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RESIDUES IN POULTRY FED WITH HIGH CONTENT OF POLYPHENOLS

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INTRODUCTION

Interest in hydroxytyrosol and other phenolic compounds is associated with their many biological benefits, especially antioxidative capacity and other effects such as low incidences of coronary heart disease and cancer in the population adopting the Mediterranean diet., which is particularly rich in food containing high percentages of polyphenols. Twenty percent of the yield of olive processing (produced from olives belonging to *Olea europaea*) is in oil and the remaining 80% is made of pulp, nut, skin and water. These olive mill wastes are a powerful pollutant and they are currently discarded, creating costs for their disposal. On the other hand, they have good lipid content and high concentration of antioxidant compounds such as tocopherols and polyphenols. For its characteristics, this by-product can be useful, after drying, in feed industry (Terramoccia et al., 2013).

An *in vivo* study was performed in poultry to assess the possible beneficial effects of polyphenols on animal health and meat characteristics. This work focuses on the analytical methods developed and validated to determine polyphenols in the administered feed and poultry meat. For the first time the residues of tyrosol, hydroxytyrosol, pinoresinol and verbascoside in control and treated chicken muscle samples are determined using liquid chromatography coupled to tandem mass spectrometry.

Experimental

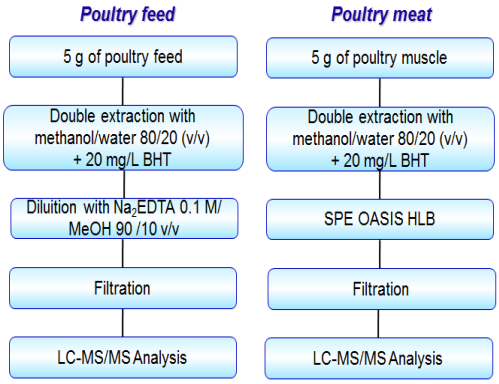

Figure 1: *Flow diagram of sample preparation*

In farm experiment. Four poultry groups (99 subjects each) received feed enriched with polyphenols using olive oil by-products (olive paste and phenolic concentrate at two different concentrations) and a fifth group was kept as control (99 subjects). The study lasted 49 days. To test the effects of integrated feeding on the slaughter performance, carcasses were weighed and the individual percentage yield was calculated on all trial subjects. Chemical-physical, chemical-bromatological and nutritional characteristics were evaluated on samples of *Pectoralis major* muscle. The detection of thermotolerant *Campylobacter* from cloacal swabs was performed by sampling 15 chickens for each group and three different age groups 21 days (before starting the experimental diets), 35 and 49 days.

LC-MS/MS method for polyphenols determination. After sample preparation (Figure 1), the four analytes were determined by LC-MS/MS technique. The separation was performed on a Gemini C18 column (100 mm x 2.0 mm, 3.0 µm) using as mobile phases water and methanol with gradient elution. The MS/MS was operated in multiple reaction monitoring mode (MRM) with negative electrospray ionization. For quantification purposes, external calibration was applied using matrix-matched curves

Method validation and sample analyses. The spiking experiments for poultry feed were performed at four concentrations: 0.1, 0.5, 5 and 50 mg/kg (six replicates per level in two different occasions: 48 experiments). For poultry meat, three different spiking levels were prepared: 0.5, 1 and 5 µg/kg (six replicates per level in two different occasions: 36 experiments).

The six feed samples administered during the in farm experiment were analysed twice to determine the polyphenol contents, whereas, for each poultry group, seven pooled samples were analysed for a total of thirty-five determinations. Also in this case, each determination was repeated twice.

Results and Discussion

Method performances were satisfactory both in feed and meat. The polyphenols levels found in enriched feed are reported in Table 1. In poultry meat pinoresinol and verbascoside were never detected. Tyrosol was determined in four samples: one belonging to group 3, two to group 4 and one to group 5. The measured concentrations ranged from 8 to 47 µg/kg. Hydroxytyrosol was present only in two samples belonging to group 5 (0.65 and 0.74 µg/kg).

The metabolite formation after the administration of polyphenols, mainly glucuronides and sulphates, is well documented in literature both in biological fluids and tissues (Lopez et al. 2015). In this study, hydroxytyrosol-3-sulphate was detected in all muscles belonging to the four supplemented groups (2, 3, 4 and 5) in the range 1-5 µg/kg (Figure 2). Actually, also in the control group (1) traces of hydroxytyrosol-3-sulphate were detected, but in most cases the identification criteria (ion ratio) were not respected due to the low signals (Figure 2). As shown in the chromatograms, an almost coeluting peak was present immediately before hydroxytyrosol-3-sulphate, which supposed identity was the other isomer, i.e. hydroxytyrosol-4-sulphate.

Finally, the results obtained from the prevalence study of thermotolerant *Campylobacter* in the five groups of chickens show that the groups 3 and 5 have a lower possibility of infection compared to the control group (P-value ≤0.05), after 49-day age (28 days after administration of supplemented diets).

Table 1: *Polyphenols levels in feed used during the in farm experiment*

Feed	Tyrosol (mg/kg)	Hydroxytyrosol (mg/kg)	Verbascoside (mg/kg)	Pinoresinol (mg/kg)	Polyphenol sum (mg/kg)
1° period	7.8	0.26	ND	ND	8.1
Group 1 (Control)	3.7	0.28	ND	ND	4.0
Group 2 (16.5% olive paste)	8.7	4.6	0.22	0.28	14
Group 3 (16% phenolic concentrate)	28	190	45	0.18	263
Group 4 (33% olive paste)	13	9.5	0.75	0.47	24
Group 5 (32% phenolic concentrate)	59	402	105	0.54	567

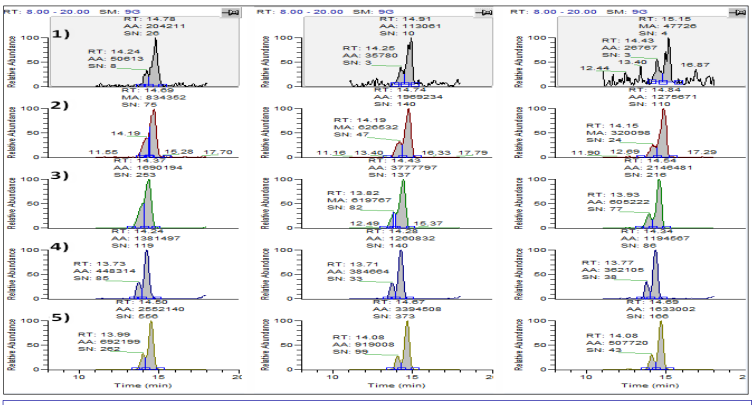

Figure 2: *LC-MS/MS chromatograms of three samples of pooled poultry muscle belonging to the five groups. The set transitions were those of hydroxytyrosol-sulphate.*

Conclusions

For the first time residues of four polyphenols were measured in poultry meat after an *in vivo* experiment. The found levels of native compounds were sporadic and generally low, whereas sulphate metabolites of hydroxytyrosol were detected in all the supplemented groups (1-5 µg/kg). Analysing the administered feed, the phenolic concentrate (groups 3 and 5) gives a significant higher content of polyphenols than olive paste (groups 2 and 4). This is probably the cause of the beneficial effects on thermotolerant *Campylobacter* prevalence, but, at the same time, also the cause of the worst animal performances observed mainly for group 5. To conclude, the incorporation of olive mill wastes in animal feed could help to obtain two important goals in the era of sustainability: the recycling of agricultural wastes and the decreasing of the use of antibiotics in farm.

Acknowledgments
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