Praca oryginalna *Original paper*

Antibiotic susceptibility of Lactococcus isolated from Turkish raw milk cheeses

FIGEN CETINKAYA, NILAY COPLU*, HUSNIYE SIMSEK*, TULAY ELAL MUS, RECEP CIBIK

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Uludag, Gorukle Campus, 16059 Bursa, Turkey
*National Reference Laboratory for Enteric Pathogens, Department of Communicable Diseases Research, Refik Saydam National Hygiene Center, 06100 Sihhiye, Ankara, Turkey

Cetinkaya F., Coplu N., Simsek H., Elal Mus T., Cibik R.

Antibiotic susceptibility of Lactococcus isolated from Turkish raw milk cheeses

Summary

Lactococcus lactis strains isolated from traditionally produced Turkish White Pickled (22 strains) and Kashar cheeses (18 strains) were examined for susceptibility to a range of antibiotics, including ampicillin, amoxicillin/clavulanic acid, vancomycin, oxacillin, penicillin G, cefotaxime, ciprofloxacin, gentamicin, streptomycin, tetracycline, erythromycin, trimethoprim, chloramphenicol, clindamycin and rifampicin, by the disc diffusion method. Susceptibility to ampicillin, amoxicillin-clavulanic acid, vancomycin and chloramphenicol, and resistance to oxacillin antibiotics were observed in all strains tested. The susceptibility to the other antibiotics was variable and strain-dependent. Only one strain exhibited resistance to erythromycin. All strains were resistant to more than one of the antibiotics tested, and the greatest number of strains were resistant to oxacillin, ceftazidime, streptomycin and clindamycin. A multiple antibiotic resistance profile revealed that most of the strains (92.5%) were resistant to three to seven antibiotics, whereas one strain demonstrated resistance to two antibiotics. The highest resistance was found in two strains isolated from White Pickled cheese, of which one was resistant to eight and the other to nine antibiotics. Our findings reveal a high level of antibiotic resistance among the strains of L. lactis and emphasize the need for prudent use of antibiotics.

Keywords: lactic acid bacteria, Lactococcus, antimicrobial susceptibility, cheese

Antimicrobial agents are commonly used in animal farming to cure or prevent bacterial infections. Their application for several years at subtherapeutic doses as growth enhancers in farm animals has led to the selection of antibiotic-resistant bacteria in the intestinal microflora (11, 28). Foodborne commensal bacterial populations may become reservoirs of antibiotic resistance genes that could be transferred horizontally to opportunistic and pathogenic bacteria (13). This is a worldwide public health problem of increasing importance. In recent years, increased attention has been given to food as a vehicle of antibiotic resistance genes (10, 16, 21, 22). Fermented dairy products may provide a vehicle for antibiotic-resistant bacteria, with a direct link between the animals' indigenous flora and the human gastrointestinal tract (22).

Lactic acid bacteria (LAB) from fermented products may act as a reservoir of antimicrobial resistance genes that could be transferred to pathogens, either in the food matrix or in the gastrointestinal tract (11, 15). *Lactococci* belonging to the lactic acid bacteria are the primary component of many industrial and artisanal starter cultures used for the manufacture of a wide range of fermented dairy products, including fresh and soft cheeses, as well as various hard and semihard cheeses (29). They naturally occur in grass and in the mouths and udders of cows. They can be transferred to milk during milking and are found in some cheese specialities made from raw milk. Since antibiotics are widely used in dairy cows to prevent or treat infectious diseases like mastitis and metritis, *Lactococcus* may acquire antibiotic resistance, survive antimicrobial treatments, and subsequently act as a reservoir of antibiotic resistance genes for other bacteria (30).

White pickled cheese, which is a soft or semi-hard cheese, is probably the most popular and economically the most important variety of traditional cheese in Turkey (18). The second most popular cheese in Turkey is Kashar, a semi-hard cheese, of which around 49,000 tons is produced every year (5). These cheese varieties are produced from raw or pasteurized ewe's milk or a mixture of cow's and ewe's milk and mostly from cow's milk (1, 17). In the traditional manufacture, cheese milks were not subjected to pasteurization, and

Tab. 1. Antibiotic susceptibility profiles of *L. lactis* from traditional raw milk cheeses

Species/Strain	Cheese	Susceptibility patterns for antibiotics															
		AMP	P	AMC	VA	OX	CTX	CAZ	CIP	CN	S	TE	E	W	C	DA	RD
L. lactis 1	White brined	S[c]	M[b]	S	S	R[a]	R	R	S	S	R	S	S	S	S	R	S
L. cremoris 2	White brined	S	M	S	S	R	M	R	S	R	R	S	S	S	S	S	S
L. cremoris 4	White brined	S	M	S	S	R	R	R	S	R	R	R	S	S	S	R	M
L. lactis 10	White brined	S	M	S	S	R	M	R	S	S	R	S	S	R	S	M	R
L. lactis 12	White brined	S	M	S	S	R	M	R	S	S	R	S	S	S	S	M	S
L. lactis 13	White brined	S	M	S	S	R	M	R	S	S	R	S	S	S	S	R	S
L. lactis 15	White brined	S	M	S	S	R	S	R	S	R	R	S	S	M	S	M	S
L. lactis 20	Kashar	S	M	S	S	R	M	R	S	R	R	S	S	S	S	R	S
L. lactis 23	White brined	S	M	S	S	R	M	R	S	R	R	S	S	S	S	M	S
L. lactis 24	White brined	S	M	S	S	R	M	R	S	S	R	S	M	S	S	S	S
L. lactis 27	White brined	S	M	S	S	R	M	R	S	S	R	S	S	R	S	S	R
L. cremoris 28	White brined	S	M	S	S	R	M	R	S	S	R	S	S	M	S	S	R
L. cremoris 30	White brined	S	M	S	S	R	M	R	S	R	R	R	S	S	S	R	S
L. lactis 31	White brined	S	M	S	S	R	M	R	S	S	R	S	M	R	S	R	R
L. lactis 32	White brined	S	M	S	S	R	M	R	S	S	R	S	S	S	S	R	S
L. lactis 34	Kashar	S	M	S	S	R	R	R	S	R	R	R	S	S	S	R	S
L. lactis 35	Kashar	S	M	S	S	R	M	R	S	S	R	S	S	S	S	R	M
L. lactis 36	Kashar	S	S	S	S	R	R	R	S	S	M	S	S	S	S	S	R
L. lactis 37	Kashar	S	M	S	S	R	R	R	S	R	R	R	S	S	S	M	S
L. lactis 39	Kashar	S	S	S	S	R	S	S	S	S	S	R	S	M	S	R	R
L. cremoris 40	Kashar	S	S	S	S	R	S	R	S	S	R	S	R	S	S	R	S
L. lactis 41	Kashar	S	M	S	S	R	M	R	S	S	R	S	S	S	S	S	S
L. lactis 42	White brined	S	M	S	S	R	M	R	M	R	R	R	S	R	S	R	R
L. lactis 43	White brined	S	M	S	S	R	M	R	S	R	R	R	S	S	S	R	R
L. lactis 44	White brined	S	M	S	S	R	M	R	M	S	R	S	S	R	S	S	R
L. lactis 46	Kashar	S	S	S	S	R	S	R	S	S	R	S	S	S	S	R	S
L. lactis 47	Kashar	S	S	S	S	R	S	R	S	S	R	R	S	R	S	M	R
L. lactis 50	Kashar	S	M	S	S	R	S	S	S	S	R	R	S	S	S	S	S
L. lactis 51	Kashar	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S
L. lactis 52	White brined	S	M	S	S	R	S	R	S	S	R	S	S	S	S	S	S
L. lactis 53	White brined	S	S	S	S	R	S	R	S	S	R	S	S	S	S	R	S
L. lactis 54	Kashar	S	M	S	S	R	R	R	S	S	R	S	S	R	S	M	R
L. lactis 55	White brined	S	M	S	S	R	S	R	S	S	R	S	S	S	S	R	S
L. lactis 56	Kashar	S	M	S	S	R	R	R	S	S	R	S	S	S	S	M	R
L. lactis 57	Kashar	S	M	S	S	R	R	R	S	S	R	S	S	S	S	M	R
L. lactis 59	Kashar	S	M	S	S	R	M	R	S	S	R	R	S	R	S	R	R
L. lactis 60	Kashar	S	M	S	S	R	M	R	S	S	M	S	S	R	S	S	S
L. lactis 61	Kashar	S	M	S	S	R	R	R	S	R	R	R	S	S	S	R	S
L. lactis 62	White brined	S	M	S	S	R	R	R	S	R	R	R	S	R	S	R	R
L. lactis 63	White brined	S	M	S	S	R	M	R	S	S	R	S	S	S	S	M	S

Explanations: AMP – ampicillin; P – penicillin G; AMC – amoxicillin/clavulanic acid; VA – vancomycin; OX – oxacillin; CTX – cefotaxime; CAZ – ceftazidime; CIP – ciprofloxacin; CN – gentamicin; S – streptomycin; TE – tetracycline; E – erythromycin; W – trimethoprim; C – chloramphenicol; DA – clindamycin; RD – rifampicin; a – resistant, b – moderately susceptible, c – susceptible the bacteria originating from raw milk and dairy environment were involved in the natural ripening process. Fermented dairy products that are not heat-treated prior to consumption may provide a vehicle for the transfer of antibiotic-resistant bacteria to humans, and therefore it is necessary to study microbial resistance to antibiotics in these products (24). The aim of this work was to evaluate the resistance of *L. lactis* strains obtained from traditionally produced Turkish cheeses to a range of antibiotics.

Material and methods

Bacterial strains and growth conditions. Bacterial strains used in this study are shown in tab. 1. A total of 40 *Lactococcus* strains, 35 belonging to *L. lactis* subsp. *lactis* and 5 belonging to *L. lactis* subsp. *cremoris*, that had been isolated from White Pickled/Kashar cheeses and identified for their technological traits were used (9). Stock cultures were kept frozen (–20°C) in M17 broth (Merck, cat. no. 1.15029) containing 20% (v/v) glycerol. The cultures were activated in M17 broth at 30°C before use.

Antibiotic susceptibility testing. Since no standards exist for the susceptibility testing of LAB, a modified version of the standard disk diffusion method (3) with M17 medium (Merck) was used (24). Single colonies of *Lactococcus* species picked from fresh cultures on M17 agar (Merck, cat. no. 1.15108) and incubated aerobically at 30°C for 48 h were used to inoculate a mixed formulation of 90% Mueller-Hinton broth (MHB, Oxoid cat. no. CM405) and 10% M17 broth (Merck, cat. no. 1.15029). When the broth culture reached the 0.5 McFarland standard turbidity at 30°C, cultures were streaked with a cotton swab over 4 mm thick agar plates containing a mixed formulation of Mueller Hinton agar (MHA, Oxoid cat. no. CM337) supplemented with 10% M17 dehydrated broth and pH adjusted to 6.7. Antibiotic disks were placed aseptically on the agar surface, and plates were then incubated at 30°C for 24 to 48 h. After incubation, the results were recorded by measuring inhibition zones and expressed as resistant (R), moderately susceptible (M) and susceptible (S) by following the cut-off levels proposed by Charteries et al. (7). *Staphylococcus aureus* ATCC® 25923 was used as a positive reference strain.

Antibiotics used and their concentrations were as follows: ampicillin (AMP; 10 µg), penicillin G (P; 10 U), amoxicillin/clavulanic acid (AMC; 20/10 µg), vancomycin (VA; 30 µg), oxacillin (OX; 1 µg), cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (CN; 10 µg), streptomycin (S; 10 µg), tetracycline (TE; 30 µg), erythromycin (E; 15 µg), trimethoprim (W; 5 µg), chloramphenicol (C; 30 µg), clindamycin (DA; 2 µg) and rifampicin (RD; 5 µg) (Oxoid, Basingstoke, UK).

The MIC of erythromycin was determined by the E test (AB Biodisk, Solna, Sweden) following the manufacturer's instructions. Since no cut-off values have been officially defined for LAB, the breakpoints established by the FEEDAP Panel of the European Food Safety Authority (EFSA) were used as a reference (4).

Results and discussion

The use of antibiotics in veterinary medicine as therapeutics, prophylactics and animal growth promoters has resulted in the appearance of resistant strains (2). Beneficial and commensal bacteria may play an important role in the transfer of antibiotic resistance elements to pathogenic and opportunistic bacteria (27). LAB have acquired the „Generally Regarded as Safe (GRAS)" status and are used as starter cultures in the fermentation process of different foods (21). Several investigators have recently speculated that LAB isolated from foods may act as reservoirs of antibiotic resistance characters (6, 13, 14, 20, 25, 30).

In the present study, 40 *L. lactis* strains isolated from raw milk cheeses have been submitted to antibiotic susceptibility test. Thirty five of the isolates examined belonged to the subspecies *lactis*, and only five were subspecies *cremoris*. Cheriguene et al. (8) reported that *L. lactis* subsp. *lactis* was more frequently isolated than *L. lactis* subsp. *cremoris* in raw goat's milk samples. In addition, it was indicated by Sanchez et al. (26) that all lactococci isolates from cheeses manufactured from raw cow's milk belonged to the subspecies *lactis*. Antibiotic susceptibility/resistance pattern of *L. lactis* strains is shown in tab. 1.

All *Lactococcus* strains displayed susceptibility to ampicillin, amoxicillin/clavulanic acid, vancomycin and chloramphenicol, whereas broad susceptibility was observed to ciprofloxacin and erythromycin antibiotics. This is consistent with reports in which susceptibility to ampicillin, amoxicillin/clavulanic acid and chloramphenicol is reported for lactococci strains (14, 19, 20, 23, 30). On the other hand, Ram et al. (23) reported that 16% of *Lactococcus* isolates recovered from raw buffalo milk were resistant to ampicillin. As confirmed previously, vancomycin susceptibility is a general attribute among lactococci strains (14, 15, 19, 24, 30).

A certain degree of resistance to some of the antibiotics tested was detected (tab. 2). The number of resistant strains was the highest for oxacillin (n = 40), followed by ceftazidime (n = 37), streptomycin (n = 37), clindamycin (n = 19) and rifampicin (n = 15). Only one *L. cremoris* strain was resistant to erythromycin. The MIC value for the erythromycin-resistant *cremoris* strain was calculated to be 256 µg/ml, which allowed us to classify this strain in the resistant category. Interestingly, only one *lactis* strain was also found to be resistant to trimethoprim.

Resistance to erythromycin, clindamycin and tetracyclin was also found by Walther et al. (30) and Florez et al. (15), and resistance to streptomycin by Florez et al. (14). The finding that most strains (n = 19) were resistant to clindamycin, differs from those of Elliott and Facklam (12), who reported that *L. lactis* strains isolated from humans were susceptible to clindamycin. This finding also differs from a previous report in

Tab. 2. Number and percentage of antibiotic resistance among *L. lactis* strains

Antimicrobials	Number (%) of resistant strains of:		
	L. lactis subsp. *lactis*	*L. lactis* subsp. *cremoris*	Total
Ampicillin	0 (0)	0 (0)	0 (0)
Penicillin G	0 (0)	0 (0)	0 (0)
Amoxicillin/ clavulanic acid	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)
Oxacillin	35 (100)	5 (100)	40 (100)
Cefotaxime	9 (25.7)	1 (20)	10 (25)
Ceftazidime	32 (91.4)	5 (100)	37 (92.5)
Ciprofloxacin	0 (0)	0 (0)	0 (0)
Gentamicin	9 (25.7)	3 (60)	12 (30)
Streptomycin	32 (91.4)	5 (100)	37 (92.5)
Tetracycline	10 (28.6)	2 (40)	12 (30)
Erythromycin	0 (0)	1 (20)	1 (2.5)
Trimethoprim	10 (28.6)	0 (0)	10 (25)
Chloramphenicol	0 (0)	0 (0)	0 (0)
Clindamycin	16 (45.7)	3 (60)	19 (47.5)
Rifampicin	14 (40)	1 (20)	15 (37.5)

which a low resistance (n = 1) to this antibiotic was reported in lactococci isolated from raw milk cheeses (24). We found that 15 out of 40 *L. lactis* strains were resistant to rifampicin. Liu et al. (20) documented that all *L. lactis* isolates were resistant to this antibiotic. Twenty percent of lactococci examined by Ram et al. (23) displayed resistance to penicillin. In contrast to this finding, we found no resistance to this antibiotic: 33 and 7 strains were classified as moderately susceptible and susceptible, respectively.

All strains displayed resistance to more than one antibiotic, and most of them (92.5%) were found to carry multiple resistance phenotypes against 3 to 7 different antibiotics (tab. 3). The *lactis* strains 42 and 62, showing resistance to 8 and 9 antibiotics respectively, had the highest resistance.

Conclusions

The present work reports information about the prevalence of antibiotic resistance in *L. lactis* isolates (belonging to the subspecies *lactis* and *cremoris*) from White Brined and Kashar cheeses, made from raw milk without the addition of starter cultures in Turkey. Our findings reveal high levels of resistance to a variety of antibiotic agents and the presence of multi-resistant bacterial isolates among natural *L. lactis* isolates, whereby raw milk cheeses can be reservoirs of antibiotic-resistant lactococci. To prevent the spread of resistance, strict control over the use of antibiotic agents in farming practice is essential.

Tab. 3. Multiple antibiotic resistances among *L. lactis* strains

Species (n)*	Number of isolates resistant to:		
	0 to 2 antibiotics	3 to 7 antibiotics	More than 7 antibiotics
L. lactis subsp. *lactis* (35)	1	32	2
L. lactis subsp. *cremoris* (5)	–**	5	–
Total (40)	1	37	2
(%)	(2.5)	(92.5)	(5)

Explanations: * – number tested;** – no isolates were resistant

References

1. *Akin N., Aydemir S., Koçak C., Yildiz M. A.*: Changes of free fatty acid contents and sensory properties of white pickled cheese during ripening. Food Chem. 2003, 80, 77-83.
2. *Ammor M. S., Florez A. B., Mayo B.*: Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol. 2007, 24, 559-570.
3. *Anon.*: Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 18th informational supplement. M100--S18, Clinical and Laboratory Standards Institute, Wayne, Pa., USA 2008.
4. *Anon.*: FEEDAP Panel. Technical guidance prepared by the Panel on additives and products or substances used in animal feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA J. 2008, 732, 1-15.
5. *Anon.*: Report of National Food and Nutrition Strategy Study Group. General Directorate of Economic Sectors and Coordination, Edition number DPT: 2670, Turkey 2003.
6. *Boguslawska J., Zycka-Krzesinska J., Wilcks A., Bardowski J.*: Intra- and interspecies conjugal transfer of Tn916-like elements from Lactococcus lactis in vitro and in vivo. Appl. Environ. Microbiol. 2009, 75, 6352-6360.
7. *Charteris W. P., Kelly P. M., Morelli L., Collins J. K.*: Antibiotic susceptibility of potentially probiotic Lactobacillus species. J. Food Prot. 1998, 61, 1636-1643.
8. *Cheriguene A., Chougrani F., Bekada A. M. A., El-Soda M., Bensoltane A.*: Enumeration and identification of lactic microflora in Algerian goats' milk. Afr. J. Biotechnol. 2007, 6, 1854-1861.
9. *Cibik R., Cetinkaya F., Ersoy M., Yibar A.*: Identification and technological characterization of Lactococcus isolated from traditional Turkish cheeses. Rev. Med. Vet. 2010, 161, 509-514.
10. *Danielsen M., Wind A.*: Susceptibility of Lactobacillus spp. to antimicrobial agents. Int. J. Food Microbiol. 2003, 82, 1-11.
11. *Devirgiliis C., Caravelli A., Coppola D., Barile S., Perozzi G.*: Antibiotic resistance and microbial composition along the manufacturing process of Mozzarella di Buffalo Campana. Int. J. Food Microbiol. 2008, 128, 378-384.
12. *Elliott J. A., Facklam R. R.*: Antimicrobial susceptibilities of Lactococcus lactis and Lactococcus garvieae and a proposed method to discriminate between them. J. Clin. Microbiol. 1996, 34, 1296-1298.
13. *Florez A. B., Ammor M. S., Mayo B.*: Identification of tet (M) in two Lactococcus lactis strains isolated from a Spanish traditional starter-free cheese made of raw milk and conjugative transfer of tetracycline resistance to lactococci and enterococci. Int. J. Food Microbiol. 2008, 121, 189-194.
14. *Florez A. B., Danielsen M., Korhonen J., Zycka J., Von Wright A., Bordowski J., Mayo B.*: Antibiotic survey of Lactococcus lactis strains to six antibiotics by Etest, and establishment of new susceptibility-resistance cut-off values. J. Dairy Res. 2007, 74, 262-268.
15. *Florez A. B., Delgado S., Mayo B.*: Antimicrobial susceptibility of lactic acid bacteria isolated from a cheese environment. Can. J. Microbiol. 2005, 51, 51-58.
16. *Franz C. M. A. P., Holzapfel W. H., Stiles M. E.*: Enterococci at the crossroads of food safety. Int. J. Food Microbiol. 1999, 47, 1-24.
17. *Halkman A. K., Yetismeyen A., Yildirim M., Yildirim Z., Halkman Z., Cavus A.*: Investigation of Kashar cheese starter culture. Turk. J. Agric. For. 1994, 18, 365-377.
18. *Hayaloglu A. A., Guven M., Fox P. F.*: Microbiological, biochemical and technological properties of Turkish White cheese 'Beyaz peynir'. Int. Dairy J. 2002, 12, 635-648.
19. *Katla A. K., Kruse H., Johnsen G., Herikstad H.*: Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. Int. J. Food Microbiol. 2001, 67, 147-152.

20. *Liu C., Zhang Y. Z., Dong K., Yuan J. P., Guo X. K.*: Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomed. Environ. Sci. 2009, 22, 401-412.
21. *Mathur S., Singh R.*: Antibiotic resistance in food lactic acid bacteria – a review. Int. J. Food Microbiol. 2005, 105, 281-295.
22. *Perreten V., Schwarz F., Cresta L., Boeglin M., Dasen G., Teuber M.*: Antibiotic resistance spread in food. Nature 1997, 389, 801-802.
23. *Ram C., Batish V. K., Grover S.*: Survey of lactic acid bacteria from buffalo raw milk for antibiotic resistance, biochemical attributes and plasmid profiles. Microbiol. Alim. Nutr. 1996, 14, 201-210.
24. *Rodriguez-Alonso P., Fernandez-Otero C., Centeno J. A., Garabal J. I.*: Antibiotic resistance in lactic acid bacteria and Micrococcaceae/Staphylococcaceae isolates from artisanal raw milk cheeses, and potential implications on cheese making. J. Food Sci. 2009, 74, 284-293.
25. *Rojo-Bezares B., Saenz Y., Poeta P., Zarazaga M., Ruiz-Larrea F., Torres C.*: Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int. J. Food Microbiol. 2006, 111, 234-240.
26. *Sanchez M. M., Delgado T., Alonso L., Mayo B.*: Phenotypic and genetic characterization of a selected set of Lactococcus lactis strains isolated from a starter-free farmhouse cheese. Food Microbiol. 2000, 17, 449-460.
27. *Teuber M.*: Spread of antibiotic resistance with food-borne pathogens. Cell. Mol. Life Sci. 1999, 56, 755-763.
28. *Teuber M.*: Veterinary use and antibiotic resistance. Curr. Opin. Microbiol. 2001, 4, 493-499.
29. *Van Hylckama Vlieg J. E. T., Rademaker J. L. W., Bachmann H., Molenaar D., Kelly W. J., Siezen R. J.*: Natural diversity and adaptive responses of Lactococcus lactis. Curr. Opin. Biotechnol. 2006, 17, 183-190.
30. *Walther C., Rossano A., Thomann A., Perreten V.*: Antibiotic resistance in Lactococcus species from bovine milk: presence of a mutated multidrug transporter mdt(A) gene in susceptible Lactococcus garvieae strains. Vet. Microbiol. 2008, 131, 348-357.

Corresponding author: Prof. Dr. Recep Cibik, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Uludag, Gorukle Campus, 16059 Bursa, Turkey; e-mail: recep_cibik@yahoo.com