Methods of Antibiotic Applications as Related to Microbial Quality of Lamb by PCA and Bioluminescence

MUSTAFA SACIT BAŞOL and UĞUR GÖĞÜŞ

ABSTRACT

The effects of different methods of application of chlortetracycline and oxytetracycline on microbial properties of lamb meat were studied. A significant antibacterial effect of antibiotic was found on total mesophilic bacteria count. The mesophilic bacteria level was determined by two methods: the conventional method using Plate Count Agar and bioluminescence. Correlation curves of the CFU and RLU values proved two methods were highly related. Thus for the microbiology quality of meat hygiene the bioluminescence method may be used as a fast and alternative method to PCA. Note that bioluminescence requires a higher degree of skill and attention.

Key Words: lamb, oxytetracycline, chlortetracycline, bioluminescence, mesophile

INTRODUCTION

INADEQUATE STORAGE CONDITIONS for meat, especially, in the eastern parts of Anatolia are major problems in Turkey regarding food hygiene and technology. The chemical and microbial quality criteria of meat which is ready for consumer markets are below the optimum hygienic standards. In Turkey many semi-modern abattoirs do not have the right microbial qualifications for hygienic trimming. Improving such conditions is slow and impractical. Therefore, using suitable antimicrobial substances such as chlortetracycline (CTC) and oxytetracycline (OTC) may provide a short-term solution to this urgent and important problem by increasing the shelf life in refrigerated conditions (4°C).

Numerous studies have shown that it is possible to improve hygienic levels by means of antibiotics. Very few studies have been carried out to indicate which species of bacteria were inhibited by which antibiotics. For example, Weiser et al. (1954) observed a higher inhibition of the Gram negative rods compared to other bacteria after treating meat products with CTC and OTC. Jay et al. (1956) observed an inhibition in *Proteus* and *Pseudomanas* in beef which was treated by CTC and OTC. Liston et al. (1957) reported a decline in *Pseudomanas* in fish meat treated by TC. Pearson (1970) reported that treating with CTC and sulphite upgraded the quality of preservation of ground beef under refrigeration conditions. In contrast to those results, Ayres et al. (1956) reported an increase from 65% to 95% in *Pseudomanas* and *Achromobacter* microflora of meat when treated with 10% CTC. DePaola et al. (1988) observed that the use of antimicrobial substances caused an increase in those bacteria that gained resistance to the antibiotics.

Because of the wide variety of microorganisms causing spoilage of meat, it has been suggested that antibiotics with a broad spectrum should be used as antibacterial substances. The most frequently used and lowest cost of such antibiotics are CTC and OTC (Kramlich et al., 1980). Nevertheless, some narrow spectrum antibiotics have also been used (Bell and Lacy, 1987) to inhibit specific microorganisms. It was concluded that the most effective applications involved combinations of antibiotics (Kramlich et al., 1980).

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Application of antibiotics to meat, however, has brought criticism and protests. Many do not agree with antibiotic application to meat as a legal hygienic procedure because many antibiotics may cause toxic and allergic reactions to consumers. Therefore a strict control on applications should be maintained. Use of antibiotics that cause toxic and allergic reactions, bacterial resistance and cumulative effects in the human body should not be allowed and irresponsible and illegal applications of antibiotics to meat should be prohibited.

Most European countries do not consume lamb and there are few studies regarding the preservation of lamb by antibiotics. Turkey has a high potential for sheep production. Therefore preservation of lamb by broad spectrum antibiotics is very important for Turkey as well as other countries.

Our objective was to investigate the effects of CTC and OTC on the total-aerobic mesophile bacterial quality of lamb. To determine the bacterial quality of lamb, not only the traditional bacteriological analyses (PCA) but also "bioluminescence" was used. The reliability and practicality of ATP-bioluminescence methods were also assessed for monitoring milk tanker cleanliness by Bell et al. (1993). Other research regarding this method was reported by Ogden (1993) for monitoring plant hygiene, and bioluminescence was described as a rapid and sensitive bacterial method.

MATERIALS & METHODS

TWELVE AKKARAMAN LAMBS of the same age (11–12 mo old), and same conditions were purchased from a local market. They were divided into four groups by block assignment. The first group (three lambs) was kept as control. They were slaughtered and their legs dissected from the sixth backbone vertebra together with the loin. These pieces were rinsed with water and chilled in a cold room (1.0 + 0.5°C) for 24 hr and transferred to 4°C storage after packing and coding (C).

Antibiotic applications

The second group of lambs was subjected to an antemortem trial. Four hours before slaughtering, they were intramuscularly injected with a mixture of CTC and OTC at different locations. As CTC source "Devamisin" tablets (Eczacıbaşı, İstanbul, Türkiye) which contains 500 mg CTC/tablet, and for OTC "Geosol" (Eczacıbaşı, İstanbul, Türkiye), a powder containing 55 mg OTC/g were used. The homogenized solution of CTC and OTC for injection was prepared in 0.9% physiological saline concentrations to provide 0.9 mg of each antibiotic/kg of live weight. Four hr after injection, the three lambs were slaughtered and their legs dissected from the sixth backbone vertebra together with the loin.

After slaughtering the third group of lambs, the legs were dissected similarly. A homogenized solution of known concentration of CTC and OTC mixture in 10L water was prepared. A suitable volume of these solutions which supplied 0.9 mg of each antibiotic/kg meat was used to spray each leg thoroughly.

The legs dissected similarly from the fourth group of slaughtered lambs were immersed for 1 min in 10L homogenized aqueous solution of CTC and OTC mixture, containing 0.9 mg of each antibiotic/kg meat.

The nine remaining legs which were treated by antibiotics by different methods were chilled in a cold room for 24 hr and then transferred to 4°C storage after packing and coding (as A = Antemortem, I = Immersion, S = Spray).

Table 1—Log values of CFU and RLU of samples for each period

Group[a]	1.4.1994 log RLU	1.4.1994 log CFU	4.4.1994 log RLU	4.4.1994 log CFU	7.4.1994 log RLU	7.4.1994 log CFU	14.4.1994 log RLU	14.4.1994 log CFU
C	1.8	1.4	2.7	2.4	4.0	3.4	*	*
A	1.4	1.4	2.1	2.4	2.3	2.4	3.8	3.3
I	1.0	0.7	1.6	1.7	1.9	1.8	2.7	2.7
S	0.8	0.5	1.5	1.8	1.8	1.8	2.7	2.8

[a] C = Control; A = Antemortem; I = Immersion; S = Spray.
* Above the legal levels, according to FAO, 1979.

Table 2—Correlation degrees and coefficients between RLU and CFU for all period

Period	Sample	Correlation Degree %	Correlation Coefficient r[a]
1.4.1994	Loin	76.9	0.876
1.4.1994	Leg	65.1	0.806
4.4.1994	Loin	19.5	0.441
4.4.1994	Leg	47.0	0.685
7.4.1994	Loin	89.4	0.945
7.4.1994	Leg	86.4	0.929
10.4.1994	Loin	82.2	0.906
10.4.1994	Leg	92.6	0.962

[a] Correlation coefficient, r, is defined as the square root of the percent correlation degree. e.g. $\sqrt{0.769} = 0.876$

Preparation of meat samples for bacteriological analysis

Meat samples for legs and loins were prepared separately. For this, purpose, areas of 1 cm^2 were marked on the surfaces of coded legs or loins. Specimens weighing 1g from these marked areas were cut and ground to obtain a bacteriologically homogeneous sample.

Bacteriological analysis

For bacteriological determination two methods were used: The traditional Plate Count Agar (PCA) and bioluminescence. These analyses were carried out for each sample four times with 3-day intervals to determine the shelf life. The analysis were repeated for the backloin and leg parts simultaneously. The results of PCA were recorded as Colony Forming Unit (CFU).

The samples for the traditional Plate Count Agar (PCA) technique were prepared by homogenizing 1g ground meat in 9 mL of 0.09 mL physiologic saline. From this 10^{-1} stock dilution, other dilutions were prepared. 1 mL of each dilution, was transferred into petri dishes (14 mm); PCA medium added and plates incubated at 37°C for 48 hr. Following incubation, colonies were counted and expressed as CFU/g ground meat sample (Harrigan and McCance 1979).

For bioluminescence, the test procedures of LUMAC's IMC (Industrial Microbial Control) kit (LUMAC Proceedings Cat. No. 9283-6, 1993, Landgraaf, The Netherlands) was used. Samples for this analysis were prepared by homogenizing 1g ground meat in 3 mL Ringer's solution (Lumac B.V., Langraaf, The Netherlands). After allowing large particles to settle, 500 µL of the supernatant were transferred into a disposable Lumacuvette (12 × 47 mm cuvette from Lumac). Then, 500 µL Nucleotide Releasing Somating Reagent (NRC-Lumac B.V.) and 20 µL Somase (an ATP-ase from Lumac) were added into the contents of the same cuvette. This mixture was incubated for 45 min at room temperature to lyse the somatic membranes and eliminate both somatic and free ATP. At the end of the incubation, 100 µL of incubated solution was transferred to the cuvette part of the biocounter (Lumac B.V.) and 100 µL of Nucleotide Releasing Bacterial Reagent (NRB from Lumac) was added to release the bacterial ATP from the bacterial cell. Finally, to produce luminescence, 100 µL of Lumit (Luciferin-Luciferase from Lumac) was added to the cuvette. Following a 10-sec-integration-time, results were recorded as Relative Light Units (RLU).

To determine the difference between the various kind of antibiotic applications, Duncan Multiple Choice test described by Scheffe (1970), was applied. To determine the correlation between the two different bacterial count methods, the statistical analysis as presented by Lyman (1988) was used.

RESULTS & DISCUSSION

DUNCAN TEST was applied on the data (Tables 1 and 2) to see the effects of antibiotic applications and the following conclusions were reached: The control and antemortem treated samples were not significantly different indicating that antemortem application of the antibiotics had little antibacterial effect. Immersion and spray applications had a greater antibacterial effect ($p<0.05$ and $p<0.01$). Therefore we concluded immersion and spraying were more effective against the mesophilic flora of lamb than an antemortem application.

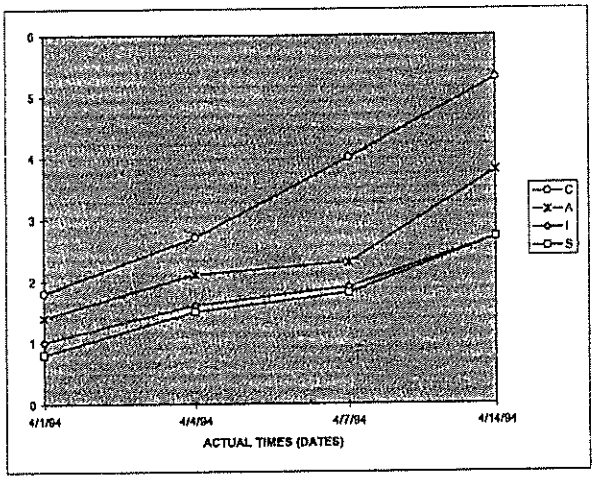


Fig. 1—Log RLU/g values of application groups and control.

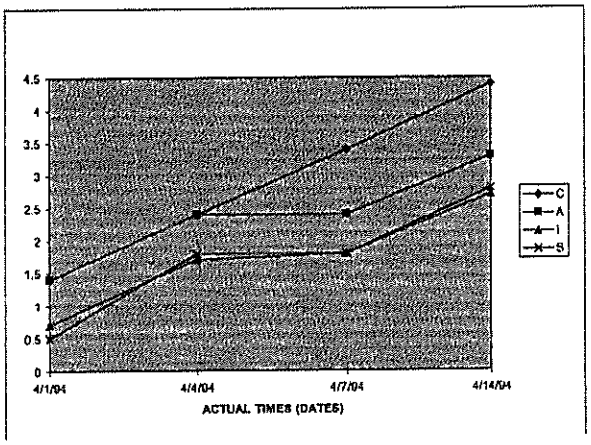


Fig. 2—Log CFU/g values of application groups and control.

The statistical analyses were made on the plots of CFU (Fig. 2) vs RLU (Fig. 1) and correlation degrees and coefficients between results on the bacteriological methods were compared (Table 2). The correlation except for the second period is between 65 and 93%. All correlation coefficients, with exception of the second period (4.4.1994), were significant ($p < 0.01$ and $p < 0.05$). Thus PCA and bioluminescence were highly correlated ($p < 0.01$ and $p < 0.05$).

The bioluminescence technique is much more practical, sensitive and faster than the traditional Plate Count. It requires <1 hr to complete an analysis compared to 24–48 hr for PCA. Therefore we concluded that the bioluminescence technique may be an alternative to conventional PCA for a fast on-line bacteriological analysis. The bioluminescence method requires a higher skill level and close attention to the storage and handling of reagents.

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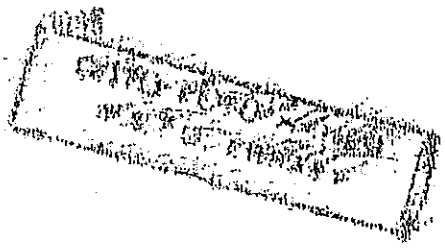
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Ms received 4/21/95; revised 9/1/95; accepted 10/12/95.

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JOURNAL OF FOOD SCIENCE

21 MAY



March-April 1996
Volume 61, No. 2
Coden: JFDAZ 61: 265–476
ISSN: 0022-1147

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A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS
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IFT

JOURNAL OF FOOD SCIENCE

March-April 1996
Volume 61, No. 2

Coden: JFDAZ 61: 265–476
ISSN: 0022-1147

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JOURNAL OF FOOD SCIENCE (formerly *Food Research*) is published six times a year (bimonthly) by Institute of Food Technologists, 221 N. LaSalle Street, Suite 300, Chicago, IL. 60601 USA. Printed in the USA. Second class postage paid at Chicago, Illinois and at additional mailing offices. POSTMASTER: Send address changes to *Journal of Food Science*, 221 N. LaSalle St., Suite 300, Chicago, IL 60601.

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A Publication of the Institute of Food Technologists

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Journal of Food Science

A Publication of the Institute of Food Technologists

Edited by:
Daryl B. Lund

Print ISSN: 0022-1147
Online ISSN: 1750-3841
Frequency: Nine times a year
Current Volume: 74 / 2009
ISI Journal Citation Reports® Ranking: 2007: 37/103 (Food Science & Technology)
Impact Factor: 1.255

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