

An Exploratory Field Study on Egg Quality and Yolk Functional Properties in Commercial and Local Laying Hen Breeds Fed Whole Hemp Seed Under Agroecological Conditions

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Supplementary File S2: In vitro digestion of egg yolks

For *in vitro* digestion, the Infogest protocol was applied as reported by Minekus et al [1] and Brodkorb et al [2]. Simulated digestion fluids for the oral (SOF), gastric (SGF), and intestinal (SIF) phases were prepared in agreement with Brodkorb et al [2]. For the oral phase, 2.5±0.05 g of each sample was diluted with SOF (1:1) and incubated for 2 min at 37°C, shaking with α -amylase (75 U/mL, pH 7.0). Subsequently, the oral bolus was diluted with SGF (1:1) and pepsin (2.000 U/mL, pH 3.0) and incubated at 37°C for 2 h under agitation. At the end of the incubation, the gastric chyme was diluted with SIF (1:1) and incubated with bile salts (10 mM, pH 7.0) and pancreatic enzymes (100 U/mL, pH 7.0) for 2 h at 37°C, under shaking condition. Before the functional profile was evaluated, the samples were stored at -20°C.

Total phenolic content (TPC) and antioxidant activity (ABTS) of hemp co-product, diets and eggs

For TPC, 200 μ L of each sample were incubated with 1.0 mL of Folin-Ciocalteu and 0.800 μ L of sodium carbonate in the dark at RT for 20 min. After incubation, the samples were read with a spectrophotometer at a wavelength of 630 nm. Data were expressed in terms of tannic acid equivalent (mg TAE/100 g). Antioxidant activity was determined using the ABTS radical cation decolorization assay according to the method of Re et al. [3] with minor modifications. Briefly, the ABTS•⁺ radical cation was generated by mixing 5 mL of ABTS solution (7 mM) with 88 μ L of potassium persulfate (K₂S₂O₈, 2.45 mM). The mixture was incubated in the dark at room temperature (RT) for 16 h prior to use. The resulting ABTS•⁺ solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm. For the assay, 10 μ L of sample were mixed with 1.0 mL of the diluted ABTS•⁺ solution and incubated for 6 min at RT in the dark. Absorbance was subsequently measured at 734 nm using a spectrophotometer. Trolox was used as the reference antioxidant standard. Calibration curves were prepared using six Trolox concentrations ranging from 0 to 2000 μ M. Antioxidant activity was calculated from the calibration curve and expressed as mg Trolox equivalents (TE) per 100 g of

sample.

References

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