# ADDITION OF AN AGRICULTURAL BY-PRODUCT RICH IN PHENOLS TO FEEDSTUFF TO INCREASE THE SHELF-LIFE OF MEAT

E. Novelli<sup>1\*</sup>, A. Taticchi<sup>2</sup>, F. Blaas3, M. Servili<sup>2</sup>, L. Fasolato<sup>1</sup>, G. Marchesini<sup>4</sup>, M. Drigo<sup>4</sup>, S.

Segato<sup>4</sup>, A. Trocino<sup>1</sup>, S. Balzan<sup>1</sup>

<sup>1</sup>Department of Comparative Biomedicine and Food Science, University of Padova, Italy;

<sup>2</sup>Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy;

<sup>3</sup>Administration of the Autonomous Province of Bolzano, Bolzano, Italy;

<sup>4</sup>Department of Animal Medicine, Production and Health, University of Padova, Italy;

\*Corresponding author email: enrico.novelli@unipd.it

Abstract - Phenolic compounds have an efficient antioxidant and antimicrobial activity. A byproduct of the oil mill rich in phenols, after a process of concentration and purification has been used as an ingredient in feedstuffs intended for chickens and heavy pigs rearing. In the flesh, hydroxytyrosol was measured in discrete concentrations up to 11 days after slaughtering, in the case of poultry. These results, in the perspective of protection against oxidative phenomena and for control of the altering microbial flora, are the subject of further evaluations.

Key words - Olive vegetation water, hydroxytyrosol, chickens and pigs

### I. INTRODUCTION

Many studies in humans and rodents have shown that dietary polyphenols exert a broad spectrum of beneficial effects with respect to health issues including anti-oxidative or anti-inflammatory properties (Fiesel et al., 2014). On the other hand, there are not many details about the effects of polyphenols in farm animals and their products. It has been estimated that more than 90% of ingested polyphenols are not absorbed in the small intestine and thus, remain in the colon at high concentration (Clifford, 2004). In the colon, the unabsorbed phenolic compounds are partially metabolized by gut microbiota and, at the same time, the interfere with the intestinal bacterial equilibrium favoring certain microbial species with respect to other (Choy et al., 2014). The virgin olive oil (VOO) production generates a considerable mass of byproducts as olive vegetation water (OVW) and the pomace. A significant amount of the phenols of the drupe, due to their intrinsic water solubility, end up in the vegetation water. The OVW has a value of BOD<sub>5</sub> and COD that are incompatible with its use in the open field. A recovery system for the phenolic fraction of fresh OVW by a membrane filtration has been developed at an industrial scale. This recovery system was equipped with three consecutive membrane-filtration steps that allowed the production of a concentrate enriched in the phenolic fraction (crude phenolic concentrate, CPC) and a permeate purified from phenolic compounds and of the major part of the organic fraction (Servili et al., 2011). The aim of this study was the enrichment of the muscular tissue of poultry and pigs with the phenolic compounds of the OVW whose antioxidant and antimicrobial action could be helpful in the shelf-life's extension.

### II. MATERIALS AND METHODS

A total of 144 male chickens, commercial hybrids of the genetic type Ross 308, were settled down in 12 pens at a density of 12 animals per pen corresponding to approximately 10 animals/m<sup>2</sup> homogeneous for initial live weight, according to three experimental groups, control L0 (commercial feed), L1 (feed supplemented with 220 mg/kg of phenols) and L2 (feed supplemented with 440 mg/kg of phenols). The phenols's addition was accomplished using the CPC whose composition is shown in Table 1. The dispensing of the experimental feedstuffs (17.2% crude protein, 5.8% ether extract) began at the 24th day of age up to the slaughter. Animals were always fed ad libitum. After a fasting of around 7 hours and a water deprivation of 2 hours, the chickens were slaughtered. After 2 hours of refrigeration in tunnel, all the carcasses immediately dissected to obtain the yield in main cuts. After boning, the breast cuts were packaged by means a PVC gas permeable film and stored at 4 °C for 12 days alternating 12 h of light and 12 h of dark.

An homogenous group of 60 pigs (Duroc x Landrace), initially maintained in a single group up to about 98 kg of live weight, was then divided homogenously by sex and weight into n=3 box. After a period of adaptation to newly formed groups, the feeding trial began at a live weight of  $127\pm11$  kg. The control (G0) received a commercial feedstuff (protein 13%, 6% cellulose, 2% fat, 5.5% ash, 40% starch) while the G1 received the feed enriched with 330 mg/kg of phenols and the G2 was fed as group G1 plus an enrichment of

unsaturated lipids (by means of extruded linseed). At the live weight of  $156\pm7$  kg pigs were slaughtered. Quantitative determination of the phenols (hydroxytyrosol as a marker) present in the muscle tissue of chicken breasts and in *L. dorsi* of the pigs was carried out on 10 g of meat submitted to extraction of phenols by SPE and measurement according to Selvaggini et al. (2006).

### III. RESULTS AND DISCUSSION

In literature, there is not much information about the phenols accumulation in muscle tissue following dietary intervention with the use of ingredients rich of these compounds. King et al. (2014) using a freezedried powder from organic olives juice extract to enrich the feed for poultry with hydroxytyrosol did not find improvements neither in vivo nor in the meat. The authors attributed the absence of effects to the fact that hydroxytyrosol is highly water-soluble and may has been unavailable as an antioxidant in the tissue of broilers that did not consume water for 4-6 h prior to slaugther. On the contrary, Fiesel et al. (2014) showed that a dietary rich in polyphenols was able to improve the gain:feed ratio in growing pigs. They postulated that an anti-inflammatory effects of the polyphenol-rich plant products in the intestine might contribute to this effect. González et al. (2007) found higher levels of phenols in the subcutaneous tissue of pigs fed with acorn if compared to pigs the had a diet with compound feed. In the present study a quantitative trend was observed in the storage of hydroxytyrosol in the muscular tissue of poultry and pigs (Tabb. 2-3). What is interesting is that phenols could be found up to 11 days after slaughter in the case of poultry.

Tab.1. Phenols composition of CPC

Tab. 2. Hydroxytyrosol concentration (ug/kg) of chicken breast at days 1 and 11 after slaugther according to different levels of phenols addition to feed

Phenols	g/L		Days after slaugther					
Hydroxytyrosol	$2.4 \pm 0.01$			1			11	
Tyrosol	$0.3 \pm 0.01$		L0	L1	L2	LO	L1	L2
Verbascoside	$5.0 \pm 0.04$	Hydroxytyrosol	$78 \pm 54$	$185 \pm 9$	$268\pm9$	nd	nd	77 ± 4
Oleuropein	$13.9\pm0.8$							

Totale phenols  $21.5 \pm 0.8$ 

Tab. 3. Hydroxytyrosol concentration (ug/kg) of pig L. dorsi according to

different levels of phenols addition to feed

	G0	G1	G2
Hydroxytyrosol	$65 \pm 6$	$194 \pm 7$	$439 \pm 25$

## IV. CONCLUSION

The CPC obtained from OVW could be seen as a by-product with an added value due to the high concentration of phenols in a purified solution. Their bioavailability in livestock nutrition can be an alternative way to improve quality and shelf-life of fresh meat.

### ACKNOWLEDGEMENTS

The study has been funded by the Italian Ministery of Instruction, University and Research - Prot. 20085FFB3H

### REFERENCES

1. Fiesel, A., Gessner, D.K., Most E. and Eder, K. (2014). Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Veterinary Research, 10:196

2. Clifford, M. N. (2004). Diet-derived phenols in plasma and tissues and their implications for health. Planta Med. 70: 1103-1114

3. Choy, Y.Y., Quifer-Rada, P., Holstege, D.K., Frese, S.A., Calvert, C.C., Mills, D.A., Lamuela-Raventos, R.M. and Waterhouse, A.L. (2014). Phenolic metabolites and substantial microbiome changes in pig feces by ingesting grape seed proanthocyanidins. Food Funct., 5: 2298–2308

4. Servili, M., Esposto, S., Veneziani, G., Urbani, S., Taticchi, A., Di Maio, I., Selvaggini, R., Sordini, B., Montedoro, G. (2011). Improvement of bioactive phenol content in virgin olive oil with an olive-vegetation water concentrate produced by membrane treatment. Food Chemistry 124: 1308–1315

5. Selvaggini, R., Servili, M., Urbani, S., Esposto, S., Taticchi, A., and Montedoro, G. (2006). Evaluation of Phenolic Compounds in Virgin Olive Oil by Direct Injection in High-Performance Liquid Chromatography with Fluorometric Detection. J. Agric. Food Chem. 54: 2832-2838

6. King, A.J., Griffin, J.K. and Roslan, F. (2014). In Vivo and in Vitro Addition of Dried Olive Extract in Poultry. J. Agric. Food Chem. 62: 7915–7919

7. González, E., Tejeda, J.F., Motilva, M.J. and Romero, M.P. (2009). Phenolic compounds in subcutaneous adipose tissue from Iberian pigs. In Audiot A. (ed.), Casabianca F. (ed.), Monin G. (ed.). 5. International Symposium on the Mediterranean Pig. Zaragoza: CIHEAM Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 76, pp. 115-118