Characterization of the animal by-product meal industry in Costa Rica: Manufacturing practices through the production chain and food safety

A. Leiva,* F. Granados-Chinchilla,* M. Redondo-Solano,† M. Arrieta-González,† E. Pineda-Salazar,† and A. Molina*,‡,[1]

*Research Center in Animal Nutrition (CINA), University of Costa Rica; †Research Center in Tropical Diseases (CIET) and Department of Microbiology, University of Costa Rica; and ‡Department of Animal Science, University of Costa Rica

ABSTRACT Animal by-product rendering establishments are still relevant industries worldwide. Animal by-product meal safety is paramount to protect feed, animals, and the rest of the food chain from unwanted contamination. As microbiological contamination may arise from inadequate processing of slaughterhouse waste and deficiencies in good manufacturing practices within the rendering facilities, we conducted an overall establishment's inspection, including the product in several parts of the process.

An evaluation of the Good Manufacturing Practices (GMP) was carried out, which included the location and access (i.e., admission) to the facilities, integrated pest management programs, physical condition of the facilities (e.g., infrastructure), equipments, vehicles and transportation, as well as critical control points (i.e., particle size and temperature set at 50 mm, 133°C at atmospheric pressure for 20 min, respectively) recommended by the OIE and the European Commission. The most sensitive points according to the evaluation are physical structure of the facilities (avg 42.2%), access to the facilities (avg 48.6%), and cleaning procedures (avg 51.4%).

Also, indicator microorganisms (*Salmonella* spp., *Clostridium* spp., total coliforms, *E. coli*, *E. coli* O157:H7) were used to evaluate the safety in different parts of the animal meal production process. There was a prevalence of *Salmonella* spp. of 12.9, 14.3, and 33.3% in Meat and Bone Meal (MBM), poultry by-products, and fish meal, respectively. However, there were no significant differences ($P = 0.73$) in the prevalence between the different animal meals, according to the data collected.

It was also observed that renderings associated with the poultry industry (i.e., 92.0%) obtained the best ratings overall, which reflects a satisfactory development of this sector and the integration of its production system as a whole.

Key words: rendering, food safety, animal by-products meal, *Salmonella*, animal feed

2018 Poultry Science 97:2159–2169
http://dx.doi.org/10.3382/ps/pey058

INTRODUCTION

Rendering is a joint series of operations, facilities, and machinery that can physicochemically transform animal by-products (including meat, bone, blood, hoofs, feathers, and other tissues) into high aggregate value feed ingredients (Sapkota et al., 2007; Meeker and Meisinger, 2015). Hence, animal by-product meals are frequently used as input in poultry, swine, and dog food (Meeker, 2006; Mekonnen et al., 2014). Hence, animal by-product rendering is envisaged not only as a revenue source but also as a means to reduce environmental pollution, as it involves waste management (Jayathilakan et al., 2012) through composting bioreactions, water treatment, and heat recirculation (e.g., using waste heat recovery evaporators during cooking). The main useful outputs of animal by-products processing to the feed industry include meat and bone meal (**MBM**), bone meal, blood meal, hydrolyzed feather meal, poultry by-products meal, fish meal, and fish oil (Meeker and Meisinger, 2015; AAFCO, 2017). Usually, broilers, pets, and pigs are fed rations that may consist of 5 g by-products/100 g feed, 25 g by-products/100 g feed, or meals made up of one-third of the protein source, respectively (Yamka et al., 2003; Badilla, 2012; Kawauchi et al., 2014). On the contrary, use of MBM in cattle, sheep, deer feed (FAO and IFIF, 2010), and cat food (de Vos and Heres, 2009; OIE, 2016) is restricted due to the risk of spongiform encephalopathy dissemination.

Received September 24, 2017.

Accepted January 24, 2018.

[1]Corresponding author: andrea.molina@ucr.ac.cr

Costa Rica allocates 2,406,418.4 hectares to the agricultural activity in all its variants. Cattle production comprises ca. 1,278,817 animals (42.1% destined to the manufacture of meat), and another 453,243 and 18,600,000 animals have been reported for the pork meat production and poultry industries, respectively (INEC, 2015).

Also, for 2006, beef consumption of 14.4 kg, 67 kg of pork, and around 23 kg of chicken per capita per year are reported (Barrantes and Jiménez, 2007; Padilla, 2008). However, it should be considered that the final meat product represents a percentage of tissue that is fit for human consumption. Meanwhile, as high as 49, 44, and 37% of the bodyweight of bovines, pigs, and chickens, respectively, are regarded as by-products, and should be adequately treated (Woodgate and Van de Veen, 2004; Meeker, 2006; Jayathilakan et al., 2012).

As mentioned above, rendering products have global importance in the feeding of production animals and some pets (especially dogs). Over 200 establishments dedicated to rendering are reported between the United States and Canada (Jekanowski, 2011), including both independent renderings and those integrated into slaughterhouses, which process up to 25 million tons per yr (Meeker and Meisinger, 2015). In Costa Rica, animal by-product meal manufacturers are distributed like so: 5 MBM (production ca. 4,500 ton/yr; 3 associated directly with slaughterhouses), 4 poultry by-product meal (production ca. 3,200 ton/yr (3 integrated into poultry meat and egg industries), and 2 fish meal producers (production ca. 2,500 ton/yr (Badilla, 2012; Molina and Granados-Chinchilla, 2015).

Given the close relationship between feed and food, the adoption of health and safety strict quality standards, and integrated vigilance models (e.g., "farm-to-fork") should be mandatory to reduce physical, chemical, and biological factors that may cause health risks (Codex Alimentarius, 2008). For example, in Costa Rica, the safety of animal meal is carried out through the official inspectors, as part of an annual program to comply with regulations recommended by the OIE (2016) regarding bovine spongiform encephalopathy (**BSE**) control.

Food safety issues govern debates about the use of animal proteins in feed; these include bacterial pathogen contamination (Meeker, 2009), especially microorganisms related to foodborne diseases such as *Salmonella* spp. (an agent capable of infecting different hosts and source severe outbreaks) (Hoelzer et al., 2011) and *Clostridium perfringens* (a pathogen that can cause substantial economic losses in the poultry industry due to necrotic enteritis) (Casagrande et al., 2013; Tessari et al., 2014). Globally, these species have presented a relatively high prevalence, especially in MBM (Papadopoulou et al., 2009; Li et al., 2012; Magwedere et al., 2015; Molina et al., 2016). Therefore, feed and feed ingredients destined for animal consumption should be regularly monitored. In fact, safety standards for sources, processing, and use of rendered products within the animal feed industry have already been discussed (Woodgate and Van de Veen, 2004). For example, the parameters used to render animal by-products (i.e., particle size and temperature set at 50 mm, 133°C at atmospheric pressure for 20 min, respectively) (European Commission, 2002; OIE, 2016) are usually sufficient to eliminate pathogenic bacteria present in the raw material. However, some pathogens are opportunistic organisms (e.g., *Salmonella* spp. and *Clostridium* spp.) and may re-contaminate products after cooking or processing and during storage, transport, and handling (Meeker, 2009). Hence, the rendering industry should be strictly controlled by the appropriate authorities, and finished products should be routinely inspected for compliance with applicable regulations. Hereafter, we aim to provide an epidemiological background of the animal meal industry in Costa Rica, including nutritional aspects of the animal meal, prevalence of relevant bacteria (*Salmonella* spp., *Clostridium* spp., total coliforms, *E. coli*, *E. coli* O157:H7), manufacturing, and food safety practices.

MATERIALS AND METHODS

Study Area and Sampling

A total of n = 89 animal meal samples of about 0.5 kg were collected in different parts of the rendering process (cooker, after extraction of fat, including a collection in the screw conveyor, and the final product) during 2015 and 2016 with the collaboration of government inspectors in 11 Costa Rican renderings as part of an annual country-wide surveillance program. Samples included poultry meal (n = 21; 23.6%), MBM (n = 62; 69.6%), and fish meal (n = 6; 6.7%). Sampling was performed following the Association of American Feed Control Officials (AAFCO, 2014). Samples were analyzed immediately upon arrival at the laboratory.

Nutritional Analysis

Dry matter (**DM**, loss on drying/moisture), crude protein (**CP**), fat (**EE**), fiber (**CF**), and ash, as well as calcium, phosphorus, and pepsin digestibility of animal protein assays were performed to assess the nutritional quality of each of the animal by-products meals collected. All tests were performed using ISO 17025 accredited methods based on AOAC 930.15, 988.05/984.13/976.06/990.02, 920.39, 962.09, 942.05, 968.08/975.03/985.35, 965.17/986.24, 935.13, and 971.09, respectively.

Microbiological Assays

As part of the microbiological evaluation of the samples collected, *Salmonella* spp., total coliform bacteria, *Escherichia coli*, and (the enterohemorrhagic) *Escherichia coli* O157:H7 analyses were performed. Assays were based on ISO 17025 accredited methods AOAC

Table 1. Factors to describe the production performance of different rendering facilities in Costa Rica.

Rendering	Equipment	Products	Production, ton d^{-1}; [final product yield, %]	Initial raw material, ton d^{-1}
A	2 crackers, 4 cookers (maximum capacity of 4,500 kg), 3 spellers, and a grinder	Mixed meat and bone meal (beef and pork)	10; [74]	13.5
B	A cracker, 4 cookers (maximum capacity between 3,000 and 4,000 kg), 2 centrifuges, and 2 grinders	Mixed meat and bone meal (beef and pork) Animal fat/3,5	12; [72]	16.6
C	A cracker, 3 cookers (maximum capacity of 5,000 kg), 2 spellers, and a grinder	Mixed meat and bone meal (beef and pork)	12; [30]	30–40
D	A cracker, 4 cookers (maximum capacity of 4,000 kg), a centrifuge, and a grinder	Animal meal by-products Poultry meal	18; [75]	24
E	A cracker, 3 cookers (maximum capacity of 3,000 kg), a centrifuge, and a grinder	Meat and bone meal Animal fat/1,1	11; [73]	15
F	6 cookers (maximum capacity of 5,000 kg), 2 spellers, and a grinder	Feathers meal Poultry meal	1.5; [30]	2 feathers:3 blood
G	2 cookers (maximum capacity of 7,000 kg), and a sage	Poultry meal	7; [52]	10–14
H	3 cookers (maximum capacity of 4,500 kg), and a sage	Poultry meal	11; [31]	35
I	A cracker, 7 cookers (maximum capacity of 2,600 kg), a speller, a grinder, and a sage	Fish meal	7; [29]	24
J	NE	Fish meal	NE	NE

NE, Not evaluated

967.25/967.28/994.04/978.24 (Molina et al., 2016) and APHA/CMMEF methods 9.91- 9.94 (based on a MPN technique) and 34.22-34.24.

Analysis for *C. perfringens* contamination was performed according to the methodology proposed by the United States Department of Agriculture´s Food Safety and Inspection Service (**USDA-FSIS**). Briefly, 25 grams of each sample were homogenized with 225 mL of Butterfield´s phosphate diluent (**BPD**). Decimal dilutions up to 10^{-5} were prepared using BPD, and 0.1 mL of each dilution was streak plated by duplicate on tryptose sulphite cycloserine (**TSC**) plates supplemented with egg yolk. After the inoculum had dried slightly, the surface was overlayed with approximately 10 mL or more of egg-yolk-free TSC agar. The plates were allowed to solidify, and they were incubated at 37°C for 24 h inside an anaerobic jar. After incubation, typical *C. perfringens* colonies (black color surrounded by a halo) were isolated for further confirmation. Analysis of suspicious colonies included Gram staining, hemolytic activity on blood agar plates, the absence of growth under aerobic conditions, and ability to grow at 42°C in chopped meat broth after 18 h of incubation. Final confirmation of suspicious *C. perfringens* isolates was performed with an API® 20A (Biomérieux, Hazelwood, MO).

Rendering Facility Description and Evaluation

Individual descriptions of the studied facilities were obtained. Data included available equipment within the premises, input for raw material, and main output metrics to assess the productivity and size of each industrial activity (Table 1).

Additionally, each of the renderings was subjected to evaluation based on recommendations by *Codex Alimentarius* (CAC/RCP 1–1969), the Central American Technical Regulation (RTCA 65.05.52:11), European Commission Directive (CE) N° 1774/2002, and the World Organization for Animal Health (OIE, 2016) to ensure feed innocuity.

Location The geographical position of the rendering facilities was taken into account, including aspects such as distance from other industrial activities, from thickets, and in an area not prone to floods. In the vehicles and transport section, the condition of the vehicles were considered, as well as their cleaning and disinfection procedures. Additionally, the availability of areas suitable for private vehicle parking far away from the primary production zone was recorded.

Infrastructure and Facilities Access to the rendering service was assessed by the adherence to hygiene practices such as change of clothes and footwear, handwashing and footwear washing, the availability of foot baths, and the route layout from the cleanest area to the dirtiest one. At the facilities, particular attention was paid to the existence of different parts for the raw material receipt, processing, and storage of the finished product, as well as an optimum process flow that minimizes cross-contamination, in addition to the materials used in the infrastructure (e.g., walls, ceilings, and floors). In the evaluation of the equipment, type of materials used, the general condition of the equipment, and the presence of devices that allow

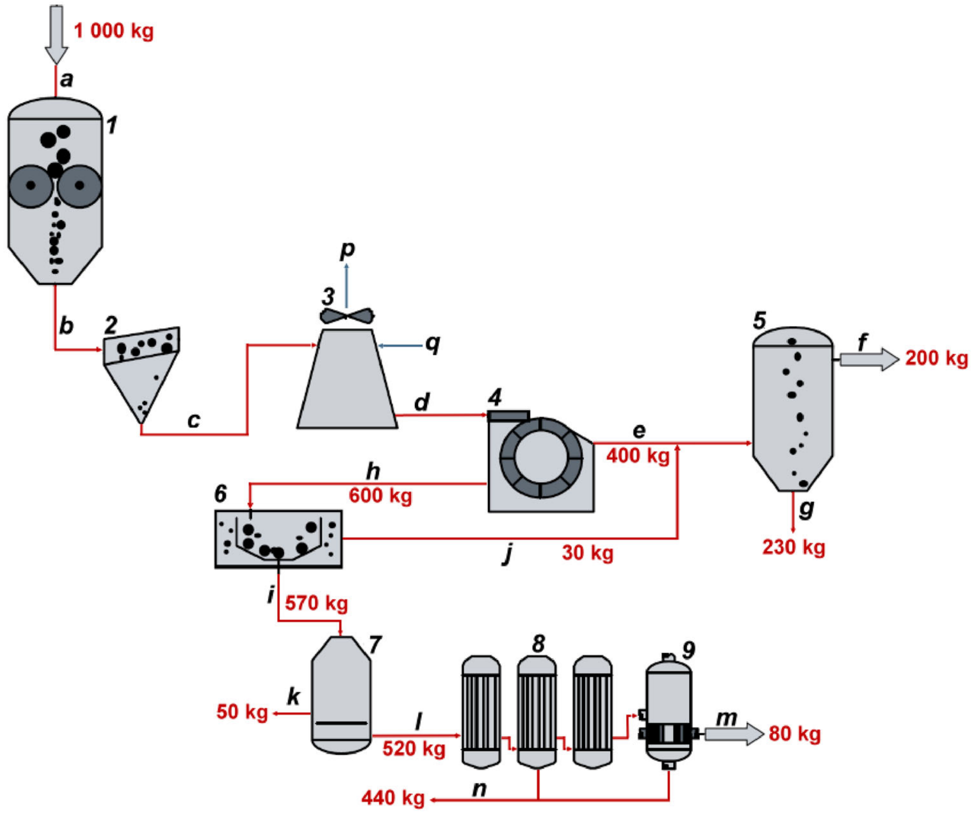

Figure 1. Process diagram describing a hypothetical fish meal production line where the mass balance is presented. Key: a. Raw fish. b. Mauled tissue. c. Sieved gum. d. Cooked material. e. Press cake. f. Fish meal. g. Waste vapors. h. Stick water. i. Fish water. j. Sludge. k. Fish oil. l. Protein water. m. Fish soluble paste. n. Waste gasses. 1. Grinder. 2. Screen. 3. Cooker. 4. Screw press. 5. Dryer. 6. Decanter. 7. Centrifuge. 8. Double-stage waste vapor evaporator. 9. Steam vacuum evaporator.

temperature and pressure monitoring during cooking were evaluated.

Pest Management and Control Integrated pest management included establishment outlining, the design of a trapping map to facilitate verification, and use of physical and chemical methods as appropriate.

Disinfection and Cleaning For the evaluation of the cleaning and disinfection procedures, some aspects including cleanup before and after the manufacturing process and disinfection methods (e.g., irrigation with boiling water, detergents, and antiseptics) were assessed. At the critical points, internationally recommended parameters, already mentioned above, were evaluated (European Commission, 2002; OIE, 2016).

Process Efficiency Calculations Mass balance was calculated for a theoretical fish meal manufacturer (Figure 1). Fish meal was chosen based on the availability of performance data for each equipment (Kirk-Othmer Encyclopedia of Chemical Technology) used during the process feed streams (e.g., material input, volumes and flows, carry-over, efficiency, performance, valve outlets, and pressures). Process thermodynamics simulation and mass balance were performed using the Peng–Robinson method. The process flowchart and all calculations were done using CHEMCAD software version 7.1.4.10142 (Chemstations, Inc., Houston, TX). Rendering schematic diagrams were drawn using Edraw Max 8.4 (EdrawSoft, Nanshan District, Shenzhen City, Guangdong Province, China)

Statistical Analysis

A variance analysis was performed with Infostat software version 12.0.0.0. The presence of microorganisms (*Salmonella* spp, *Clostridium* spp, total coliforms, and *E. coli*) was used as the dependent variable, and the type of meal of animal origin (MBM, poultry by-products, and aquaculture by-products) was used as the independent variable. When normal distribution assumptions were not met, the Kruskal–Wallis test was used. All assays were executed using Infostat software version 12.0.0.0 (Universidad de Córdoba, Argentina). Results were considered to differ significantly if $P < 0.05$.

RESULTS

Nutrition and Composition

We observed considerable variations in CP (avg 45.9; 37.0 to 51.5 g/100 g), calcium (avg 9.9; 5.4 to 12.9 g/100 g), and phosphorus (avg 5.4; 2.9 to 7.3 g/100 g) in MBM. Poultry by-products returned the most mutable values for crude protein (avg 71.6; 46.3 to

Table 2. Nutritional components (expressed in g/100 g) obtained from animal by-product meals collected in Costa Rican renderings from 2015 to 2016.

Nutrient	Dry matter	Crude protein	Crude fat	Crude fiber	Ash	Ca	P	Protein digestibility
Meat and bone meal (n = 23)								
Mean ± Standard deviation	95.3 ± 1.8	45.9 ± 3.8	18.2 ± 3.4	1.5 ± 1.0	29.4 ± 4.9	9.9 ± 1. 9	5.4 ± 1.1	81.7 ± 10.3
Median	95.8	46.3	17.6	1.3	28.9	10.0	5.3	82.4
Max	97.4	51.5	25.1	4.0	37.8	12.9	7.3	95.1
Min	90.7	37.0	13.4	0.3	16.4	5.4	2.9	62.2
Poultry by-products (n = 44)								
Mean ± Standard deviation	92.9 ± 4.0	71.6 ± 14.9	13.1 ± 7.8	1.0 ± 0.8	9.1 ± 7.4	2.8 ± 1.6	1.7 ± 1.2	49.1 ± 19.3
Median	93.7	76.0	10.4	0.7	7.4	2.5	1.7	40.6
Max	97.9	90.3	31.2	3.0	31.1	6.2	4.7	89.5
Min	85.2	46.3	2.8	0.2	2.9	0.5	0.5	25.2
Fish meal (n = 4)								
Mean ± Standard deviation	96.5 ± 0.3	58.1 ± 1.0	14.2 ± 3.3	1.1 ± 1.1	19.8 ± 2.3	5.9 ± 0.5	3.9 ± 2.4	–
Median	96.5	58.1	13.3	1.1	19.8	5.7	3.0	–
Max	96.7	59.1	17.9	1.8	21.4	6.7	7.4	–
Min	96.3	57.0	11.4	0.3	18.1	5.5	2.3	–

90.3 g/100 g), calcium (avg 2.8; 0.5 to 6.2 g/100 g), and phosphorus (avg 1.7; 0.5 to 4.7 g/100 g). Values for fish meal were steadier: CP (avg 58.1; 57.0 to 59.1 g/100 g), calcium (avg 5.9; 5.5 to 6.7 g/100 g), and phosphorus (avg 3.9; 2.3 to 7.4 g/100 g) (Table 2). On the other hand, Table 2 shows pepsin digestibility values for both MBM and poultry by-products meal with average values of (81.7 ± 10.3) g/100 g and (49.1 ± 19.3) g/100 g, respectively.

Rendering Safety Evaluation

Several rendering facilities evidenced serious deficiencies in safety protocols and basic good manufacturing practices, as recommended by FAO and IFIF (2010), for feed processing establishments (Table 3). Each graded aspect considered within the scope of our assessment is determinant to guarantee a safe product (Table 3). Overall, location (avg 75.0) and pest management (avg 71.4) are among the best-scored sets. Conversely, entry procedures (avg 48.6) and infrastructure (avg 42.2) are among the lowest (Table 3).

Location Regarding location, most renderings comply with evaluation criteria (avg 75.0). Rendering A: (avg 75.0); renderings E (avg 75.0) and I (avg 75.0): must improve access and entry points for both pedestrians and vehicles, as the main route is made of gravel. On the other hand, rendering D (avg 25.0) is near an area prone to floods.

Infrastructure and Facilities Outdated infrastructure (over 40 yr old, except for F and G), with no evidence of recent amendments, porous surfaces, or without waterproofing represent occupational hazards for employees and justify the overall low score (avg 42.2) for the infrastructure category (Table 3). In addition [except for E (avg 77.8) and G (avg 100.0)], general rendering construction has been done with non-hermetical features allowing the product to be exposed to external agents (e.g., dust). Some cases, such as renderings B (avg 33.3) and C (avg 22.2), have common areas in which raw material reception, processing, and final product storage are occurring simultaneously and relatively near each other. Nevertheless, (except for renderings B, F, and G) current process flows are inconvenient for general cleanup, as they hamper access to some equipment (conveyor screws, expeller, among others), or the equipment is very close to each other. In broad terms (except for renderings F, G, and I), no clear procedure was found for access to the facilities, and there was no extra change of clothing for the workers nor hand or foot wash areas. Noteworthy, rendering E fails to comply with most of the evaluated points in this section, but the distance between the processing areas and the strict personnel flow between the different areas are sufficient to obtain a *Salmonella* spp. free product.

Disinfection and Cleaning Three different scenarios were found with respect to cleaning after cooking the product, i.e., establishments with 1) programmed procedures (production is halted during scrubbing), 2) weekly rinse using hot water, detergents, and disinfectants (this is the most common in Costa Rica), and 3) screw conveyor dry cleansing and use of hot water and detergents only in the raw material receiving docks (e.g., rendering E). Deficient practices observed within other renderings that can contribute to product contamination included equipment that is not dried before each batch, equipment spatial location hindering cleaning procedures, and, particularly, failure to remove residual organic matter from the screw conveyors.

Pest Management and Control Pest control (including snare setting, establishment mapping, staff training, and recommendation of disinfectant products) is usually relegated to third parties. We found facilities that are pest proofed (avg 71.4) (Table 3). In this regard, rendering D (avg 42.9) is a notable exception, because it presents faults in the infrastructure, which facilitate the access of birds, insects, and rodents to the installation, and therefore to the product.

Table 3. Animal by-products rendering facility assessment according to aggregate marks based on safety compliance.

Key points[a,b]/Rendering	A	B	C	D	E	F	G	H	I	J
Location[75.0]	75.0	100.0	100.0	25.0	75.0	100.0	100.0	NE	75.0	100.0
Admission to the facilities [48.6]	71.4	57.1	57.1	0.0	14.3	100.0	100.0	NE	85.7	NE
Integrated pest management[71.4]	100.0	85.7	85.7	42.9	85.7	71.4	100.0	85.7	57.1	NE
Physical condition of the facilities[42.2]	55.6	33.3	22.2	33.3	77.8	66. 7	100.0	NE	33.3	NE
Equipment[62.5]	75.0	75.0	100.0	50.0	75.0	75.0	100.0	NE	75.0	NE
Cleaning procedure[51.4]	71.4	71.4	57.1	57.1	28.6	85.7	85.7	NE	57.1	NE
Vehicles and transportation[57.1]	71.4	85.7	85.7	57.1	57.1	42.9	100.0	NE	71.4	NE
Critical points[51.7]	66.7	66.7	66.7	66.7	66.7	50.0	50.0	NE	83.3	NE
Total[57.5]	*73.3*	*71.9*	*71.8*	*41.5*	*60.0*	*74.0*	*92.0*	*10.7*	*67.3*	*12.5*

[a]Key points are equal to each one of the sections that were evaluated.
[b]Number in brackets represents the average of compliance for each of the points chosen as a determinant of safety.
NE, Not evaluated. A grading system based on scores (from 0 to 100).

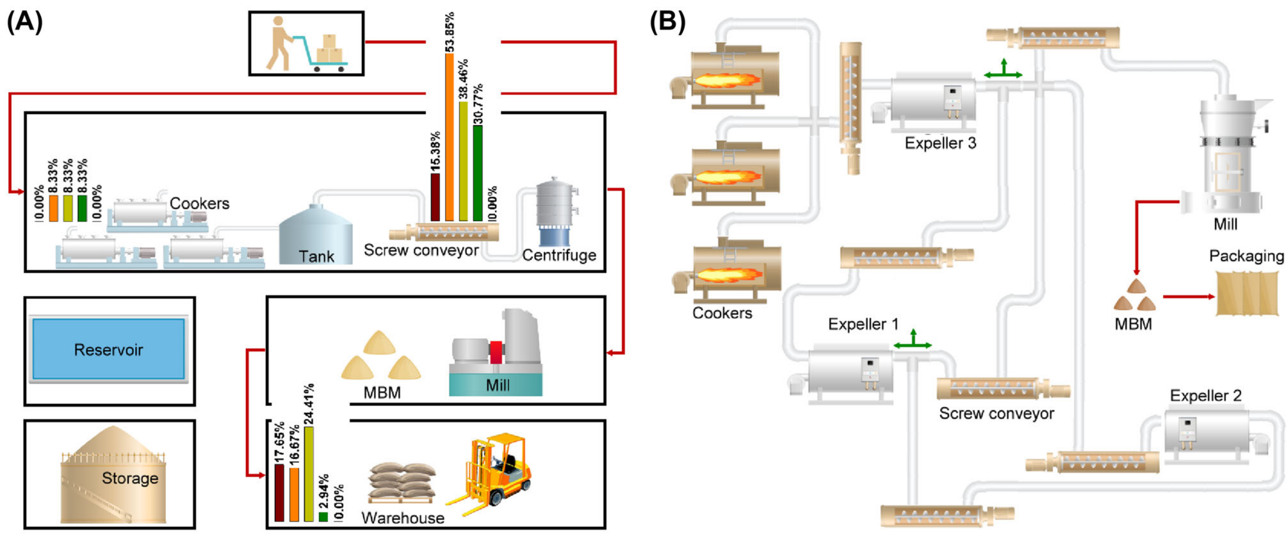

Figure 2. Continuous dry rendering processes schematic diagrams. Examples of (A) adequate and (B) unfitting distributions within MBM facilities found in the country. Black outlines represent the physical divisions among different process areas. Graphs within panel (A) represent microbiological contamination of specific points sampled during production (i.e., after cooking, after defatting, and in the final product. ■*Salmonella* spp., ■*Clostridium* spp., ■Total coliforms, ■*Escherichia coli*, ■*E. coli* O157:H7).

Vehicles and Material Transport Overall, establishments kept load vehicles in good condition. Particular trucks destined for raw materials and finished product transportation were regularly disinfected and cleaned following proper procedures. Only rendering G (avg 100.0) has vehicle disinfection arcs, both at the entrance and at the exit of the establishment. On the other hand, rendering F (avg 42.9) production transport is achieved through hoists and conveyors from the raw material to the finished product, due to its integrated system in poultry production. Rendering F is unique as it is associated with a processing plant of chicken for human consumption (Table 3).

Regulatory Critical Thresholds All renderings (except those destined to process poultry by-products) comply with international recommended critical points, including temperature and cooking time. Interestingly, manufacturers of poultry meal use a lower temperature (120 to 124°C) than their MBM and fish meal counterparts. Nevertheless, regarding meal particle size, a simple visual inspection suffices to evidence that none of the renderings complies with this parameter (i.e., 50 mm). However, samples seized directly after the product left the cookers showed no *Salmonella* spp. prevalence (Figure 2A).

Rendering Production

In Costa Rica, the MBM rendering works on average with 21.8 tons/d of initial raw material (max 40, min 13.5). In the case of the poultry meal, there is an average of 18.0 ton/d and the fish meal 29.5 ton/d (Table 1).

Renderings with the best yields (i.e., the final percentage of the finished product) are producers of MBM (30 to 75% product yield, Table 1), and fish renderings were the lowest (29% product yield, Table 1).

Microbiological Evaluation

The prevalence for indicator microorganisms specified by international regulations ranged from 0.0 to 33.3% (Table 4). Overall, the data indicate *E. coli* (0.0

Table 4. Microbial safety indicator organisms for each animal by-product meal.

Animal meal/ indicator organism	*Salmonella* spp. (presence in 25 g)	*Clostridium* spp. (presence in 10 g)	*Bacillus* spp. (presence in 10 g)	*E. coli* (>3 MPN/g)	Total coliform bacteria (>3 MPN/g)
Meat and bone meal ($n = 62$)	12.9% ($n = 8/62$)	27.4% ($n = 17/62$)	32.2% ($n = 20/62$)	9.7% ($n = 6/62$)	25.8 ($n = 16/62$)
Poultry by-products ($n = 21$)	14.3% ($n = 3/21$)	33.3% ($n = 7/21$)	4.8% ($n = 1/21$)	14.3% ($n = 3/21$)	28.6% ($n = 6/21$)
Fish meal ($n = 6$)	33.3% ($n = 2/6$)	16.7% ($n = 1/6$)	16.7% ($n = 1/6$)	0.0% ($n = 0/6$)	33.3% ($n = 2/6$)
Accepted minimum parameters	Absence in 25 g[a,b]	Absence in 10 g[a]	Absence in 10 g[a]	Absence in 1 g[c]	For every 5 samples max 2 between 10 and 300 CFU/g, and none with >300 CFU/g[a]

[a]European Commission (2002).
[b]FDA (2013).
[c]FDA (2005).

to 14.3%) and total coliform bacteria (25.8 to 33.3%) presence in $n = 89$ samples (Table 4). Additionally, *E. coli* O157:H7 was found to be absent in all samples ($n = 89$, Figure 2A).

The overall presence of *Clostridium* spp. and *Bacillus* spp was of 28.1% ($n = 25/89$) and 24.7% ($n = 22/89$), respectively. *Clostridium* spp. and *Bacillus* spp. were found in several parts of the process (Figure 2A), even after thermal processing; the presence of these microorganisms may be caused by re-contamination of product due to poor sanitary conditions of the facilities, or from the workers themselves.

In addition, the presence of *Salmonella* spp. was found for $n = 8$ MBM samples (Table 4). Furthermore, 37.5% ($n = 3/8$) of these positive MBM samples were recollected from different points of the rendering process (i.e., transport through conveyors and defatting by expellers); directly after cooking, the samples were negative (Figure 2A). It is interesting to mention that one of these samples is directly related to the finished product of the same rendering, i.e., it can be inferred that there is obviously cross-contamination of pathogenic microorganisms when there is a failure following good manufacturing practices (GMPs). However, *C. perfringens* was found just in $n = 1$ MBM sample (1.61% prevalence).

On the other hand, poultry by-product meal demonstrated the lowest prevalence of *Bacillus* spp ($n = 1$, 4.8%), while MBM showed the highest ($n = 20$, 32.2%). All poultry by-products samples ($n = 21$) represent the final product.

Fish meal reported the highest prevalence of *Salmonella* spp. and total coliforms bacteria (33.3% for both accounts), but these samples ($n = 6$) exhibited the lowest incidence for *Clostridium* spp. (16.7%) and were negative for *E. coli* (Table 4).

Interestingly, no significant differences ($P = 0.73$) were found between the different animal meals regarding the presence of *Salmonella* spp. Similar results were obtained for *Clostridium* spp. ($P = 0.68$), *Bacillus* spp. ($P = 0.21$), total coliforms ($P = 0.70$), and *E. coli* ($P = 0.44$).

Furthemore, directly after cooking, there was no presence of *Salmonella*, however, this changes as the production process moves forward. This change occurs during the production process, as shown in Figure 2A; also, the levels of *Clostidium* spp., total coliforms, and *E. coli* augmented through the rendering process (Figure 2A). The previous statements support the conclusion that microbes post cooking can come only from re-contamination.

DISCUSSION

Nutrition and Composition

By-products of animal origin and the final rendering product are characterized by the contribution of the protein of high biological value (2.59% lys and 0.69% met), in addition to calcium and phosphorus, during the production of feedstuff (Rostagno et al., 2011; NRC, 2001; Meeker, 2009). Exceptions include diets formulated for ruminants and felines, due to the possibility of BSE transmission (OIE, 2016).

When data for different animal by-product meals are compared (Table 2), it is evident that the nutritional quality of the national products is similar to the data of South America and the United States (Rostagno et al., 2011; NRC, 2012). It is apparent that the procedures, work equipment, and the raw materials used for meal elaboration allow complying with the international guidelines. The few deviations found are to be expected, as rendering associated with slaughterhouses produces a mixed product (swine and beef, mainly, Table 1). Those rendering facilities that are not associated with slaughterhouses must acquire their raw materials elsewhere and have the disadvantage of using a mostly bone input, which in turn, reduces the percentage of CP and raises the ash contents (Jayathilakan et al., 2012; Kawauchi et al., 2014; Hendriks et al., 2012).

Even so, the animal meal is still considered a good option to lower the costs of animal feed production (Meeker and Meisinger, 2015), as the local production is available and, compared to other protein sources, is cheaper [e.g., distillers' dried grains (28.9 g CP/100 g, 35 profat): 148 dollars per metric ton, and soybean meal (45.1 g CP/100 g): 408 dollars per metric ton. Both values calculated on a free-on-board transport to

New Orleans, LA) (NRC, 2001)]. Additionally, evidence suggests that inclusion of vegetable origin meals, when substituting animal ones in the feed, may need further study (Zhang et al., 2014). Recent data have even described that inclusion of vegetable-based diets negatively affects growth and tissue quality in fish (Liang et al., 2017). During the preparation of animal feed, animal by-product meals can be included up to a maximum of 5, 15, and 25 g per 100 g of poultry, swine, and dog food, respectively (Murray et al., 1997; Rostagno et al., 2011).

In this regard, thermal processing may be necessary for safety but must be surveilled, as overcooking can have a deleterious effect on protein quality (e.g., amino acid bioavailability; Meeker, 2006; Meeker and Meisinger, 2015; Hendriks et al., 2012). It is recommended that such assays be incorporated in the routine nutritional analysis for animal by-product meals. On the other hand, moisture content (≤10 g/100 g) should be guarded as an increased a_w favors pathogen growth (Badilla, 2012).

There are 2 main premises regarding animal by-product meals: 1) the cooking process favors the loss of amino acids and the digestibility of the protein, and 2) animal meal protein is a raw material that provides protein of high biological value (Table 2). Animal by-products must be as digestible as possible so that the animal has an optimal yield in weight gain and egg or milk production (Meeker, 2009; Meeker and Meisinger, 2015). Therefore, despite being a relative measure [e.g., other enzymes, in addition to pepsin, act in the gastrointestinal tract], *in vitro* digestibility with pepsin is still an accepted method (Nieto et al., 2005), allowing a relatively swift evaluation of animal meal (Bellaver et al., 2000).

AAFCO (2017) reports acceptable values for protein digestibility of 88.00 g/100 g and 75.00 g/100 g for MBM and hydrolyzed feather meal, respectively. Both MBM and poultry by-product meals do not comply with the above guidelines. It can be inferred that the thermal process may contribute to the low quality of the final meal, which in turn, may reflect negatively on the productive performance of the animal (Hendricks et al., 2012). However, these treatment conditions comply with other established procedures (European Commission, 2002; OIE, 2016), and they are efficient to eliminate pathogens (Table 4, Lambertini et al., 2016). Lastly, Nieto et al. (2005) reported that on average the pepsin digestibility of the meal of aquatic animal by-products is 65.00%.

Rendering Safety Evaluation

Location Rendering A's neighboring industry is dedicated to the manufacture of computer equipment, which was considered not to be a safety issue for the final product. On the other hand, as rendering D is near an area prone to floods, pest infestations are a real possibility (COMIECO, 2012; National Renderers Association, 2015). Furthermore, facilities lacking clear access procedures and that are surrounded by underground may have a higher incidence of biological contamination (FAO and IFIF, 2010; National Renderers Association, 2015).

Infrastructure and Facilities Different parts of the process should be carried out in dissimilar zones (FAO and IFIF, 2010; Meeker and Meisinger, 2015). As raw materials could become a significant source of contamination for the final product (they are usually contaminated with high amounts of bacteria), the division of areas is a critical point to guarantee safety. High humidity of slaughterhouses could also favor recontamination, as it promotes the replication of this initial bacterial load (Meeker and Meisinger, 2015; Jayathilakan et al., 2012). On the other hand, external thermometers and manometers are used to ensure critical equipment performance, especially for cookers, which have a primary role in tissue transformation and pathogen elimination.

Disinfection and Cleaning Screw conveyor dry cleansing and additional hot water and detergent steps for the raw material receiving docks (e.g., rendering E) seem to be the most efficient methods based on the absence of microbial pathogens (i.e., *Salmonella* spp.) during the assay of samples ($n = 7$) collected from facilities applying this procedure. Nonetheless, for rendering E, $n = 2$ samples exhibited a total coliform bacteria concentration >110,000 MPN/g, which is usually used as a hygiene marker, reflecting inadequate sanitation conditions and the risk for the presence of other pathogens. Otherwise, at least one *Salmonella* spp. contamination incident was found for each rendering after fat extraction (expeller) and carried all the way through the finished product. Biofilms or already established "in-house" strains may be responsible for repetitive and stubborn contamination (Vestby et al., 2009; Shi and Zhu, 2009; Joseph et al., 2001). Several practices observed within other renderings that can contribute to final product contamination included equipment that is not dried before each batch, equipment spatial location hindering cleaning procedures, and, particularly, failure to remove residual organic matter from the post-cooker screw conveyors.

Pest Management and Control Based on the evidence recollected during facility inspection, the control system cannot be efficient if the facilities are not proofed against insects, rodents, and birds, which are known vectors of pathogenic microorganisms.

Vehicles and Material Transport The general average of this section was 57.1% (Table 3); however, of the 8 evaluated renderings, only one obtained a rating below 50% (rendering F, avg. 46.9) because the transport points of raw material were not assessed, since screws transport this from the broiler slaughterhouse.

Regulatory Critical Thresholds During poultry meal preparation, it is assumed that lower temperature during cooking when compared with other meal processing procedures is still sufficiently high enough for the elimination of pathogens, while the nutritional quality of the product is maintained (Lambertini et al., 2016b; Lambertini et al., 2016a). On the other hand, since poultry meal is not considered a raw material with BSE risk, it is not necessary to retain the temperature of 133°C (Meeker and Meisinger, 2015; Lambertini et al., 2016b). Currently, in Costa Rica, raw material particle size, as measured previous to cooking, is not a parameter being evaluated by government officials. Rendering non-compliance of the regulatory framework declaring maximum particle size does not seem to affect the prevalence of pathogens, meaning that regulatory compliance does not often impact contamination.

Rendering Production

A theoretical analysis using machinery efficacy, outputs, and turnover demonstrate that, e.g., a fish rendering produces 200 kg fish meal (20% theoretical yield) and 80 kg fish oil (8% theoretical yield) from 1,000 kg raw material (Figure 1). Though rendering already is a waste recycling procedure, it is critical to note that residue production, after the meal is obtained, can range from 71 to 25% in mass (Table 1, Figure 1). Interestingly, this is in line with the yields found *in situ*, i.e., 29% yield (data from rendering I, Table 1).

Microbiological Evaluation

The absence of *Clostridium* spp. and *E. coli* in fish meal must be assessed with caution, as sample *n* may be too small to reach relevant conclusions (Table 4). Though the presence of *E. coli* and total coliform bacteria in several samples is not considered worrisome per se, it does speak about failure in general cleaning procedures (Table 4). In fact, higher levels of coliform bacteria and *E. coli* in poultry by-products concurred with a higher prevalence of *Salmonella* and *Clostridium* spp. in comparison with MBM samples. Lower DM content in poultry meal, when compared with MBM samples (Table 2), could result in higher humidity or water activity values in the final product; this situation may favor the contamination rates and the establishment of foodborne pathogens. More studies are necessary to understand why poultry meal samples have higher contamination rates.

Contamination found in the final product may reflect cross-contamination, which, as stated before, can be caused by having the entire process in a single general area (Figure 2B). Spore-forming bacteria, such as *Clostridium* and *Bacillus* species, can withstand harsh environmental conditions, such as high temperatures (Freedman et al., 2016). However, cooking parameters used in rendering facilities should be sufficient to eliminate microbial contamination; this means that these spore formers may reach the final product due to cross-contamination. As it is observed in Figure 2A, this contamination may occur very soon after cooking. This situation is evidenced by the number of samples positive for both clostridia and bacilli spores and, most importantly, the presence of the pathogenic *C. perfringens* isolated from a MBM sample. Low incidence of *C. perfringens* suggests that the risk of contamination with these bacteria from animal feed is very low. Nevertheless, higher contamination rates with other clostridia indicate a high risk of pollution for the final product, as these species are ubiquitous (Ferreira et al., 2003) and they may share common environmental niches. Also, low *C. perfringens* incidence in the samples may be a consequence of a more moderate sporulation capacity of this species in the environment as compared with other clostridia (de Jong et al., 2002); this situation may limit the sensitivity of the methodology to actually isolate *C. perfringens* from samples heavily contaminated with other spore formers.

Animal by-product meals can be vehicles for transmission and contamination with *Salmonella*. Table 2 shows that all 3 types of products analyzed in the study are prone to contamination with this bacterium. The presence of *Salmonella* in animal by-products is a confirmation that there is a high rate of cross-contamination of the final product, as the regular cooking process for animal meals should be sufficient to eliminate this pathogen. Higher prevalence of *Salmonella* in samples taken at the end of the process (Figure 2A) confirms that there is contamination from the production environment. Presence of *Salmonella* in animal by-products must be taken into account, as this microorganism is widely recognized for being able to survive for extended periods in low water activity foods (Santillana Farakos et al., 2014). Other studies have reported a high prevalence of *Salmonella* spp. in MBM samples (Li et al., 2012; Molina et al., 2016; Jiang, 2016). The rendering industry should take proper steps to control the microbial contamination of animal by-products with *Salmonella*,to reduce the risk for live animals and humans.

CONCLUSIONS

Differences among integrated meat, poultry, and fish systems are transferred to the rendering process as well. Enterprises such as avian product processing have evolved into a safety-centric industry involving all production areas. Other production systems (cattle beef) remain undeveloped in Costa Rica regarding production, animal nutrition, and welfare. Most renderings are still considered secondary productive activities, and they are seen just as a means to dispose of the slaughterhouse's waste instead of using them as productive recycling facilities, as other countries regard them. The confirmation of high risk for microbial contamination justifies the need to increase control measures and upkeep vigilance programs, regarding meal safety and facilities post-cooking disinfection; this is relevant considering the industrial and economic relevance of this activity. Also, it is pertinent to identify the primary contamination sources for the product within the processing environment. Updating renderings' equipment and facilities should be mandatory to ameliorate hazards and improve feed safety critical point compliance. Even though animal by-product meals have been wholly discarded or restricted just to particular applications in several countries (e.g., fertilizers), they can still be considered a valuable and relatively inexpensive protein and mineral source in diets for productive animals. Rendered products could be used to improve the sustainability of food production. Hence, animal by-products can still be a good option for such places where other protein sources may be deemed too expensive or are not available at all. Noteworthy, thanks to the data collected, national meal manufacturers were able to apply immediate corrective actions for some of the deviations found during the inspection. Finally, besides the issues covered above, we suggest further control must be enforced on other aspects of increasing notoriety, such as odor and noise control, biowaste management, and occupational hazards.

ACKNOWLEDGMENTS

The authors would like to acknowledge the collaboration of Costa Rican Renderings, for allowing entry to facilities, inspection visits and sampling of its product. The Office of the Vice Provost for Research of the University of Costa Rica supported this project financially, grant B6074. Special thanks to Carolina Sibaja for diagramming the process flow for Figure 2 and to Leticia Badilla, Alejandra Jiménez, and Francisco Corrales for their assistance during the rendering inspections.

REFERENCES

AAFCO. 2017. Official Publication. Association of American Feed Control Officials. 591.

AAFCO. 2014. Feed Inspector´s Manual 5th.ed. Association of American Feed Control Officials Inspection and Sampling Committee. 220.

Badilla, A. 2012. Calidad nutricional de la harina de carne y hueso producida en Costa Rica y evaluación del cumplimiento de requisitos mínimos de inocuidad y manufactura. Tesis de maestría, Universidad de Costa Rica, San José, Costa Rica. p 106.

Barrantes, J., and R. Jiménez. 2007. Caracterización de la agrocadena de carne bovina. Ministerio de Agricultura y Ganadería Región Pacífico Central, Costa Rica. p 61.

Bellaver, C., D. Zanotto, A. Guidonu, and C. Klein. 2000. *In vitro* solubility of meat and bone meal protein with different pepsin concentrations. Cienc. Rural. 30:489–492.

Casagrande, M. F., M. V. Cardozo, M. C. Beraldo-Massoli, L. Boarini, F. A. Longo, A. C. Paulilo, and R. P. Schocken-Iturrino. 2013. *Clostridium perfringens* in ingredients of poultry feed and control of contamination by chemical treatments. J. Appl. Poult. Res. Arch. 22:771–777.

Codex Alimentarius. 2008. CAC/RCP 54–2004 Code of Practice on Good Animal Feeding Rome, Italy, *Codex Alimentarius*. Accessed May. 2017. www.fao.org/input/download/standards/10080/CXP_054e.pdf.

[COMIECO] Consejo de Ministros de Integración Económica Centroamericana. 2012. RTCA 65. 05. 63: 11 Reglamento Técnico Centroamericano Productos utilizados en la alimentación animal, Buenas Prácticas de Manufactura. 48.

de Jong, A. E. I., R. R. Beumer, and F. M. Rombouts. 2002. Optimizing sporulation of *Clostridium perfringens*. J. Food Prot. 65:1457–1462.

de Vos, C. J., and L. Heres. 2009. The BSE risk of processing meat and bone meal in nonruminant feed: A quantitative assessment for The Netherlands. Risk Anal. 29:541–557.

European Commission. 2002. Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Brussels, Belgium, Official Journal of the European Communities. L 273/1-95. Accessed May. 2017. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002R1774&from=ES.

[FAO/IFIF] Food and Agriculture Organization/International Feed Industry Federation. 2010. FAO Animal Production and Health Manual. Good Practices for the Feed Industry. Implementing the *Codex Alimentarius* Code of Practice on Good Animal Feeding. FAO, Rome. 110. Accessed Jul. 2017. http://www.ifif.org/uploadImage/2012/1/17/0a9131729326f9a2a111ae84024a851f1326840707.pdf.

[FDA] United States Food and Drug Administration. 2005. Feed Contaminants Program. FDA, Rockville, Maryland, USA. Accessed Oct. 2016. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/ComplianceEnforcement/UCM113409.pdf.

[FDA] United States Food and Drug Administration. 2013. Compliance Policy Guide Sec. 690.800 Salmonella in Food for Animals. FDA, Rockville: Maryland, USA. Accessed Oct. 2016. http://www.fda.gov/downloads/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/UCM361105.pdf.

Ferreira, C. E. A., V. Nakano, E. L. Durigon, and M. J. Avila-Campos. 2003. Prevalence of *Clostridium spp.* and *Clostridium difficile* in children with acute diarrhea in São Paulo City, Brazil. Mem. Inst. Oswaldo Cruz 98:451–454.

Freedman, J., A. Shrestha, and B. McClane. 2016. *Clostridium perfringens* enterotoxin: Action, genetics, and translational Applications. toxins 8:73.

Hendriks, W. H., C. A. Butts, D. V. Thomas, K. A. K. James, P. C. A. Morel, and M. W. A. Verstegen. 2012. Nutritional quality and variation of meat and bone meal. Asian Australas. J. Anim. Sci 15:1507–1516.

Hoelzer, K., A. I. Moreno Switt, and M. Wiedmann. 2011. Animal contact as a source of human non-typhoidal salmonellosis. Vet. Res. 42:1–27.

INEC. 2015. Pages 146, VI Nacional Agropecuario: Resultados Generales. Instituto Nacional de Estadística y Censos: Costa Rica.

Jayathilakan, K., K. Sultana, K. Radhakrisna, and A. Bawa. 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: A review. J. Food Sci. Technol. 49:278–293.

Jekanowski, M. 2011. Survey says: A snapshot of rendering. Render. 40:58–61.

Jiang, X. 2016 Prevalence and characterization of *Salmonella* in animal meals collected from rendering operations. J. Food Prot. 6:1026–1031.

Joseph, B., S. Otta, I. Karunasagar, and I. Karunasagar. 2001. Biofilm formation by *Salmonella* spp. On food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol. 64:367–372.

Kawauchi, I., N. Sakomura, C. Pontieri, A. Rebelato, T. Putarov, E. Malheiros, M. Gomes, C. Castrillo, and A. Carciofi. 2014. Prediction of crude protein digestibility of animal by-product meals for dogs by the protein solubility in pepsin method. Journal of Nutritional Science. 3:1–5.

Lambertini, E., A. Mishra, A. Guo, H. Cao, R. Buchanan, and A. Pradhan. 2016b. Modeling the long-term kinetics of Salmonella survival on dry pet food. Food Microbiol. 58:1–6.

Lambertini, E., R. Buchanan, C. Narrod, R. Ford, R. Baker, and A. Pradhan. 2016a. Quantitative assessment of human and pet exposure to *Salmonella* associated with dry pet foods. Int. J. Food Microbiol. 216:79–90.

Li, X., L. Bethune, Y. Jia, R. Lovell, T. Proescholdt, S. Benz, T. Schell, G. Kaplan, and D. McChesney. 2012. Surveillance of salmonella prevalence in animal feeds and characterization of the salmonella isolates by serotyping and antimicrobial susceptibility. Foodborne Pathogens and Disease. 9:692–698.

Liang, X. F., L. Hu, Y. C. Dong, X. F. Wu, Y. C. Qin, Y. H. Zheng, D. D. Shi, M. Xue, and X. F. Liang. 2017. Substitution of fish meal by fermented soybean meal affects the growyh performance and flesh quality of Japanese seabass (*Lateolabrax japonicus*). Animal Feed Science and Technology. 229:1–12.

Magwedere, K., D. Rauff, G. De Klerk, K. H. Keddy, and F. Dziva. 2015. Incidence of nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012–2014. CID 61.

Meeker, D., and J. Meisinger. 2015. Companion animals symposium: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. Anim. Sci. 93:835–847.

Meeker, D. 2009. North American rendering: Processing high quality protein and fats for feed. R. Bras. Zootec. 38:432–440.

Meeker, D. 2006. Essential Rendering. All About The Animal By-Products Industry. National Renderers Association, USA.

Mekonnen, T., P. Mussone, and D. Bressler. 2014. Valorization of rendering industry wastes and co-products for industrial chemicals, materials, and energy: A review. Crit. Rev. Biotechnol. 2014:1–12.

Molina, A., F. Granados-Chinchilla, M. Jiménez, M. Acuña, M. Alfaro, and G. Chavarría. 2016. Vigilance for salmonella in feedstuffs available in Costa Rica: Prevalence, serotyping and tetracycline resistance of isolates obtained from 2009 to 2014. Foodborne Pathogens and Disease. 13:119–127.

Molina, A., and F. Granados-Chinchilla. 2015. Inocuidad microbiológica de los alimentos para animales en Costa Rica. Nut. Anim. Trop. 9:13–31.

Murray, S. M., A. R. Patil, G. C. Fahey, N. R. Mechen, and D. M. Hughes. 1997. Raw and rendered animal by-products as ingredients in dog diets. J. Anim. Sci.. 75: 2497–2505.

National Renderers Association. 2015. FSC36 SAFE FEED/SAFE FOOD: Guidance for Developing, Documenting, Implementing, Maintaining and Auditing the Program. p 93. Accesed March. 2016. http://www.safefeedsafefood.org/images/pdf/FSC36%20Guidance%20Document%20FINAL%2050218.pdf.

Nieto, M., L. Cruz, D. Ricque, and M. Ezquerra. 2005. Técnica de digestibilidad invitro en ingredientes y alimentos para camarón. Ciencia Uanl. 8:65–73.

NRC. 2001. Pages 406, Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press: Washington, D.C.

NRC. 2012. Pages 424, Nutrient Requirements of Swine. 11th rev. ed. National Academy Press: Washington, D. C.

OIE. 2016. Bovine Spongiform Encephalopathy, Volume II. Recommendations applicable to OIE Listed diseases and other diseases of importance to international trade. Chapter 11.4. Terrestrial Animal Health Code. Accessed Jul. 2017. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_bse.pdf.

Padilla, M. 2008. Comportamiento de la Actividad Porcina en Costa Rica 2000–2006. Ministerio de Agricultura y Ganadería: Programa Nacional de Cerdos, Costa Rica. p 17. Accessed May. 2016. http://www.mag.go.cr/informacion/inf.tecnica%20pro.cerdos/prog-nac-cerdos-actividad-2000-2006.pdf

Papadopoulou, C., J. J. Carrique-Mas, R. H. Davies, and A. R. Sayers. 2009. Retrospective analysis of *Salmonella* isolates recovered from animal feed in Great Britain. The Veterinary Record. 165:681–688.

Rostagno, H., L. Teixeira, J. Lopes, P. Cezar, R. Flávia De Oliveira, D. Lopes, A. Soares, S. Toledo, and E. Frederico. 2011. Pages 259, Tablas Brasileñas Para aves y Cerdos. ed. Universidad Federal de Viçosa, Departamento de zootecnia. Viçosa, Brasil.

Santillana Farakos, S. M., D. W. Schaffner, and J. F. Frank. 2014. Predicting survival of Salmonella in low–water activity foods: An analysis of literature data. J. Food Prot. 9:1448–1461

Sapkota, A., L. Lefferts, S. McKenzie, and P. Walker. 2007. What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. Environ. Health Perspect. 115:663–670.

Shi, X., and X. Zhu. 2009. Biofilm formation and food safety in food industries. Trends in Food Science & Technology. 20: 407–413.

Tessari, E. N. C., A. L. S. P. Cardoso, A. M. I. Kanashiro, F. Greice, Z. Stoppa, R. L. Luciano, and A. G. Machado de Castro. 2014. Analysis of the presence of *Clostridium perfringens* in feed and raw material used in poultry production. FNS 5: 614–617.

Vestby, L., T. Moretro, S. Langsrud, E. Heir, and L. Nesse. 2009. Biofilm forming abilities of Salmonella are correlated with persistence in fish meal and feed factories. BMC Vet. Res. 5:20.

Woodgate, S., and J. Van der Veen. 2004. The role of fat processing and rendering in the European Union animal production industry. Biotechnol. Agron. Soc. Environ 8:283–294.

Yamka, R., U. Jamikorn, A. True, and D. Harmon. 2003. Evaluation of low-ash poultry meal as a protein source in canine foods. J. Anim. Sci. 81:2279–2284.

Zhang, Y. Q., Y. B. Wu, D. L. Jiang, J. G. Qin, and Y. Wang. 2014. Gamma-irradiated soybean meal replaced more fish meal in the diets of Japanese seabass (*Lateolabrax japonicus*). Anim. Feed Sci. Technol. 197:155–163.