HEALTH BENEFITS OF MEAT PRODUCTS WITH REDUCED NITRITE LEVELS: THE PHYTOME PROJECT

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Abstract – The European PHYTOME project (Phytochemicals to reduce nitrite in meat products), aims to develop innovative meat products in which the food additive nitrite has been replaced by natural compounds originating from fruits and vegetables. These biologically active compounds (or phytochemicals), are known to contribute to improved gut health and are added to the meat as natural extracts. A range of products with reduced nitrite levels have been produced and the health promoting effects of these products are currently being evaluated in a human dietary intervention study with 80 healthy volunteers. After consumption of a fully controlled diet with either relatively high amounts of the traditional meat products or products produced following the new concept, faeces and colonic material are collected and investigated for markers of colorectal cancer risk. Preliminary data from a feasibility study (n=20) show that increased consumption of traditional processed meat products increase the capacity of faecal water to induce genetic damage to colonic cells, but does not increase faecal levels of N-nitroso compounds (NOC), a class of compounds hypothesized to explain the link between meat consumption and cancer risk. Faecal NOC levels were however increased by the combination of increased drinking water nitrate and meat consumption.

Key Words – Health, Phytochemicals, nitrite reduction.

I. INTRODUCTION

Trend analyses indicate that the consumption of meat products in most EU countries is stagnating, with the exception of the market segment 'light and healthy'. However, consumer perception is increasingly influenced by messages in the media that consumption of nitrite preserved meat products contributes to human cancer risk. The World Cancer Research Fund (WCRF) has classified the evidence for a causal relationship between intake of processed meat and risk of colorectal cancer as convincing and has recommended consumers to limit red meat and avoid processed meat consumption [1].

The proposed mechanism to explain this association involves the formation of N-nitroso compounds (NOC) in the intestinal tract which is stimulated by the combination of nitrite and haem iron, both factors known to be present in processed meat (Vermeer et al., 1998; Cross et al., 2003). On the other hand, nitrite is added for good reasons: it is important to control pathogenic microbes, to control oxidation and rancidity and to ensure an appealing pink meat colour, which is also desired by the consumers.

Therefore, the aim of the PHYTOME project is to develop new meat processing technologies, resulting in innovative products that have no or strongly reduced nitrite levels and that have been shown to contribute to improved gut health. The new meat products are enriched with carefully selected biologically active compounds, so called phytochemicals, present in various natural plant extracts. Some of these bioactive natural compounds possess antimicrobial activity that may allow replacement of nitrite without hampering microbiological safety [4]. Phytochemicals are also known to protect the gut from the induction of genetic damage and adverse health effects [5-7].

The industrial partners in the PHYTOME consortium have produced a range of meat products in which nitrite has been replaced or reduced by natural extracts containing a complex mixture of bioactive ingredients. In order to evaluate the positive impact on human health, a human dietary intervention study has been designed focussing on cancer risk markers in colonic tissues using the newest genomics techniques available. Here we present the design and first outcomes of this health impact evaluation. The final meat products resulting from the meat sciences studies as well as the outcome of the health benefit assessment studies will be used for elaborate consumer studies to establish the response to the newly developed products.

II. MATERIALS AND METHODS

This human intervention study has a parallel design with only healthy volunteers, divided over 2 groups. Subjects receive a completely controlled diet with 2 different types of meat products, with our without the addition of natural compounds, with either normal levels, or low levels of nitrite, separated by a wash-out period were only white meat is consumed. As drinking water nitrate may be converted to nitrite in the body, also intake of drinking water nitrate is controlled and the effect on relevant endpoints is evaluated. The meat products include a combination cooked ham, cooked sausage, dried sausage and dry cured ham, the latter two both produced according to northern and southern style. These products are provided by SSICA (The Experimental Station for the Food Preserving Industry, Parma, Italy), Vanden Borre (Oudenaarde, Belgium) and Henri van de Bilt (Beuningen, Netherlands), all partners of the PHYTOME consortium.

After each of the three intervention periods of 15 days where participants consume 300 grams meat per day (corrected for body weight), blood, urine, saliva, mouth wash and faeces are sampled. Previous intervention studies on NOC formation indicate that a period of 15 days for each part of the study is sufficient to detect changes in the markers that are measured [8]. Additionally, colonic biopsies are collected during an endoscopic examination. In order to establish the impact of natural compounds in processed meat products on the formation of NOC in humans faecal and urinal level of NOC is measured as Total Apparent Nitroso Compounds (ATNC) and used as an indicator of colonic exposure to endogenous nitrosation products [9]. Also the genotoxic potential of faecal water is assessed by means of the COMET assay for DNA breakage. Additionally, DNA damage, gene expression profiles and DNA methylation will be measured in colonic tissues, which can be interpreted as an indicator of potential cancer risk.

All data analyses are done group wise to examine the overall effects of nitrite levels in meat. This is done for each intervention period (normal meat products and meat products enriched with natural compounds) and the wash-out period (white meat) serves as a control.

III. RESULTS AND DISCUSSION

Preliminary results from a pilot study population (n=20) to test the feasibility of the study design show that increased meat consumption has a stimulating effect on the genotoxicity of faecal water. The combined intake of red meat products with increased drinking water nitrate was not significantly influencing genotoxicity scores as compared to the base line measurements (Table 1). On the other hand, there was no statistically significant difference in N-nitroso compounds between baseline samples and after the intervention (t-test). In contrast to this, nitroso compounds were significantly higher with high nitrate drinking water ($p=0.0001$ as compared to meat intervention, $p=0.0002$ when compared with baseline levels). This difference was more pronounced in participants with a red meat diet than the white meat diet (Table 1). In participants with a red meat diet, faecal water nitroso compounds were higher after consumption of high nitrate water as compared to participants on a white meat diet, although this difference was only marginally significant ($p=0.07$).

The impact of replacement of nitrite by natural bioactive compounds was not established in this pilot study.

Elaborate analyses of all markers in the main study, which is still ongoing, will reveal differences in transcriptomic and epigenomic markers after consumption of meat products enriched with natural compounds. These markers can be interpreted as an indicator of reduced cancer risk.

Furthermore, correlating gene expression changes to changes in genotoxic endpoints (DNA damage, reduction in N-Nitroso compounds) will provide insight in the

molecular processes involved in cancer risk reduction. The identification of molecular pathways that are crucial in the carcinogenic process will demonstrate a causal association between dietary changes and markers of carcinogenic risk.
The outcome of these studies will be used in further consumer acceptance studies.

Table 1 Fecal water genotoxicity (%) and ATNC (μM of $NaNO_2$ eqv) levels after 1 week high meat diet following 1 week high meat diet combined with high nitrate drinking water consumption

Genotoxicity	Baseline	Meat	Drinking water
All participants	100 ± 0	175.1 ± 103.4	157.7 ± 127.9
White Meat	100 ± 0	191.4 ± 129.4	187.2 ± 190.2
Red Meat	100 ± 0	163.6 ± 86.6	138.0 ± 70.2
ATNC	Baseline	Meat	Drinking water
All participants	14.7± 9.2	15.5± 10.4	35.63 ± 21.10
White Meat	12.7± 9.4	14.5± 11.0	28.2 ± 18.6
Red Meat	17.4± 8.7	17.0± 10.1	45.9 ± 21.0

IV. CONCLUSION

A range of new processed meat products has been produced by the PHYTOME consortium for evaluation of expected beneficial health effects. A human dietary intervention study has been designed to establish markers of exposure and molecular responses in human colonic tissue. The first results have demonstrated that the intake of traditional meat products can stimulate the capacity of the colonic content to induce genetic damage which could ultimately result and cancer development, but also that the evaluation of drinking water nitrate as an alternative source of nitrite may have a strong influence on the colonic exposure to potentially carcinogenic NOC. Further evaluation of the gene expression responses to the intervention and the comparison between traditional and new products will provide insight in the beneficial effects of the (partial) replacement of nitrite by natural extracts and which molecular mechanisms are involved in these effects.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from the European Commission. This research is the result from work package 6 of the SME Associations project PHYTOME financed under the 7th Framework programme (EU-FP7 grant agreement no. 315683), investigating the replacement of nitrite in processed meat products by natural compounds.

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