PROCESSING, PRODUCTS, AND FOOD SAFETY

Chicken feet bacteriological quality at 4 steps of technological processing

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ABSTRACT The production of chicken feet is primarily intended for foreign markets, and there is still no specific legislation in Brazil that determines the quality standard of these products. The bacteriological quality of chicken feet was evaluated as a product for human consumption at different steps of the technological processes. Eighty broiler feet from 20 lots at 4 steps of processing were collected for quantitative analysis, total count of aerobic mesophilic bacteria, and determining the most probable number of coliforms and fecal coliforms. Thirty-eight pools of 15 broiler feet each from 19 lots were used for qualitative analysis and the isolation of *Salmonella enterica* spp. and *Escherichia coli* O157:H7. *Escherichia coli* O157:H7 was not found in any of the samples. *Salmonella* spp. were isolated in 68% (13/19) of the lots. The *Salmonella* Schwarzengrund serotype was found in 12 of the 13 lots of positive samples and the *Salmonella* Anatum and *Salmonella* Corvallis serotypes were identified in the remaining lot. Processing is effective in reducing contamination by mesophilic bacteria, coliforms, and *Salmonella* spp. in these products. This work constitutes the first study in Brazil on microbiological quality of chicken feet.

Key words: chicken feet, *Salmonella* spp., *Escherichia coli*, coliform, mesophilic

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INTRODUCTION

Although chicken feet are considered to be byproducts in Brazil, they are exported as cuts to other countries, with the same commercial value as other edible parts. This product has been exported to Eastern countries for several years; however, with the opening of the Chinese market in recent years, this process has been intensified. The export of chicken feet from Brazil to China amounted to 66.91% of the total chicken exports in 2010 (USDA/FAS, 2010).

As there is no specific legislation for chicken feet, the microbiological standards used for chicken feet are the same for chicken meat. Thus, the industry is inspecting and sorting this product according to the requirements of the consumer market (Teixeira, 2008). China, the world's main importer of chicken feet, established new standards for the acceptance of chicken meat, including the absence of *Salmonella* spp. and *Escherichia coli* O157:H7 in 25 g of chicken meat (USDA/FAS, 2006). Although *Salmonella* spp. are often isolated from chicken meat (Lopes et al., 2007; Ribeiro et al., 2007; Matias et al., 2010), *E. coli* O157:H7 is rarely isolated from this product (Nataro and Kaper, 1998; Silva et al., 2001).

Despite the great volume marketed and the differences among countries related to microbiological standards of chicken feet, there are no scientific publications demonstrating the real risks to human health related to their consumption. The aim of this work was to assess the microbiological quality of chicken feet as a product for human consumption at different steps of the technological process at industrial facilities under Federal Inspection Service using quantitative and qualitative tests.

MATERIALS AND METHODS

Chicken feet were collected in a poultry processing plant under Federal Inspection Service, at Minas Gerais State (Brazil) during 5 visits from May to July 2009. Eighty broiler feet from 20 separate lots were collected individually from each processing step to determine the total count of aerobic mesophilic bacteria (**TA**), the most probable number of coliforms (**TC**), and the most probable number of fecal coliforms (**FC**). The sampling was performed at 4 steps: after cutting at the tibiametatarsus joint (**P1**), after scalding between 65 and

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70°C for 28 s and withdrawing of the cuticle (**P2**), after chilling (**P3**), and after freezing at a temperature of approximately −30°C (**P4**). All samples were packed in plastic bags and isothermal boxes and transported to the laboratory.

A portion of 25 g was taken from each foot, rinsed in 250 mL of 0.1% buffered peptone water and manually shaken for 1 min. The pour plate technique was used for TA and the most probable number (**MPN**) technique was used for TC and FC (USFDA, 1998).

For *Salmonella* spp. and *E. coli* O157:H7, isolation was conducted as described by USFDA (1998). From each of the 19 lots studied (1 lot was missed), 15 feet were collected and tested as a single composite unit. For each step, at P1 and at P4, a total of 570 feet was rinsed in 400 mL of buffered peptone water in a plastic bag and manually shaken for 1 min. After isolation procedures, the antigenic characteristics of *Salmonella* spp. were analyzed with somatic and flagellar antisera (Oswaldo Cruz Institute, Rio de Janeiro, Brazil).

Escherichia coli strains were tested serologically for the O157 somatic and H7 flagellar antisera (Denka Seiken Co., Tokyo, Japan). The isolates that were H7-negative were examined for motility by agar stab methods to confirm nonmotile variants (Fields et al., 1997). *Escherichia coli* O157 culture supernatant toxicities for Vero cell were evaluated for the specific cytopathic effects. Ten, 100, and 1,000 50% cytotoxic doses of control Stx2 per mL were added to the filtrates to confirm that no inhibitors of cytotoxicity were present (Gentry and Dalrymple, 1980). To detect the O157 *stx1*, *stx2*, *eae*, *ehxA*, and *saa* genes, a multiplex PCR was performed according to the procedure described previously (Paton and Paton, 2002), and amplified DNA fragments were resolved by gel electrophoresis using 2% agarose, and then stained with ethidium bromide.

The results of TA, TC, and FC were expressed in logarithms to base 10 ($\log_{10}$) and subjected to Bartlett's test for variance similarities as prerequisites of further use of parametric or nonparametric tests. The values of TA were subjected to ANOVA, followed by Tukey's test to differentiate among steps, whereas for TC and FC we used Kruskal-Wallis nonparametric analysis followed by Dunn's test for differentiation among steps, both at the significance level of 5%. A Student's *t*-test at the 5% level was also used to confirm step similarities in the case of the nonparametric test. The chances of contamination by *Salmonella* spp. at the P1 and P4 steps of processing were quantified by calculating the odds ratio and its CI at 5% significance. All statistical analyses followed procedures described previously (Thrusfield, 1995). The program InStat, version 3.1 (GraphPad, 2009) was used for the calculations.

RESULTS AND DISCUSSION

The results of Bartlett's test revealed that the variances of the TA averages at steps P1 to P4 were similar ($P > 0.05$), meaning that the data followed a normal distribution (Thrusfield, 1995), whereas for TC and FC, variances at the same steps were different ($P < 0.05$).

Regarding TA, the contamination level decreased after processing ($P < 0.05$) from P1 to P4 (Figure 1), but

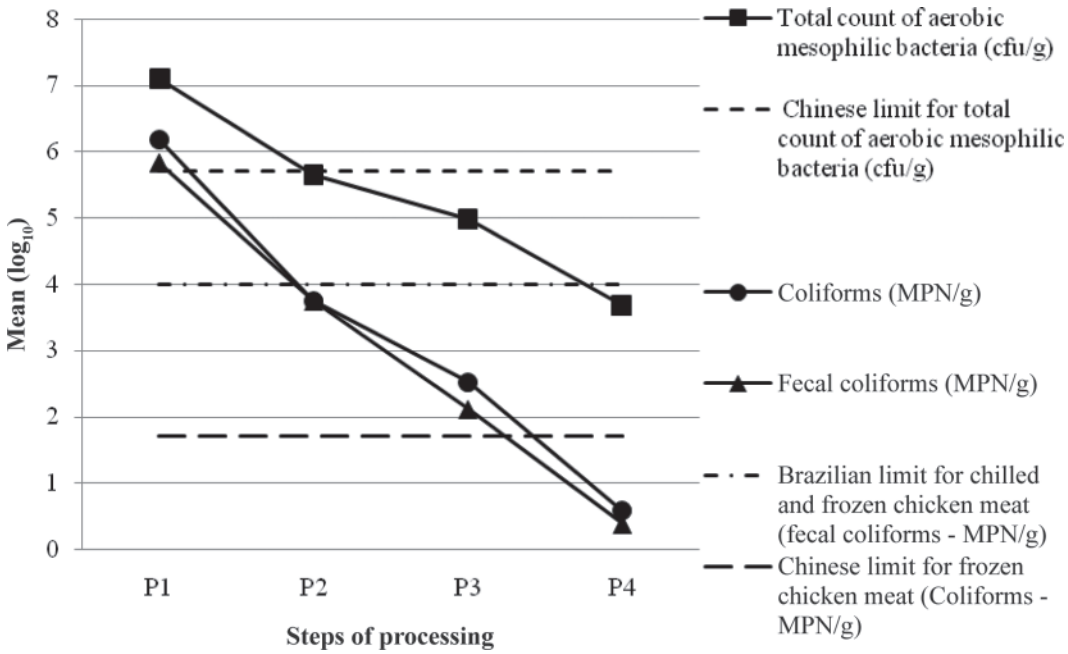

Figure 1. Mean total count of aerobic mesophilic bacteria (TA) and most probable number (MPN) of coliforms (TC) and fecal coliforms (FC) of feet samples collected at 4 steps of processing vs. Chinese and Brazilian legal limits (n = 20). P1 = after cutting at the tibia-metatarsus joint; P2 = after scald and withdrawal of cuticle; P3 = after chilling; and P4 = after freezing.

Table 1. Mean total count of aerobic mesophilic bacteria (TA) and most probable number (MPN) of coliforms (TC) and fecal coliforms (FC) of feet samples collected at 4 steps of processing

Step[1]	TA[2]	TC[3]	FC[3]
P1	7.1 ± 0.2^{a}	6.2 ± 0.2^{a}	5.8 ± 0.2^{a}
P2	5.6 ± 0.2^{b}	3.7 ± 0.2^{b}	3.7 ± 0.3^{b}
P3	5.0 ± 0.2^{b}	2.5 ± 0.2^{b}	2.1 ± 0.2^{b}
P4	3.7 ± 0.1^{b}	0.6 ± 0.1^{c}	0.4 ± 0.1^{c}

[a–c]Means within a column lacking a common superscript differ ($P < 0.05$).

[1]P1 = after cutting at the tibia-metatarsus joint; P2 = after scalding and withdrawal of cuticle; P3 = after chilling; and P4 = after freezing.

[2]n = 20; values are means ± SE ($\log_{10}$ cfu/g).

[3]n = 20; values are means ± SE ($\log_{10}$ MPN/g).

there was only a statistical difference between P1 and the other steps (Table 1).

Before scalding, Kotula and Pandya (1995) found a TA average for chicken feet of 6.2 ± 0.1 cfu/g, which is lower than the value we found in our present study. Higher values were obtained by Lopes et al. (2007) with chicken carcasses when compared with the steps after scalding and after chilling. Göksoy et al. (2004), working with chicken carcasses, found a higher value after scalding, but a similar result after chilling. These differences between results obtained in the analysis of feet and carcasses might be credited to different technological processing of these products, such as the higher temperatures used for feet scalding and the larger predisposition to contamination of carcasses because of evisceration.

For the first time in Brazil, chicken feet for human consumption were examined microbiologically. The numbers for *Salmonella* spp., TA, and coliforms were low and similar to those obtained by Bilgili et al. (2003). There is no legislation in Brazil to regulate the maximum limit for the TA in poultry meat, but the legislation of China set a maximum limit of 1.0×10^6 cfu/g for fresh poultry and 5.0×10^5 cfu/g for frozen poultry (USDA/FAS, 2006). Therefore, both fresh and frozen feet analyzed in this study showed values in accordance with Chinese regulations (Figure 1).

In the Kruskal-Wallis test, the TC averages were higher at the beginning of the feet processing, before scalding (P1), but only with statistical differences ($P < 0.05$) between this step and the others. After scalding (P2) and after chilling (P3), the averages decreased but did not differ significantly ($P > 0.05$) among themselves (Table 1). The Kruskal-Wallis test was also significant ($P < 0.05$) for differences among FC samples. By the Dunn test, samples collected at P1 were different from those at the other steps. The P2 and P3 steps were equal ($P > 0.05$), and P3 differed from P4 ($P < 0.05$), but P4, judged the same as P2, was found different by the Student's *t*-test ($P < 0.05$), used as confirmatory (Table 1).

Lopes et al. (2007) and our group found similar values for TC and FC at step P2 by analyzing chicken carcasses after scalding. All samples studied in this work were in accordance with the national standard for fecal coliforms in fresh and frozen chicken meat (Figure 1; Ministério da Saúde, 2001), which is the only existing microbiological standard in Brazil for chicken meat.

When compared with Chinese standards, presented by USDA/FAS (2006), the average of FC in fresh feet samples were higher than the allowed value. However, all samples were in accordance with the Chinese standards for FC analysis on frozen poultry, even being 100 times lower than the Brazilian standard (Figure 1).

The values found for TC and FC in the feet studied showed a more expressive reduction than the TA values during technological processing. Between P1 and P4, a reduction of 5.6 $\log_{10}$ MPN/g was observed for TC and 5.4 $\log_{10}$ MPN/g for FC, but only 3.4 $\log_{10}$ cfu/g was found for TA. One possible explanation is the lower resistance of these bacterial groups to sudden temperature changes during processing at the steps of scalding (P2), chilling (P3), and freezing (P4).

The results for *E. coli* O157:H7 yielded 2 strains that were positive against the O157 antiserum, among the samples collected in P1. None was confirmed as O157:H7 strains. Similarly, Silveira et al. (1999) did not find this serotype among 17 strains of *E. coli* O157 isolated in hamburgers produced in Brazil. Doyle and Schoeni (1987) reported the isolation of *E. coli* O157:H7 from chicken meat in the United States. However, in research conducted in Brazil, positive results for this bacterium have not been found (Silva et al., 2001; Jakabi et al., 2004; Silveira, 2010).

Of the 19 lots of feet investigated for *Salmonella* spp., 68% (13/19) were positive before scalding. This result is similar to that found by Kotula and Pandya (1995), who reported that 55% of feet were contaminated by this organism.

After processing, *Salmonella* spp. was isolated from only 1 lot (5.26%), which was also positive before processing. The value of the odds ratio was 39.00 ($P < 0.05$), which means that the chance of the bacteria being present before processing was 39 times greater than that after processing (CI = 4.17–363.64). Bilgili et al. (2003) analyzed processed chicken feet and found 2.78% positivity for *Salmonella* spp. Data from the Brazilian National Program for Monitoring the Prevalence and Resistance of Bacteria in Chicken indicate a *Salmonella* spp. prevalence of 4% in frozen chicken carcasses marketed at retail (Ministério da Saúde, 2008), whereas a prevalence of 2.3% was found in several products of poultry originated from Europe (EFSA, 2010).

Other research has demonstrated a higher prevalence of *Salmonella* spp. in chicken carcasses, such as 19% (17/90) reported by Cason et al. (1997), 39.3% (24/61) by Ribeiro et al. (2007), and approximately 59% by Chen et al. (2010). Consequently, the contamination

rates for chicken feet are smaller than those for chicken carcasses.

Salmonella Schwarzengrund was found in 12 of the 13 positive lots investigated. This serotype was the second most prevalent in research conducted by Chen et al. (2010). *Salmonella* Anatum and *Salmonella* Corvallis were both isolated from the remaining positive lot. This demonstrates the possibility of contamination by different serotypes in the same sample, and the importance of serotyping all suspicious colonies.

Juneja et al. (2003) studied the thermal resistance of *Salmonella* spp. and showed that a temperature of approximately 65°C is not enough to eliminate these bacteria in 28 s, which is the time used to scald the feet. According to the authors, 71°C would be a sufficient temperature for a 7.0 $\log_{10}$ reduction of *Salmonella* spp., considering the processing time used. In Brazil, there are no legal requirements for time and temperature for scalding chicken feet, but legislation (Ministério da Agricultura, Pecuária e Abastecimento, 1952) suggests a temperature range of between 80 and 90°C; however, this range is not used by the industry, in order to save on steam. After processing, there was a 92.31% reduction in *Salmonella* spp. occurence in the lots studied. Considering the indicator microorganisms, a 99.98% reduction (3.4 $\log_{10}$) occurred with TA, and a 99.99% reduction occurred (5.6 $\log_{10}$ and 5.4 $\log_{10}$) with TC and FC, respectively. From these results, it appears that, despite the different quantities, there was a trend of reducing contamination.

Conclusions

Technological processing of chicken feet used by the poultry industry was effective for the reduction of TA, TC, FC, and *Salmonella* spp. As there is no standardization for the technological processing of chicken feet, other studies that determine the appropriate processing techniques and define the limits to be followed are demanded. Thus, a raw product with a high hygienic-sanitary standard can be obtained, even with low-cost processing, as was used in this work.

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REFERENCES

Bilgili, S. F., D. Zelenka, and J. E. Marion. 2003. Microbiological quality of broiler chicken feet (paws). Int. Poultry Sci. Forum 82:21. (Abstr.)

Cason, J. A., J. S. Bailey, N. J. Stern, A. D. Whitemore, and N. A. Cox. 1997. Relationship between aerobic bacteria, *Salmonellae*, and *Campylobacter* on broiler carcasses. Poult. Sci. 76:1037–1041.

Chen, M. H., S. W. Wang, W. Z. Hwang, S. J. Tsai, Y. C. Hsih, C. S. Chiou, and H. Y. Tsen. 2010. Contamination of *Salmonella* Schwarzengrund cells in chicken meat from traditional marketplaces in Taiwan, and comparison of their antibiograms with those of the human isolates. Poult. Sci. 89:359–365.

Doyle, M. P., and J. L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394–2396.

EFSA. 2010. Community summary report on trends and sources of zoonoses, zoonotic agents, and foodborne outbreaks in the European Union in 2008. EFSA J. 8:1496 10.2903/j.efsa.2009.1438.

Fields, P. I., K. Blom, H. J. Hughes, L. O. Helsel, P. Feng, and B. Swaminathan. 1997. Molecular characterization of the gene encoding H antigen in *Escherichia coli* and development of a PCR-restriction fragment length polymorphism test for identification of *E. coli* O157:H7 and O157:NM. J. Clin. Microbiol. 35:1066–1070.

Gentry, M. K., and J. M. Dalrymple. 1980. Quantitative microtiter cytotoxicity assay for *Shigella* toxin. J. Clin. Microbiol. 12:361–366.

Göksoy, E. O., S. Kirkan, and F. Kök. 2004. Microbiological quality of broiler carcasses during processing in 2 slaughterhouses in Turkey. Poult. Sci. 83:1427–1432.

GraphPad. 2009. InStat 3.0 User's Guide. GraphPad Software Inc., San Diego, CA.

Jakabi, M., D. S. Gelli, C. A. Ristori, A. M. R. De Paula, H. Sakuma, G. I. S. L. Lopes, S. A. Fernandes, and R. B. Luchesi. 2004. Occurrence of *Salmonella* spp. and *Escherichia coli* O157:H7 in raw meat marketed in São Paulo city, Brazil, and evaluation of its cold tolerance in ground beef. Rev. Inst. Adolfo Lutz 63:238–242.

Juneja, V. K., H. M. Marks, and T. Mohr. 2003. Predictive thermal inactivation model for effects of temperature, sodium lactate, NaCl, and sodium pyrophosphate on *Salmonella* serotypes in ground beef. Appl. Environ. Microbiol. 69:5138–5156.

Kotula, K. L., and Y. Pandya. 1995. Bacterial contamination of broiler chickens before scalding. J. Food Prot. 58:1326–1329.

Lopes, M., J. A. Galhardo, J. T. Oliveira, R. Tamanini, S. F. Sanches, and E. E. Muller. 2007. Research of *Salmonella* spp. and indicator microorganisms in poultry carcasses and chilling tanks water in poultry slaughterhouse. Semina: Ciênc. Agric. 28:465–476.

Matias, B. G., P. S. A. Pinto, M. V. Cossi, and L. A. Nero. 2010. *Salmonella* spp. and hygiene indicator microorganisms in chicken carcasses obtained at different processing stages in 2 slaughterhouses. Foodborne Pathog. Dis. 7:313–318.

Ministério da Agricultura Pecuária e Abastecimento. 1952. Regulamento da inspeção industrial e sanitária de produtos de origem animal. Decreto 30.691, seção 1. Diário Oficial da União, Brasília, DF, Brazil.

Ministério da Saúde, Agência Nacional de Vigilância Sanitária. 2001. Aprova o regulamento técnico sobre padrões microbiológicos para alimentos. RDC 12, seção 1. Diário Oficial da União, Brasília, DF, Brazil.

Ministério da Saúde, Agência Nacional de Vigilância Sanitária. 2008. Relatório do Monitoramento da prevalência e do perfil de susceptibilidade aos antimicrobianos em enterococos e salmonelas isolados de carcaças de frango congeladas comercializadas no Brasil. ANVISA, Brasília, DF, Brazil.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli.* Clin. Microbiol. Rev. 11:142–201.

Paton, A. W., and J. C. Paton. 2002. Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *stx1, stx2, eae, ehxA*, and *saa.* J. Clin. Microbiol. 40:271–274.

Ribeiro, A. R., A. Kellermann, L. R. Santos, M. C. Bessa, and V. P. Nascimento. 2007. *Salmonella* spp. in raw broiler parts: Occurrence, antimicrobial resistance profile, and phage typing of the *Salmonella* Enteritidis isolates. Braz. J. Microbiol. 38:296–299.

Silva, N., N. F. A. Silveira, C. Contreras, N. J. Beraquet, F. Yokoya, C. A. Nascimento, V. M. Oliveira, and C. L. Tse. 2001. Occurrence of *Escherichia coli* O157:H7 in meat products and sensibility of the detection methods. Ciênc. Tecnol. Aliment. 21:223–227.

Silveira, J. B. 2010. Evaluation of *Escherichia coli* O157:H7 in ground beef samples collected in Rio Grande do Sul State, Brazil. MSc Diss. Univ. Fed. Rio Grande do Sul, Porto Alegre, RS, Brazil.

Silveira, N. F. A., N. Silva, C. Contreras, N. J. Beraquet, F. Yokoya, C. A. Nascimento, V. M. Oliveira, and C. L. Tse. 1999. Occurrence of *Escherichia coli* O157:H7 in hamburgers produced in Brazil. J. Food Prot. 62:1333–1335.

Teixeira, V. Q. 2008. Anatomopatologia e bacteriologia da pododermatite em frangos de corte sob inspeção sanitária. MSc Diss. Univ. Fed. Fluminense, Niterói, RJ, Brazil.

Thrusfield, M. V. 1995. Veterinary Epidemiology. 2nd ed. Blackwell Science, Edinburgh, UK.

USDA/FAS. 2006. Fresh and frozen poultry product standard. GAIN Report: CH6001. Accessed August 2009. http://www.fas.usda.gov/gainfiles/200601/146176574.pdf.

USDA/FAS. 2010. Poultry and products annual 2010. GAIN Report: CH10056. Accessed Nov 2010. http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Poultry%20and%20Products%20Annual_Beijing_China%20-%20Peoples%20Republic%20of_9-30-2010.pdf.

USFDA. 1998. Bacteriological Analytical Manual Online. 8th rev. ed. Accessed May 2009. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.