NOTA TÉCNICA:

MICROBIOLOGICAL QUALITY OF ANIMAL MEALS PROCESSED IN UNITS LOCATED IN THE MINAS GERAIS STATE, BRAZIL

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ABSTRACT

Animal meals present potential for microbiological contamination, however they are still widely used in animal feeds. The objective of this study was to evaluate the microbiological quality of these meals processed in eight units distributed in the Minas Gerais state. Samples of viscera meals (248), meat and bone meals (224), and feather meals (216) were collected for microbiological evaluation from the receiving area of a feed mill located in the city of Viconde do Rio Branco, MG, Brazil. The results showed a high index of bacterial contamination of the species *Salmonella* sp. ($5.3x10^2$) and *Staphylococcus* sp. ($4.5x10^5$), total coliform ($2.8x10^5$), thermotolerant coliforns ($3.3x10^5$), total mesophiles ($4.7x10^5$), and fungi colonies ($3.6x10^5$). The animal meals processed in the unit "A" showed the highest levels of microbiological contamination. Additionally, the viscera meals were the by-products with lowest quality. Furthermore, processing units of animal meals cannot guarantee the microbiological control of products and may contaminate the final feed. It was concluded that the animal meal does not meet the minimum quality for commercialization in feed mills.

Keywords: byproducts, control, feed, ingredients, safety.

RESUMO

QUALIDADE MICROBIOLÓGICA DAS FARINHAS DE ORIGEM ANIMAL PROCESSADAS EM UNIDADES LOCALIZADAS NO ESTADO DE MINAS GERAIS, BRASIL

As farinhas de origem animal apresentam potencial de contaminação microbiológica, no entanto, são ainda muito utilizadas na formulação de dietas para animais. O objetivo deste estudo foi avaliar a qualidade microbiológica das farinhas de origem animal processados em oito unidades distribuídas no estado de Minas Gerais. As amostras de farinhas de vísceras (248), de carne e ossos (224), e farinhas de penas (216) foram coletadas para avaliação microbiológica no recebimento de uma fábrica de ração, localizada na cidade de Viconde do Rio Branco, MG. Os resultados demonstraram um elevado índice de contaminação bacteriana pelas espécies *Salmonella* sp. ($5,3x10^2$) e *Staphylococcus* sp. ($4,5x10^5$), coliformes totais ($2,8x10^5$), coliformes termotolerantes ($3,3x10^5$), mesófilos totais ($4.7x10^5$), e colônias de fungos ($3,6x10^5$). As farinhas de origem animal processadas na unidade "A" apresentaram os maiores níveis de contaminação microbiológica. Além disso, as farinhas de vísceras foram os subprodutos com qualidade inferior. As farinhas de origem animal obtidas nas unidades de processamento estão fora do padrão exigido pela legislação no que se refere a qualidade microbiológica e colocam em risco a qualidade final da ração. Concluiu-se que as farinhas de origem animal não atendem a qualidade mínima para serem comercializadas para as fábricas de rações.

Palavras-chave: controle, ingredientes, rações, segurança. subprodutos.

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INTRODUCTION

In the chicken slaughtering process, portions of the birds are discarded because they are unfit for human consumption. These materials should be sent to a destination that does not cause risk to the environment and most importantly, that complies with laws regulating waste disposal. However, an alternative to normally discarding the waste is to transform these wastes into products to alleviate the shortage of raw materials for poultry feed, permitting that they are reutilized. In Brazil the first scientific studies on the use of poultry slaughterhouse by-products as a protein source in diets for broilers began from the 1960s and this information was used for a long time in feed formulations, but the need for improved livestock has fueled the search for improved technologies for precise formulations of diets.

Animal meals, resulting from the processing of residues not consumed (meat, viscera, bones, feathers and etc.), are dietary supplements rich in nutrients, constituted of high biological value proteins, minerals, salts and vitamin B compounds. The meals are considered a primordial nutritional ingredient in feed elaboration for domestic animals, with a growing market (ANDRIGUETTO *et al*. 1990; TAUXE, 2002).

Microbiological control of animal meals destined for animal nutrition is of great importance, because the ingestion of bacteria-polluted raw material may cause serious problems for the animals that ingest them (MURRY et al., 2004). The microbiological quality of animal meals basically depends on contamination of the raw material, final product contamination and of storage conditions (PATRICK *et al*., 2004).

The characteristics of meat and bone meals make the them susceptive to physic-chemical alterations by several photogenic microbes (MACIOROWSKI *et al*., 2005). The *Salmonella* sp. has selectivity for different animal species and they present variability resistance to environmental conditions (RAMIREZ *et al*., 2005). Oliveira *et al*. (2003), when evaluating critical points of contamination by *Salmonella* sp. in the process of viscera meal production, observed that thermal treatment utilized in industrial processing is capable of eliminating the bacteria in viscera meal, but contamination can occur again during the cooling and storage phases. Moreover, the author highlighted that utilizing polluted viscera meal could favor the persistence of *Salmonella* sp. Therefore, the use of viscera in the meal form should be submitted to permanent control in order to prevent that meals leave the factory contaminated with *Salmonella* sp. The practice employed for alimentary products in the determination of hygienic quality of the foods is the determination of indicative organisms. In relation to the microorganisms most indicative or representative of sanitary quality, the group of fecal coliforms stands out and, in the case of the rations, the presence of salmonellas.

The objective of this study was to evaluate the microbiological quality of animal meals processed in eight different units located at Minas Gerais State and used as ingredients in feed formulations. The specifics objectives of the study were to:

- Characterize the animal meal processing units with respect to microbiological quality;
- Identify the types of microbial contamination present in animal meals;
- Provide microbiological quality parameters for animal meals to feed manufacturing industries.

MATERIAL AND METHODS

This study was conducted at the company Pif Paf Alimentos S/A and its suppliers of animal meals (eight processing units) located in the different regions of the state of Minas Gerais (Figure 1), in partnership with the Department of Agricultural Engineering (DEA), Federal University of Viçosa (UFV).

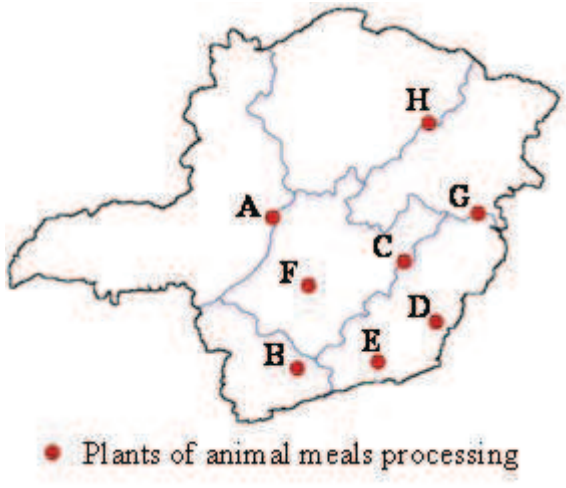

Figure 1. Locations of the animal meals processing units, Minas Gerais, Brazil.

As described in Figures 2 and 3, the basic process of animal meal production consisted of recovery of non-edible wastes from animal slaughter.

The by-products are crushed and then processed in digesters for pressure cooking. In the processing of animal meals, the inedible offal, feathers, bones and meat are sent to a warehouse and then passed through a rotating screen where the solid and liquid phases are separated (Figure 3).

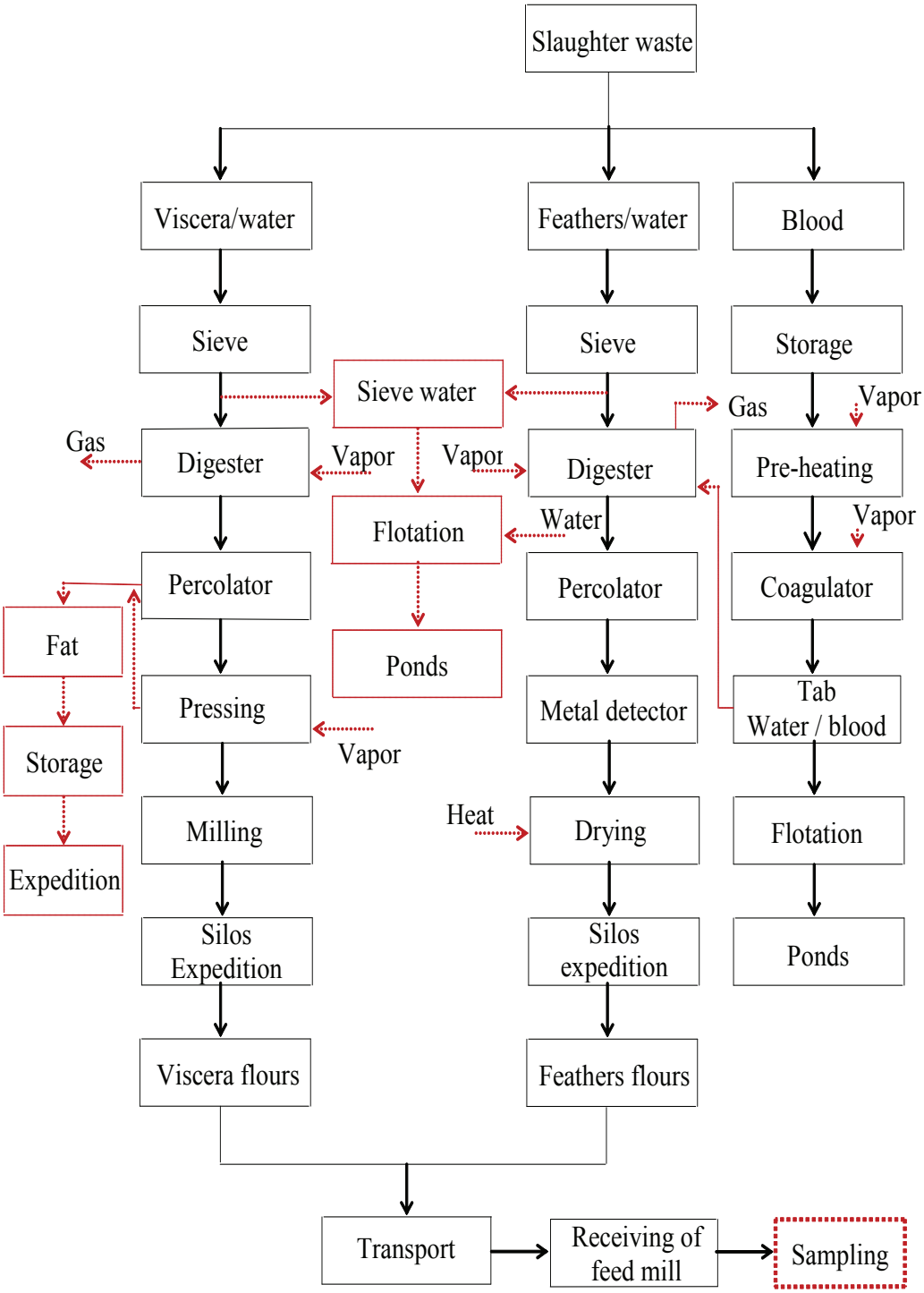

Figure 2. Processing of viscera and feather meals.

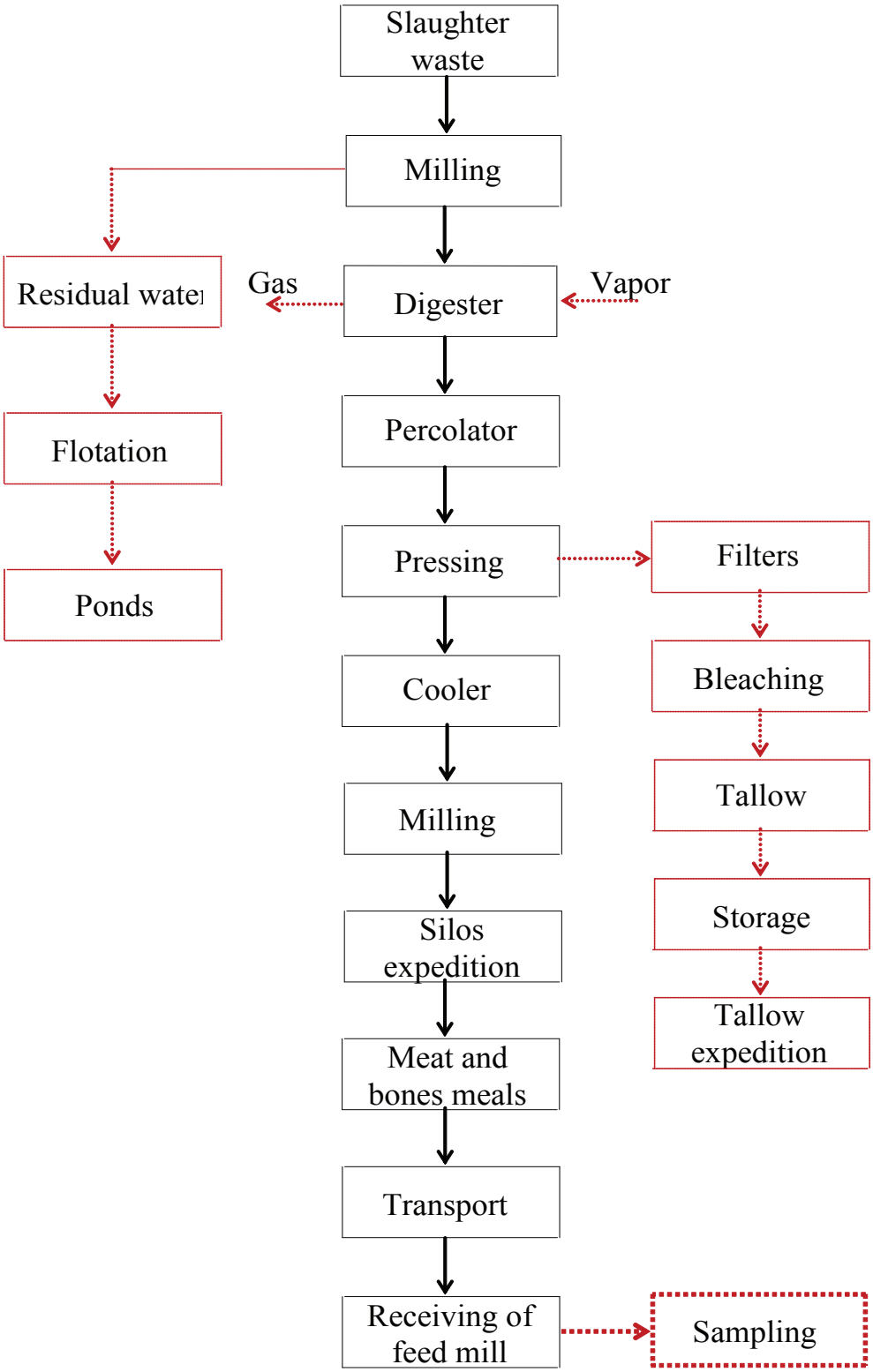

Figure 3. Processing of meat and bone meals.

The liquids resulting from the process are sent by pipe to a gas scrubber, while the solid portion is directed to a storage silo. In the digester, the separated residue is subjected to autoclave sterilization, at a temperature of 121 °C for 20 minutes at a pressure of 5.5 $kgf.cm^{-2}$. The supernatant, composed of water and fat, is separated in a funnel and the water discarded. After decanting the fat, the sludge accumulated on the bottom is sent back to the digester. Fats are stored and later transported to the feed mill. The solid material is cooled to room temperature and milled to pass through 6 mm sieves.

Sampling of the animal meals was performed at the raw materials receiving area of the feed mill. Lots of meals in bulk samples were collected from the transport trucks before unloading with the aid of a mechanical auger. The size of the lots was determined randomly according to the number of sampling points in the load. For every ten tones of product, ten meal samples were obtained. The samples collected were mixed and reduced to three sub samples of 1 kg, packed in plastic bags, certified and sent for analysis. In total, 248 samples of viscera meal, 224 samples of meat and bone meal, and 216 samples of feather meal were evaluated.

The microbiological analyses of *Salmonella* sp. ($CFU.g^{-1}$), *Staphylococcus* sp. ($CFU.g^{-1}$), total Coliforms ($MPN.g^{-1}$), thermotolerant coliforms ($MPN.g^{-1}$), total Mesophiles ($NMP.g^{-1}$), and fungi ($CFU.g^{-1}$) were performed in the laboratory of the feed industry (Pif Paf Alimentos S/A). Counting of fungi colonies was determined by the Dhingra and Sinclair (1995) method. For this, 25 g of the animal meals was weighted in a sterile stomacher bag and then mixed with 225 mL of the saline water and peptone 0.1%, yielding a 10^{-1} dilution.

After homogenization of the sample, serial dilutions were performed up to 10^{-6}, using test tubes with 9.0 mL of the same diluents. For the cultivation process the surface plating technique was used, utilizing acidified potato dextrose agar medium (acidified P.D.A.) with 10% tartaric acid to a pH of 3.5. Each plated was inoculated with 0.1 mL of each sample dilution pipetted onto a sterile petri dish containing 20 mL of the solidified acidified P.D.A. culture medium. Using a drigalski loop, the inoculums were spread over the entire surface of medium. Samples were then incubated at 25 ºC ± 1 ºC for four days. The analyses of *Salmonella* sp. and *Staphylococcus* sp. were performed via the general method of the American Public Health Association-A.P.H.A. (SPECK, 1984).

For counting of *Salmonella* sp. colonies, the samples placed in Muller Kauffmann tetrathionate broth were incubated at 37 ºC for 18 h. Counting of *Staphylococcus* sp. colonies was done in a specific baird-parker culture medium (B.P.) incubated at 35 °C ± 1 ºC for 48 h. The total count of Mesophiles was conducted in plate count agar (P.C.A.) at 37 ºC ± 1 ºC within 24 h of incubation. The total thermotolerant coliforms was determined by the multiple tubes method, utilizing the products of sodium lauryl sulphate broth (S.L.S.) and brilliant green bile lactose broth (B.G.B.L.) (Biobrás S.A.), respectively. For determination of thermotolerant coliforms, aliquots of 1 mL of each dilution (up to 10^{-6}) were transferred to three sets of tubes containing S.L.S., with inverted Durham tubes. Next, the tubes were incubated at 35 ºC ± 1 ºC for 24 h. A loop of each tube with growth and gas production was inoculated in tubes containing 10 mL of E.C., with inverted Durham tubes. The tubes containing B.G.B.L. were incubated at 35 ºC ± 1 ºC for 24 and 48 h, while E.C. tubes were incubated for 24 and 48 hours in a 45 ºC water bath. The formation of gas in the tubing indicated the presence of total Coliforms. All analysis was performed in triplicate.

RESULTS AND DISCUSSION

In accordance with auditing standards of foods (ANVISA RDC nº.12, 2001), a standard was elaborated for evaluating the microbiological quality of animal meals (Table 1), depending on the quality requirements of the feed processing industries.

Animal meals are derived from processing wastes not used for direct human consumption, but are rich in nutrients, composed primarily of proteins, minerals, salts and vitamins. Thus, they have been widely used in the formulation of diets for animals. However, some factors, mainly microbial contamination, limit its use. Table 2 characterizes the percentage of animal

Table 1. Microbiological quality of animal meals used in the formulation of feeds

Types of microorganisms	Good	Acceptable	Unacceptable
Salmonella sp. (CFU.g^{-1})	-	Ausent	Present
Staphylococcus sp. (CFU.g^{-1})	$<10^3$	10^3 to 10^5	$>10^5$
Total Coliforms (MPN.g^{-1})	$<10^3$	10^3 to 10^5	$>10^5$
Thermotolerant coliforms (MPN.g^{-1})	$<10^3$	10^3 to 10^5	$>10^5$
Total Mesophiles (NMP.g^{-1})	$<10^6$	10^6 to 10^7	$>10^7$
Fungi (CFU.g^{-1})	$<10^4$	10^4 to 10^5	$>10^5$

ANVISA (RDC nº. 12, 2001).

Table 2. Percentage (%) of animal meal samples contaminated by bacteria and fungi

Types of Microorganisms	Viscera meals[a] (%)	Feather meals[b] (%)	Meat and bone meals[c] (%)
Salmonella sp. (CFU.g^{-1})	72.00	80.00	71.43
Staphylococcus sp. (CFU.g^{-1})	44.00	66.67	77.14
Coliforms (MPN.g^{-1})	56.00	60.00	62.86
Thermotolerant coliforms (MPN.g^{-1})	40.00	46.67	54.28
Mesophiles (NMP.g^{-1})	64.00	80.00	68.57
Fungi (CFU.g^{-1})	68.00	80.00	80.00

248 samples of viscera meals[a], 224 samples of meat and bone meals[b] and 216 samples of feather meals[c].

meal samples contaminated by fungi and bacteria species (P>0.05). The meat and bone meals presented the lowest indexes of microbiological quality. *Salmonella* sp. and fungi species were the microorganisms most observed in the samples.

In the results obtained (Table 3 and 4) it was observed that regardless of the processing unit where the animal meals were produced, the indexes of microbiological contamination are generally below the recommended standards of food safety and for use in animal feed formulation (Table 1).

There may be many factors that affect the quality of animal meals, however, during the stages of production, processing conditions, storage form and hygiene were the main critical points. Nevertheless, it was also observed that when utilizing heat treatment for processing of animal meals, it can eliminate most of the microorganisms. Contrarily, the cooling step maintains the presence of bacteria especially for a longer period, which then develop during storage. It is estimated that these were the reasons for increased levels of microbial contamination in animal meal samples analyzed from the receiving unit of the feed mill (Table 4). Thus, the use of animal meals of low microbiological quality should be avoided in the feed. Preliminary inspection should be performed at the output of the processing units or upon receipt at the feed mill, giving preference to meals subjected to special treatment with high temperatures and adequate safety measures.

Although this study showed that the processing units generate animal meals that are not subjected to careful evaluation of the levels of microbial contamination, the feed manufacturing units accept these products in function of their low cost, and they are used in feed formulations without further control. According to some authors the economic

losses caused by contaminated animal meals are high, and directly affect the production of animals and quality of final products. Some of the most cited are the diseases caused by *Salmonella* sp., especially avian salmonellosis (DAVIES et al., 2004), where were observed over time in poultry houses in the animal feed factory farms evaluated. They caused pillories in young broilers (white diarrhea), typhoid aviary in adult broilers (greenish diarrhea) and avian paratyphoid in adult and young broilers.

Table 3. Average count of bacteria and fungi in viscera, meat and bone, feathers meals from different processing units within the State of Minas Gerais

Flours of aninal origin	Processing units	*Salmonella* sp.	*Staphylococcus* sp.	Fungi
		CFU.g^{-1} S.D.	CFU.g^{-1} S.D.	CFU.g^{-1} S.D.
Viscera meals[a]	A[nv]	1.6x10^{1} 3 u	2.2x10^{5} 70 u	3.1x10^{5} 84 u
	B[nv]	0.9x10^{2} 10 u	3.1x10^{5} 80 u	6.1x10^{4} 67 a
	C[nv]	1.4x10^{2} 15 u	4.4x10^{4} 55 a	5.6x10^{3} 38 g
	D[nv]	3.1x10^{1} 5 u	4.5x10^{5} 67 u	6.4x10^{4} 45 a
	E[nv]	2.8x10^{2} 20 u	2.0x10^{5} 71 u	3.6x10^{5} 40 u
	F[nv]	3.1x10^{2} 23 u	2.9x10^{5} 64 u	2.9x10^{5} 42 u
	G[nv]	4.0x10^{1} 4 u	3.6x10^{4} 78 a	5.2x10^{3} 40 g
	H[nv]	5.3x10^{2} 25 u	3.2x10^{3} 82 a	2.4x10^{4} 62 a
Meat and bone meals[b]	A[nmb]	3.6x10^{1} 4 u	1.2x10^{4} 72 a	3.3x10^{4} 45 a
	B[nmb]	2.3x10^{2} 12 u	4.6x10^{4} 38 a	2.1x10^{5} 67 u
	C[nmb]	4.1x10^{1} 5 u	4.1x10^{3} 48 a	3.8x10^{4} 36 a
	D[nmb]	5.1x10^{1} 6 u	5.5x10^{4} 57 a	2.4x10^{4} 45 a
	E[nmb]	1.4x10^{1} 3 u	1.2x10^{4} 73 a	3.3x10^{4} 56 a
	F[nmb]	3.1x10^{1} 4 u	4.6x10^{4} 38 a	2.1x10^{5} 60 u
	G[nmb]	2.7x10^{2} 13 u	4.1x10^{3} 52 a	3.8x10^{4} 63 a
	H[nmb]	4.6x10^{1} 2 u	5.5x10^{4} 59 a	2.4x10^{4} 63 a
Feathers meals[c]	A[nf]	1.0x10^{1} 2 u	1.3x10^{3} 27 a	3.1x10^{3} 45 a
	B[nf]	0.5x10^{2} 3 u	2.2x10^{4} 34 a	3.2x10^{4} 47 a
	C[nf]	1.1x10^{1} 3 u	3.3x10^{2} 43 g	2.7x10^{4} 48 a
	D[nf]	0.9x10^{1} 4 u	4.1x10^{3} 44 a	1.2x10^{3} 36 g
	E[nf]	0.4x10^{1} 3 u	1.6x10^{3} 42 a	3.6x10^{4} 39 a
	F[nf]	0.1x10^{1} 2 u	2.1x10^{4} 51 a	2.6x10^{5} 72 u
	G[nf]	1.0x10^{1} 3 u	3.3x10^{3} 30 a	2.9x10^{4} 54 a
	H[nf]	0.9x10^{1} 2 u	2.5x10^{3} 47 a	1.4x10^{4} 60 a

[nv]Samples of viscera meals evaluated (31), [nmb]Samples of meat and bone meals evaluated (28), [nf]Samples of feather meals evaluated (27). Score of evaluation: [g](Good), [a](Acceptable), [u](Unacceptable). Colony Forming Units per gram (CFU.g^{-1}). Standard Deviation (S.D.).

Table 4. Average count of bacteria and fungi in viscera, meat and bone, feathers meals from different processing units within the State of Minas Gerais

Flours of aninal origin	Processing units	Total Coliforms	Thermotolerant coliforms	Total Mesophiles
		MPN.g^{-1} S.D	MPN.g^{-1} S.D	MPN.g^{-1} S.D
Viscera meals[a]	A^{nv}	3.6x10^{3} 67 a	4.7x10^{4} 66 a	2.3x10^{4} 48 g
	B^{nv}	4.2x10^{4} 76 a	2.3x10^{3} 55 a	3.5x10^{5} 42 g
	C^{nv}	3.8x10^{3} 45 a	5.1x10^{2} 21 g	3.5x10^{3} 36 g
	D^{nv}	2.8x10^{5} 62 u	4.2x10^{4} 34 a	3.6x10^{4} 52 g
	E^{nv}	2.7x10^{4} 68 a	3.3x10^{5} 89 u	4.3x10^{4} 50 g
	F^{nv}	0.9x10^{4} 72 a	1.2x10^{4} 96 a	3.1x10^{4} 62 g
	G^{nv}	3.5x10^{3} 29 a	4.1x10^{3} 33 a	2.6x10^{4} 43 g
	H^{nv}	3.2x10^{4} 26 a	2.6x10^{4} 46 a	5.0x10^{4} 41 g
Meat and bone meals[b]	A^{nmb}	3.8x10^{4} 48 a	1.7x10^{5} 66 u	2.6x10^{5} 71 g
	B^{nmb}	2.6x10^{5} 67 u	3.4x10^{4} 54 a	4.3x10^{4} 67 g
	C^{nmb}	2.7x10^{4} 73 a	4.5x10^{3} 43 a	4.3x10^{4} 58 g
	D^{nmb}	3.4x10^{4} 74 a	6.1x10^{3} 41 a	4.7x10^{5} 65 g
	E^{nmb}	3.8x10^{4} 64 a	1.7x10^{5} 56 u	2.6x10^{5} 73 g
	F^{nmb}	2.6x10^{5} 76 u	3.4x10^{4} 67 a	4.3x10^{4} 66 g
	G^{nmb}	2.7x10^{4} 82 a	4.5x10^{3} 39 a	4.3x10^{4} 63 g
	H^{nmb}	3.4x10^{4} 85 a	6.1x10^{3} 40 a	4.7x10^{5} 54 g
Feather meals[c]	A^{nf}	3.4x10^{3} 32 a	1.3x10^{4} 63 a	2.0x10^{4} 45 g
	B^{nf}	2.8x10^{3} 36 a	2.5x10^{4} 57 a	1.2x10^{4} 53 g
	C^{nf}	1.8x10^{3} 30 a	3.4x10^{4} 63 a	2.1x10^{4} 54 g
	D^{nf}	2.2x10^{4} 53 a	4.2x10^{3} 62 a	1.5x10^{4} 56 g
	E^{nf}	2.9x10^{4} 55 a	2.4x10^{4} 67 a	2.6x10^{4} 58 g
	F^{nf}	2.1x10^{4} 43 a	2.7x10^{3} 38 a	3.9x10^{4} 48g
	G^{nf}	1.8x10^{3} 46 a	3.6x10^{3} 38 a	4.3x10^{4} 49 g
	H^{nf}	2.4x10^{3} 38 a	4.0x10^{3} 41 a	3.7x10^{5} 62 g

[nv]Samples of viscera meals evaluated (31), [nmb]Samples of meat and bone meals evaluated (28), [nf]Samples of feather meals evaluated (27). Score of evaluation: [g](Good), [a](Acceptable), [u](Unacceptable). Colony Forming Units per gram (CFU.g^{-1}). More Probable Number per gram (MPN.g^{-1}). Standard Deviation (S.D.).

Salmonella causes toxicity in humans upon consumption, mainly in the form of poultry products such as meat, eggs and egg products. The process of *Salmonella* sp. contamination in the production chain of poultry involves the transmission in the egg, triggering infection of the chicks. Transmission may also occur to reservoir animals with bacteria, such as rodents and insects.

In horizontal transfer of the feed, the poultry farm industries were evaluated according to the addition of animal meal contaminated by bacteria in the feed formulation. The presence of fungi in animal meals (Table 3) accounted for major losses in terms of quality. According to Bennet and Klich (2003), aflatoxins are one of the main toxins produced by the fungi species *Aspergillus flavus*

found in samples. Its action is toxic when present in animals and results in poorer performance, including reduced activity of pancreatic enzymes and decreased concentration of bile (BENNET; KLICH, 2003), increased incidence of injury to the sciatic nerve (WHITAKER, 2003) and antagonism to the metabolism of vitamins, proteins and amino acids, lipids and carbohydrates, coenzymes, or acting on enzyme complexes, mainly in the liver and affect the chemical structure of DNA (HALLOY *et al*., 2005).

Bacteria of the genus *Staphylococcus* found in high levels in animal meal (Table 3 and 4) require various nutrients for their development. This species occurred more in meat products (meat and viscera meals). The origin of this bacterial contamination was the raw material and feed handler. The consequences of *Staphyloccous aureus* and *Aspergillus* sp. contamination in animal meals have been the formation of toxins in broilers, observed over time. The presence of Coliforms in animal meal was due to fecal contamination and low sanitary conditions observed in the processing of by-products.

CONCLUSION

It was concluded from this study that:

- Animal flours showed variability in their microbiological quality;
- Animal flours processed in different units within the State of Minas Gerais did not meet the microbiological quality standard set for the feed industry;
- It was concluded that the animal meal does not meet the minimum quality to be commercialization for feed mills.

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