

# Beneficial Effect of Dietary Bioactive Compounds from Residual Nuts, a By-Product of Pastry on Antioxidant Defense in Pigs after Weaning

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## Abstract

The walnuts of *Juglans regia* are rich source of a variety of bioactive compounds like polyunsaturated fatty acids (PUFA omega-6, -9, -3), polyphenols, etc known as antimicrobial and antioxidant substances. In the present study the antioxidant potential of a residual walnuts resulting from the pastry processing was used as a dietary alternative source of antioxidants in pigs after weaning. Thirty crossbred TOPIG hybrid weaned pigs were randomly assigned (n=10) to three experimental treatments: a normal diet (control group) and two diets included 3 and 5% walnuts residual for 35 days.

A high concentration of total polyunsaturated fatty acids,  $\omega$ -6 (61.0g/100g fat),  $\omega$ -9 (17.89g/100g fat oleic acid) and  $\omega$ -3 (11.06g/100g fat) as well as a total phenolic content (PT) of 15.66g GAE/L extract was found in residual walnut. Results indicated an increase of total antioxidant capacity and of the activity of antioxidant enzymes catalaza (CAT) and superoxid dismutaza (SOD) in plasma and liver of pigs fed walnut diets, without effect on glutation peroxidaza (GPx) and biochemical parameters. Further work is necessary to provide data about the effect of other percentage of walnut dietary inclusion and to select the optimum.

**Keywords:** antioxidant status, pig, walnut by-product

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## 1. Introduction

Many plant sources contain bioactive compounds such as natural antioxidants (polyphenols, mono- and polyunsaturated fatty acids, vitamins, minerals, etc.) which have well-known anti-inflammatory, antimicrobial, antioxidative, hypoglycaemic, hypolipidemic and immunomodulating effects [1-3] in animals and humans. The industrial processing of these sources for foods or other products produce significant amounts of by-products/wastes that preserve much

of their bioactive compounds, and which can be used as alternative sources of feed in farm animal diets.

The walnuts (*Juglans regia*) and walnut oil are known for their high content of oil (50-70%) rich in mono- and polyunsaturated fatty acids, particularly linoleic acid (omega-6) and oleic acid (omega-9), [4]. The walnuts are also rich in tannins and polyphenols, vitamins (tocopherols) and minerals (magnesium), [5, 6] antioxidant compounds which can influence the activity of important enzymes that play a key-role in the oxidative stress, responsible for inflammations, cancers, atherosclerosis and early ageing, by the release of free radicals and by the initiation of oxidation reactions which lead to imbalances in cell metabolism and in the enzymatic activity [7]. The nut cakes are the main by-product resulting

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from walnut processing for oil production. Beside this, other by-product/waste results from the use of walnuts for pastry and cakes, which has the advantage to preserving the entire nutritive value of the nuts. The use of walnut by-products in farm animal feed has been poorly studied. In the present study the antioxidant potential of a residual walnuts resulting from the pastry processing was used as a dietary alternative source of antioxidants in pigs after weaning. As it is well-known, weaning and the post-weaning are very difficult periods for this species, when the enzyme and immune systems are still under development, which makes the piglets particularly sensitive to infections with *E. coli* and *Rotavirus* [8]. The gastro-intestinal inflammations and the oxidative stress are characteristic processes of the weaning period. The inclusion of walnut waste in the compound feed for growing pigs might be an alternative source of nutrients and antioxidants suitable for feeding strategies aiming to enhance the immune status and health state of the farm animals, thus avoiding bacterial infections and decreasing the need for antibiotics by the use of natural products.

Being a new product, studies are needed on the level of inclusion in compound feeds formulations, on its inflammatory/anti-inflammatory and antioxidant effect, etc. Hence, during this stage of the project we conducted a feeding trial on weaned piglets which received a compound feed with 3-5% walnut waste.

## 2. Materials and methods

### *Experimental design*

#### *Animals, diet, samples collection*

The feeding trial was carried out on three groups of TOPIG hybrid piglets (10 piglets/groups) housed in pens and fed *ad libitum* with a normal commercial diet for pigs (control diet CD), 3% residual walnut diet (WD 3%) and 5% residual walnuts diet (WD 5%) respectively. At the end of the feeding trial (35 days) blood samples were aseptically collected on lithium heparin (Vacutainer tubes) from each animal and centrifuged at 775xg for 25 min to obtain plasma. Pigs were sacrificed and organs samples were collected on ice. Liver aliquots were stored at -80°C until analyzed. Biochemical and antioxidant and immune markers (total antioxidant

capacity, TCA, antioxidant enzymes activity and immunoglobulin IgA, IgM and IgG) were determined from plasma and liver aliquots.

#### *Extraction of polyphenols and total phenolic content of GP and GS cakes*

The polyphenols from grape pomace and grape seed cakes were extracted using 80% acetone (ratio sample; solvent 1:7 w/v) for 20 h at 37°C with continuous shaking. The total polyphenols were determined using Folin–Ciocalteu method and a gallic acid standard calibration curve. The absorbance was read at 750 nm on a UV-VIS diode array spectrophotometer (Specord 250, Analytic Jena), and the total polyphenol concentration was calculated using the correlation between the sample absorbance and gallic acid concentration. The results were expressed as Gallic acid equivalents (mgGAE)/100g grape by-product sample.

#### *Total polyphenols and fatty acid analysis*

The polyphenols content of walnut residue were determined according to Folin–Ciocalteu method and a gallic acid standard calibration curve as described by [9] while the fatty acid composition from walnut by-product was determined by gas chromatography using a Perkin Elmer gas chromatograph (Clarus 500, USA) and a BPX70 capillary chromatographic column for fatty acid methyl esters (60 m×0.25 mm i.d.×0.20 µm, Agilent, column flux being 50 mL/min, and the split ratio 1:100) as described by [10].

#### *Biochemical and immunological parameters analyses*

Blood biochemical markers and immunoglobulin subssted IgA, IgM and IgG were determined from plasma of blood collected at the end of the experiment on an automatic BS-130 Chemistry analyzer (Bio-Medical Electronics Co., LTD, China) and according to Bethyl manufacturer's instructions (Bethyl, Montgomery, TX, USA) respectively using a micro plate reader (Tecan Infinite M200 PRO, Austria).

#### *Total antioxidant capacity and antioxidant enzymes (SOD, CAT, GPx) activity analyses*

Antioxidant level in liver was determined with the total antioxidant capacity (TAC) kit (QuantiChrom–BioAssay Systems, USA) according with manufacturer's instructions as described by [11]. Antioxidant enzyme activity of superoxide

dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) was measured by using Cayman kits and their recommendations. Briefly, 1g of frozen liver tissue was homogenised in chilled phosphate buffer for each specific enzymes and centrifuged at 1500 or 15000xg for 15 min at 4°C. The resulted supernatants were used for the activity determination described by Cayman kit (Ann Arbor, MI). The absorbance was measured using a Tecan micro plate reader (Tecan, SunRise, Austria).

#### *Statistical analyses*

One way ANOVA analysis was used to investigate the statistical differences between groups for all parameters analysed. Significant differences between means were determined by the least square difference Fisher procedure (StatView software 6.0, SAS Institute, Inc., Cary, NC). Values of  $P < 0.05$  were considered significant.

### **3. Results and discussion**

The biochemical analyses revealed a high concentration of total polyunsaturated fatty acids (PUFA) especially  $\omega$ -6 fatty acids (61.0g/100g fat from which 60.78 g/100g fat for linoleic acid), followed by  $\omega$ -9 (17.89g/100g fat oleic acid) and  $\omega$ -3 (11.06g/100g fat from which 10.96g/100g fat for  $\alpha$ -linolenic acid). The total phenolic content was 15.66g GAE/L extract.

#### *Effect of the walnut waste on the general health status (in vivo systemic response–blood biochemical parameters)*

The effect of the walnut meal on the general health status of the animals was evaluated via some blood biochemical parameters which reflect its influence on the carbohydrate, lipid, protein and mineral metabolism, as well as on the hepatic and renal metabolism. The plasma concentrations of glucose, cholesterol and triglycerides in the piglets treated for 35 days with walnuts diets showed no significant influence neither for the diet with 3% nor for that with 5% residual nuts (data not shown). A slight decrease of the plasma urea and creatinine concentrations was noticed in the piglets treated with 3% nut meal, but the differences from the control group were not statistically significant. The piglets treated with 5% nut meal showed a higher concentration of TGO (42.56 vs 36.11 U/L) and a lower concentration of alkaline phosphatase (151.77

vs 16.15 U/L). No effect on Ig subssted IgA, IgM and IgG were noticed (data not shown).

By contrast, the inclusion of other nut by-product (cashew nut bran) at levels of 7.5 and 15% in broiler diets decreased the levels of cholesterol, palmitic (C16:0) and linoleic (C18:2) acids and increased oleic acid (C18:1) content of abdominal fat [4].

#### *Effect of the walnut waste on the total antioxidant capacity in liver*

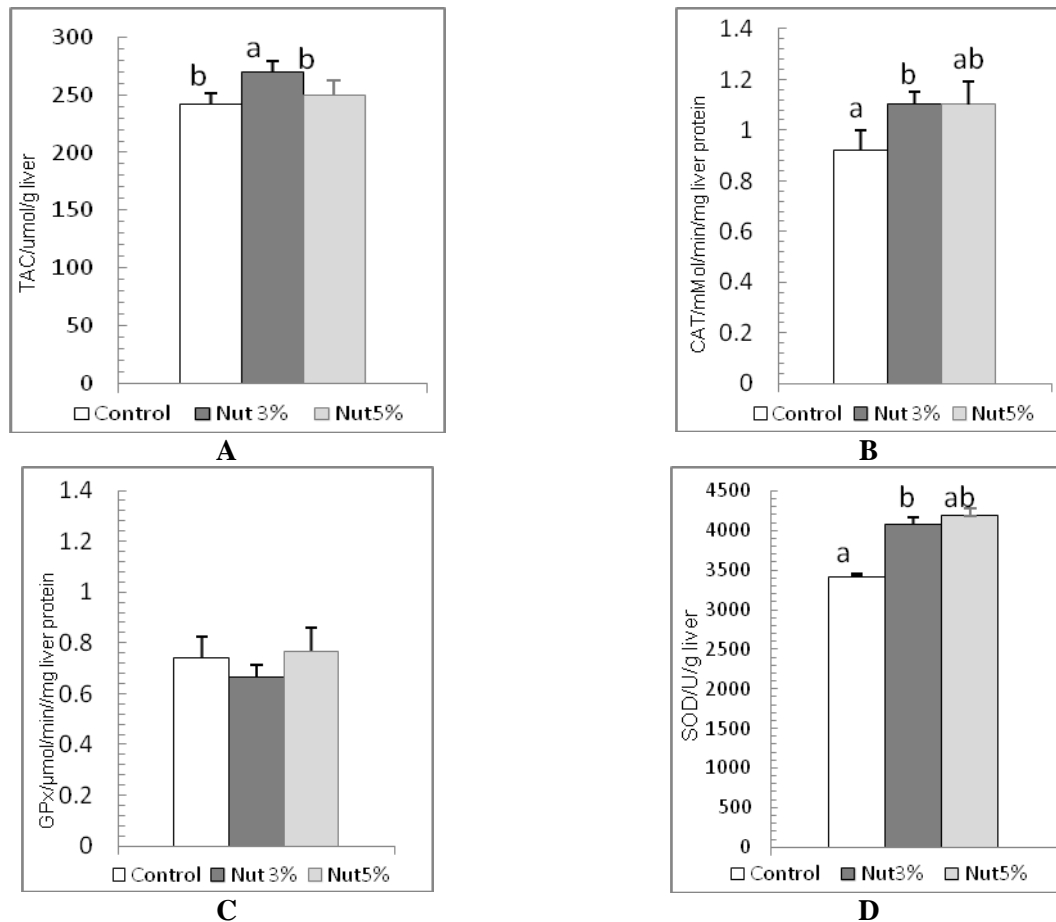
Feeding the piglets for 35 days with 3% and 5% walnut diet enhanced the antioxidant capacity of the blood plasma, particularly in the 3% walnut diet (270.0-Nut3% vs 249.6-Nut5% and 241.5-Control trolox equivalents) (Figure 1A).

#### *Influence of the walnut waste on the antioxidative enzymatic response in the liver*

Oxidative stress enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in the liver samples collected in the end of the feeding trial. Data processing showed that the diet with residual walnut increased significantly the activity of CAT (0.919-C vs 1.103-Nut3% and 1.103-Nut5%) and that of SOD (3415.99-control vs 4075.2-Nut3% and 4190.0-Nut5%) and had no effect on the activity of GPx (0.743-control vs 0.664-Nut3% and 0.768-Nut5%) compared to the control (Figure 1B, 1C, 1D). The higher enzymatic activity of the catalase suggests that walnut can be a suitable way to stop the oxidative stress in the organism. Similarly, recent study of [12] showed that a mixture of plant polyphenols from apple, grape seed, green tea and olive leaf improved plasma antioxidant activity by decreasing malondialdehyde concentration in plasma. Also, [13] showed that dietary supplements of polyphenols from grape waste (grape pomace) activate the antioxidant enzyme system and prevent hypercholesterolemia and [10, 14] reported that antioxidants and other bioactive compounds from grape waste (grape seed cakes and grape pomace) reduced inflammatory markers in pig spleen and liver. By contrast there are studies showing that dietary supplementation with grape seed and grape marc extract as sources of polyphenols had no effect on hepatic pathways linked to inflammation, the antioxidant and cytoprotective system, stress of the endoplasmic reticulum and the xenobiotic system in healthy piglets [2].

Further studies are required, though, to investigate other levels of walnut waste inclusion in the formulations for piglets particularly after the difficult period of weaning.

Correlations with other parameters which are important for animal health and growth performance, with economic impact in pigs have to be done.



**Figure 1.** Effect of dietary bioactive compounds from residual nuts on antioxidant status in pigs after weaning

Pigs received three different diets: 1) control, 2) 3% residual walnut (Nut-3%), 3) 5% residual walnut (Nut-5%) for 35 days. At the end of the experiment, blood and liver samples were collected and analyzed for total antioxidant capacity and antioxidant enzymes activity using Cayman method.

A) Total antioxidant capacity is expressed as trolox equivalent. B), C), D) Enzymes activity was reported per g of tissue.

All values are represented as mean with their standard errors. ANOVA (one-way) followed by Fishers tests were performed to analyze the effect of the diets.

<sup>a,b</sup>=Mean values within a row with unlike superscript letters were significantly different (P<0.05).

#### 4. Conclusions

The walnuts residual from pastry or other industrial processing represent an alternative source of antioxidant compounds which could be use in animal diet in certain period like for example in pigs after weaning. Diet included walnut enhanced the hepatic antioxidant status by increasing the total antioxidant capacity and antioxidant enzyme activity).

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