MICROBIOLOGICAL CONTAMINATION OF LAMB CARCASSES: EFFECT OF ABATTOIR SIZE, SAMPLING SITE AND TIME OF STORAGE

Vergara, H.[1 2] , Berruga, M.I.[1,2], Linares, M.B.[1,2] and Gallego, L.[1,2]

[1]Departamento de Ciencia y Tecnología Agroforestal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, 02071 Albacete, Spain

[2]Sección de Calidad Alimentaria, Instituto de Desarrollo Regional, Universidad de Castilla-La Mancha, 02071 Albacete, Spain

Background

The interest in meat hygiene has increased in recent years, mainly due to the current crisis suffered by the meat sector. These emergencies have alerted the European Union (EU) to perform an extensive normative for controlling hygienic-sanitary quality of meat "from the farm to the fork", therefore including slaughtering and dressing procedures in this slogan. The European regulatory authorities have set up the microbiological assessment of carcasses before chilling in the abattoirs (Directive EC/471/2001). However, carcass quality during chilled storage has only been recommended by the ICMSF, but not by EU requirements. On the other hand, in the products with undisputed prestige that have attained their own official label, such as Manchego Spanish Breed Lamb, it is necessary for their organoleptic quality also to be accompanied by optimum microbiological quality during the pre-sale period.

Objectives

The aim of this work was to evaluate the effects on microbiological development of carcass lamb chilled at 4ºC of the next factors: size of the abattoir (number of animals slaughtered per week), the sampling site (flank, neck and rump), and the time of storage (at 24 h and 6 days post-slaughter which is the maximum time permitted for sale in "Manchego Lamb Denomination of Origin").

Materials and methods

In June, forty lambs of the Manchega breed were slaughtered at 25 kg live weight using standard commercial procedures. The slaughter was carried out in two commercial abattoirs of different size (located in the same city): a small abattoir and a big abattoir, (less than 2000 and more than 8000 animals slaughtered per week respectively, including pigs, lambs and beef). In each abattoir 20 lambs were slaughtered and the carcasses remained at 4ºC for 6 days post-slaughter in a chilling room. None of the carcass exhibited 24-h pH values higher than 5,7.

In all carcasses, the sampling (according to EC Decision 471/2001) was carried out by swabbing areas of 100 cm^2 in three different sites of carcass (rump, flank and neck), and at 1 and 6 days post-slaughter. Samples were stored at 4ºC in sterile tubes containing 10 ml of peptone water until examination, after no more than 3 hours.

Each sample was homogenised for 60 seconds, and additional serial 10-fold dilutions of homogenates were made in peptone water and were inoculated in Petri dishes for enumerations of: Mesophiles, pouring on Plate Count Agar (Scharlau Chemie, Barcelona, Spain) at 32°C for 2 days; psychrotrophs, pouring on PCA at 7ºC for 10 days; enterobacteria, using pour plates of Violet Red Bile Dextrose agar (Scharlau Chemie, Barcelona, Spain) and incubated at 32°C for 48 h; and Yeasts and Moulds, were determined on Rosa Bengala agar (Scharlau Chemie, Barcelona, Spain) incubated at 25ºC for 3 days. All microbial counts were expressed as base –10 logarithms of colony forming units per cm^2 of surface area (log CFU cm^{-2}).

Effects of abattoir, time of storage, sampling site and their interactions, on each microbial group was determined using a general lineal model (GLM). Factors and interactions that did not achieve significance were excluded from the analysis to increase the degrees of freedom of the residuals. Differences between sites of carcass in each abattoir were examined using a analysis of variance (ANOVA). When the differences among sites were significant ($p< 0.05$), Tukey's test was carried out to check the differences between pairs of groups. The effect of time of storage in refrigeration for each site was analysed using ANOVA at a significance level of $P< 0.05$. The correlation between pairs of microbial groups were examined using a simple correlation. Data were analysed using the SAS (1998) statistical package.

Results and discussion

GLM shows that the number of microorganisms found on carcasses varied significantly between abattoirs, sampling sites and time of storage (Table 1). In general for all microbial groups we found significant interactions between these factors. Other works (Sumner et al., 2002) reported that there were little difference in the mean values of aerobic viable count of the carcasses produced in abattoirs and very small plants.

After 1 day post-mortem in both, small and big abattoir (Table 2 and 3, respectively), the highest microbial recovery were observed on the flank ($P < 0.05$). After 6 days post-slaughter in the small abattoir the differences among sites only remained for enterobacteria ($P < 0.05$), and the lowest recovery was in the rump. However after this time in the big abattoir there were significant differences among sites for all microorganisms groups analysed.

In general, all microbial groups increased with time in both abattoirs. But in the small abattoir significant differences were not found in mesophiles neither psychrotrophs in flank site with time.

The correlations between the microbial groups assayed achieved significance in the four groups. Thus, mesophiles showed a positive correlation with psychrotrophs ($R= 0.881$, $P < 0.01$), enterobacteria ($R= 0.641$, $P < 0.01$), and yeasts and moulds ($R= 0.711$, $P < 0.01$); psychrotrophs were positively correlated with enterobacteria ($R= 0.674$, $P < 0.01$), and yeasts and moulds ($R= 0.702$, $P < 0.01$); and enterobacteria with yeasts and moulds ($R= 0.626$, $P < 0.01$).

The mean of mesophiles per cm^2 in this work ranges from 1.37 to 4.91 log, whereas the means counts of psychrotrophs varied from 0.85 to 3.91 log. According to these results, it could be concluded that the slaughtering practices in both abattoirs were acceptable. Moreover, the counts after 6 days of chilled storage are in agreement with the current EU requirements for ovine carcasses immediately after slaughter. Both, mesophiles and psychrotrophs were recovered from 95% of samples analysed in both abattoirs after 1 day at counts inferior than 3.5 log CFU/cm^2. After 6 days only 17.8% and 15.6% of samples recovered for mesophiles and psychrotrophs, respectively, showed counts superior than this level.

Our results are in the range found by other authors in a similar period of time. Along this line, Sumner *et al.* (2002) found that 364 chilled lamb carcasses from 17 Australian abattoirs had a mean value of aerobic viable counts of 2.59 log CFU/cm^2. In three Swiss abattoirs the median counts for aerobic total counts ranged from 2.5 to 3.8 log CFU/cm^2 (Zweifel and Stephan, 2003). Other studies showed mean values of mesophiles on chilled carcasses which ranged from 3 to 5 log CFU/cm^2 (Prieto et al., 1991; Vanderlinde et al., 1999; Duffy et al., 2001).

Enterobacteria values were less than 1 log CFU/cm^2 in 90% of samples after 1 day of storage. This group was only presented in 50% of samples at this time. After 6 days the 75% of samples showed count inferior than 2 log CFU/cm^2 . The EU requirements establishes count above 2.5 log CFU/cm^2 as unacceptable for this microbial group in carcasses before chilling.

The results also showed a low occurrence of yeasts and moulds. In 75% of samples analysed overnight this microbial group was not recovered. Counts were inferior to 3 log CFU/cm^2 in 95% of the samples stored for 6 days. These results suggest that yeasts and moulds contamination is apparently not a problem.

Conclusions

In general, the microbial counts for the four microbial groups analysed were higher in the big abattoir. In both abattoirs the mean values in the flank were higher than in the rump and in the neck. After six days post-slaughter there was an increase in all microbial groups analysed, however these levels were similar to those observed in other European or non abattoirs. According to these results, it could be concluded that the slaughtering practices in both abattoirs were good and the counts fell within the recommendations of International Commission on Microbiological Specifications for Foods (ICMSF).

Table 1. Factors (abattoir, sampling site, storage time) and interactions affecting mean level (log CFU/cm^2) of mesophiles, psycrotrophs, *Enterobacteriaceae,* and yeasts and moulds.

Model[a]	Mesophiles	Psychrotrophs	Enterobacteria	Yeasts & moulds
R^2	58.70	47.34	66.69	54.33
S.E.E. [b]	0.69	0.75	0.59	0.64
P value	0.000	0.000	0.000	0.000
Constant	2.64 ± 0.05	2.21 ± 0.05	0.82 ± 0.04	0.72 ± 0.05
Abattoir	- 0.50 ± 0.05***	- 0.33 ± 0.05***	- 0.09 ± 0.04*	- 0.38 ± 0.04***
Time	- 0.38 ± 0.05***	- 0.44 ± 0.05***	- 0.54 ± 0.04 ***	- 0.42 ± 0.04 ***
Site[1]	- 0.14 ± 0.07***	- 0.23 ± 0.08 ***	- 0.53 ± 0.06 ***	- 0.02 ± 0.06 ***
Site[2]	0.65 ± 0.06***	0.66 ± 0.08***	0.74 ± 0.06***	0.42 ± 0.06***
Site[1] x Time	-	- 0.01 ± 0.08 *	0.34 ± 0.06 ***	-
Site[2] x Time	-	0.18 ± 0.08*	- 0.33 ± 0.06***	-
Site[1] x Abattoir	0.10 ± 0.04***	0.04 ± 0.08 *	- 0.05 ± 0.06 ***	- 0.08 ± 0.06 ***
Site[2] x Abattoir	- 0.34 ± 0.07***	- 0.20 ± 0.08*	- 0.21 ± 0.06***	- 0.23 ± 0.06***
Time x Abattoir	-	-	-	0.14 ± 0.04 **
Site[1] x Time x Abattoir	- 0.21 ± 0.07	- 0.20 ± 0.08 ***	- 0.02 ± 0.06 ***	-
Site[2] x Time x Abattoir	0.30 ± 0.07***	0.29 ± 0.08***	0.23 ± 0.06***	-

[a] Significance levels for each factors are indicated as follows: *P< 0.05,** P< 0.01, and *** P< 0.001. The model shows only interaction that were significant at least for one microbial group.
[b] S.E.E., standard error of the estimate.

Table 2. Effect of sampling site (rump, flank or neck) on the microbial recovery (log CFU/cm^2) after 1 or 6 days post-slaughter in carcasses from the small abattoir.

Microbial group	Time	Rump	Flank	Neck	ANOVA
Mesophiles	1 day	1.57 ± 0.58^{ab}	2.29 ± 0.77^{b}	1.37 ± 1.30^{a}	**
	6 days	2.62 ± 0.86	2.62 ± 0.55	2.35 ± 0.69	NS
	ANOVA	***	NS	**	
Psychrotrophs	1 day	0.99 ± 0.95^{a}	2.34 ± 0.82^{b}	0.85 ± 1.04^{a}	***
	6 days	2.36 ± 1.02	2.31 ± 0,79	2.32 ± 0.84	NS
	ANOVA	**	NS	***	
Enterobacteria	1 day	nd	0.68 ± 0.95^{b}	0.07 ± 0.32^{a}	***
	6 days	0.31 ± 0.52^{a}	1.85 ± 0.68^{b}	1.47 ± 0.74^{b}	***
	ANOVA	**	***	***	
Yeasts & moulds	1 day	nd	0.188 ± 0.51^{a}	nd	*
	6 days	0.46 ± 0.89	0.89 ± 0.85	0.49 ± 0.80	NS
	ANOVA	*	**	**	

Significance levels for each factors are indicated as follows: *P< 0.05, ** P< 0.01, and *** P< 0.001; NS: not significance; $^{a, b, c}$ in the same row are significantly different (P< 0.05); nd: no growth were detected.

Table 3. Effect of sampling site (rump, flank or neck) on the microbial recovery (log CFU/cm^2) after 1 or 6 days post-slaughter in carcasses from the big abattoir.

Microbial group	Time	Rump	Flank	Neck	ANOVA
Mesophiles	0 days	2.82 ± 0.42^{a}	3.38 ± 0.25^{b}	2.13 ± 0.48^{c}	***
	6 days	2.96 ± 0.38^{a}	4.91 ± 0.78^{b}	2.66 ± 0.42^{a}	***
	ANOVA	NS	***	**	
Psychrotrophs	0 days	2.04 ± 0.58^{a}	2.87 ± 0.57^{b}	1.46 ± 0.45^{c}	***
	6 days	2.49 ± 0.41^{a}	3.91 ± 0.85^{b}	2.44 ± 0.44^{a}	***
	ANOVA	*	***	***	
Enterobacteria	0 days	0.17 ± 0.29^{a}	0.68 ± 0.64^{b}	0.02 ± 0.07^{a}	***
	6 days	0.69 ± 0.41^{a}	3.05 ± 0.86^{b}	0.86 ± 0.67^{a}	***
	ANOVA	***	***	***	
Yeasts & moulds	0 days	0.53 ± 0.81^{a}	1.10 ± 0.65^{b}	nd	***
	6 days	1.81 ± 0.77^{a}	2.42 ± 0.66^{a}	0.76 ± 0.85^{b}	***
	ANOVA	***	***	***	

Significance levels for each factors are indicated as follows: *P< 0.05, ** P< 0.01, and *** P< 0.001; NS: not significance; $^{a, b, c}$ in the same row are significantly different (P< 0.05); nd: no growth were detected.

References

Commission Decision (2001/471/EC) of 8 June 2001 laying down rules for the regular checks on the general hygiene carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. Official Journal L 165 , 21/06/2001 P. 0048 – 0053.

Duffi, E.A., Belk, K.E., Sofos, J.N., LeValley, S.B., Kain, M.L., Tatum, J.D., Smith, G.C. and Kimberling, C.V. 2001. Microbial contamination occurring on lamb carcasses processed in de United States. J. Food Prot. 64: 503-508.

ICMSF, *Microorganisms in foods. 2. Sampling for microbiological analysis: Principles and specific applications* (2nd edn.). University of Toronto Press, Toronto (1986).

Prieto, M., García, M.L., García, M.R., Otero, A. and Moreno, B. 1991. Distribution and evolution of bacteria on lamb carcasses during aerobic storage. J. Food Prot., 54: 945-949.

SAS Institute.1998.SAS users guide: Statistics, version 6.12.Cary, NC, USA:SAS Institute Inc.

Sumner, J., Petrenas, E., Dean, P., Dowsett, P., West, G., Wiering, R. and Raven, G. 2003. Microbial contamination on beef and sheep carcases in South Australia. Int. J. Food Microbiol. 81: 255-260.

Vanderlinde, P.B., Shay, B. and Murray, J. 1999. Microbiological status of Australians sheep meat. J. Food Prot. 62: 380-385.

Zewifel, C. and Stephan, R. 2003. Microbial monitoring of sheep carcass contamination in three Swiss abattoirs. J. Food Prot. 66: 946-952.