Olive cake dietary supplementation in rabbit: immune and oxidative status

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ABSTRACT - The aim of the present study was to study the effect of three different types of dehydrated olive cakes on immunitary and oxidative status of growing rabbits. One hundred and sixty New Zealand White weaned rabbits, 30 day old, were divided into four homogeneous groups. To experimental groups was administered a similar diet integrated with 5% of three types of olive cake (A, B, C) obtained from local olive oil mills. The olive cakes A and C were both of high quality (C better than A), while the by-product B had the lowest level of total polyphenols, o-diphenols and oleic acid and the highest peroxide number. The aspecific immunitary and oxidative status was improved in animals fed olive cakes richer in antioxidant components.

Key words: Rabbit, Olive cake, Immune, Oxidative status.

INTRODUCTION - The olive cultivation is widely spread in the Mediterranean area, where the olive oil is used as a dietary food. Following the extraction of oil from olives, a considerable amount of by-products known as olive cake is obtained. Olive cake is a mixture of skins, pulp, woody endocarps and seeds and represents about 35% by weight of the processed olives. Amro et al. (2002) investigated the antioxidative activity of olive cake and isolated four fractions (coumaric, fenolic, cinnamic acids and oleuropein) that showed good hydrogen donating activity, indicative of their effective action as radical scavengers.

Given its long period of degradation, olive cake is the main pollulant in the Mediterranean basin (Gil and Hai, 1997). However, its particular chemical composition makes this waste material a valuable feed source for many animal species, especially ruminants and rabbits too.

Olive cake contains variable amounts of oil (3.5-5%), crude protein (2.5-6%) and fibre (22-35%), depending on fruit quality and technological process employed in the oil extraction (Rupić et al., 1999). The aim of the study was to establish the effect of three different types of dehydrated olive cakes on immunitary and oxidative status of the growing rabbits.

MATERIAL AND METHODS - The study was carried out in the experimental rabbit farm of the Animal Production Department (University of Perugia). One hundred and sixty New Zealand White weaned rabbits, 30 days old, were divided into four homogeneous groups: Control and three experimental groups (A, B, C).

All rabbits were housed in biccellular cages located in the same air-conditionated room. Feed and water were available ad libitum. The Control group was fed a standard diet established according to the current recommendations for growing rabbits. To experimental groups were administered isoenergetic and isoproteic diets containing 5% of three types (A, B, C) of olive cake and integrated with 50 mg/kg of α-tocopheryl acetate.

The dried olive cakes derived from different cultivar and production processes: the A and B by-products derived from Frantoio and Coratina olive cultivar, respectively, while C was a mixture of different olive cultivars. The for-
mer olive cakes were obtained by oil mechanical extraction from stoned olives, whereas B resulted from pomaces stoned after oil extraction and dried after 5 days of storage. Fatty acid composition of olive cakes was determined by the method of Folch et al. (1957).

At 15 days of age, when pups were still ingesting only milk, blood samples were obtained by cardiac puncture for the evaluation of the basal immune parameters (lysozyme, Serum Bactericidal Activity: SBA, Haemolytic Complement Assay: HCA) and oxidative status (antioxidant power - AOP; reactive oxygen molecules – ROMs; thiobarbituric acid reactive substances – TBARs).

Blood samples were taken again at 45 and 75 days of age. The serum lysozyme was measured by the lyso-plate assay (Osserman and Lawlor, 1966) and results were expressed as µg/mL. The SBA was performed according to Amadori et al. (1997) method. The HCA (Barta and Barta, 1993) was carried out in microtitre plates and values were expressed as CH50.

The level of ROMs, expressed as mmol H2O2, and the antioxidant power (AOP), expressed as µmol HClO neutralized, were determined by using commercial kits (Diacron, Grosseto, Italy). TBARS were determined by HPLC according to the method of Halliwell and Chirico (1993) and expressed as µmol MDA/L. Statistical analysis was done with a linear model (STATA, 2005) taking into account the fixed effect of feed and the age of animal.

RESULTS AND CONCLUSIONS - The main characteristics of the olive cakes are summarized in Table 1. The olive cakes A and C were both of high quality, with C better than A. Coratina cultivar (C) is known to have high phenolic concentration. Contrarily, the by-product B had the lowest level of total polyphenols, o-diphenols and oleic acid and the highest value of peroxide number.

These results, taken together, highlight the positive effect of the stoning process: stone removal reduces the oxidative degradation of phenols, considering that seed has the highest peroxidative activity. The drying process performed on the fresh pomaces could also contribute to their preservation from oxidative damage.

The native immunity and the oxidative status of animals were both affected by the presence of olive cake in feed (Table 2) and the differences increased with the age.

The native immunity indexes of A and C group improved and only group B showed a poor HCA and SBA. This lower activity is related to animal’s difficulty to counteract infective agents and thus determines a higher susceptibility to diseases.

Lysozyme concentration at baseline was in normal range indicating no inflammation (Bonnafous and Raynaud, 1980). Lysozyme was lower in group B and higher in group A at both ages (45 and 75 days; P<0.01).

Regarding oxidative status, group C had the best antioxidant power and, accordingly, the lowest ROMs and TBARs concentration.

Opposite results were found in group B most likely due to the lower content in polyphenols and to its high content in peroxides. Andreadou et al. (2006), evaluating the efficacy of the oleuropein on the rabbit metabolic profile, found a strong antioxidant protection.

Regarding productive performance, all the dietary treatments slightly reduced growth rate, feed efficiency, carcass weight and yield (data not shown; Dal Bosco et al., 2007).

In conclusion, the qualitative characteristics of olive cake is a peculiar factor for its use as a dietary component for growing rabbits. Rabbits fed a high quality of this by-product result more protected against oxidative damage. Further studies needed to define the effect of olive cake integration on the performance and meat quality of rabbit.

Table 1. Main characteristics of olive cakes.

|                  | A       | B       | C       |
|------------------|---------|---------|---------|
| Total polyphenols| mg/100 g dry weight | 1662.5 | 958.8   | 2135.0  |
| o-diphenols      | "       | 798.8   | 410.1   | 987.0   |
| Peroxide number  | meq O2/kg | 15.3   | 21.4    | 9.8     |
| C16:0            | % fatty acid | 12.97  | 13.84   | 11.81   |
| C18:0            | "       | 2.59    | 2.24    | 2.62    |
| C18:1n-9         | "       | 73.97   | 69.37   | 76.27   |
| C18:2n-6         | "       | 7.55    | 9.52    | 7.73    |
| C18:3n-3         | "       | 1.10    | 0.95    | 0.94    |
Table 2. **In vivo** immunitary and oxidative status.

|                      | Mean ± standard deviation |
|----------------------|---------------------------|
| Lysozyme μg/mL       | 15.19±4.20                |
| SBA %                | 10.51±2.34                |
| HCA 30CH50/150μL     | 30.12±9.29                |
| AOP μmol HCLO neutr./mL | 221.61±64.05          |
| ROMs mMoH2O2         | 4.16±2.81                 |
| TBARs μMol/L         | 6.86±3.51                 |

45 days of age

|                      | CONTROL | A | B | C | Pooled SE |
|----------------------|---------|---|---|---|-----------|
| Lysozyme μg/mL       | 17.58C  | 15.32B | 12.96A | 15.25B | 1.68      |
| SBA %                | 10.74A  | 13.22B | 10.14A | 11.26A | 1.25      |
| HCA 30CH50/150μL     | 53.23   | 53.04   | 54.42   | 52.50   | 2.03      |
| AOP μmol HCLO neutr./mL | 287.33b | 242.80a | 267.00a | 282.18b | 50.23     |
| ROMs mMoH2O2         | 6.33C   | 5.06A   | 6.50B   | 3.97A   | 0.89      |
| TBARs μMol/L         | 8.48A   | 15.54B  | 14.81B  | 6.90A   | 1.05      |

75 days of age

|                      | A | B | C | Pooled SE |
|----------------------|---|---|---|-----------|
| Lysozyme μg/mL       | 22.20B | 20.36B | 13.68A | 15.63A | 1.95      |
| SBA %                | 26.23B | 29.42C | 67.77a | 84.59a | 2.58      |
| HCA 30CH50/150μL     | 70.28b | 72.64c | 67.77a | 272.70a | 48.79     |
| AOP μmol HCLO neutr./mL | 331.16b | 351.79b | 272.70a | 382.18c | 48.79     |
| ROMs mMoH2O2         | 6.15B  | 6.58B  | 6.97B  | 3.18A  | 0.57      |
| TBARs μMol/L         | 9.75B  | 17.42C | 25.20D | 7.50A  | 1.24      |

N=20 per group and age; a..b: P<0.05; A..D: P<0.05.

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