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# Effect of hop β-acids as dietary supplement for broiler chickens on meat composition and redox stability

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ABSTRACT

Addition of β-acids extracted from hop at different levels (0, 30 mg $kg^{-1}$, 60 mg $kg^{-1}$, 240 mg $kg^{-1}$) to the diet of broiler chickens demonstrated significant effects on the average concentration of polar metabolites and fatty acids of relevance for meat quality. The largest metabolic differences between control group and chicken fed different levels of β-acids were achieved using 30 mg $kg^{-1}$ of supplement. As determined by EPR spin-trapping, increased redox stability was also obtained for meat from chicken fed 30 mg $kg^{-1}$ of β-acids which also had highest level of endogenous antioxidants, especially anserine, carnosine, NADH and PUFAs. Diet and storage period were found to affect protein oxidation and myosin and actin were recognized as the main targets of protein oxidation. Myofibrillar proteins from chicken fed hop β-acids showed to be less susceptible to oxidation. A moderated level of hop β-acids as dietary supplement accordingly improve the overall redox stability, protecting myofibrillar proteins and fatty acids against oxidation and improve the nutritional properties of meat from broiler chickens.

## 1. Introduction

Oxidative pathways involving lipids and proteins are implicated in nutritional loss and quality deterioration of most food products. Especially for meat based products, the addition of phytogenic additives has been shown to efficiently prevent lipid and protein oxidation (Devatkal, Narsaiah, & Borah, 2010; Jongberg, Torngren, Gunvig, Skibsted, & Lund, 2013; Shah, Bosco, & Mir, 2014). However, addition of plant extracts directly to the meat may influence negatively the consumer acceptance. The use of plant extracts in animal feeding trials has been considered as a potential alternative for improving the redox stability without affecting the sensory aspects of meat (Arihara, 2006; García, Catalá-Gregori, Hernández, Megías, & Madrid, 2007; Greathead, 2003; Madrid, Garcı, Orengo, & Megı, 2004; Shah et al., 2014).

Meat is recognized as the most important nutritional source of high quality proteins. However, the redox stability of meat, depending on the lipid profile and on the content of bioavailable iron as a prooxidant, has been associated to an increased risk of many diseases such as cancer (Oostindjer et al., 2014; Valsta, Tapanainen, & Männistö, 2005). Considering that the content of heme iron in white meat is 10 fold lower than in red meat, particular attention has been given to substitute red meat with other protein sources like chicken. Additional health concerns of the consumers related to the preference for meat from animals raised without using growth promoters and synthetic antibiotics are also increasing (Castellini, Muginai, & Dal Bosco, 2002; Verbeke & Viaene, 2000).

Phytogenic additives for non-ruminant animal feed have been receiving attention due to their pharmacological properties holding the potential for replacing antimicrobial agents and growth promoters (Bortoluzzi, Menten, Romano, Pereira, & Napty, 2014; Racanicci, Danielsen, & Skibsted, 2007; Racanicci, Menten, Alencar, Buissa, & Skibsted, 2011). For broiler chickens and pigs, several studies have demonstrated that the diet has an effective impact on animal performance, intestinal health, fat content, lipid metabolism, meat composition and the oxidative stability of meat (Bortoluzzi et al., 2014; García et al., 2007; Madrid et al., 2004; Rabie & Szilágyi, 1998; Racanicci et al., 2007, 2011; Zhan, Li, Xu, & Zhao, 2006). When added to the animal diet, the absorbed active compounds from plant materials can directly reach the muscle tissue or can be metabolized into biological precursors of endogenous antioxidants. In both cases, the bioactive compounds can provide the antioxidant mechanisms required to

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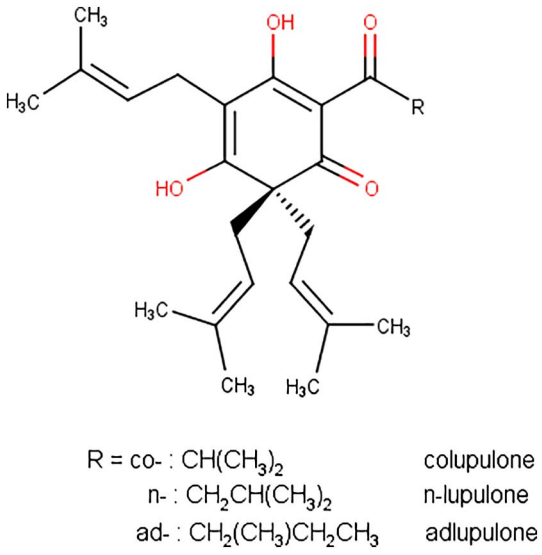


R = co- : $CH(CH_3)_2$ colupulone
n- : $CH_2CH(CH_3)_2$ n-lupulone
ad- : $CH_2(CH_3)CH_2CH_3$ adlupulone

**Scheme 1.** Chemical structure of β-acids.

improve animal health and welfare and to protect meat against oxidation during storage and processing (Verbeke & Viaene, 2000). Many studies reported that use of botanicals as dietary supplement in animal feed effectively modulates animal metabolism affecting animal welfare and meat quality (Patra & Saxena, 2011; Zawadzki et al., 2017).

Widely recognized for their use in beer manufacturing, hop (*Humulus lupulus*) is one example of phytogenic additive with antimicrobial activity that has been studied as a dietary supplement to enhance animal performance especially for broiler chickens (Bortoluzzi et al., 2015, 2014; Bozkurt, Küçükyılmaz, Çatlı, & Çınar, 2009; Cornelison et al., 2006). Pharmacological properties and antioxidant effects have been associated with the extract of hop, which has two principal classes of bioactive compounds: β-acids (lupulones, colupulones and adlupulones; Scheme 1) and α-acids (humulones and isohumulones). β-acids, GRAS notice, have antimicrobial activity and are alternatives to replace antibiotics and growth promoters in animal feed (Bortoluzzi et al., 2015). Positive effects of dietary hop on animal performance and intestinal health of broiler chickens and weanling pigs have already been demonstrated (Bortoluzzi et al., 2014; Sbardella et al., 2016). Despite the fact that many reports in literature have been comparing the bacteriostatic efficacy of hop β-acids to the most used synthetic antibiotics in animal feeding trials for the evaluation of performance, there is little information regarding the metabolic effects following the use of hop as supplement for animal diet. Herein, we report the impact of increasing dietary levels of hop β-acids on the metabolic profile and redox stability of meat from broilers.

## 2. Materials and methods

### 2.1. Animals and sampling

Animal experiments were conducted at the College of Agriculture "Luiz de Queiroz" (ESALQ), University of São Paulo, under supervision of Professor J.F.M. Menten and all the procedures were approved by the institutional animal care and use committee of the institution. One-day-old male Cobb 500 broiler chicks (a total of 1440 animals, initially averaging 42.3 ± 0.4 g in weight) were randomly distributed into five treatments with six repetitions for treatment and forty animals for experimental unity. Birds were fed *ad libitum* during 42 days according to the four experimental treatments: T1 – negative control basal diet; T2 – basal diet supplemented with β-acids at 30 mg $kg^{-1}$; T3 – basal diet supplemented with β-acids at 60 mg $kg^{-1}$; T4 – basal diet supplemented with β-acids at 240 mg $kg^{-1}$. The nutritional programme was divided into four-phase feeding program: prestarter (1–7 d), starter (7–21 d), grower (21–35 d), and finisher (35–42 d), providing, respectively, 2950; 3000; 3100 and 3150 kcal $kg^{-1}$ of metabolizable energy; 23.3; 21.5; 20.0; and 19.0% of crude protein; and 1.31; 1.17; 1.08; and 1.01% of lysine. The basal diets consisted of corn, soybean meal, and the inclusion of 5% poultry by-products meal and 5% wheat bran, in agreement to the proposed nutritional requirements of the chickens (Rostagno et al., 2011). Also, 20 mg of α-tocopherol acetate per kg of food were added to a pre-mixture of vitamins in order to provide the required levels of vitamin E.

After 42 days of feeding trial, one chicken per replicate was euthanized by cervical dislocation. After slaughter, *pectoralis* major muscle was collected, deboned and the samples were vacuum-packed and stored at a freezer at − 80 °C until proceed to the analyses used in this study.

### 2.2. Extraction of polar metabolites

All the samples were kept on ice during the extraction of the polar metabolites in order to prevent meat degradation. Approximately 0.10 g of frozen meat was homogenized with 1.0 mL of cold methanol/water solution (1:1 *v*/v) for 1 min using a commercial cell disruptor (Fast Prep®, MP Biomedicals, Solon, OH, USA) (Prema et al., 2015). Meat homogenates were centrifuged (10 min at 10,000 × *g* at 10 °C) to remove precipitated protein, fat and connective tissue. Supernatants were collected and transferred to Eppendorf tubes. After drying in a centrifugal concentrator (Speed-Vac, Thermo Savant, Holbrook, NY, USA), meat extracts containing the polar metabolites were re-suspended with deuterium oxide phosphate buffer (0.10 M, pD = 7.2) containing 0.050% *w*/w of sodium 3-trimethylsilyl-2,2,3,3-d4-propionate (TMSP-d4, from Cambridge Isotopes, Leicestershire, UK) as internal chemical shift standard. 550 mL of the solution containing the polar metabolites were transferred to a 5 mm NMR precision tube (Vineland, NJ, USA).

### 2.3. NMR quantitative profile of the polar metabolites of meat

Extracts of meat were analyzed by high-resolution $^{1}$H NMR spectroscopy using a Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) with magnetic field of 9.4 T (600.00 MHz for hydrogen frequency) equipped with a 5 mm PABBI probe head with gradients, automated tuning and matching accessory (ATMATM), BCU-I for regulation of temperature, and a Sample-Xpress sample changer. NMR experiments were performed using a pulse of 90° and 32 repeated scans were accumulated into 32,768 complex points spanning a spectral width of 20.55 ppm and relaxation delay of 4 s. The pulse sequence NOESY-presaturation (Bruker 1D noesygppr1d pulse sequence) was employed to suppress the water peak with irradiation at the water frequency (O1 = 1881.95 Hz) during the recycle and mixing time delays. FIDs were processed with a line broadening of 0.3 Hz by using exponential multiplication before Fourier transformation. Spectra were processed by phase and baseline correction and by the calibration of the internal chemical shift standard TMSP-d4 at 0 ppm using the software TopSpin 2.1 (Bruker BioSpin, Rheinstetten, Germany).

### 2.4. Data analyses and statistical methods

The database present in the Chenomx NMR suit 8.1 software (Chenomx Inc., Edmonton, AB, Canada) was used for the identification and the assignment of peaks in the $^{1}$H NMR spectra related to the polar metabolites of the meat extracts. Further NMR experiments ($^{1}$D JRES, 2D $^{1}$H-$^{1}$H TOCSY and 2D $^{1}$H-$^{13}$C HSQC) were performed on the meat extracts in order to confirm the peak assignments that were obtained from the Chenomx software database. For quantification of the polar metabolites of meat extracts, TMSP-d4 concentration was referenced as 1 mM and the 600-MHz library from Chenomx NMR Suite 8.1 was used