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Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds

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ABSTRACT

A partial substitution (25 or 50%) of dietary protein with *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) meals as protein sources in the diet of Barbary partridge (*Alectoris barbara*) has been tested in terms of raw and cooked meat quality. Twelve partridges per feeding group (control - SBM, HI25, HI50, TM25 and TM50) were slaughtered. The peeled carcasses of the HI25, HI50, TM25 and TM50 groups were heavier than those of the SBM group, both raw and cooked. The pH, color and shear force of the raw meat were not affected to any greater extent by the diet, whereas the presence of insect meal seemed to increase the yellowness index of the cooked meat. The proximate composition was unaffected by both the species and the level of insect meal, although the fatty acid profile was changed considerably. The HI and TM groups had significantly higher C18:1n-9 and lower C16:0 contents than SBM. Furthermore, *Hermetia illucens,* added as 50% of the dietary protein, induced a significant increase in C12:0 and C16:1n-7. As a result, the highest AI and TI were obtained for the HI50 diet (0.38 and 0.75, respectively), whereas the TM groups both had intermediate AI values (around 0.35) and the lowest TI (0.67). Finally, the cholesterol content of the birds was not affected by insect inclusion in the feeds.

1. Introduction

Birds such as pheasant, quail, and partridge are currently considered part of the poultry industry, and they are reared for different purposes, such as for game, hunting preserves or commercial meat production. As game birds, their ecological importance is linked to improving biodiversity, restocking and habitat maintenance (Scandura & Apollonio, 2010). In addition, although its consumption originates from hunting, game bird meat, especially that of partridge, has recently become more sought after by gourmet markets (Özek, Yazgan, & Bahtiyarca, 2003). As a consequence of the growing economic value as a result of their commercial production, the number of specialized farms in which game birds are raised has been increasing.

The Barbary partridge (*Alectoris barbara*) is an avian species (*Galliformes*) that is found throughout Spain, the Canary Isles and North-Western Africa. In Italy, this species is only found in the Island of Sardinia, where it is mainly reared for restocking and as game birds. Information provided by Alonso et al. (2008) suggests that partridge is not a species that is particularly suitable for raising in captivity, due to its difficulties in adapting to cages, and its susceptibility to intestinal diseases (Khaksar, Veldkamp, & Hashemipour, 2013). Furthermore, a recent study has reported on the effects of different rearing systems, such as barn and free-range, on the carcass composition and meat quality of *Alectoris chukar* (Yamak, Sarica, Boz, & Ucar, 2016). In this context, feeding strategies should be adopted to allow the birds to adapt morphologically and functionally to the naturally available feeding sources in the environment and to minimize the changes in meat composition.

Insects are part of the natural diet of *A. barbara*, as they are for poultry in general, and successful attempts to substitute soybean with insect meals as feeds for the poultry sector have recently been made. Black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) are two of the most promising insect species for commercial exploitation and for use in poultry feeds (Józefiak et al., 2016), thanks to their composition and the relative easiness of farming them. These insects have been tested as ingredients in diets, and interesting results have been achieved (Biasato et al., 2016; Bovera et al., 2016; Cullere et al., 2016).

The effects of these diets on the growing performances and health status of *A. barbara* reared in captivity were reported by Loponte et al. (2017). However, no information is available in the literature on the differences in meat quality and carcass characteristics among partridges

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fed with insects and raised in captivity. Moreover, Barbary partridge meat quality has rarely been investigated. For this reason and continuing the work of Loponte et al. (2017), the aim of the present study was to assess whether a partial substitution (25 or 50%) of soybean protein with *T. molitor* and *H. illucens* meals as protein sources in the diet affected the fresh and cooked meat quality and performance of Barbary partridge.

2. Material and methods

The trial was carried out on a private partridge farm in Sardinia (Italy). All the birds were treated humanely, according to the principles stated by European Directive 2010/63/UE, put into law in Italy with D. Lgs. 26/2014, regarding the protection of animals used for experimental and other scientific purposes. The experimental procedures were approved by the Ethical Animal Care and Use Committee of the University of Naples Federico II (Prot. no. 2017/0017676).

Ninety 7-day old Barbary partridges (average weight 25.16 ± 2.98 g) were randomly assigned to 5 experimental groups (18 birds per group) and were fed 5 isoproteic (22.74 ± 0.24% as feed) and isoenergetic diets (2627.4 ± 24.4 Kcal/kg) as reported in Loponte et al. (2017), where some other results obtained in the aforementioned trial can be found. The diets mainly differed as far as the ingredients used as main protein source are concerned: soybean meal, *Hermetia illucens* larva meal (HI, purchased from Hermetia Deutschland GmbH & Co KG, Amtsgericht Potsdam, Germany), or *Tenebrio molitor* larva meal (TM, purchased from Gaobeidian Shannon Biology Co., Ltd., Shannong, P. R. China). The control group (SBM) was fed a corn-soybean meal-based diet; 25 or 50% of the dietary protein was substituted with protein from the *Hermetia illucens* or *Tenebrio molitor* larva meals in the HI25, HI50, TM25, and TM50 diets, respectively. The ingredients and the chemical-nutritional characterization of the diets are reported in Loponte et al. (2017). Since insect meals were characterized by different lipid content, their inclusion in the isoenergetic diets required an adjustment in the vegetable oil (maize oil) content.

The birds in each group were raised in 2 cages, each one divided into 3 sections, hosting 3 birds each (6 replicates with 3 partridges each for a total of 18 birds per group) for 57 days. Feeds and fresh water were administered *ad libitum,* and, where necessary, an adequate temperature was guaranteed for the chicks by means of infrared lamps; moreover, natural lighting (12–13 h light/day) was guaranteed. At 64 days of age (live weight 248.66 ± 17.24 g, SBM; 272.70 ± 18.57 g, HI25; 269.64 ± 15.70 g, HI50; 267.33 ± 20.97 g, TM25; 262.32 ± 17.16 g, TM50), 2 partridges were randomly selected from each replicate (6 partridges for each cage) and 12 partridges per group were slaughtered in a specialized slaughterhouse. The birds were weighed, degutted and plucked, and the carcasses were then transferred to the laboratory, where the carcass traits and meat quality of raw and cooked samples were examined.

2.1. Carcass traits and cooking trial

The carcasses were peeled, weighed and cut into two symmetric parts: the right and left sides. The right sides were weighed and subsequently divided into the neck, breast, leg and wing. The incidence of these parts was calculated. The breasts were then subdued for physical analyses (see Section 2.2). Once concluded, all the parts were deboned and minced before the chemical analysis was performed (see Section 2.3).

The left sides were weighed then allotted to a cooking trial, after which the cooking yield was calculated. Cooking was performed in an oven for 20 min at 225 °C (72 °C in the center for 15 min). Baking paper was placed between the baking tray and the meat. No salt or oils were added before or during cooking. Physical analyses (see Section 2.2), such as color and texture evaluations, were conducted on the baked carcasses prior to deboning and mincing the samples for chemical analyses (see Section 2.3).

2.2. Physical analyses

The pH, color and texture were assessed. The pH values were monitored using a pH-meter (Columbus, OH, USA) in three different points of the raw breasts. Meat color (L*, a*, and b*; CIE, 1976) was measured at 3 points of both the raw and cooked breasts, using a Konica Minolta colorimeter (Chiyoda, Tokyo, Japan). The shear force of both the raw and cooked breast samples (3 × 3 cm area) was taken into consideration as a texture parameter and it was measured using a Zwick Roell® texturometer model KAF-TC 0901279 (Zwick GmbH & Co. KG, Ulm, Germany), equipped with a blade and a 1kN load cell. Data were collected and analyzed using Test-Xpert2 by Zwick Roell® software, version 3.0 (Zwick GmbH & Co. KG, Ulm, Germany).

2.3. Chemical analyses

2.3.1. Proximate composition

The moisture, crude protein (N × 6.25) and ash contents were determined on 6 right sides per group using the 950.46, 976.05 and 920.153 AOAC, Association of Official Analytical Chemists (2012) methods.

2.3.2. Lipid content and fatty acid profile

The total lipid content was determined on the other 6 half sides of the partridges, according to the Folch, Lees, and Sloane Stanley (1957) method; the fatty acids (FAs) in the lipid extracts were trans-esterified, and the FA composition was determined by gas chromatography (Varian GC 430 gas chromatograph; Varian Inc., Palo Alto, CA, USA), according to Secci, Borgogno, Mancini, Paci, and Parisi (2017). Tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) was utilized as the internal standard for FA quantification through calibration curves (standard Supelco 37 component FAME mix; Supelco, Bellefonte, PA, USA). The atherogenicity index (AI) was calculated according to the [C12:0 + (4 × C14:0) + C16:0]/(ΣPUFAn-3 + ΣPUFAn-6 + ΣMUFA) formula, and the thrombogenicity index (TI) was calculated according to the [C14:0 + C16:0 + C18:0]/[0.5 × ΣMUFA) + (0.5 × ΣPUFAn-6) + (3 × ΣPUFAn-3) + (ΣPUFAn-3/ΣPUFAn-6)] formula for cooked meat, as suggested by Ulbricht and Southgate (1991); the hypocholesterolaemic/Hypercholesterolaemic FA ratio (h/H) was calculated as (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)/(C14:0 + C16:0) (Santos-Silva, Bessa, & Santos-Silva, 2002). The fatty acid profile of the experimental diets was also determined, and the results are reported in the Supplementary material (Table S1).

2.3.3. Cholesterol determination

Five-hundred μL of lipid extract of both the meat and diets was saponified in order to obtain the unsaponifiable fraction for cholesterol determination. Briefly, 500 μL of α-cholestane (0.2 mg/mL in chloroform) was added to the extract as an internal standard and then evaporated using a Rotavapor®. Five mL of KOH in methanol (0.5 M) was utilized for the saponification, which took place at 93 °C for 40 min. Cholesterol extraction was promoted by adding 3 mL of distilled water and 2 mL of n-hexane. The upper phase was transferred directly into a vial for GC analysis, which was performed using a Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Supelco SAC™ fused silica capillary column (30 m × 0.25 mm i.d., 0.25-μm film; Supelco, Bellefonte, PA, USA). One- μL of sample was injected at a 1:100 split ratio at 300 °C. The oven temperature was programmed in order to rise from 130 to 290 °C in 8 min (20 °C/min) and was then left at 290 °C for 11 min; the detector was set at 300 °C. Helium was utilized as the carrier gas and was kept at a constant flow of 1.3 mL/min. The cholesterol content was calculated through a calibration curve obtained with a