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Dietary effect of dried bay leaves (*Laurus nobilis*) meal on selected productive performances and on quality meat traits in growing rabbits

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Abstract

Laurus nobilis, the leaves of which are used as a seasoning in cooking, is rich in active compounds such as phenols, flavonols and flavones, and has antioxidant and antimicrobial effects. This study evaluates the effects of the dietary supplementation with fat and dried bay leaves on growth performance and meat quality parameters of weaned rabbits. The trial lasted 56 days. At weaning (35 ± 2 days of age), 120 rabbits were divided into four groups (n=30) and fed four experimental diets: a control diet (CON); the same diet with 2.5% lard supplementation (CF); a control diet supplemented with 1 g/kg feed of dried bay leaf meal (BL); and a fat-enriched diet with 1 g/kg of dried bay leaf meal (BLF). Dietary fat negatively affected growth performance and final weight ($P<0.01$) and no effects of dried bay leaves were observed ($P>0.05$). The chemical composition, retinol and alpha-tocopherol content and lipid oxidative stability of *Longissimus lumborum* (LL) muscle were not affected ($P>0.05$) by lard or dried bay leaf supplementation. Dietary treatment with dried bay leaves reduced ($P<0.01$) the cholesterol content in the LL muscle compared with CF groups. An improvement in the sensory characteristics (juiciness and fibrous texture) was also observed in LL muscle from rabbits fed dried bay leaves. Further studies are required to determine the optimal dosage of dietary bay leaves and the length of dietary supplementation in order to clarify the mechanism of action of the active principles and to improve rabbit growth performance and meat quality.

Keywords: bay leaves, lard, growth performance, growing-rabbit, meat quality

1. Introduction

Dietary supplementation with natural substances may positively affect animal welfare and growth performance (Abd-El-Hady, 2013), due to the prevention of several gut disorders, thus improving consumption and feed efficiency (Krieg et al., 2009). In intensive rabbit farming, especially in the weaning period, gastro-intestinal disorders may occur, worsening health with an increase in the mortality rate from 20% to 50% (Gidenne and Garcia, 2006; Gidenne et al., 2005; Lebas et al., 1998). Adding plant supplements in feed may improve animal immune parameters, reducing the oxidative stress status and mortality (Corbi et al., 2018; Fortun Lamothe and Boullier, 2007).

There is a growing interest in the use of plant extracts in rabbit feed in order to improve growth performances, and modulate the fatty acid composition and meat oxidative stability (Vizzarri et al., 2017; Palazzo et al., 2015; Rossi et al., 2020). Dietary supplementation with plants may be a sustainable strategy to enhance exogenous antioxidants that may enhance meat oxidative stability (Botsoglou et al., 2004), improving the shelf-life and sensory characteristics (Palazzo et al., 2015; Rossi et al., 2017).

Bay leaves (*Laurus nobilis* L.) come from an evergreen plant that is mainly cultivated in the Mediterranean area (Derwich et al, 2009). Its alcoholic extract or dried meal leaves have a high antioxidant activity towards lipid peroxidation, due to the content of phenols, flavonoids and flavonols (Casamassima et al 2017; Elmastas et al., 2006). Several *in vitro* studies have also reported an antimicrobial activity of bay leaf extract and essential oils, with a possible reduction in gastrointestinal disease (Erturk, 2006; Ozcan et al., 2010; Qnais et al., 2012).

Data on rabbit feed supplementation with aromatic plants are scarce and often contradictory. Ayala et al. (2011) showed that supplementation with dried oregano leaves improved growth performance, while Pogany-Simonova et al. (2010) found that oregano and sage extracts improved animal welfare and amino acidic composition. Other studies have reported that dietary oregano essential oils did not affect rabbit growth performance, but did inhibit carcass microbial growth during storage and increased the meat oxidative stability (Botsoglou et al 2004; Soultos et al, 2009).

Environmental conditions may affect rabbit feed intake and growth performance, thus fat dietary supplementation is one approach to increasing the energy intake, reducing the heat increment, and is also feasible due to the lower cost (Cervera et al., 1997; Casado et al, 2010).

Several authors observed an improvement in growth performance after fat inclusion in the rabbit diet with lard and animal fat which showed a high digestibility (71 to 75%) (Fernández & Fraga, 1996; Fernández-Carmona et al., 2000). In fact, it is reported that fat dietary integration may affect the histological structure of the small intestine, improving villi height in growing rabbits (Trebušak et al., 2019). In addition, the fatty acid composition of rabbit meat should be influenced by dietary fat, and the inclusion of lard may improve meat quality parameters, by decreasing lipid peroxidation (Fernández-Carmona et al., 2000).

Considering the interest in high energy formulations and that data are lacking on the use of dried bay leaves meal in the literature as a dietary supplement in rabbits, the aim of the present study was to investigate the

effects of the dietary supplementation with lard and dried bay leaves meal on growth parameters and meat quality in growing rabbits.

2. Material and Methods

2.1 Animal and experimental design

The animals used in this experiment were treated according to the European Union guidelines (2010/63/EU) and approved by the Italian Ministry of Health (DL 26, 2014).

The trial lasted 56 days and was conducted on a local farm in southern Italy (Molise region, 41°35'42.5"N 14°41'06.5"E) on 120 clinically healthy California x White New Zealand weaned male rabbits (35 ± 2 days of age). Rabbits were reared in cages (two rabbits per cage) with feeders and automatic water dispensers. The temperature (18 ± 4 °C) and relative humidity (70 ± 5%) were recorded throughout the experiment using a digital thermograph positioned at the height of the animal cages.

Rabbits were randomly divided into four experimental groups of 30 animals, balanced for age and body weight (1.415 ± 0.111 kg; expressed as mean ± SEM). The rabbits received four experimental diets: a control diet (CON), the same diet supplemented with 2.5% lard (CF); the control diet supplemented with 1 g/kg feed of dried bay leaf meal (BL), and a fat-enriched diet supplemented with 1 g/kg feed of dried bay leaves (BLF). The experimental diets were supplied by Agrizoo (Miranda, Isernia, Italy) and the bay leaves were obtained from Herboristeria Erbamea (San Giustino, Perugia, Italy).

The experimental diets were analysed in triplicate according to AOAC (2002), following the recommendations of the European group on rabbit nutrition (EGRN, 2001). The fatty acid composition of feed samples was determined after chloroform–methanol extraction (Folch et al., 1957), and fatty acids were determined as methyl esters using a gas chromatograph (Thermo Quest TRACE 2000, Thermo Scientific, USA) and a capillary column (SAC^{tm}-5 column 300 cm × 0.25 mm, Supelco, USA). Fatty acids were identified on the basis of standard elution times (FAME PUFA2, Supelco, Bellefonte, PA, USA). The fatty acid percentages were calculated with Chrom-Card (Thermo-Fisher, Milano, Italy, v. 1.17). Data on the ingredients and chemical composition of experimental diets are reported in Table 1.

Throughout the study, feed was available *ad libitum* and animals were monitored daily to assess their health conditions. They were weighed at the beginning (0 day), at 28 days, and at the end of the experimental trial (56 days). The daily feed intake was calculated from the amounts of feed offered and refused weekly. These data were used to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

2.2 Polyphenol quantification of bay leaves

The polyphenol composition was determined following Russo et al. (2017). This was performed using an Agilent 1260 Infinity HPLC (Agilent Technologies GmbH, Waldbronn, Germany) quaternary solvent manager coupled with degasser (G1311B), sampler manager (G1329B), Diode Array Detector (G1315C),

column manager (G1316A). The analytical column was a Waters Cortecs endcapped RP-C18 column (150 mm × 4.6 mm × 2.7 μm particle size; Waters Corp., Milford, MA, USA).

The analyses were carried out at 30 °C by a gradient system with a mobile phase of 0.1% ortho-phosphoric acid in deionised water (C) and acetonitrile gradient grade (D) at a flow rate of 0.60 mL/min and the injection volume was 5 μL. The gradient elution was as follows: 0–1 min (90% C and 10% D), 1–5 min (85% C and 15% D), 5–10 min (80% C and 20% D), 10–12 min (80% C and 20% D), 12–20 min (30% C and 70% D), and 20–25 min (30% C and 70% D). The post-run was set at 3 min. The samples were kept at 4 °C in the sampler manager. The detection wavelengths were set at 265 nm for flavonoids and 320 nm for hydroxycinnamic acid and tannins. Data were analyzed by Agilent Open Lab Chem Station software (Waldbronn, Germany) for LC 3D systems. The polyphenol composition of the bay leaves is reported in Table 2.

2.3 Slaughter parameters

At 90 ± 2 days old, all rabbits were weighed and after 6 h fasting, randomly selected and slaughtered at the slaughterhouse. The rabbits were electrically stunned (100 V, 50 Hz, 2-3 seconds) and sacrificed by exsanguination from the jugular vein following the guidelines established by the European Community (1099/2099/EC 2009) and national legislation (Law n. 116/92) on the protection of animals at slaughter. The carcasses were prepared according to Blasco and Ouhayoun (1996) by removing the skin, distal part of the limbs, genital organs, bladder and the gastrointestinal tract. Hot carcasses were then weighed and the dressing percentage was calculated. After a storage at 4° C for 24 h, the right *Longissimus lumborum* (LL) muscle (n=15) was removed from each carcass. Samples were vacuum packed and stored at −20 °C until lab analyses.

2.4 Physical parameters

The pH was measured on LL muscle at 45 minutes (pH0), at 24h (pH24) and 48h (pH48) after slaughter. The pH was performed using a pH meter (HI-98191 microcomputer; Hanna Instruments Woonsocket, United States) equipped with an electrode. The pH probe was calibrated using standard buffers of pH 4.0 and 7.0. The maintenance of calibration was monitored between samples.

2.5 Chemical parameters

Dry matter (DM), protein, ether extract (EE), and ash contents of the LL muscle were determined according to AOAC (2002) methods. Dry matter was determined by the oven drying method at 105 °C until constant weight (method 950.46), protein by the Kjeldahl method (method 990.03) using a 6.25 factor to convert the nitrogen content into total protein, ether extract by Soxhlet extraction (method 920.39), and ash using a muffle furnace for 12 h at 550 °C (method 920.153).

The cholesterol content of the LL muscle was determined in accordance with Du and Ahn (2002). Lipids were extracted from 1.5 g of minced meat homogenate with 33% KOH (ratio of 94:6), using Ultra-Turrax T18 Homogenizer (IKA, Cincinnati, USA) and were kept on ice to prevent oxidation. Cholesterol was extracted with 5 ml of hexane, and 1 µl was injected into the gas chromatograph. The cholesterol was identified based on the retention time of the standard (Sigma Aldrich, St. Louis, USA), and quantified with Chrom-Card v. 1.17 by comparing the peak area with the reference standard curve. All samples were analysed in triplicate.

2.3 Oxidative stability

Meat oxidative stability was measured by evaluating the thiobarbituric acid-reactive substance (TBARS) content of LL meat sample content in 4 °C chilled LL meat samples at 0 h, 24 h, 48 h and at 120 h, and also after 60d of frozen storage following Meineri et al. (2010) as follows: 500 mg of meat was homogenized with 10 mL of distilled water using anUltra Turrax T25 homogenizer (IKA, Cincinnati, USA), and 2.5 ml of 25% trichloroacetic acid was added to the homogenized sample, cooled at 4 °C for 15 min, and then centrifuged at 4000g at 4 °C for 5 min. The supernatant was filtered through Whatman 52 filter paper, and an aliquot of 3.5 ml was added to 1.5 ml of 0.67% thiobarbituric acid and incubated at 70 °C for 30 min. Immediately after cooling, the absorbance of the sample was read in a spectrophotometer at 532 nm and compared to a standard curve of malondialdehyde (MDA; Sigma Aldrich, St. Louis, USA). All analyses were performed in duplicate and the results were expressed as mg of MDA per kg of meat.

2.4 Alpha-tocopherol and retinol content

Alpha-tocopherol and retinol were determined in the LL muscle using a modifiedversion of Zaspel and Csallany's procedure (1983). The muscles were analyzed using an HPLC system (Kontron Instruments, Milan, Italy) consisting of an autosampler (HPLC autosampler 360, Kontron Instruments, Milan, Italy) with a loop of 20 µl, a high-pressure pump and a C18 column 5 µm, 150mm×4.6mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and methanol (75: 25 v/v), and a flow rate of 1 ml per min was used. Alpha-tocopherol and retinol were identified using a fluorimeter detector and by comparing the samples' retention time with the pure standards (97%) purchased from Sigma Aldrich (St. Louis, Missouri, USA). The quantification was carried out using Geminyx v. 1.91 (Goebel Instrumentelle Analytik GmbH, Hallertau, Germany) by comparing the area sample peak with that of the reference standard curves.

2.5 Sensory analysis

The LL preparation for sensory analysis was performed after thawing the LL muscle for 24 h at 4 ◦C and preparing it as one piece in an uncovered stainless-steel dish in a conventional oven (REX, Italy) at 150 °C. A thermocouple (Pentronic AB, Gunnebobruk, Sweden) was inserted into the centre of each piece of meat to register the core temperature. The LL was removed from the oven at 80 °C to allow for post-heating rise.

After cooling, the entire LL muscle was cut into 1.5 cm thick slices (Electrolux 50, 220–24, kW 0.2; AB Electrolux, Stockholm, Sweden). The LL slices were warmed to 60 °C before the evaluation. A trained sensory panel was chosen, consisting of eight members familiar with descriptive analysis procedures (EN ISO 13299, 2010),. All assessments were carried out in a sensory laboratory equipped according to ISO 8598 (1989) recommendations. The list of descriptors, definitions, and standards are reported in Palazzo et al. (2015).

The sensory profile was assessed according to EN ISO 13299 (2010) and the panel evaluated the samples on different days in triplicate. Four samples (one per experimental treatment: CON, CF, BL, BLF) were evaluated in each session on the same day. Within each session, the design was balanced in terms of order and carry-over effects (MacFie et al., 1989). During training and sampling, the panel had access to unlimited water and unsalted crackers. They were requested to evaluate the intensity of each attribute by assigning a score of between 0 (no sensation) and 10 (extremely intense).

2.6 Statistical analysis

Statistical analysis was performed with SPSS/PC Statistics v. 25.0 (SPSS Inc., IBM corp., Armonk, New York, USA), considering the cage as the experimental unit for growth performance and the rabbit for the meat quality parameters. Data on growth performance, carcass characteristics and meat quality parameters were analyzed with factorial ANOVA, where dietary fat and bay leaves were the main factors. A repeated measures ANOVA was used to assess differences in TBARS between treatment groups at different storage times and to identify interactions between treatment and time. The sensory data were submitted to ANOVA with samples, judges, replicates, and their interactions as effects (EN ISO 13299, 2010). The significance of these effects was tested with F tests. Post-hoc pairwise contrasts were evaluated by Duncan's test. Data are reported as mean ± SEM. Differences among treatments were considered significant at $P < 0.05$.

3. Results

3.1 Productive performance and carcass characteristics

Data on the growth performance of the rabbits are reported in Table 3. The weight of the rabbits at 28 and 56 days was reduced ($P < 0.05$) by the fat-enrich diets (CF and BLF), whereas at 28 days, a higher trend (P = 0.072) was observed from the dietary supplementation with bay leaves. A lower average daily gain ($P < 0.05$) was thus observed in the first period of the trial (0-28 d) and throughout the study (0-56 d) in the groups fed the fat diets (CF and BLF).

In the first period (0-28 d), the bay leaf meal increased ($P < 0.05$) the average daily gain compared to the added-fat diets. The feed intake in the second period of the trial (28-56 d) tended to be higher ($P = 0.077$) in rabbits receiving ($P > 0.05$) the fat-enrich diets (CF and BLF). Feed conversion was higher ($P < 0.05$) in the fat supplemented diet, in particular in the BLF group.

Table 4 reports the slaughter parameters and the LL muscle pH. The carcass parameters were negatively affected ($P < 0.05$) by the dietary supplementation with fat. Supplementation with bay leaves did not influence the carcass traits ($P > 0.05$). No effects of dietary treatments ($P > 0.05$) were observed on skin weight. There was an interaction between dietary-fat and bay leaf meal ($P < 0.05$) on carcass weight and dressing percentage, with a lower value in the BLF group. The pH showed no statistical differences ($P > 0.05$) between the groups at the different sampling times. An interaction ($P < 0.05$) between dietary-fat and bay leaf meal was observed on pH at 24 and 120 h.

3.2 Meat quality parameters and oxidative stability

Table 5 reports the data on the chemical composition, cholesterol content and retinol and alpha-tocopherol of LL muscle. The chemical composition was not affected ($P > 0.05$) by lard supplementation, whereas the moisture and crude protein were affected by bay leaf supplementation. The BL and BLF groups showed a reduced ($P < 0.05$) cholesterol content in comparison with the added-fat diet (CF). The retinol content was not affected by dietary treatments ($P > 0.05$). Dietary supplementation with lard and bay leaves (BLF group) tended to improve ($P = 0.072$) the muscle alpha-tocopherol content.

Table 6 reports the oxidative stability of the LL muscle during the five days of storage at 4 °C, and after 60 days of frozen storage. No effects of dietary treatment with bay lead meal were observed ($P > 0.05$), however the dietary supplementation with lard, negatively affected ($P < 0.001$) this parameter at 24 hours and after 60 days of frozen storage. As expected, the storage time negatively affected ($P < 0.001$) the oxidative stability of the LL muscle. A significant interaction between the fat and bay leaves was observed for the MDA content after 60 days of frozen storage.

3.3 Sensory analysis evaluation

Table 7 reports the F values for the aroma, taste, flavour and texture parameters of the LL muscle sensory profile. Sensory analysis revealed a difference ($P < 0.05$) between treatments in terms of rancid aroma, salty taste and texture. The mean values of sensory attributes of the *Longissimus lumborum* muscle from rabbits fed the experimental diets are reported in Table 8. The rancid aroma was higher ($P < 0.05$) in the BLF group than the CON and CF groups (mean value 0.1), however this parameter did not influence the global sensory profile because the value was around 0.7 in a scale ranging from 0 to 10. The salty taste was higher ($P < 0.05$) in the LL muscle from rabbits fed supplemental fat (CF and BLF) than the CON and BL groups. The juiciness was higher ($P < 0.05$) in LL from rabbits receiving the bay leaf meal (BL and BLF) than the other groups, and the fibrous was negatively affected ($P < 0.05$) in the CF group than the others. The other parameters regarding aroma and flavour were comparable in the experimental groups. The judges recorded differences ($P < 0.001$) for all the descriptors. This is common in sensory evaluations due to the different use of the scale (Lea et al., 1997). There were no differences ($P > 0.05$) between the sample, repetition and

interactions (except for judges x samples for texture parameters), which indicated that the mean scores for each descriptor can be assumed to be satisfactory for the LL muscle sensory profile.

4. Discussion

4.1 Growth performance

To the best of our knowledge, no previous study has reported the effects of dietary supplementation with animal fat and bay leaf meal on the productive performance and meat quality parameters of growing rabbits. Dietary fat and in particular the BLF group showed a lower ADG, body weight, and feed conversion compared to the other groups. Surprisingly, this indicates that the addition of the lard in the diet of growing rabbits had no positive effect on productive performance in our trial conditions. Although there were no differences between groups in feed intake, rabbits fed lard had lower ADG and greater feed conversion ratios compared with the other groups.

Data on dietary supplementation with fat on growth performance in rabbits are conflicting. Our date are not in line with previous studies reporting that dietary fat positively affects rabbit growth performance (Alhaidary et al., 2010; Van Manen et al., 1989). Maertens (1998) reported that dietary fat did not affect growth. De Endrade et al (2018) reported no effects on performance and meat quality when 2.5 % of animal fat was included in the rabbit diet. However, other authors have reported a lower growth performance when linseed or linseed oil was included in the diet of growing rabbits (Bianchi et al., 2009; Casado et al.,2013). One recent study hypothesized that fish oil reduced nutrient absorption in rabbits, resulting in a lower daily gain (Rodríguez et al., 2019). It is possible that the dietary level of fat and the dietary fatty acid composition are implicated in the different response of growth performance in rabbits detected in several studies. Moreover, breeding techniques, environmental temperature, and genetic type may also be involved.

Our data on the dietary supplementation of bay leaves revealed only slight effects on growth parameters, in agreement with previous studies in other species. Karaalp et al. (2011) reported no effect of dried bay leaf supplementation in the Japanese quail diet (2 and 4 g/ kg feed). Bulbul et al. (2015), also found no differences in body weight, feed intake and feed conversion in quails fed a diet supplemented with essential oils of sage, bay leaves and a mixture of the two. In other trials on quails, positive effects on growth performance were found with the integration of pure essential oils of thyme, black seed and rosemary (Denli et al., 2004, Ciftci et al., 2013), or following the administration of a mixture of these extracts in broilers (Alcicek et al 2003, Kucukyilmaz et al., 2012).

4.2 Carcass characteristics and meat quality parameters

In our experiments, the saughter and carcass weight of animals were negatively affected by fat supplementation. As reported by Rodríguez et al. (2019), this is possibly related to the effects of the dietary PUFA/SFA ratio and nutrient uptake in the intestinal tract.

The physical and chemical parameters of LL muscle were not affected by dietary supplementation with fat, in accordance with previous studies on rabbits fed different fat sources (Peiretti et al., 202; Rodríguez et al., 2017).

We found high cholesterol content in the CF group. Dietary supplementation with dried bay leaf meal did not affect meat physical parameters, however the muscle cholesterol content was significantly affected. This result is probably related to the action of flavonoids, tannins and saponins on bay leaf meal, which likely affected the biochemical mechanisms of cholesterol synthesis. In fact, in ducks fed a diet supplemented with bay leaves, Ismoyowati et al. (2016) showed a lower incidence of cholesterol and fat in muscle. According to Ismoyowati, flavonoids prevent lipid oxidation by reducing the muscle cholesterol and triglyceride content. Moreover, tannins are able to reduce the intestinal absorption of cholesterol, lowering its concentration (Zhang et al., 2010). A previous study of ours showed a lower blood cholesterol and triglyceride concentration in rabbits fed with dried bay leaves (Casamassima et al., 2017). This data is related to the lower content of these components in the LL muscle.

As expected, the oxidative stability of the LL muscle was negatively affected by lard supplementation, probably due to the high fat content of the diet, in accordance with Tres et al. (2009).

We found that dietary integration with dried bay leaves did not improve the oxidative stability of the LL muscle during refrigerated storage at 4°C, and after 60 days of storage at -20°C. Wen et al. (1996) observed that the oxidative lipid stability of poultry meat is linked to the alpha-tocopherol concentration in the phospholipids of cell membranes. In our study no difference was observed in LL muscle retinol and alpha-tocopherol, which were comparable among groups. Karaalp and Genc (2013) reported that a quail diet supplemented with bay leaves (2 and 4 g/kg of feed) decreased breast muscle MDA content after 8 days of storage at 4° C, while they found no differences after 2 and 5 days of refrigeration. They hypothesized that *Laurus nobilis* leaves need a longer period of time to reveal the antioxidant effect. It is also possible that the bay leaf meal content that we tested was not sufficient to affect the oxidative stability and antioxidants in LL muscle.

4.3 Sensory parameters

The sensory parameters of the LL muscle were affected by dietary supplementation with fat and bay leaf meals. In particular, the texture parameters of the LL muscle were positively affected by dietary bay leaf meal (BL and BLF) with a positive effect on juiciness and fibrousness. These results are in accordance with a previous study reporting that the tenderness and juiciness was improved in the muscle of quails fed bay leaves (Alrubaee, 2018). It is possible that the improvement in sensory traits might be related to a high water-holding capacity of the LL muscle of rabbits fed bay leaf meal, due to its polyphenol content. Palazzo et al. (2015) also showed that rabbit dietary supplementation with *Lippia spp,* containing polyphenols, improved the texture parameters of LL muscle. A recent study by our group (Rossi et al., 2020) reported an improvement in the juiciness, tenderness and stringiness of thighs from rabbits fed brown seaweed and a

plant polyphenol mixture. In contrast, Meineri et al. (2016) found no improvement in the sensory characteristics of rabbit meat after dietary supplementation with *Salvia hispanica.*

Although the rancid aroma was much lower in the BL group and did not influence the overall sensory profile, it is likely due to the high content of PUFA.

The sensory parameters of rabbit meat are related to the genetic type, breeding techniques, type of muscle (Szkucik and Pyz-Łukasik, 2008), and slaughter age (Corino et al., 2004). They are also related to the type, dosage and length of dietary supplementation of the natural extract.

5. Conclusions

Dietary supplementation with fat in our experimental conditions negatively affected the growth performance of growing rabbits, without any improvement in meat quality parameters. Dietary supplementation with dried bay leaves did not affect growth performance and improved the nutritional quality of LL muscle, decreasing the cholesterol content. An enhancement in the sensory parameters related to texture was also observed.

Further studies are needed to determine the optimal dosage and length of dietary bay leaf supplementation in order to clarify the mechanism of action of the active principles and to improve rabbit growth performance and meat quality.

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Conflict of interest

The authors declare that they have no conflict of interest.

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506 **Table 1**. Ingredient and chemical composition of experimental diets.

Item	Experimental diet[1]			
	CON[1]	CF	BLF	BL
Ingredient, %				
Alfalfa hay	39.8	39.8	39.8	39.8
Wheat bran	24.8	22.3	22.2	24.7
Alfa alfa meal dehydratated	9.7	9.7	9.7	9.7
Barley	7.9	7.9	7.9	7.9
Beet pulp	7.8	7.8	7.8	7.8
Soybean meal	7	7	7	7
Lard	-	2.5	2.5	-
Dicalcium phosphate %,	1.96	1.96	1.96	1.96
Calcium carbonate	0.49	0.49	0.49	0.49
Vitamin and mineral premix[2]	0.3	0.3	0.3	0.3
Sodium chloride	0.25	0.25	0.25	0.25
Bay leaves meal	-	-	0.1	0.1
Chemical composition:				
Moisture, % wet weight	5.8	5.9	5.7	5.9
Crude protein, % DM[3]	16.8	17.0	16.8	17.1
Crude fat, % DM	2.6	4.7	4.8	2.9
Crude fiber, % DM	13.5	12.5	12.8	13.4
Ash, % DM	5.8	5.7	6.0	5.7
NDF[4]	31.1	28.6	30.7	30.6
ADF[5]	13.8	14.8	14.8	12.6
Fatty acid composition, %				
C14:0	0.76	0.78	0.78	0.76
C16:0	24.71	25.13	25.19	24.68
C16:1	0.49	0.57	0.59	0.46
C18:0	3.50	3.83	3.88	3.49
C18:1 n-9	15.40	16.30	16.18	15.36
C18:2 n-6	41.05	40.97	41.36	41.09
C18:3 n-3	12.17	10.71	10.55	12.16
C20:0	0.74	0.77	0.80	0.74
C20:4 n-6	0.05	0.04	0.04	0.05
C20:5 n-3	0.79	0.63	0.63	0.79
C22:6 n-3	0.30	0.26	0.26	0.33
∑SFA[3]	29.72	30.51	30.65	29.67
∑MUFA[3]	15.89	16.87	16.77	15.82
∑PUFA[3]	54.39	52.62	52.85	54.51

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508 [1] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard
509 plus 1 g/kg feed of dried bay leaves meal.
510 [2] The vitamin and mineral premix supplied per kg of feed: vitamin A 2000 I. U., vitamin D3 320 I. U., vitamin E 4.0 mg, vitamin B2 0.52
511 mg, vitamin B6 0.40 mg, vitamin B12 0.006 mg, vitamin K 0.32 mg, vitamin H 0.020 mg, vitamin PP 3.2 mg, folic acid 0.10 mg, D-
512 pantotenic acid 2.4 mg, copper 5.6 mg, manganese 4.0 mg, iron 12.0 mg, zinc 16.0 mg, iodine 0.060 mg, selenium 0.040 mg.
513 [3] DM: dry matter
514 [4]NDF: neutral detergent fiber
515 [5]ADF: acid detergent fiber
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Table 2. Phenolic compounds in bay leaves (*Laurus nobilis* L.): concentrations (mg/kg of dry weight) and class.

Phenolic compound	Concentration	Class
Cynaroside	430.3±9.62	Flavone
Rutin	303.4±17.25	Flavonol
Vitexin	231.9±4.08	Flavone
Trans sinapic acid	120.0±2.37	Hydroxycinnamic acid
Neochlorogenic acid	109.2±4.49	Hydroxycinnamic acid
Ellagic acid	90.0±2.29	Ellagitannin
Trans p-coumaric acid	45.5±1.08	Hydroxycinnamic acid
Chlorogenic acid	27.9±6.71	Hydroxycinnamic acid
Syringic acid	20.7±1.46	Hydroxybenzoic acid

Table 3. Growth performance of rabbit fed a control diet (CON) and diets containing lard (CF), dried bay leaves meal (BL) and lard plus dried bay leaves meal (BLF).

Item[1]	Experimental diet[2]				SEM	*P*-value[3]		
	CON	CF	BLF	BL		F	B	F*B
Body weight, kg								
0d	1.44	1.42	1.39	1.41	0.23	0.747	0.501	0.981
28d	2.33	2.21	2.01	2.25	0.44	0.048	0.072	0.577
56d	2.91	2.81	2.62	2.89	0.39	0.017	0.167	0.235
ADG[3], g/d								
0d-28d	31.90	28.06	22.07	29.28	1.14	0.008	0.033	0.379
29d-56d	20.71	21.67	22.05	24.05	0.68	0.700	0.180	0.283
0-56d	26.31	24.86	22.07	26.67	0.51	<0.001	0.100	0.038
Feed intake, g/d								
0-28d	129.5	130.0	131.0	129.9	0.61	0.552	0.591	0.816
29-56d	143.1	146.4	145.8	144.4	0.69	0.077	0.776	0.452
0-56d	136.3	138.2	138.2	137.2	0.58	0.200	0.692	0.714
FCR[4]								
0-28d	4.16	4.73	6.09	4.49	0.21	0.007	0.028	0.178
29-56d	6.95	6.89	6.73	6.14	0.19	0.572	0.262	0.515
0-56d	5.56	5.81	6.41	5.32	0.12	<0.001	0.093	0.054

[1] Data are reported as mean and pooled SEM. n=15

[2] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard plus 1 g/kg feed of dried bay leaves meal.

[3] F: P-value of lard-enriched diets; B: P-value of dry bay leaves diets; F*B: P value of the interaction of the two main factors.

Table 4. Slaughter parameters and pH values of rabbits of rabbit fed a control diet (CON) and diets containing lard (CF), dried bay leaves meal (BL) and lard plus dried bay leaves meal (BLF).

Item[1]	Experimental diets[2]					*P* – value3		
	CON	CF	BLF	BL	SEM	F	B	F*B
Slaughter weight, kg	2.95	2.82	2.63	2.90	0.039	0.025	0.138	0.312
Hot carcass weight, kg	1.86	1.78	1.64	1.91	0.068	0.002	0.138	0.044
Dressing percentage, %	63.28	63.29	62.33	65.96	0.712	0.016	0.224	0.015
Head weight, g	200.83	194.00	182.60	186.00	2.728	0.015	0.312	0.731
Internal organ weight[4], g	154.50	149.17	121.80	134.33	3.759	0.001	0.135	0.536
Skin weight, g	515.17	495.50	460.20	494.17	8.980	0.120	0.137	0.684
pH								
0h	6.21	6.05	6.15	6.12	0.037	0.400	0.917	0.189
24h	6.18	5.91	6.10	6.04	0.041	0.195	0.778	0.040
48h	5.92	5.85	5.98	5.88	0.040	0.833	0.574	0.307
120h	5.94	5.75	5.89	5.84	0.028	0.206	0.667	0.032

[11] Data are reported as mean and pooled SEM. n=15

[2] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard plus 1 g/kg feed of dried bay leaves meal.

[3] F: P-value of lard-enriched diets; B: P-value of dry bay leaves diets; F*B: P value of the interaction of the two main factors.

[4] Internal organs: heart, liver, spleen and lung.

Table 5. Chemical composition, cholesterol and fat-soluble vitamins content of *Longissimus lumborum* muscle from rabbit fed a control diet (CON) and diets containing lard (CF), dried bay leaves meal (BL) and lard plus dried bay leaves meal (BLF).

Item[1]	Experimental diets[2]				SEM	*P* – value[3]		
	CON	CF	BLF	BL		F	B	F*B
Moisture, %	72.1	71.3	71.9	73.3	0.040	0.151	0.022	0.483
Crude protein, %	23.15	22.06	22.02	23.27	0.003	0.941	0.072	0.883
Ether extract, %	3.42	2.91	3.80	2.80	0.036	0.860	0.760	0.342
Ash, %	1.04	1.04	0.95	0.92	0.027	0.872	0.107	0.812
Cholesterol, mg/100g	51.3	60.9	55.0	51.9	0.963	0.011	<0.001	0.003
Vitamin E, mg/100g	0.182	0.171	0.179	0.174	0.002	0.664	0.189	0.072
Vitamin A, mg/100g	0.040	0.035	0.034	0.037	0.001	0.472	0.670	0.142

[1] [1] Data are reported as mean and pooled SEM. n=15

[2] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard plus 1 g/kg feed of dried bay leaves meal.

[3] F: P-value of lard-enriched diets; B: P-value of dry bay leaves diets; F*B: P value of the interaction of the two main factors.

Table 6. Oxidative stability of *Longissimus lumborum* muscle from rabbit fed a control diet (CON) and diets containing lard (CF), dried bay leaves meal (BL) and lard plus dried bay leaves meal (BLF) in relation to refrigerated storage time.

Malondialdehyde, mg/kg	Experimental diets[2]				SEM	*P* – value[3]		
	CON	CF	BLF	BL		F	B	F*B
24 hours	0.90	1.26	1.27	0.92	0.066	<0.001	0.809	0.978
48 hours	1.53	1.50	1.84	1.37	0.078	0.067	0.429	0.043
120 hours	2.08	2.13	2.23	2.19	0.097	0.779	0.530	0.999
60 days, after tawing	0.78	1.27	0.92	1.10	0.027	<0.001	0.832	0.051

[1] [1] Data are reported as mean and pooled SEM. n=15;

[2] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard plus 1 g/kg feed of dried bay leaves meal.

672 **Table 7.** The F value and statistical significance of dietary treatments, judges (n = 8), replicates (n = 3) and
673 their interaction for each sensory descriptor.

Descriptors	F value					
	Treatments	Judges	Replicates	T x J	T x R	J x R
Rabbit aroma	1.56	2.58	0.10	0.52	1.38	0.62
Liver aroma	0.91	11.00***	0.68	1.04	0.87	0.70
Rancid aroma	3.92*	5.26**	0.20	2.21	1.42	0.83
Salty	3.02*	28.81***	0.65	1.23	2.63*	2.31
Rabbit flavor	2.36	4.59 **	0.37	1.95	1.06	1.31
Liver flavor	1.66	6.64**	0.81	0.77	1.08	0.75
Tender	1.70	11.00***	0.10	3.62**	2.15	2.15
Juicy	6.20**	19.91***	1.94	2.38**	2.17	1.02
Fibrous	6.97**	33.99***	2.94	6.39***	0.55	0.86

674 T x J, treatments ∗ judges; T x R, treatment ∗ replicates; J x R, judges x replicates.
675 *** for $P < 0.001$; ** for $P < 0.01$; for * $P < 0.05$.
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Table 8. Mean values of sensory attributes of *Longissimus lumborum* muscle from rabbit fed a control diet (CON) and diets containing lard (CF), dried bay leaves meal (BL) and lard plus dried bay leaves meal (BLF).

Descriptors	Experimental diets[1]			
	CON	CF	BLF	BL
Rabbit aroma	4.4	3.8	5.1	4.8
Liver aroma	1.1	0.8	1.4	1.4
Rancid aroma	0.1^{b}	0.1^{b}	0.3^{ab}	0.7^{a}
Salty	2.5^{ab}	3.3^{a}	3.3^{a}	2.2^{b}
Rabbit flavor	5.2	5.0	4.9	3.9
Liver flavor	0.8	0.8	1.4	1.6
Tender	5.3	4.5	4.9	5.3
Juicy	2.3^{b}	2.3^{b}	3.1^{a}	3.5^{a}
Fibrous	1.6^{b}	3.2^{a}	1.7^{b}	2.3^{b}

[1] CON: control diet; CF: 2.5% lard-enriched diet; BL: control diet with 1 g/kg feed of dried bay leaves meal; BLF: diet with 2.5% of lard plus 1 g/kg feed of dried bay leaves meal;

[a, b] means within rows with different superscript letters differ significantly for $P < 0.05$.