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## Incidence of *Enterobacteriaceae* and *Salmonella* spp. in Poultry By-Product Meal for Sale on Retail Market in Adana and Mersin\*

*Adana ve Mersin İlleri Perakende Yem Mağazalarında Satılan Tavuk Unlarında Enterobacteriaceae ve Salmonella spp. Araştırılması*

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**Öz:** Mersin ve Adana İlleri yem perakende piyasasından, Mayıs-Ağustos 2012 tarihleri arasında toplanan 95 adet tavuk unu numunesi mikrobiyolojik kaliteleri açısından araştırıldı. Toplam mezofilik aerobik bakteri (TMAB), *Enterobacteriaceae* ve *Salmonella* spp. sayımları sırasıyla FDA/BAM (2001), ISO 21528-2 (2004) ve ISO 6579-2005 yöntemlerine göre yapıldı. *Salmonella* spp. sayımları Vidas (SLM) REF 30702 yöntemine göre doğrulandı. TMAB, *Enterobacteriaceae* ve *Salmonella* spp. sırasıyla 29 (%30,53), 19 (%20) ve 5 (%5,26) örnekte tespit edilmiştir. Gıda zincirinde “çiftlikten sofraya” ilkesine göre hayvan yemleri birincil aşamadır. Bu yüzden, yem endüstrisinde Tehlike Analizi ve Kritik Kontrol Noktaları (HACCP) programı düzenli olarak kontrol edilmelidir.

**Abstract:** From May to August 2012, 95 samples of poultry by-product meal for sale on the retail market in Mersin and Adana were analyzed for the microbial quality. Total Mesophilic Aerobic Bacteria (TMAB), *Enterobacteriaceae* and *Salmonella* spp. were enumerated by FDA/BAM (2001), ISO 21528-2 (2004) and ISO 6579-2005 procedures, respectively. *Salmonella* spp. analyses were confirmed by Vidas (SLM) REF 30702 procedure. TMAB, *Enterobacteriaceae* and *Salmonella* spp. were found in 29 (30.53%), 19 (20%) and 5 (5.26%) samples, respectively. Animal feed is at the beginning of the food safety chain in the “farm-to-fork” model. Therefore, a Hazard Analysis and Critical Control Point (HACCP) program should be checked regularly for the animal feed industry.

**Anahtar sözcükler:** *Enterobacteriaceae*, *Salmonella* spp., tavuk unu, yem endüstrisi.

**Key words:** *Enterobacteriaceae*, feed industry, poultry by-product meal, *Salmonella* spp.

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## Introduction

Bacteria that belongs *Enterobacteriaceae* family enter the animal feed chain as normal contaminants of raw materials used in the manufacture of animal feeds. The family *Enterobacteriaceae* encompasses 30 established genera, including *Salmonella* spp, *Escherichia* spp, *Shigella* spp and *Yersinia* spp. Many of the genera exhibit pathogenicity towards man, animals, insects and plants and many of the pathogenic forms produce toxins (Maciorowski et al., 2004).

A number of the genera in this family occur regularly in association with animals; they are found as indigenous members of the gut microflora where they may either produce no harmful effects, or are capable of causing disease in both endothermic and ectothermic animals. *Enterobacteriaceae* are opportunistic organisms and may re-contaminate products after cooking or processing and during storage, transport and handling (Veldman et al., 1995). Post process contamination is of concern for all feed ingredients and not restricted only to animal proteins. Despite this fact, animal proteins continue to be more closely scrutinized for *Enterobacteriaceae* contamination than other feed ingredients (Hamilton, 2002). There is a recognized association between the risk of isolation of salmonella and degree of *Enterobacteriaceae* contamination (Veldman et al., 1995). This has led to the consideration of recording *Enterobacteriaceae* contamination levels in feedstuffs as an indicator of feed hygiene and potential limits to the degree of contamination being set by the major retailers.

Poultry by-product meal (PBM) is a high-protein commodity. It is made from grinding clean, rendered parts of poultry carcasses and can contain bones, offal and undeveloped eggs and intestines. Poultry by-product meal quality and composition can change from one batch to another. Poultry by-product meal (along with meat and bone meal, blood meal, feather meal and fish meal) is one of the most important sources of animal protein used to feed livestock, poultry, pet and aquaculture animals (Meeker and Hamilton, 2006). However, it is only banned in ruminant rations by The European Union Regulation (Regulation (EC) No 1774/2002) as a preventive measure against the spread of Bovine Spongiform Encephalopathy (Anonymous, 2009).

There is evidence that controlling microbial contamination in livestock feed produces significant health benefits in both human and animal populations. Humans can be exposed to the bacteria by consuming improperly prepared eggs, meat or milk from infected animals or

from foods contaminated by the feces of infected animals (Veldman et al., 1995). The microbial quality of poultry by-product meal being available in retail markets of Adana and Mersin provinces of Turkey was evaluated in this study. For this purpose, total mesophilic aerobic bacteria, *Enterobacteriaceae* and *Salmonella* spp. were studied in samples.

### Material and Methods

From May to August 2012, a total of 95 poultry by-product samples for sale in Adana and Mersin retail market were collected into sterile nylon bags for microbial analyses by cold chain procedures.

TMAB was enumerated by the procedures of FDA/BAM (2001) according to Maturin and Peeler (2001). *Enterobacteriaceae* was detected and enumerated by the procedures of ISO 21528-2:2004 (Anonymous, 2004). *Salmonella* spp. contaminations were analyzed by ISO 6579-2005 (Anonymous, 2005) and verified by Vidas Salmonella (SLM) Reference 30702 (Vidas, 2010) methods.

### Results and Discussion

Total mesophilic aerobic bacteria counts were between  $2 \times 10^2$  -  $8.0 \times 10^4$  cfu g<sup>-1</sup> in 29 (30.53%) samples and *Enterobacteriaceae* counts were between  $1 \times 10^2$  -  $4 \times 10^3$  cfu g<sup>-1</sup> in 19 (20%) samples (Table 2). *Salmonella* spp. was detected in 5 out of 95 samples (Table 1).

**Table 1.** *Salmonella* spp. detected samples of the total of 95 poultry by-product meal samples collected from retail market of Adana and Mersin Provinces

| Sample                  | Tested Samples | <i>Salmonella</i> spp. positive | Positive sample ratio, % |
|-------------------------|----------------|---------------------------------|--------------------------|
| Poultry by-product meal | 95             | 5                               | 5.26                     |

**Table 2.** Total Mesophilic Aerobic Bacteria (TMBA) and *Enterobacteriaceae* colony formed samples of the total of 95 poultry by-product meal samples collected from retail market of Adana and Mersin Provinces (cfu g<sup>-1</sup>)

| Number # | TMAB                | <i>Enterobacteriaceae</i> | Number <sup>#</sup> | TMAB                | <i>Enterobacteriaceae</i> |
|----------|---------------------|---------------------------|---------------------|---------------------|---------------------------|
| 1        | 7.6×10 <sup>2</sup> | 4×10 <sup>3*</sup>        | 16                  | 1.1×10 <sup>3</sup> | 7.2×10 <sup>2*</sup>      |
| 2        | 8.0×10 <sup>4</sup> | 1×10 <sup>2</sup>         | 17                  | 6.7×10 <sup>2</sup> | -                         |
| 3        | 4×10 <sup>3</sup>   | 2.2×10 <sup>2</sup>       | 18                  | 2.3×10 <sup>3</sup> | -                         |
| 4        | 5×10 <sup>2</sup>   | 4×10 <sup>3*</sup>        | 19                  | 4×10 <sup>2</sup>   | 5.5×10 <sup>2*</sup>      |
| 5        | 4.7×10 <sup>3</sup> | 2×10 <sup>2</sup>         | 20                  | 2×10 <sup>2</sup>   | 4×10 <sup>3*</sup>        |
| 6        | 2.3×10 <sup>2</sup> | 4×10 <sup>3*</sup>        | 21                  | 2.5×10 <sup>2</sup> | -                         |
| 7        | 9×10 <sup>2</sup>   | 7×10 <sup>2*</sup>        | 22                  | 1.9×10 <sup>3</sup> | 4×10 <sup>3*</sup>        |
| 8        | 7.9×10 <sup>3</sup> | 4×10 <sup>3*</sup>        | 23                  | 3.3×10 <sup>2</sup> | 4×10 <sup>3*</sup>        |
| 9        | 7.0×10 <sup>4</sup> | -                         | 24                  | 3.1×10 <sup>2</sup> | -                         |
| 10       | 6.3×10 <sup>3</sup> | 3.4×10 <sup>2*</sup>      | 25                  | 2.7×10 <sup>3</sup> | -                         |
| 11       | 7×10 <sup>3</sup>   | -                         | 26                  | 3.5×10 <sup>2</sup> | -                         |
| 12       | 8.5×10 <sup>2</sup> | 4×10 <sup>3*</sup>        | 27                  | 7.6×10 <sup>3</sup> | 5×10 <sup>2*</sup>        |
| 13       | 5.0×10 <sup>4</sup> | 7.9×10 <sup>2*</sup>      | 28                  | 6×10 <sup>2</sup>   | -                         |
| 14       | 4.2×10 <sup>2</sup> | 4×10 <sup>3*</sup>        | 29                  | 3.9×10 <sup>2</sup> | 5×10 <sup>2*</sup>        |
| 15       | 5.7×10 <sup>3</sup> | -                         |                     |                     |                           |

- : not found

\*: inedible feedstuff according to Turkish Microbial Standards of Animal Basis Feedstuffs, 2008

# : colony formed samples of the total of 95 samples

Mesophilic aerobic bacteria counts of poultry by-product meal samples reflect the post-contamination. On the basis of Turkish Microbiological Standards of Animal Origin Feedstuffs, maximum permissible *Enterobacteriaceae* counts of 1 g sample must be 300 or below and Salmonella must not be found in 25 g of analyzed sample. In the present study, 16 (16.84%) of 95 poultry by-product meal samples were found as inedible feedstuff (Table 1) according to Turkish Microbiological Standards of Animal Origin Feedstuffs (Anonymous, 2008). 5 (5.26%) of 95 samples (Table 1) were also found to be contaminated by *Salmonella* spp. Similarly, regulations in a number of countries and organizations already use

*Enterobacteriaceae* and *Salmonella* as an evaluation tool for microbial quality of feedstuffs like our country (Van Schothorst and Oosterom, 1984; Veldman et al., 1995).

The microbiological safety and quality of poultry by-product meal is equally important to producers, retailers, animals and humans. The control of bacteria in feed has been shown to improve production performance in poultry and to reduce the incidence of *Salmonella* in breeding animals in the farm environment, on carcasses and in eggs. In rendering technology, the temperatures (115° to 145° C) are sufficient to kill bacteria, viruses and many other microorganisms to produce an aseptic protein product that is free of potential biohazards and environmental threats (Meeker and Hamilton, 2006). However, inadequate heat application and biofilm generation on rendering units, using high risk material during rendering or storage, and inadequate transport and handling conditions after rendering may be responsible for recontamination (Hamilton, 2002; Maciorowski et al., 2004). Similarly to current study, Cox et al. (1983) detected *Enterobacteriaceae* and *Salmonella* in 92% of meat and bone meal samples collected from commercial mills. Bensink (1979) also reported that 114 out of 164 meat and bone meal samples from 8 rendering plants (69.5%) were contaminated with salmonella.

Processing poultry by-products for feed use is a good way to mitigate the environmental problems caused by poultry processing. If not properly managed, vermin, bacteria and viruses from poultry offal could be released in the environment and may result in water contamination (leaching of nutrients and pathogenic microorganisms) and air pollution (Anonymous, 2011). However, it is important that a HACCP program is needed to monitor contaminations both pre- and post-handling of ingredients and manufactured feed in animal feeds as part of a comprehensive “farm to fork” control systems. These "critical" points may occur during cooling, draining, drying, milling, screening, sorting, mixing, storing and bagging.

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