from control animals.

## II. MATERIALS AND METHODS

A batch of 54 pigs, from Piétrain sires, were divided into three groups: a group fed a basal diet and two supplemented groups, fed a basal diet supplemented at 2g/kg (Ax1: 100 ppm of vitamin E + polyphenol with 8.6 ppm gallic acid equivalent [5]) or at 4g/kg with the same supplementation (Ax2) during the finishing period. The pH1 (45 min *post-mortem (pm)*) and ultimate pH (pH24 ext., at 24h pm) were performed on *Semimembranosus* and *Long head triceps brachii* (LHT). Meat colour (colorimeter Konica Minolta, Japan) and drip loss (EZ method) were measured on LHT. Three batches of cooked ham models (2.5 kg/batch) were produced with pork shoulder from the standard group (DCNO: Dark Cooked with Nitrite and Oxidized) or from antioxidant-supplemented groups (DCNOAx1 and DCNOAx2). A single brine was made with salt (17g/kg), sodium nitrite (102 ppm), sodium erythorbate (500 ppm), and dextrose (5g/kg). After mixing and tumbling for one night, pieces of meat were cooked to an internal temperature of 68.5°C for 48 min and stored in vacuum packaging for 14 days at 4°C under UV lamp. At day 0, cooked hams were analysed for: vitamin E (NF 12822), fat, protein, moisture (NF V04-403 - NF V04-407 - NF V04-401) and, total and nitrosylated heme iron (Hornsey). Lipid oxidation was assessed by the TBARS method (Thiobarbituric Acid Reactive Substances) at day 0 and day 14. Twenty-four rats (Fischer 344) were assigned to 4 groups and received experimental diets for 15 days: *1)* diet without cooked ham (CON), *2)* diet with DCNO ham, *3)* diet with DCNOAx1 ham, and *4)* diet with DCNOAx2 ham. Faeces were collected for 24h and stored at -20°C until analyses: haem, luminal aldehydes (TBARS), cytotoxicity, ATNC (Apparent Total Nitroso Compounds).

## III. RESULTS AND DISCUSSION

Supplementation had no effect on pH, lightness (L*) and drip loss. The vitamin E content increased respectively by a factor of 2.57 and 3.03 in DCNOAx1 and DCNOAx2 (Table 1). The predominant form was α -tocopherol. The moisture, fat and protein amounts were similar as those of a cooked, derinded and defatted ham. Total heme iron was higher, as desired due to the use shoulder. Supplementation had no impact on nitrosylation. Lipid oxidation was reduced in DCNOAx2 ($p < 0.03$).

**Table 1.** Chemical composition of cooked ham models.

| | **Vit. E** (mg/100g) | **Moisture** (%) | **Fat** (%) | **Protein** (%) | **Heme** (mg/kg) | **Heme-NO** (mg/kg) | **TBARS** (mg/kg) D0 | D14 |
|---|---|---|---|---|---|---|---|---|
| **DCNO** | 0.225 | 74.43 | 3.72 | 19.02 | 111±4.9a | 70±4.6 | 0.309±0.03 | 0.266±0.03 |
| **DCNOAx1** | 0.579 | 74.09 | 4.04 | 19.24 | 94±5.1b | 69±2.6 | 0.314±0.05 | 0.295±0.01 |
| **DCNOAx2** | 0.683 | 74.60 | 3.33 | 19.16 | 104±2.1ab | 73±1.2 | 0.269±0.02 | 0.247±0.03 |

Heme: Total heminic pigment; Heme-NO: nitrosylated heme iron; Means values ± SE (n=4); Values with different letters (a–i) are significantly different ($p< 0.05$).

The DCNO diet induced a significant increase of three endogenous biomarkers: ATNC, Heme-NO and TBARS (Table 2). The antioxidant-enriched rearing in DCNO-Ax1 and DCNO-Ax2 diets had no effect on ATNC and Heme-NO. Interestingly, the DCNOAx2 diet significantly reduced the formation of luminal aldehydes (MDA). This effect significantly reduced cytotoxic and genotoxic activities of faecal biomarkers in DCNO-Ax1 and DCNO-Ax2 groups (↗ cellular viability) compared to the DNCO group.

**Table 2.** Effect of dietary supplementation during pig rearing on fecal biomarkers modulation in rats.

| **Diets** | **Rat Numbers** | **Nitroso-compounds** ATNC (µmol/L) | Heme-NO (µmol/L) | **TBARS** µmol/L Eq. MDA | **Cellular viability** (%) |
|---|---|---|---|---|---|
| **CON** | 6 | 4.06±3.81 | 4.71±2.93 | 13±0.9 | 47.2±4 |
| **DCNO** | 6 | 18.47±4.25* | 11.35±3.01* | 32.8±5.2** | 19.8±6.5** |
| **DCNOAx1** | 6 | 18.50±5.78* | 12.71±3.06* | 29±8.6** | 36.5±5.6# |
| **DCNOAx2** | 6 | 19.00±5.45* | 17.67±3.46* | 25.5±1.4# | 35.7±7.4# |

Data are mean ± SEM (n=10); * significantly different of CON ("*"$p$ <0.05, "**" $p$ <0.01); # significantly different of DCNO ($p$ value <0.05).

## IV. CONCLUSION

This study shows that the enrichment in α-tocopherol and plant extracts during pork rearing decreases the formation of faecal aldehydes in rats fed a diet based on cooked hams from supplemented pigs. This protective effect was observed on cellular viability increasing when meat was enriched with an antioxidant during animal rearing. It should be investigated (i) whether these endogenous changes are associated with a protective effect against the promotion of colorectal carcinogenesis and (ii) whether effects on the formation of luminal TBARS are also observed in the context of the reduction or removal of nitrites recently associated with high luminal peroxidation [6].

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