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EFFECT OF DIETARY SUPPLEMENTATION WITH HYDROPONIC GREEN BARLEY FODDER ON RABBIT PERFORMANCE, ANTIOXIDANT CAPACITY, AND TOTAL PHENOLIC COMPOUNDS IN MEAT

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RESEARCH ARTICLE

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ABSTRACT: Rabbit meat stands out as a nutritious low-fat protein source rich in essential amino acids, vitamins, and minerals, yet its production faces challenges from high feed costs and environmental impacts. Hydroponic green barley fodder (HGBF) emerges as a promising sustainable alternative, offering rapid growth, enhanced nutrient profiles including antioxidants, and reduced resource demands. This study aimed to evaluate the effects of dietary HGBF supplementation, with or without hydrogen peroxide (H_2O_2) seed pre-treatment, on rabbit performance, meat antioxidant capacity, and total phenolic compounds. Sixty-three post-weaned New Zealand rabbits (initial weight: 606 g) were divided into three groups for 42 days: T1 (100% pellets, control), T2 (50% pellets + 50% HGBF at 75 mM H_2O_2 pre-treatment), and T3 (50% pellets + 50% HGBF), All treatments were ad libitum. Carcass yields were assessed post-slaughter, while meat samples underwent 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) assays for antioxidant activity, enzymatic evaluations (catalase, superoxide dismutase, phenylalanine ammonia-lyase), and Folin-Ciocalteu method for phenolics. Results revealed no significant differences in hot or cold carcass weights across treatments, averaging below export standards due to the rabbits' young age. However, T2 diet significantly boosted total phenolic content ($P < 0.05$) and antioxidant capacity via DPPH (97.52% inhibition) and $ABTS^{\bullet+}$ (84.07% inhibition) compared to T1 and T3 groups with lower catalase activity indicating reduced oxidative stress. Enzymatic activities for superoxide dismutase and phenylalanine ammonia-lyase remained unaffected. These findings underscore HGBF with H_2O_2 pre-treatment as an effective strategy to enhance meat quality through elevated phenolics and antioxidants, fostering oxidative stability and nutritional value while promoting eco-friendly rabbit farming practices for broader livestock sustainability.

Keywords: Antioxidants, Carcass yield, Hydroponic green barley fodder, Oxidative stability, Rabbit meat, Total phenolic compounds.

INTRODUCTION

Rabbit meat is consumed all over the world, being delicious, appetizing and high quality. It is composition is mainly lipids, proteins and water, with high levels of micronutrients, essential amino acids, vitamins and minerals. Additionally, it has low uric acid, purines, and fat (Dalle Zotte and Szendro, 2011). However, it has been studied that the composition of the lipids depends on the type of rabbits feeding, being reflected in carcass composition (Oo et al., 2018). Furthermore, rabbit production is economically relevant for their sporting utility and the manufacture of skins that are used in multiple products (Pelletier et al., 2019). Furthermore, this species, the New Zealand rabbit, is commonly utilized in biological experiments within educational institutions and hospitals, serving as a valuable model for nutritional and antioxidant research (Song et al., 2020). Rising rabbits demand large volume of raw plant material to fattening (Wang et al., 2020). Fattening rabbits requires substantial plant-based feed, including tubers, cereals, fruits, and vegetables. Wild rabbits mainly feed of herbaceous and grasses, which contain considerable quantities of cellulose (Grioui et al., 2021).

In 2015, global cereal production reached 10 million tons; about 60% of world consumption of coarse grains (barley, maize, oats, quinoa, rye, sorghum, tef, and millet) used for livestock feed (FAO, 2021). Cereals also are produced for fodder livestock, since they can be converted directly into protein usable by animals, lowering the cost of feed, accelerating around 25% of growing and diminish time of fattening (Miah et al., 2020). Barley, constituting 65% of

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livestock feed. Whole grain barley includes flavonoids, folates, lignans, phenolic acids, phytosterols and tocoles, with the predominant vitamin E (tocol), flavonoids, phenols, sterols and vitamins. These phytochemicals offer strong antioxidant capacity, useful for reducing free radicals. In addition, this specie is rich in dietary fiber made up of soluble and insoluble fibers (Abouelezz et al., 2019). Hydroponic Green Barley Fodder (HGBF) yields 7–10 kg per kg of grain in 8–15 days, with increased crude protein, and calcium content; additionally, acid detergent fiber, neutral detergent fiber, calcium content and crude protein are increased (Singh et al., 2019).

Furthermore, rabbit meat production requires diets that mixture between 100 to 200 g of HGBF and a daily average of 50 g of concentrates, per animal, for optimal production (Miah et al., 2020). Furthermore, HGBF includes great fiber content, that it is necessary for rabbits' digestion and nutrition (Delis-Hechavarria et al., 2021). Besides, Selim et al. (2021) obtained a final live weight gain (1,533 g) with a supplementation of 15 g/kg with *Moringa oleifera*. Additionally, Abouelezz et al. (2019) had the highest body weight in male rabbits using concentrated plus HGBF and the feed intake was similar in all treatments. Indeed, the mixture of two feeds allowed the absorption of nutrients for growth, which could have a benign effect on the characteristic of rabbit meat (Dalle Zotte et al., 2018). Meat is susceptible of losing its nutritional properties due to the action of the oxidation process on lipids to rather than the degradation process by bacteria (Chen et al., 2017). This deterioration is observed in the discoloration of the meat. For example, Beefs are most susceptible to this oxidation process, reducing quality and shelf life (Bouzaida et al., 2021). The chemical processes involved in this process are the water retention capacity, rancid odors due to the formation of aldehydes and ketones, reduced solubility of proteins (Kim et al., 2019). However, studies on the concentrated diet plus HGBF are very scarce. Therefore, in this manuscript the effect of two combinations of barley fodder one with seeds pre germination on 75 mM hydrogen peroxide (H_2O_2) and barley fodder with water supplemented plus 50% pellet, were evaluated on yield carcass and rabbit meat antioxidant capacity.

MATERIALS AND METHODS

Plant material and growth conditions

Hydroponic Green Barley Fodder (HGBF) was cultivated at Innovation and development Green (IDGREEN) farm, La Norita, Queretaro, México (20°32'58.6"N -100°30'10.6"W; altitude 1823 m) in January-February 2021. Barley seeds (*Hordeum vulgare* L var. Cantabria) were soaked in water and 75 mM hydrogen peroxide concentration for 24 h. It drained and placed on trays of 54 × 35 × 7 cm. The trays were stored in a chamber fodder for 11 days at 25°C, 14 h light:10 h dark photoperiod, and relative humidity of 80%. Fodder was watered only with water, six times daily for two min each one. Rabbits were fed with HGBF (leaves, roots and seeds) immediately after harvesting, including (Morales et al., 2010).

Animal and materials

Sixty-three post-weaned New Zealand rabbits (average initial body weight (BW): 0.606 ± 0.01 kg) were housed in four wire cages (76 × 48 × 30 cm) containing three rabbits for each treatment. The temperature in the rabbitry was 15 ± 2°C, 41.10 ± 10.68% relative air humidity and 12-h light: 12-h dark photoperiod. Rabbits had free access to clean water. The 42 days study included three fed diets: T1 (100 % pellets), T2 (50% pellets + HGBF at 75mM H_2O_2), T3 (50% pellets + HGBF *ad libitum*). All treatments were ad libitum. Rabbits were weighed and tranquillized with azaperone (2 mg/kg BW), anaesthetized with isoflurane (inhaled) and euthanized using a cardiac overdose of sodium pentobarbital (50 mg/kg BW) (Gómez Soto et al., 2018).

Ethical regulations

The study was performed at DGREEN farm. The protocol was reviewed and approved by the Autonomous University of Queretaro's Bioethics Committee (authorization: 90-FI-2017) of the Engineering Science Faculty's. The handling of the experimental animals was carried out conforming to the Mexican Official Norm 'NOM-062-ZOO-1999' guidelines (Norma Oficial Mexicana, 1999) and the International Guiding Principles for Biomedical Research Involving Animals (CIOMS/ICLAS 2012).

Sampling

Analyses were carried out at the Animal Nutrition Laboratory, Faculty of Natural Sciences, Autonomous University of Queretaro, Queretaro, Mexico. The stomach, jejunum, ileum, cecum, and colon were dissected, and emptied contents measured a potentiometer (Model PH211, Hanna Instruments®). Jejunum-ileum, liver, gallbladder, stomach, colon-rectum and cecum sections were collected, washed with saline solution, weighed, and preserved in 10% neutral formalin solution for further analyses (Gómez Soto et al., 2018).

Antioxidant activity (AOx)

Antioxidant properties of rabbit meat from each treatment were assessed using the 1,1-diphenyl-2-picrylhydrazyl

(DPPH•) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) assays.

DPPH• Radical Scavenging Activity (DRSA) Assay

The DPPH• assay was adapted from Chen et al. (2012) with modifications. A 0.5 mL sample solution's aliquot (600 µg/mL) was combined with 0.5 mL of 0.1 mM DPPH• in methanol. The mixture was vigorously mixed, incubated in darkness at ambient temperature for 30 min, and absorbance was recorded at 515 nm. Methanol replaced DPPH• for the blank, and deionized water replaced the sample for the control. The DRSA percentage was calculated using the equation (1): $DRSA(\%) = [1-(A_{sample}-A_{blank}/A_{control})] \times 100$ (1)

where A_{sample} is the sample absorbance, A_{blank} is the blank absorbance, and $A_{control}$ is the control absorbance.

$ABTS^{\bullet+}$ radical scavenging activity (ARSA) assay

The ARSA assay was conducted following Re et al. (1999) with adjustments. $ABTS^{\bullet+}$ radicals were produced by reacting 7 mM $ABTS^{\bullet+}$ with 2.45 mM potassium persulfate, incubating in darkness for 12 h at room temperature. The $ABTS^{\bullet+}$ solution was diluted in 0.15 M phosphate-buffered saline (PBS, pH 7.4) to achieve an absorbance of ~0.7 at 734 nm. Then, 3 mL of diluted $ABTS^{\bullet+}$ was mixed with 150 µL of sample (600 µg/mL), and absorbance was measured under dark conditions. PBS replaced $ABTS^{\bullet+}$ for the blank, and deionized water served as the control. ARSA was calculated as (2): $ARSA(\%) = [1-(A_{sample}-A_{blank}/A_{control})]x100$ (2)

where A_{sample} is the sample absorbance, A_{blank} is the blank absorbance, and $A_{control}$ is the control absorbance.

Enzyme activity assays

Enzyme activities in rabbit meat were evaluated to assess oxidative stress responses, focusing on catalase (CAT), superoxide dismutase (SOD), and phenylalanine ammonia-lyase (PAL).

Samples preparation of enzymatic assays

Lyophilized rabbit meat samples (0.5 g) were homogenized in 1 mL of chilled extraction buffer and centrifuged at 13,000 rpm for 20 min at 4°C. The resulting supernatant was used for enzyme activity measurements. Protein content was quantified using the Bradford (1976) method, with bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as the standard.

Catalase activity assay

Catalase (CAT; EC 1.11.1.6) activity was measured spectrophotometrically by monitoring the rate of H_2O_2 decomposition at 240 nm, following Afiyanti and Chen (2014). The reaction mixture contained 0.95 mL of 50 mM potassium phosphate buffer (pH 8.0), 0.05 mL of enzymatic extract, and 0.1 mL of 100 mM H_2O_2. Absorbance changes were recorded for 1 min at 25°C. One CAT unit decomposes 1 µmol of H_2O_2 per minute at pH 8.0. Results were expressed as U/mg protein.

Superoxide dismutase activity assay

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by measuring the inhibition of nitro blue tetrazolium (NBT) photochemical reduction, as described by Hayat et al. (2018). The reaction mixture included 1.5 mL of 50 mM potassium phosphate buffer (pH 7.8), 0.3 mL of 0.1 mM EDTA, 0.3 mL of 0.13 M methionine, 0.3 mL of 0.02 mM riboflavin, 0.05 mL of enzymatic extract, and 0.25 mL of distilled water. The mixture was exposed to fluorescent light (86.86 µmol/m^2·s) for 20 min, and absorbance was measured at 560 nm. One SOD unit inhibits 50% of NBT reduction at pH 7.8 and 25°C. Results were reported as U/mg protein.

Phenylalanine ammonia-lyase (PAL) activity

PAL activity (EC 4.3.1.5) was assessed using the method of Toscano et al. (2018). The assay measured cinnamic acid production from L-phenylalanine at 290 nm. The reaction mixture consisted of 2.3 mL of 0.1 M borate buffer (pH 8.8) with 10 mM L-phenylalanine and 0.2 mL of enzymatic extract. After incubation at 40°C for 1 h, the reaction was stopped with 0.5 mL of 5 N HCl. Absorbance was recorded at 290 nm. One PAL unit releases 1 µmol of cinnamic acid per minute at pH 8.8 and 40°C. Results were expressed as U/mg protein.

Total polyphenol content (TPC)

Lyophilized rabbit meat samples (1 g) were pulverized in liquid nitrogen and extracted with a methanol:water mixture (1:10 w/v). The suspension was sonicated (Bransonic M2800-CPX-HE, Emerson, Ferguson, MO, USA) for 30 min, then centrifuged at 10,000 rpm for 15 min at 4°C. Supernatants were stored at 4°C in dark vials. Total phenols were quantified using the Folin-Ciocalteu method (Vergara-Castañeda et al., 2010) with gallic acid as the standard. A 140 µL extract was mixed with 460 µL of distilled water and 250 µL of 1 N Folin-Ciocalteu reagent. After 5 min, 1250 µL of 20% (w/v) sodium carbonate was added. The mixture was vortexed, incubated in darkness for 2 h, and absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Statistical analysis

Rabbits were randomly divided into three dietary treatment groups, 21 animals being assigned to each one of the treatments (T1, T2, and T3, described in Animal and materials section) and each group having three replications with seven rabbits in each replication. Data were analyzed as a randomized complete block design. Data were subjected to analysis of variance (ANOVA) and Tukey tests ($\alpha < 0.05$) were using the Statgraphics® Centurion XVI statistical software (StatPoint Technologies Inc., Bedford, MA, USA, 2010).

RESULTS AND DISCUSSION

Productive behavior of rabbit meat

The study determinates the productive performance of rabbit meat across three dietary treatments—100% pellets, 50% pellets + 50% HGBF at 75 mM H_2O_2, and 50% pellets + 50% HGBF—over a 42-day period, with results presented in Table 1. Live weight no was significantly different compared to the HGBF-supplemented groups, indicating a potential influence of pellet-based diets on growth. Stomach pH and organ weight showed no significant differences across treatments. Jejunum pH and length exhibited slight variations, though not statistically significant ($P > 0.05$). Cecum pH and length, colon-rectum pH, length, and liver relative weight remained consistent across groups, with no significant treatment effects ($P > 0.05$), suggesting that HGBF supplementation maintains gastrointestinal and organometric stability.

Table 1 – Productive behavior of rabbit meat (x ± SD, n=21).

Parameters	Pellets 100%	Pellets 50% + 50% HGBF at 75 mM H_2O_2	Pellets 50% + 50% HGBF
Alive weight (kg)	1.71 ± 0.09[a]	1.63 ± 0.16[a]	1.66 ± 0.15[a]
Stomach pH	1.59 ± 0.34[a]	2.07 ± 1.22[a]	1.91 ± 0.37[a]
Stomach organ empty weight (g)	19.58 ± 1.87[a]	20.45 ± 0.82[a]	20.85 ± 1.82[a]
Jejunum-ileum pH	7.33 ± 0.21[a]	7.91 ± 1.07[a]	7.43 ± 0.36[a]
Jejunum-ileum organ empty weight (g)	34.50 ± 5.66[a]	34.73 ± 1.66[a]	38.27 ± 2.17[a]
Jejunum-ileum length (cm)	205.50 ± 46.75[a]	225.50 ± 16.05[a]	207.00 ± 49.28[a]
Cecum pH	5.85 ± 0.08[a]	6.02 ± 0.18[a]	5.96 ± 0.14[a]
Cecum organ empty weight (g)	24.60 ± 4.47[a]	21.30 ± 2.85[a]	25.13 ± 4.02[a]
Cecum length (cm)	41.25 ± 3.03[a]	40.25 ± 2.86[a]	39.25 ± 0.83[a]
Colon-rectum pH	6.41 ± 0.27[a]	6.57 ± 0.14[a]	6.46 ± 0.24[a]
Colon-rectum organ empty weight (g)	28.93 ± 5.36[a]	27.85 ± 1.12[a]	28.95 ± 2.49[a]
Colon-rectum length (cm)	95.25 ± 3.34[a]	95.75 ± 6.26[a]	96.50 ± 4.56[a]
Liver relative weight (g)	63.00 ± 11.42[a]	64.15 ± 11.23[a]	62.83 ± 14.48[a]
Gallbladder weight (g)	0.53 ± 0.13[a]	0.70 ± 0.27[a]	0.65 ± 0.32[a]

SD=Standard deviation. [a], Means within a column with different superscripts differ significantly ($P<0.05$).

In recent years, interest in the consumption of nutraceutical products has increased in the market (Cesari et al., 2018). Meat is considered a functional food with many bioactive compounds; specifically, rabbit meat has important nutritional and dietary properties to improve and support health (Dal Bosco et al., 2018). Figure 1 illustrates the hot and cold carcass weights and yields for the three treatments.

No significant differences were observed in hot or cold carcass weights across the treatments (T1, T2, T3). Average carcass weights were below typical export standards, attributed to the rabbits' young age at slaughter (initial age: 35 days; final age: ~77 days). This suggests that partial substitution of pellets with HGBF (with or without H_2O_2 pre-treatment) maintains carcass yield without compromising growth performance, despite the shift to a more sustainable feed option. It has been seen that alternative rabbit feeds variations with alternative products such as herbs, spices, barley, corn, alfalfa, vegetables, among others, produce lower yields in the meat of different species for meat production (Dalle Zotte et al., 2014).

Studies such as those of Mattioli et al. (2016) reported higher carcass performance in rabbit meat with supplementation with Digestarom® or 15% Gliricidia leaf meal with plus multi-enzymes (1 g/Kg) in the hot carcass weight in the diet of the 35-day-old rabbits (Oloruntola et al., 2018). El-Kady et al. (2021) achieved a carcass weight of 1,591.67 ± 49.50 g in white New Zealand rabbit with the diet of 33% of corn stalks treated with *Trichoderma reesei* fungi. Diets supplemented with organic products waste have also been evaluated, as is the case of Dabbou et al. (2017); they evaluated the cranberry waste as an alternative resource to the rabbit diet; carcass performance was not affected by the inclusion of this diet. While, Grioui et al. (2021) showed no significant effect of dried tomato waste in the feeding of growing rabbits was evaluated on the characteristics of the carcass and the meat quality. In addition, they determined that the weight of the carcass was between 1.27 and 1.185 Kg with the rabbits fed with 10 and 20% of the dried tomato

waste, and obtaining a higher percentage of yield (62.21%) with the treatment of 10% of the hot runner while in the cold runner the yield was 59.31% with the same treatment.

However, it should be noted that these supplemented diets types provide 2% more crude protein than diets based on concentrates (Mattioli et al., 2016). Likewise, it has been studied that factors such as ionization, acidification, and land use directly influence the weight of the carcass of rabbit meat production (Cesari et al., 2018). Some studies indicate that the meat 'softness' is associated with multiple factors involving chemical composition and muscle changes, stress from protein breakdown during slaughter, processing time, diet and refrigeration conditions of the meat canal. As well as, it varies between species and races, the different muscles and the cuts distances due to the change in temperature between them when the muscle becomes meat. Additionally, after cooking, the mechanical evolution of the meat is the result of the relative contribution of two components (myofibrillar and conjunctiva) in addition to their interaction (Grioui et al., 2021).

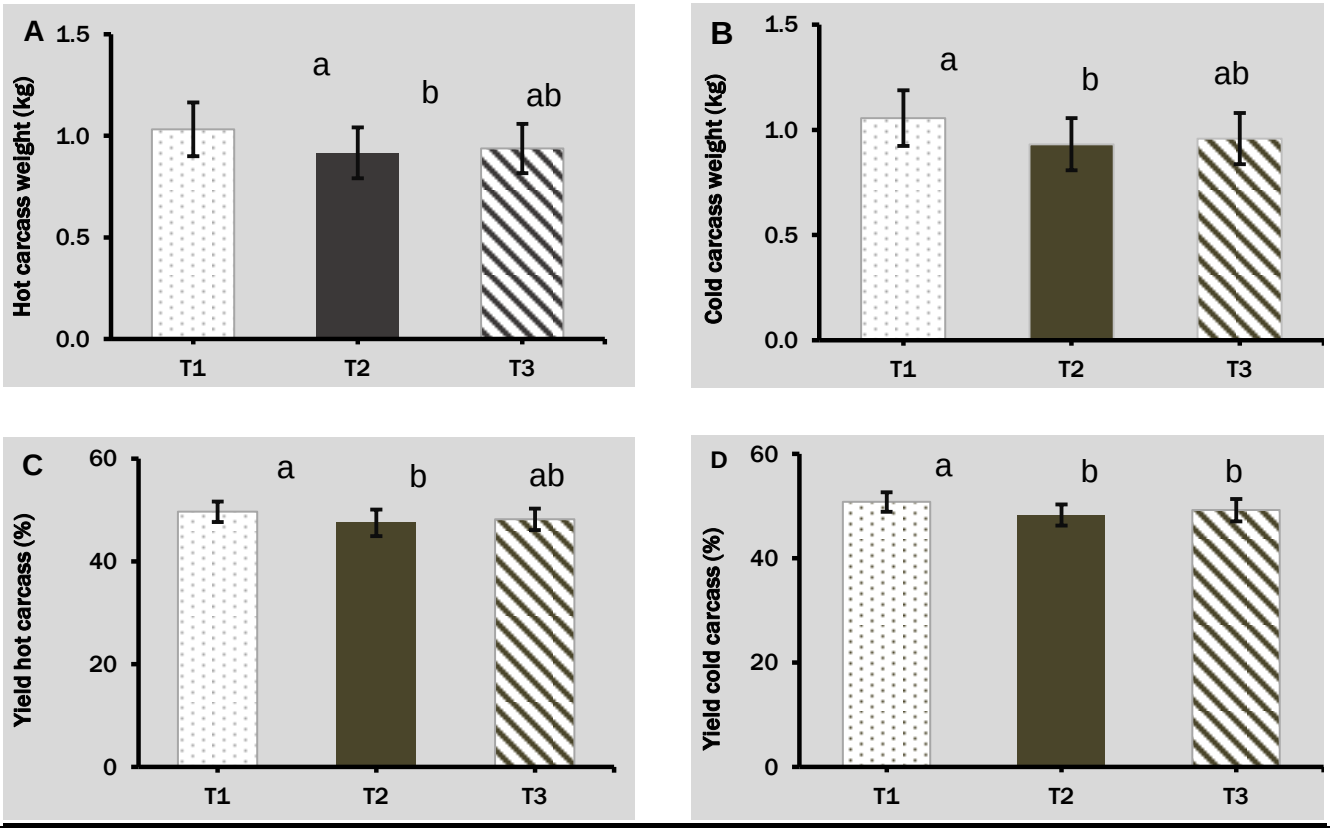

Figure 1 - The hot and cold carcass weights and yields for the three treatments. All treatments were ad libitum. Different letters indicate significant difference according to ANOVA and Tukey test (α = 0.05).

Antioxidant capacity of rabbit meat fed with three different treatments

Bioactive compounds present in functional foods are absorbed by animals enhance tissue antioxidant capacity (Mattioli et al., 2016). Similarly, the antioxidant capacity of plants and spices are derived from phenolic compounds (flavonoids, tannins, phenolic acids) and some vitamins such as E, C and A can prevent liperoxidation through trapping free radicals (Oloruntola et al., 2018). Table 2 presents the result of the antioxidant capacity of the rabbit meat evaluated in this study.

No significant differences were observed between treatments, suggesting consistent antioxidant activity. Some phytochemicals in plants improve the antioxidant, antimicrobial capacity, taste and palatability of foods, which could result in higher feed consumption and performance in animals (Hashem et al., 2017; Oloruntola et al., 2018). Phytochemicals in HGBF improve antioxidant and antimicrobial properties, potentially increasing feed intake. The antioxidant activity benefits are observed in the tissue's oxidation status, the intensification of the protein's metabolism, fats and carbohydrates; as well as better health and immunity of the animal fed with diets supplemented with nutraceutical characteristics (Mattioli et al., 2016). Hashem et al. (2017) in their study with supplementation of vitamin E (150 mg vitamin E/Kg dry matter), dried roots of *Moringa oleifera* (150 or 300 mg roots of moringa /kg dry matter) and propolis (150 or 300 mg propolis /Kg matter seca) obtained a greater significant difference (1.93 - 1.99 mM/L) compared to the diet without supplementation. For their part, Dal Bosco et al. (2015) in their study with 30-day-old rabbits, supplemented the diet with 20 g/d with alfalfa and flax sprouts, they found the presence of γ-tocopherol and lutein plus Zeaxanthin that was not present in the meat of rabbits fed with the control diet. Additionally, El-Desoky et al. (2017) orally

applied 50 mg/Kg of the ethanolic extract of *Moringa oleifera* leaves in seven-month-old rabbits; this dose increased serum albumin concentration, total antioxidant capacity (132%) and testosterone. Dal Bosco et al. (2018) modified the environment in addition to the inclusion of 3% linseed oil, once stored they found that liperoxidation was reduced with this treatment after 10 days. Including the study by Mattioli et al. (2016), in which the inclusion of Digestarom® at 300 mg/kg increased the levels of α-tocopherol that were absorbed by the animals, preventing lipid peroxidation through the extinction of free radicals.

Study findings support the inclusion of HGBF in improving the oxidative state of growing rabbits. Based on the HGBF chemical constitution, these effects could be attributed to the antioxidant capacity that is similar to cereals such as wheat and corn (Narwal et al., 2016), and to its content of phenolic acids, flavonoids, lignans, tocoles (vitamin E), phytosterols and folates. Most of these active metabolites have a strong antioxidant capacity (Abouelezz et al., 2019). Reports on the effects of this food category additives on rabbit growth performance, antioxidant and antibacterial activity (Narwal et al., 2016), meat quality, blood biochemical parameters (Shanti et al., 2017) and reproductive performance (El-Desoky et al., 2017) were compared with this study.

Table 2 - Quantification of the inhibitory activities of DPPH• and ABTS•+ in equivalent moles of Trolox/mg MS, and the inhibition ratio.

Rabbit meat treatments	ARSA		DRSA	
	Inhibition (%)	Polyphenols (trolox/mg)	Inhibition (%)	Polyphenols (trolox/mg)
Pellets 100%	80.47±11.84	1,986.84±266.92^{a}	37.51±17.24	1.13±0.52
Pellets 50% + 50% HGBF at 75 mM H_2O_2	79.76±10.37	1,789.80±212.27^{b}	40.77±18.52	1.12±0.51
Pellets 50% + 50% HGBF	79.48±9.90	1,858.95±211.23^{ab}	44.04±16.69	1.25±0.48

DM: dry matter, HGBF: Barley Hydroponic Green Fodder. a,b,c, Means within a column with different superscripts differ significantly (P<0.05).

Tests of the enzymatic activity of rabbit meat fed with three different treatments

Similarly, the plants and spices antioxidant capacities are derived from phenolic compounds (flavonoids, tannins, phenolic acids) and some vitamins such as vitamin E, C and A, which mitigate liperoxidation through trapping free radicals or from the antioxidant enzymes activation such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Mattioli et al., 2016; Selim et al., 2021). Similarly, these enzymes can prevent oxidation by stabilizing initiated free radicals such as superoxide and hydrogen peroxide, one of the most reactive free radicals in vivo. Figure 2 shows the quantification of the stress-related enzymes and the enzyme of the phenylpropanoid pathway.

The enzymatic activity related to CAT HGBF-supplemented groups (T2 and T3) exhibited lower catalase activity compared to T1, suggesting reduced oxidative stress as the body relied less on CAT to neutralize reactive oxygen species, likely due to the antioxidant-rich HGBF diet. In addition, no significant differences were observed in SOD and PAL activities across all treatments (T1, T2, and T3). This stability indicates that HGBF supplementation did not disrupt these enzymatic defenses, maintaining baseline oxidative stress management, which distribute and retained in the tissues. However, this bioavailability has not yet been directly demonstrated due to the lack of equipment and the analytical technique developed (Mattioli et al., 2016). Oloruntola et al. (2018) studied the superoxides degradation in rabbit meat in diets supplemented with 15% Gliricidia leaf flour plus multi-enzymes (1 g/Kg). They promoted the elimination of the superoxide ion by increasing the activity of SOD. This trend is not reflected in the results of Figures 2A and 2B. In the study done by Vizzari et al. (2021) polysaccharides was used from brown algae (*Laminaria digitate* and *hyperborea*, ratio 1:1 at 0.6%) in the diet, increased SOD and glutathione peroxidase. A benefit of the use of food supplements rich in antioxidant enzymes is the ideal animal development since these enzymes block oxidative stress, delay or prevents the oxidation of proteins, lipids, DNA and carbohydrates. The PAL enzymatic activity (Figure 2C) did not show a significant difference between treatments. Therefore, it can be deduced that the phenylpropanoid pathway was not affected by the inclusion of the rabbit's diet variants in this study. Mattioli et al. (2016) found with the supplementation of Digestarom® (300 mg/kg) that lipid peroxidation was reduced by activating antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, because these components were distributed in the muscles through blood.

In mammals, the antioxidant defense system includes enzymes (superoxide dismutase, glutathione peroxidases and catalase), extracellular proteins that bind to iron and copper (albumin, transferrin, lactoferrin, haptoglobin and ceruloplasmin), antioxidant vitamins (C, E and β-carotene) and other cellular compounds (quinones, glutathione, uric acid and bilirubin) (Jiang and Xiong, 2016). In addition, several exogenous phenolic compounds derived from fruits, vegetables, legumes or supplementary ingredients of the diet added to food, such as spices and herbs, contribute to the antioxidants reserve (Loganayaki et al., 2013; Narwal et al., 2016; Castrica et al., 2019). These antioxidants dietary sources are essential when the body is exposed to a high degree of radical stress. Likewise, recent advances in antioxidant studies have allowed the possibility of mitigating chemical toxins in meat products through different strategies; such as,

moderate thermal processing conditions to reduce toxin formation, bioaccessibility restriction technology, and antioxidant interventions (Jiang and Xiong, 2016).

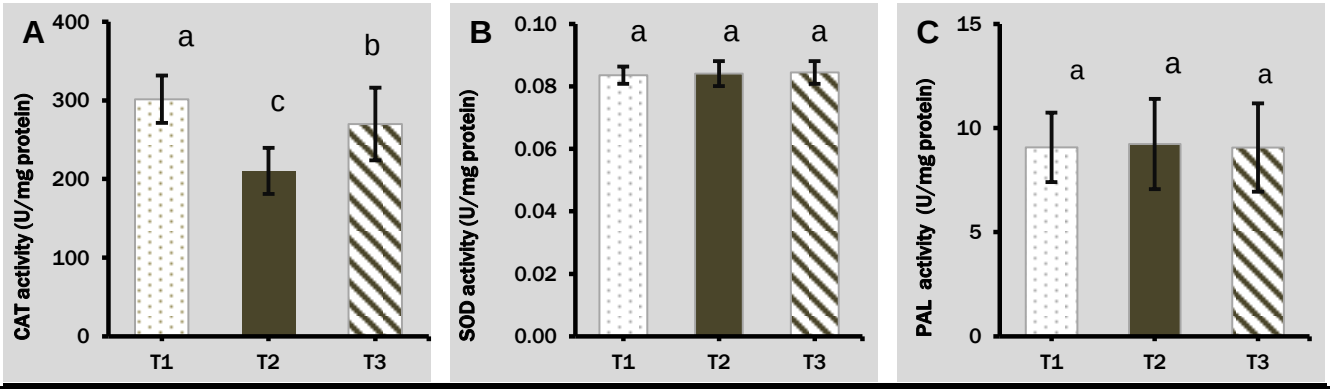

Figure 2 - Quantification of enzymatic activity in rabbit meat fed: T1- 100% Pellets, T2- 50% Pellets + 50% HGBF at 75 mM H_2O_2, T3- 50% Pellets + 50% HGBF. HGBF: Hydroponic Green Barley Fodder. SOD= Superoxide dismutase, CAT= Catalase and PAL= Phenylalanine ammonia-lyase. Different letters indicate significant differences according to the ANOVA and Tukey tests (α = 0.05).

Total phenol content of rabbit meat fed with three different treatments

Phenolic compounds are key antioxidants role (Alagbe and Akintayo-Balogun, 2020). The content of phenols quantified in rabbit meat supplemented in the diet with HGBF is shown in Figure 3. The phenols presence in rabbit meat is an important indicator of antioxidant capacity, with fat-soluble antioxidants being those with the highest presence in tissues (Mattioli et al., 2020). Phenols have antimicrobial effects in general (Branciari et al., 2021). Vizzari et al. (2021) supplemented with 0.6% polysaccharides from brown algae (*Laminaria digitate* and *hyperborea*, 1: 1 ratio), finding an important effect on the growth, health and development of animals in general. Branciari et al. (2021) supplemented with 280 mg/Kg of the olive residue polyphenol extract in the rabbit diet. As expected, polyphenol sulfate metabolites were detected in the rabbits meat, in addition to the reduction of Pseudomonas spp. in meat during storage (Branciari et al., 2021). However, the 20% introduction of grape wastes that were incorporated into the diet; there was no significant difference in total phenolic content, antioxidant capacity, antioxidant inhibition power and lipid oxidation in meat (Bouzaida et al., 2021). Selim et al. (2021) fed rabbits for 42 days with *Moringa oleifera* leaves, and related the low presence of cholesterol in the longissimus lumborum muscles with the presence of phenols in the moringa leaves, since they act as a natural hypocholesterolemic agent. In addition, this diet can contribute to a high level of components such as phenols, tannins and phytate in meat (Selim et al., 2021). Menchetti et al. (2020) supplemented the New Zealand rabbits diet with Goji berries (3%) increased the phenolic content (24.96 mg GAE/100 g) and the antioxidant properties in the muscle (Castrica et al., 2020; Menchetti et al., 2020). The both radical systems antioxidant activity (DPPH• and $ABTS^{\bullet+}$) and total phenolic compounds were influenced by the genotype and the environment. Likewise, the inclusion of foods high in phenolic compounds and antioxidant capacity for livestock feeding can modulate the content of phenolic compounds in the muscles and that the food rich in phenols can increase the level of antioxidants in the tissues (Menchetti et al., 2020). Therefore, both the genotype and the environment play an important role in determining the antioxidant activity and phenolic content of rabbit meat (Dal Bosco et al., 2018).

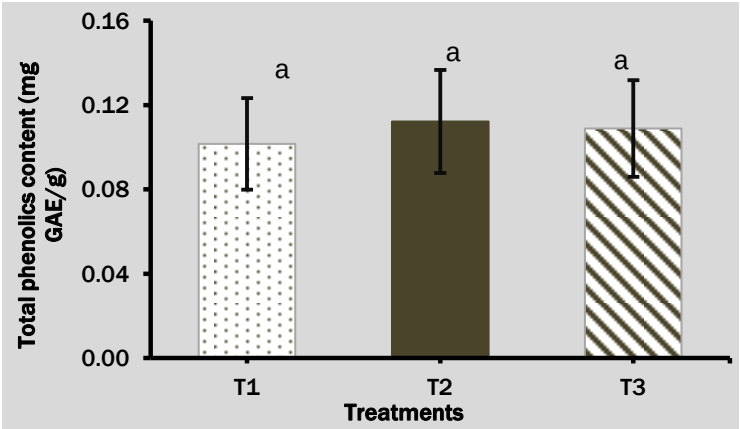

Figure 3- Total phenol content (CFT, mg GAE/g) of methanolic extracts from rabbit meat fed with: T1= Pellets 100%, T2= Pellets 50% + 50% HGBF at 75 mM H_2O_2, T3= Pellets 50% + 50% HGBF. GAE: Gallic Acid Equivalents, HGBF: Hydroponic Green Barley Fodder. Results plotted as means. Different letters indicate significant differences (α = 0.05).

CONCLUSION

This study demonstrates that dietary supplementation with Hydroponic Green Barley Fodder (HGBF), particularly with hydrogen peroxide seed pre-treatment (T2), significantly enhances the antioxidant capacity and total phenolic content of rabbit meat after 42 days of fattening. These improvements, driven by HGBF's rich profile of bioactive compounds (e.g., polyphenols, flavonoids, and vitamins), contribute to superior meat quality and oxidative stability. Additionally, HGBF's sustainable production reduces greenhouse gas emissions and feed costs, offering an environmentally friendly alternative to conventional feeds. These findings support the adoption of HGBF in rabbit production to improve meat functionality and promote sustainable livestock systems, with potential applications in broader animal nutrition strategies.

DECLARATIONS

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Ethics approval
The authors followed all applicable international, national, and institutional guidelines for the care and use of rabbits. The authors complied with the ARRIVE guidelines.

Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution
All authors contributed equally to the study.

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Consent to publish
All authors have reviewed and approved the final manuscript for publication.

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Competing interests
The authors declare no competing interests in this research and publication.

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