Chapter

Microbiological Quality of Chicken Meat Fed with Olive Leaves (*Olea europaea L.*)

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Abstract

This study evaluated the antimicrobial activity of olive leaves in vitro and meat chicken fed with percentages of 5 and 10 g of olive leaves for each kg feed. This is justified by the relevance of obtained safe products, with emphasis on the use of natural additives. The olive leaves presented antibacterial activity in all tested bacteria. For the bacteria *Yersinia enterocolitica*, *Escherichia coli*, and *Shigella*, the minimal inhibitory concentration varied from 0.6 to 1.5 mg/ml. The treatment with an addition of olive leaves showed better microbiological stability of the thighs and drumsticks of chickens than treatment without an addition of olive leaves. The use of 5 g/kg diet inhibited the growth of *Staphylococcus aureus* and *aerobic psychrotrophic* and aerobic mesophilic, while the use of 10 g/kg of diet inhibited the growth of *Enterococcus* spp., lactic bacteria, thermotolerant coliforms, *Pseudomonas*, *Clostridium perfringens,* and *Escherichia coli*.

Keywords: antimicrobial activity, olive leaves, natural additive, chicken meat

1. Introduction

In order to inhibit microbial growth of raw materials or cuts, it is often more effective than the direct addition of preservatives to add compounds into the diet of the growing animals [1]. The phenolic compounds occurring naturally in the plants have the ability to inhibit the growth of microorganisms, including bacteria, viruses, and fungi, maintaining the quality of meat for a long time.

In a study conducted by Bisignano et al. [2], the in vitro antimicrobial activity of oleuropein and hydroxytyrosol extracted from olive leaves was evaluated, and the efficiency for the pathogenic bacterium *Staphylococcus aureus* was identified. In another study Bisignano et al. [3] identified the antimicrobial components of olive leaves, and they discovered the effectiveness of long-chain aliphatic aldehydes against Gram-positive and Gram-negative bacteria.

In a study conducted by ERBAY and ICIER [4], the main compound found in olive leaves was oleuropein, being 24.54% in dry leaves. In a study realized by Paiva-Martins [5] with an objective of to assess the influence of OL supplementation at a lower level on feed digestibility and meat quality, the results indicated that olive leaves may be included in pig diets at 25 g/kg in order to improve the tocopherol content of meat without excessively compromising growth performance.

Upon investigating the in vitro activity of a commercial extract of olive leaf (*Olea europaea* L.) containing 4.4 mg/ml oleuropein, against a wide range of microorganisms, Sudjana et al. [6] determined that the compound has an inhibitory activity for *Helicobacter pylori*, *Campylobacter jejuni*, and *Staphylococcus aureus*. The in vitro activity of olive leaves was also studied by Markin et al. [7], who observed efficiency especially against *Klebsiella* and *Pseudomonas*.

Botsoglou et al. [8] evaluated the effect of the use of olive leaves in turkey's supplement diet in quantities of 5 and 10 g of leaves/kg diet in relation to microbiological quality of breast fillets that were stored at 4°C during 12 days. The turkey fillets that received olive leaves in the diet have had lower numbers of colonies of lactic acid bacteria, psychotropic, mesophilic, and enterobacteriaceae.

This study was designed to evaluate the effects of supplementation of the percentages of 5 and 10 g of olive leaves/kg of feed in the diet of broilers, on microbiological, of the meat the thighs and drumsticks stored at 4°C (±1°C) for 12 days.

2. Materials and methods

2.1 Extraction and chemical composition of olive leaves

Olive leaves (*Olea europaea* L.) of the variety Ascolana were collected between January and March 2012; drying in a tray dryer, at 45°C with air circulation for 72 hours, was realized. The leaves dried were milled in razor mill type Willey in 1 mm. The material was stored in paper and plastic packaging at 4°C until use.

The determination of total phenolics in olive leaves before followed the methodology described by Swain and Hills [9], which used as pattern the gallic acid, in concentrations of 50, 100, 150, 200, and 250 mg/l to build the calibration curve. Liquid chromatographic analysis of olive leaves.

We evaluated the oleuropein content present in olive leaves according to the method proposed by Guimarães et al. [10], and the chromatography conditions were based on Quirantes-pine et al. [11]. The separation of oleuropein was realized by using a HPLC Agilent 1260 Infinity (Agilent Technologies, Germany) liquid chromatography with a diode array detector (DAD).

2.2 Evaluation of different concentrations of olive leaves for antibacterial activity in vitro

The minimum inhibitory concentration (MIC) analysis for the in natura olive leaves (after harvest) and after drying for microorganisms was realized: *Escherichia coli* (ATCC8739), *Salmonella typhimurium* (ATCC14028), *Shigella dysenteriae* (NCTC7919), *Yersinia enterocolitica* (CDC175), *Clostridium perfringens* (NCTC8798), *Listeria monocytogenes* (ATCC19117), *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC14502), and *Enterobacter aerogenes* (ATCC13048). The lyophilized bacteria were activated and replicated, and the suspension turbidity was standardized according to the nephelometric scale of McFarland in 0.5 which corresponds to the concentration of 1.5×10^8 CFU/ml (colony-forming units per milliliter).

The plant extract obtained from the olive leaves was evaluated according to microdilution in all concentrations: 20; 10; 5; 2.5; 1.25; 0.625; 0.312; and 0.156 mg/ml. The extract was put in plaques, and all the plaques were incubated in a greenhouse at 35°C for 24 hours and read with revealing. The read had objective show what concentrations the olive leaves had better effect on microorganisms.

2.3 Animals and diets

The chickens were created in the farm for 42 days and fed with the following diets: T1 (traditional diet without addition of olive leaves), T2 (diet with addition of 5 g of olive leaves for each kg feed), and T3 (diet with addition of 10 g of olive leaves for each kg of feed).

The broilers were slaughtered, and thighs and drumsticks, with skin and bone, were collected, stored in plastic bags of polyethylene without barrier, at 4°C (±1°C), for 12 days to monitor the growth microbiological.

2.4 Microbiological analysis

The poultry meat was analyzed microbiologically on days 0 (zero), 3, 6, 9, and 12 of storage.

Clostridium perfringens was performed with culture medium TSC and pour plate sowing depth and reading after 24 hours of incubation at 36°C (±1°C), according to the methodology described by IN 62, August 26, 2003, of the Ministry of Agriculture [12].

The analysis of fecal coliform, *Staphylococcus aureus*, aerobic mesophilic, *Escherichia coli*, *Enterococcus* spp., coliform bacteria, aerobic psychrotrophic, lactic acid, *Pseudomonas* spp., *Campylobacter* (*jejuni, coli,* and *lari*), *Salmonella*, and *Listeria monocytogenes* was performed according to the AOAC method [13].

Shigella, Streptococcus, Yersinia, and *Klebsiella* were determined in VITEK 2 [12].

2.5 Statistical analysis

All analyses took place in triplicate runs. Results were statistically analyzed by mean standard deviation, variance, and Tukey test at 95% significance, using the software Statistica 6.1 (Statsoft Inc., USA).

3. Results and discussion

3.1 Extraction and chemical composition of olive leaves

The average for the analysis of olive leaves was 4.65% moisture in dry basis, 4.69% of fixed mineral residue, 1.38% fat, 23.3% crude fiber, and 12.73 g/NT 6.25 × 100 g protein. This result is in accordance with that found by Botsoglou et al. [8]. The low percentage moisture of olive leaves ensures your quality, because it is not favorable to the development of fungi, molds, and yeasts.

The total phenolic content found in olive leaves, before and after the drying, was 12,275 and 9525 mg/g leaves, respectively. Similar values were found by Makris et al. [14] who reported 40.27 mg of gallic acid equivalents/g of dried olive leaves, and by Botsoglou et al. [8] who found phenol content of 26 mg of gallic acid equivalents/g of dried leaves.

The oleuropein tenor found in the olive leaves was 15.0 (±0.8) g/kg (CV de 5.1%, n = 3). This value was similar to the one found by Paiva-Martins et al. [15] which obtained 22.3 (±0.18) g/kg oleuropein in olive leaves.

3.2 In vitro antibacterial activity

Table 1 presented the values of inhibitory minimum concentration (MIC) in mg/ml from the olive leaf (*Olea europaea* L.) gross extract in natural and after drying.

Minimum inhibitory concentration—MIC (mg/ml)		
Microorganism	**In natural leaf extract**	**Dry leaf extract**
Salmonella typhimurium	20	>20
Staphylococcus aureus	20	>20
Pseudomonas aeruginosa	20	>20
Listeria monocytogenes	>20	>20
Enterobacter aerogenes	10	>20
Clostridium perfringens	5	>20
Shigella dysenteriae	1	0.156
Yersinia enterocolitica	0.625	0.156
Escherichia coli	0.625	0.078

Table 1.
TTest results for MIC determination for olive leaves (Olea europaea L.) extract.

All the bacteria tested presented sensibility for olive leaves, some with more intensity and others with less. For the bacteria *Yersinia enterocolitica*, *Escherichia coli*, and *Shigella*, both the olive extracts presented moderated action, with values between 0.6 and 1.5 mg/ml.

For the microorganisms *Salmonella typhimurium, Staphylococcus aureus*, *Pseudomonas aeruginosa, Listeria monocytogenes, Enterobacter aerogenes*, and *Clostridium perfringens*, a lower inhibitory action from olive extracts, with MIC above 1.5 mg/ml, was found. For the microorganisms that showed results >20, less capacity of inhibition was verified.

3.3 Effect of olive leaves on microorganisms in meat

The safety and quality of fresh broiler beef can be estimated by counting microorganism indicators aerobic mesophilic and psychrotrophic [16]. **Table 2** shows the number of colonies ($\log_{10}$ CFU/g) of aerobic mesophilic, lactic acid bacteria, aerobic psychrotrophic, and *Pseudomonas*.

Analyzing the growth of the microorganism aerobic mesophilic (**Table 2**), the treatments that received diet supplemented with olive leaves (T2 and T3) remained within the quality standards during the 12 days storage at 4°C reaching a maximum counting of 5.63 and 5.87 $\log_{10}$ CFU/g, respectively, while control treatment showed a counting of 6.07 $\log_{10}$ CFU/g from the third day of storage. The aerobic mesophilic counting of 10^7 CFU/g or 7 $\log_{10}$ CFU/g is considered an indicator for the end of shelf life of cooled broiler meat [17]. Some studies that are more precise indicate outside the ideal sanitary conditions broilers with a counting of mesophilic 10^6 CFU/g [18]. According to these parameters, the counting between 10^6 and 10^7 CFU/g was considered a limit to end the shelf life, and it can be said that the diet with added olive leaves of broilers provided an increase in the shelf life of meat compared with the control treatment.

The number of colonies of lactic acid bacteria, in the treatments with olive leaves (T2 and T3) in all analyzed days, was lower than that of the control treatment (T1) and differed significantly ($P < 0.05$) among themselves. These results show that olive leaves present an inhibitory effect on the growth of lactic acid bacteria (**Table 2**). Between treatments with olive leaves, T3 had throughout the period fewer colonies of lactic acid bacteria than T2, and this result was significantly different ($P < 0.05$). Botsoglou et al. [8] added 5 and 10 g olive leaves/kg in the

		Analysis			
		Aerobic mesophilic bacteria (log CFU/g)	**Lactic acid bacteria (log CFU/g)**	**Psychrotrophic bacteria (log CFU/g)**	***Pseudomonas* spp. (log CFU/g)**
Storage time (days)					
0	T1	7.30E + 05[a]	1.00E + 02[a]	1.10E + 03[a]	3.20E + 03[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	3.90E + 03[b]	4.40E + 01[b]	7.80E + 01[c]	7.01E + 02[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	1.30E + 03[c]	3.20E + 01[c]	6.20E + 02[b]	8.00E + 01[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
3	T1	1.20E + 06[a]	5.20E + 03[a]	2.30E + 04[a]	1.30E + 04[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	5.60E + 04[b]	7.32E + 02[b]	1.97E + 02[c]	3.80E + 03[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	2.90E + 04[c]	1.80E + 02[c]	2.10E + 03[b]	2.10E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
6	T1	2.50E + 06[a]	4.20E + 05[a]	2.81E + 04[a]	5.30E + 04[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	9.70E + 04[c]	3.50E + 03[b]	2.10E + 03[c]	4.70E + 03[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	6.60E + 05[b]	3.10E + 03[c]	1.71E + 04[b]	3.10E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
9	T1	1.91E + 07[a]	1.70E + 06[a]	1.30E + 05[a]	1.80E + 05[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	2.30E + 05[c]	2.30E + 04[b]	6.72E + 03[c]	2.30E + 04[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	6.81E + 05[b]	5.80E + 03[c]	2.40E + 04[b]	5.80E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
12	T1	4.51E + 07[a]	3.50E + 06[a]	3.80E + 05[a]	4.90E + 06[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	4.30E + 05[b]	3.10E + 04[b]	7.42E + 03[c]	3.80E + 04[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	7.51E + 05[b]	6.00E + 03[c]	3.60E + 04[b]	6.20E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)

T1 (control diet), T2 (diet supplemented with 5 g olive leaves/kg feed), and T3 (diet supplemented with 10 g olive leaves/kg feed). a, b, and c are scanned horizontally between T1, T2, and T3 intervals for analysis. Different letters show significant difference (P < 0.05) by Tukey test.

Table 2.
Number of colonies (log_{10} CFU/g) of aerobic mesophilic bacteria, lactic acid bacteria, psychrotrophic bacteria, and Pseudomonas spp. in thighs and drumsticks of broiler storage at 4°C for 12 days.

turkey feed and evaluated microbial growth in breast fillets, which were stored at 4°C for 12 days. On the twelfth day of storage, the number of lactic acid bacteria in the control treatment was 6.5 log_{10} CFU/g, and treatments with 10 and 5 g olive leaves were 4 and 5 log_{10} CFU/g, respectively. In this study, the number of lactic acid bacteria in meat on day 12 was 3.77 and 4.49 log_{10} CFU/g for treatments T3 and T2,

respectively, and these values were lower than those found by Botsoglou et al. [8] in the same storage time and the same temperature.

Although the counting of aerobic psychrotrophic microorganisms indicates the degree of deterioration of refrigerated foods, the Brazilian legislation establishes no standard for these microorganisms. However, the International Commission on Microbiological Specifications for Foods [17] establishes 10^6–10^7 CFU/g as a standard. Considering these microbiological standards, the chicken meat in this study was fit for consumption during the 12 days at 4°C (**Table 2**). Throughout the storage period, T2 had the lowest number of colonies, with a significant difference ($P < 0.05$) of T3 and T1. The number of colonies of T3 was lower than that found in T1 also with significant difference ($P < 0.05$), indicating that olive leaves had significant effects on the growth of the counting of aerobic psychrotrophic microorganisms. In breast turkey fillets that received olive leaves in the diet, Botsoglou et al. [8] found the number of colonies of aerobic psychrotrophic on the twelfth day of storage of 4.6 and 5.7 $\log_{10}$ CFU/g for tests that received 10 and 5 g of olive leaves/kg diet. This counting were largest found that in this study, 4.55 to T3 and 3.87 $\log_{10}$ CFU/g to T2 (**Table 2**).

The analysis of total aerobes and *Pseudomonas* spp. are good indicators of spoilage of poultry meat [19]. The counting of *Pseudomonas* spp. is defined by several authors as indicating the end of useful life values when they reach 6–7 $\log_{10}$ CFU/g [20]. Considering these values and the results shown in **Table 2**, the three treatments were acceptable for consumption during the 12 days. The treatments T2 and T3 showed that a number of colonies of *Pseudomonas* spp. were significantly lower ($P < 0.05$) than that found in T1, on all days of storage. There were significant differences ($P < 0.05$) between treatments T2 and T3 throughout the study period, indicating that supplementation with olive leaves 5 g/kg feed showed better inhibitory capacity for this microorganism.

Table 3 shows the results of the microbiological analysis of total coliforms, *Enterococcus* spp., *Staphylococcus aureus*, thermotolerant coliforms, *Clostridium perfringens*, and *Escherichia coli*.

The Brazilian legislation does not establish microbiological parameters of coliforms. The treatments were subjected to this analysis to know the microbial load and so evaluate the sanitary conditions of the broiler meat of the three treatments, since these parameters reflect the quality of the raw material. The results vary between treatments (**Table 3**), where T2 and T3 had lower levels of total coliforms than T1 during the 12 days of storage.

The *Escherichia coli* presence in foods indicates microbial contamination of fecal origin [21]. The *Escherichia coli* (**Table 3**) started with similar values among the three treatments 3.07, 2.79, and 2.73 $\log_{10}$ CFU/g for T1, T2, and T3, respectively. After 9 and 12 days of monitoring, T2 and T3 had significant reductions ($P < 0.05$) compared to T1, demonstrating that the use of olive leaves at both concentrations had better effect inhibitory which T1. The use of 10 g/kg olive leaves in diet had greater inhibitory effect than the use of 5 g/kg for this microorganism.

The use of olive leaves in the amount of 10 g/kg feed showed a better inhibitory effect than the use of 5 g/kg feed for *Clostridium perfringens*, and both showed an inhibitory effect significantly ($P < 0.05$) better than T1.

According the Resolution no 12/2001, National Agency for Sanitary Surveillance [22], cuts of broiler cooled or frozen can have a tolerance limit for counting coliforms 45°C/g of 10^4 or 4 $\log_{10}$ CFU/g. According to this tolerance, T1 would be inappropriate for marketing and consumption because its initial counts were 4.5 $\log_{10}$ CFU/g, while the treatments with olive leaves have had initial counts of 1.93 and 2.36 for T3 and T2, respectively, and had presented counts within the tolerance limit of the legislation until the sixth day of storage at 4°C. The analysis

		Analysis					
		Total coliforms (log CFU/g)	***Enterococcus* spp. (log CFU/g)**	***Staphylococcus aureus* (log CFU/g)**	**Thermotolerant coliforms (log CFU/g)**	***Clostridium perfringens* (log CFU/g)**	***Escherichia coli* (log CFU/g)**
Storage time (days)							
0	T1	4.40E + 02[a]	3.31E + 03[a]	3.80E + 01[a]	3.20E + 04[a]	5.00E + 00[a]	1.20E + 03[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	1.12E + 02[b]	1.80E + 02[b]	1.00E + 00[c]	2.31E + 02[b]	3.00E + 00[a]	6.30E + 02[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	8.60E + 01[c]	1.10E + 02[b]	2.30E + 01[b]	8.70E + 01[b]	2.00E + 00[a]	5.41E + 02[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
3	T1	6.10E + 03[a]	4.20E + 04[a]	4.21E + 02[a]	5.50E + 05[a]	1.07E + 01[a]	3.70E + 04[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	2.81E + 02[b]	2.30E + 03[b]	1.00E + 00[c]	9.31E + 02[b]	1.00E + 01[a]	9.30E + 03[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	1.52E + 02[c]	3.41E + 02[c]	4.83E + 01[b]	9.51E + 02[b]	1.00E + 01[a]	7.30E + 03[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
6	T1	9.71E + 03[a]	1.60E + 05[a]	7.80E + 03[a]	7.40E + 05[a]	1.20E + 04[a]	7.40E + 05[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	4.80E + 02[b]	4.10E + 03[b]	2.10E + 01[c]	1.40E + 03[c]	8.40E + 02[b]	1.60E + 04[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	1.50E + 02[c]	1.20E + 03[c]	2.83E + 02[b]	3.80E + 03[b]	2.30E + 02[c]	1.10E + 04[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)

		Analysis					
		Total coliforms (log CFU/g)	***Enterococcus* spp. (log CFU/g)**	***Staphylococcus aureus* (log CFU/g)**	**Thermotolerant coliforms (log CFU/g)**	***Clostridium perfringens* (log CFU/g)**	***Escherichia coli* (log CFU/g)**
9	T1	5.50E + 04[a]	6.30E + 05[a]	1.90E + 04[a]	1.50E + 06[a]	6.21E + 04[a]	1.30E + 06[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	3.10E + 03[b]	4.20E + 04[b]	5.01E + 02[c]	7.50E + 04[b]	3.20E + 03[b]	1.90E + 05[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	2.80E + 03[c]	3.10E + 03[c]	3.90E + 03[b]	2.92E + 04[c]	6.12E + 02[c]	4.10E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
12	T1	7.40E + 04[a]	8.80E + 05[a]	5.60E + 04[a]	5.30E + 06[a]	8.41E + 04[a]	5.20E + 06[a]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T2	4.10E + 03[b]	5.20E + 04[b]	7.01E + 02[c]	9.60E + 04[b]	5.40E + 03[b]	2.20E + 05[b]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)
	T3	3.20E + 03[c]	4.20E + 03[c]	4.40E + 03[b]	3.12E + 04[c]	7.22E + 02[c]	5.10E + 03[c]
		(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)	(±0.0002)

T1 (control diet), T2 (diet supplemented with 5 g olive leaves/kg feed), and T3 (diet supplemented with 10 g olive leaves/kg feed). a, b, and c are scanned horizontally between T1, T2, and T3 intervals for analysis. Different letters show significant difference ($P < 0.05$) by Tukey test.

Table 3.
Number of colonies (log_{10} CFU/g) of total coliforms, Enterococcus spp., Clostridium perfringens, Staphylococcus aureus, thermotolerant coliforms, and Escherichia coli in thighs and drumsticks of broiler storage at 4°C for 12 days.

of fecal coliform indicated that T2 and T3 have had significant inhibitory effect ($P < 0.05$) compared to T1, demonstrating that both concentrations of olive leaves are inhibitory.

The research of *Enterococcus* spp. is not mandated by legislation, and few studies investigated these microorganisms. The counting showed that T3 had the best inhibitory effect than T2 and T1, and in the twelfth day, values of 3.61, 4.71 and 5.94 $\log_{10}$ CFU/g to T3, T2, and T1, respectively, were found. The broiler meat that received an addition of olive leaves (T2 and T3) showed significant reductions ($P < 0.05$) in the counts of *Enterococcus* spp. from the third to twelfth day of storage, demonstrating efficient reduction of this microorganism regarding the treatment of broilers that received a traditional diet (T1).

The current legislation in Brazil does not set a standard for *Staphylococcus aureus* in broiler meat; however, there are reports that are required between 10^5 and 10^6 CFU/g of *Staphylococcus aureus* per gram of food so that the toxin is formed at levels that can cause intoxication [21]. Considering this pattern, and analyzing the growth (**Table 3**), one can say that during the 12 days of storage at 4°C, the broilers meat of the treatments broilers fed the diet supplemented with the olive leaves have had significantly lower count ($P < 0.05$) than the T1, which is indicative of safer conditions. The maximum counts found for the broiler thighs and drumsticks were 2.84, 3.64, and 4.74 $\log_{10}$ CFU/g for T2, T3, and T1, respectively. Inhibition of *Staphylococcus aureus* was significantly lower ($P < 0.05$) in the treatment which received the addition of olive leaves in 5 g/kg of diet than T1 and T3. At the twelfth-day follow-up, the difference between T1 and T3 was 1.1 $\log_{10}$ CFU/g, demonstrating the inhibitory effect of olive leaves for this microorganism.

The federal legislation provides the absence of *Salmonella* in 25 g for poultry meat cooled. In the broiler thighs and drumsticks analyzed, the *Salmonella* wasn't present. This result confirms the microbial quality of the products, since the absence of *Salmonella* in samples attests to the hygienic and sanitary conditions.

The samples of broiler meat analysis of the three treatments showed absence of *Listeria monocytogenes*, proving the safety of the product for listeriosis.

The research for *Campylobacter* (*coli*, *jejuni*, and *lari*), *Shigella*, *Klebsiella*, *Yersinia*, and Streptococcus indicated the absence of these microorganisms for the three treatments during the study period.

4. Conclusions

The use of 5 g/kg olive leaves reduced the growth of *Staphylococcus aureus* and psychrotrophic total aerobic count, while 10 g/kg of diet reduced the growth of count of Enterobacteriaceae, lactic acid bacteria, total coliforms, *Pseudomonas* spp., *Clostridium perfringens*, and *Escherichia coli*. The samples of broiler meat analysis of the three treatments showed the absence of *Listeria monocytogenes.*

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