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Article

Effect of the addition of aqueous extract of garlic (*Allium sativum*) to the diet of rabbits (*Oryctolagus cuniculus*) on the productivity and on the physical and microbiological quality of the meat

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Abstract:

Garlic (*Allium sativum*), as a natural antimicrobial, has favored animal welfare, as well as the safety and quality of the meat. The objective of this study was to assess the production indicators and the physical and microbiological quality of the meat of rabbits fattened with the addition of aqueous extract of garlic (AEG) to their diet. A completely randomized design was carried out with three treatments of 28 New Zealand rabbits X Chinchilla (*Oryctolagus cuniculus* X *Chinchilla chinchilla*) each (LW 1 ± 0.6 kg, 30 ± 5 d); control group (food only),

treatment 1 (0.9% AEG) and treatment 2 (1.8% AEG, sprinkled on the food every 3 d). Daily weight gain and food conversion were determined to occur during four weeks after weaning. Aerobic mesophiles, fecal coliforms, and psychrophiles were quantified in the meat, and the pH and color (B*, a* and b*) were determined, all in *Longissimus dorsi*, at 1, 3, 5, 7 and 9 d of storage in refrigeration. A multivariate variance analysis ($P \leq 0.05$) and a Tukey test at 5 % were performed for the production indicators and the physical and microbiological variables. Unlike in psychrophiles and mesophiles, there were no significant differences ($P \geq 0.05$) in the production indicators throughout the time of conservation. No fecal coliforms were observed in any of the samples. The addition 1.8% of aqueous extract of garlic improved the shelf life by two days (total: 9 d) by reducing the content of psyschrophiles, without affecting the production indicators or the physical quality of the meat.

Key words: *Allium sativum*, Shelf life, Microbiological analysis, Meat quality.

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Introduction

The meat industry employs various methods to delay the changes that impair the meat and prolong the period of acceptability —changes that are directly related to the presence of microorganisms— . Today, it is common to seek the combination of two or more factors (physical, chemical or biological, among others) that will interact additively or synergistically to control the microbial population and avoid the severe application of a single conservation factor; this to improves the quality of sensory and nutritional status of the food and allows the production of minimally processed foods[(1)]. The use of non-natural antimicrobials is common in the industry of processed meat; however, these are currently being rejected by the consumers due to the effects that they can cause to health. Therefore, the need has emerged to search for other antimicrobial substances of natural origin[(2)]. Garlic (*Allium sativum*) is a natural antimicrobial with a wide range of nutraceutical properties, due to its content of sulfur compounds, among them allicin. Equally, the beneficial effects of garlic extract on the health of animals have been demonstrated, as in the case of the rabbits and their meat, prolonging its shelf life and, therefore, consumer safety[(3-6)]. The deterioration of rabbit meat in refrigeration is due to the activity of endogenous enzymes, along with the activity of microbial contaminants in the product during slaughtering and carving up. When the product is distributed at refrigeration temperatures, the meat has a shelf life of 6 to 8 d,

as some reports have mentioned[7-10]. Other authors[11] marinated pork with garlic juice and onion in order to determine its effect on the quality during storage in refrigeration. As for their sensory effect, the juices of garlic and onion provided the meat with greater tenderness and a better taste. Although research has been carried out on the use of garlic extract in the meat of different species, the microbial load in the meat when adding the extract to the diet of rabbits has not been assessed. Therefore, the objective of this study was to evaluate the effect of the addition of aqueous extract of garlic to the diet of rabbits on the production indicators, as well as on the physical and microbiological quality of stored meat.

Materials and methods

Biological material

There were used 84 male and female weaned New Zealand rabbits X Chinchilla (*Oryctolagus cuniculus* X *Chinchilla chinchilla*) (1.0 ± 0.6 kg, 35 ± 5 d), housed in the interior of ships with natural ventilation and in a temperate climate (22 ± 2°C), in a modular system of cages on a floor with automatic nipple-type water dispensers and feed chutes, during June-July, 2015.

Study site

The study was carried out in the head farm of the Distributor of Nezahualcoyotl Rabbits (DISCONNEZA), located in the Municipality of Nezahualcoyotl, State of Mexico. It is located between the parallels 19° 24' 02" N and 99° 00' 53" W, at an average altitude of 2,235 m asl.

Preparation of the extract

The aqueous extract of garlic (AEG) was developed from a mother dilution of 0.125 g/ml[12], for which garlic was liquefied without the husk during 5 min (Oster 6630-13), and this extract was strained twice through gauze pads. The resulting AEG was stored in refrigeration at (4 °C) until its use (7 d)[13]. The animals were divided into three treatments: control group CG (without added AEG), treatment 1 T1 (0.9 %), and treatment 2 AEG T2 (1.8 % AEG).

The extracts were sprayed on commercial food (Union Tepexpan Plus® rabbit feed; crude protein: 16.5%; crude fat: 3%; crude fiber: 15%; ashes: 9% and moisture: 12%) every three days from the beginning of the assay. The selected doses correspond to those reported by Mariezcurrena-Berasain[13] for the best production of gas and fermentative parameters as the best power available for producing short-chain fatty acids (SCFA) and metabolizable energy (ME), in her study on gas production *in vitro*. In order to evaluate the production indicators, the rabbits were weighed on a weekly basis (with a Dibatec digital scale), removing the food 12 h before, and the total weight gain was registered, along with the daily weight gain (individually) and the food intake during four weeks. Food and water were provided *ad libitum*.

Slaughter

The rabbits were deprived of food 24 h before being slaughtered. They were desensitized through atlanto-occipital dislocation[14]; they were slaughtered and bled to death through a cut in the jugular vein and the carotid artery, and eviscerated through a cut in the *linea alba* for removal of the abdominal and thoracic viscerae. Finally, the limbs were severed, and the temperature of the carcass decreased to 4 °C. The carcasses were identified and transported, at refrigeration temperature, to the Agricultural Products Quality Laboratory of the Faculty of Agricultural Sciences of the Autonomous University of the State of Mexico, for the corresponding analyses (August-December 2015).

Physical analyses

A first reading of the pH (Hanna Instruments, model HI 99163) and color (Minolta Chroma meter CR 400, with lighting D65 and 10° observer) of samples of the right *Longissimus dorsi* muscle of the carcasses was taken *in situ* at 45 min *post mortem* (in hot carcass). Subsequent analyses were performed on the samples taken from the same muscle during the d 1, 3, 5, 7 and 9 in samples preserved in trays and covered with film at 4 °C and in duplicate.

Microbiological analyses

It was quantified by duplicate the colony-forming units (CFU) of fecal, mesophilic, and psychrophilic coliforms throughout the conservation period. The French standard AFNOR-

NF-V0860-1996[15] was used for the quantification of fecal coliforms, as there is no Official Norm for these microorganisms in Mexico[16].

Statistical analysis

A variance analysis ($P \leq 0.05$) was carried out for the production indicators, and when significant differences were found, a comparison was applied using the Tukey test at 5%. The study variables were the three treatments (CG, T1 and T2), and the response variables were weekly weight, weekly weight gain, and conversion efficiency, during a period of four weeks. A multivariate analysis of variance ($P \leq 0.05$) was applied to the results obtained from the microbiological and physical-chemical study in order to determine the effect of the treatments and the days of conservation. The response variables were UFC of fecal coliforms, aerobic mesophiles, psychrophiles, pH, brightness, red index, and yellow index. The Tukey test at 5 % was carried out for those values that showed significant differences, using Stat Graphics Centurion XV. I

Results and discussion

Production indicators

The results of the productive variables are shown in Tables 1, 2 and 3, in which, as can be observed, there were no significant differences for any of them. The effect of garlic on the productive variables in rabbits remains controversial; some authors have reported that its bioactive compounds have a positive effect on these aspects[17,18]. More recent reports are in opposition, and, according to them, garlic reduces the plasma levels of cholesterol, the blood pressure, and platelet aggregation, or promotes the immune response without affecting these variables, although few studies cited good production results when garlic is supplied together with other aromatic plants. The present work agreed that there was no significant effect ($P \geq 0.05$) for weekly weight, weekly weight gain, or food conversion efficiency. In broiler chickens, the garlic extract has been reported as a stimulator for weight gain; in rabbits, it is suggested that it is conditioned by the digestive physiology[19-22].

Table 1: Variable weight of rabbits per week (kg/LW)

	Teatment			*P*
	CG	**1**	**2**	
Week 1	1.14±0.02	1.09±0.03	1.03±0.04	0.2617
Week 2	1.37±0.03	1.3±0.04	1.29±0.05	0.5028
Week 3	1.59±0.03	1.50±0.04	1.53±0.05	0.1182
Week 4	1.84±0.04	1.75±0.05	1.81±0.06	0.1721

CG= control group (without added AEG); treatment 1= 0.9 % AEG; treatment 2= 1.8 % AEG. AEG= aqueous extract of garlic.

Table 2: Variable weight gain per week (kg/LW)

	Treatment			*P*
	CG	**1**	**2**	
Week 1	0.26±0.01	0.25±0.01	0.25±0.01	0.4845
Week 2	0.23±0.01	0.24±0.01	0.26±0.02	0.3897
Week 3	0.22±0.01a	0.16±0.0a	0.23±0.01a	0.0050
Week 4	0.24±0.01	0.25±0.01	0.28±0.01	0.8360
Total	0.96±0.02	0.91±0.02	1.24±0.05	0.1790

CG= control group (without added AEG); treatment 1= 0.9 % AEG; treatment 2= 1.8 % AEG. AEG= aqueous extract of garlic.

a,b,c Means with different letters in the same row indicate statistically significant differences.

Table 3: Variable feed conversion per week (kg/LW)

	Treatment			*P*
	CG	**1**	**2**	
Week 1	2.5±0.16	2.76±0.21	4.42±0.49	0.3474
Week 2	3.15±0.56	3.14±0.73	5.21±1.52	0.9950
Week 3	2.89±0.73	4.62±0.95	5.28±0.47	0.1541
Week 4	2.88±0.34	2.96±0.44	3.95±0.72	0.8903
Total	2.84±0.1	3.1±0.13	4.29±0.47	0.1308

CG= control group (without added AEG); treatment 1= 0.9 % AEG; treatment 2= 1.8 % AEG. AEG= aqueous extract of garlic.

In relation to the weekly weight gain, Table 2 shows that the treatment 2 with 1.8% of AEG resulted in a greater weekly weight gain. The foregoing is consistent with other studies[23,24], which showed that allicin in garlic promotes the performance of the intestinal flora, thus improving digestion and energy use, which leads to a better growth in broiler chickens.

In relation to the conversion (Table 3), after four weeks, no differences were observed between treatments. However, a tendency to increase the variable when adding a greater dose of extract is apparent.

Physical and microbiological analyses

After the variance analyses (per day and per treatment), significant differences ($P \leq 0.05$) in shelf life were found for aerobic mesophiles and psychrophiles. When significant differences were found for these variables, a Tukey test at 5 % was applied, as shown in Table 4.

Table 4: Physical and microbiological profile during the shelf life of rabbit meat

	Treatment			*P*	EEM
	CG	**1**	**2**		
MA (log_{10} UFC/ cm^2)					
Day 1	1.50x	2.15	2.23	0.092	0.208
Day 3	2.08xy	2.35	2.18	0.675	0.210
Day 5	2.58xy	2.65	2.54	0.960	0.258
Day 7	2.46xy	2.51	2.68	0.642	0.167
Day 9	3.01y	2.99	2.40	0.460	0.371
P	0.009	0.097	0.781		
PSI (log_{10} UFC/ cm^2)					
Day 1	1.57x	1.65x	2.01x	0.348	0.207
Day 3	2.43xy	2.30y	2.77xy	0.255	0.182
Day 5	2.90y	2.85z	2.99y	0.805	0.156
Day 7	2.01xy	2.21y	3.15y	0.064	0.286
Day 9	3.19y	3.40z	3.02y	0.474	0.206
P	0.015	≤0.001	0.016		
pH					
Day 1	6.64ab	6.26aw	7.01bx	0.0058	0.101

Day 3	6.66	6.49x	6.97x	0.0995	0.101
Day 5	6.86ab	6.55ay	7.01bx	0.0278	0.090
Day 7	6.38a	7.11bz	6.21ay	0.0108	0.147
Day 9	6.64a	7.08bz	6.54az	0.0137	0.937
P	0.469	0.000	0.000		
L*					
Day 1	61.17	59.76	61.38	0.762	1.645
Day 3	58.41	59.76	57.42	0.683	1.842
Day 5	58.11	56.79	56.51	0.680	1.326
Day 7	58.13	57.17	57.55	0.901	1.478
Day 9	58.68	56.94	58.37	0.588	1.218
P	0.789	0.065	0.255		
a*					
Day 1	2.04	1.99	1.25	0.167	0.280
Day 3	1.21	1.63	2.17	0.205	0.330
Day 5	3.75	1.57	2.38	0.422	1.106
Day 7	3.16	1.58	1.805	0.345	0.756
Day 9	2.80	1.73	1.916	0.589	0.752
P	0.582	0.783	0.360		
b*					
Day 1	4.27	3.69	4.17	0.436	0.320
Day 3	3.48	4.46	2.78	0.376	0.784
Day 5	5.10	3.14	4.53	0.349	0.901
Day 7	5.17	3.76	4.14	0.443	0.754
Day 9	5.30	3.71	4.02	0.289	0.683
P	0.599	0.263	0.550		

CG= control group (without added AEG), treatment 1 (0.9 % AEG) and treatment 2 (1.8 % AEG). AEG= aqueous extract of garlic; SEM= standard error of the mean.

AM= aerobic mesophiles; PSY= psychrophiles; B*= brightness; a*= intensity of red; b*= intensity of yellow.

a,b,c Means with different letters in the same row indicate significant differences ($P<0.05$).

x,y,z Means with different letters in the same column are significantly different ($P<0.05$).

Significant differences ($P \leq 0.05$) were found in aerobic mesophiles between days of exposure only in the CG, in which d 9 exhibited a larger population than d 1 (Table 4). The range at which the shelf life for this microbial population began in the three treatments was 1.50 to 2.23 log10 CFU/cm^2. Although the Mexican Norm does not indicate a reference value for this microbial population in raw meats of any kind, it is suggested that the European Union, according to the European Commission's Guideline 2001/471/EC[19], reports acceptable values of less than 3.50 log10 CFU/cm^2. There are no reports for rabbit meat. Thus, on the last day of the shelf life of the meat used in the present experiment (d 9), all treatments were found to be within the permissible limits although there were no significant differences ($P > 0.05$) between them. The presence of aerobic mesophiles is used as a general indicator of hygiene and of the population of microorganisms for estimating the quality of the handling and manipulation of the meat; it includes bacteria, molds and yeasts that can thrive at 30 °C[25]. Other studies in rabbit meat without supplementation with antimicrobials in the diet reported higher values of for aerobic mesophiles (5.87 log10 CFU/cm^2) that exceed the allowable limit at 7 d of exposure[26]. In the present study, there was no significant difference ($P \geq 0.05$) for this variable at the end of the shelf life, which suggests that the handling and hygiene was adequate in all three treatments.

In the case of psychrophiles, significant differences were found ($P \leq 0.05$) between days of exposure, but not between treatments. The range at the beginning of the shelf life was 1.57 to 2.01 log10 CFU/cm^2. In the shelf life, the kinetics of growth showed that the T2 started with a greater burden (2.01 log10 CFU/cm^2) than the CG and T1. However, on d 9 of the shelf life of T2, the number of UFCs (3.02 log10 CFU/cm^2) was lower than in the CG and in T1 (3.19 and 3.40 log10 CFU/cm^2, respectively). Therefore, the growth of psychrophiles was lower in the T2 (1.8 % AEG), compared with the control group (CG) and the lowest dose of AEG (T1). These microorganisms are important for predicting the stability of the product under conditions of refrigeration, and it is suggested that T2 (1.8 % AEG) exhibited the greatest stability, although there are no regulations determining permissible limits for stability in rabbit meat. It is problematic to establish maximum allowable limits in aerobic mesophiles and psychrophiles, since meat is a product that, in most cases, undergoes a cooking process before being consumed, reaching high temperatures that eliminate these microorganisms. Another research paper[27] mentions that the 24 h fast improves the microbiological quality, as the presence of undesirable microorganisms in the carcass decreases when the digestive tract is empty. No growth of fecal coliforms was observed in any of the treatments.

The initial load is proportional to the final population reached by a meat during its shelf life. Bacteria reproduce exponentially, and therefore, a high initial population will result in less time to reach those levels at which the meat breaks down[27,28]. Although there are no significant differences, in another research on the effects of added garlic[28] in which the antimicrobial capacity of extracts of garlic obtained using solvents added to minced pork

medallion was evaluated showed that all the extracts inhibited the growth of *Listeria monocytogenes* and *Escherichia coli* 0157:H7. Similarly, another study researched the antimicrobial potential of certain sulfur compounds present in garlic against microbial growth in beef. The results showed that these compounds inhibited the growth of five strains intentionally inoculated in the meat (*Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Campylobacter jejuni*)[29]. Thus, the microbial growth behaved similarly to other results[28,29] when fresh and dry powdered garlic were added directly to camel meat, and a delay in microbial growth during its conservation was reported. Other studies have tested the effectiveness of extracts of garlic in the conservation of carcasses of fresh poultry stored in refrigeration and have shown a significant reduction in microbial contamination, inhibiting the growth of mesophilic microorganisms and reducing the growth of total and fecal coliforms[30], consistently with the inhibition of fecal coliforms in this study. On the other hand, it has been reported that aqueous solutions of garlic on slices of golden catfish (*Brachyplatystoma rousseauxii*) stored at 4°C showed an improvement in the microbiological quality through inhibition of psychrotrophic bacteria and lactic acid bacteria, among others, for at least 15 d[31]. Allicin in garlic has been reported to be a successful antioxidant and antimicrobial agent worth researching, given its benefits in terms of the shelf life of rabbit meat through prevention or reduction of the oxidation of lipids and proteins. The presence of allicin in the carcass may have slowed the growth of microorganisms that contaminate the meat during the quartering[21,32]; therefore, this study —like others on the use of thyme (*Thymus vulgaris*), lactic acid or sumac (*Rhus coriaria* L.) in the meat of this species— proposes that the shelf life can be extended under the tested conditions. However, further research is required.

Significant differences ($P \leq 0.05$) were found for the variable pH, both in the treatments and in the shelf life. The range was 6.26 to 7.01 between treatments at the beginning of the shelf life. At its end (at d 9), the range between treatments was 6.54 to 7.08. The pH of the muscle of healthy animals ranges between 7.04 and 7.30, reaching values of 5.50 to 5.70 at 24 h *post mortem*[33,34,35]. The present results for d 9 were slightly higher than those of other values reported[34] (pH 6.0); however, the authors report only for one day of shelf life. Therefore, it is once again suggested that the slight alkalization presented above improves the shelf life. The pH value is affected by the content of glycogen in the muscles, which, in turn, is affected by stress prior to slaughter. As shown in Table 4, the pH values were high during the shelf life, consistently with other reports that suggest that low concentrations of glycogen raise the pH, and that the meat is more susceptible to the microbiological alteration by an early use of amino acids[36,37]. However, the present study does not evaluate whether or not glycogen prevented the pH from increasing; this is, therefore, an area of opportunity for future research. As in other protein foods kept in refrigeration and in aerobiosis, the pH of rabbit meat increases as the storage progresses, due to bacterial activity[38]; this is consistent with the

results of the present research, in which the pH values exhibited an increase as the shelf life evolved in all the analyzed treatments.

There were no significant differences ($P \geq 0.05$) between treatments for the variables brightness, intensity of red and intensity of yellow, or shelf life, (Table 4). The values obtained for b* ranged from 59.76 to 61.38 on the first day of shelf life, and between 56.94 and 65.20 for d 9. Thus, on day 9, the values were found to be slightly below those determined in rabbit meat by other assessments, which mentioned a brightness value of 59.48[39,40]. The present values are slightly above those reported in other studies, of 54.9[41]. In the case of a*, the range of these reports was 1.21 to 3.75, similar to those indicated by the authors mentioned above (2.49 and 2.84, respectively). In the case of b*, the meat analyzed in the present study had a slightly more yellow color, since the values of this variable ranged between 2.78 and 5.17, consistently with those of other studies, of 4.3[41]. Finally, the color was not affected by the treatments, which suggests that the quality of meat treated with aqueous extract of garlic may not modify the purchase decision of the consumers.

Conclusions and implications

The addition of aqueous extract of garlic in the diet of rabbits had an effect mainly on the shelf life variable, as it reduced the account of psychrophiles, whereby the quality of the meat was improved, its shelf life, increased by 2 d (adding up to a total of 9 d). However, it had no effect on the production indicators or on the physical quality of the meat (pH and color).

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