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SURVEILLANCE OF SOME ENTERIC PATHOGENS IN COMMERCIAL FEED MILLS

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ABSTRACT

To study the role of feed mills as a source of infection of poultry and fish through contamination by different microorganisms, samples were obtained from different feed ingredients and from finished products. Bacteriological examination of different feed ingredients revealed that isolation % of salmonella species, *E. coli* and *Clostridium perfringens were* (49.3%, 53.6% and 4.3%, respectively). Soya meal had the highest salmonella and mould counts (74×10^2 and 47×10^3 CFU/g respectively), Yellow corn had the highest coliform count ($12x10^2$ CFU/g) as well as, poultry offal powder only showed *Clostridium perfringens* (50×10^5 CFU/g). Improvements in the safety of animal and poultry feed should include strengthening the surveillance of the feed for bacterial contamination in order to reduce their potential hazard after feed consumption.

Keywords: Feed mills, ingredients, *Salmonella, E. coli, Clostridium*, Mould

INTRODUCTION

The microbiology of animal feeds became imperative in views of recent bird infections and disease outbreaks in Egypt. A potential and more deadly hazard has been associated with the consumption of microbial toxins of bacterial and fungal

origin in feed (**1).** Mold and mycotoxin contamination of feed and feed ingredients occurs worldwide and because of the ubiquitous nature of these micro-organisms, they cannot be totally eliminated from feeds and feed ingredients (**2).** Feed ingredients remain a source of feed contamination. Dust must be considered a major contamination source. It would appear that dust accumulation around the pellet mill could effectively negate the sanitizing effects of pelleting **(3).** Formic acid at 0.5–1.5% in feed was associated with reduced counts of *S. gallinarum* (introduced continually in the feed) throughout the alimentary tract of young broilers **(4)**. Feed may be contaminated during processing, storage or transport. Contaminated feed frequently causes zoonozes and for that reason, it is necessary to establish surveillance programs for microbiological feed hazards. Microbiological quality control programs are increasingly applied throughout food chain production in order to minimize the risk of infection for the consumer. Even with improved methods for detecting pathogens in foods, microbiologists so mandated often face a "needle-in-a-haystack" challenge **(5; 6; 7).** The effects of organic acids on bacterial virulence and acid tolerance have led some to express concern that the use of such compounds in feed may enhance the pathogenicity of surviving bacteria **(8; 9)**.Animal feed ingredients, particularly animal and plant-derived protein meals, are frequently contaminated with Salmonella species either from the source or from the processing plant. Recontamination in compounding mills is an additional problem. For ease of application and safe handling of treated feed, organic acids may be used in the form of stabilized preparations, salts, or appropriate mixtures of salts and straight acids. The organic ions will exert much of their effect only when the feed is ingested, that is, in the dissolved state and associated with protons in a low pH environment **(10).** Considering the health hazard posed to animal/poultry and the unsuspecting consumers of such contaminated feeds and its overwhelming socioeconomic impact, it is penitent to undertake this study. The present work was designed to identify the bacterial and fungal flora of commercially available poultry and fish feed ingredients and finished feeds sold in Egypt.

MATERIALS AND METHODS

Traditional and standardized analysis of animal feed ingredient for the presence of bacteria relies on culture enrichment step, selective and differential plating, followed by subsequent identification by morphological, biochemical and/or

immunological tests. These methods have low limits of detection and can be used in complex food sample matrices, but they are labour-intensive and typically require days from initiation to readout **(6).**

1-Sample collection:

Samples either of different feed ingredients or finished feeds were obtained from three feed mills located at Giza Governorates, Egypt during the period extended from January 2013 to January 2014 **(Table 1**) according to the method described by **(11)**. The samples were transported in a cooler to the laboratory within 2 hr.

2. Bacteriological isolation:

The samples were analyzed within 2-6 hours from collection. The different media such as nutrient agar (NA), nutrient broth (NB), SS agar (Salmonella-Shigella Agar), BGA (Brilliant Green Agar), EMB **(**eosin methylene blue) and McConkey were prepared separately.

For culturing, 10 g feed samples were taken and ground. 90 ml peptone water was poured into the beaker and mixed with the samples. Then, 900µl PBS was taken to each of the small bottles accordingly. 100µl mix sample from beaker was taken to one of the small bottles for serial dilution. Serial dilution was made up to 10^{-4}. All the feed samples were measured in 1 g and taken to the nutrient broth in the test tube separately and kept in the incubator at 37^{o}C overnight. These appropriate dilutions were cultured by spread plate technique on the nutrient agar plates .Inoculated nutrient agar plates were incubated overnight at 37^{o}C. The serial dilution of other samples were done by the same way and incubated overnight at 37^{o}C. The colony forming units on the nutrient agar media were counted as Total viable count (TVC). The colonies of the nutrient agar were sub-cultured on the selective media and inoculated for the identification of *Salmonella, E. coli and Clostridium species* bacteria from the different feed samples and were incubated at 37^{o}C overnight. *Salmonella, Clostridium species* and *E. coli* were identified by the color of the colonies on the selective media. According to **(12),** the bacteria were counted in the particular media by the mean of their colonies of the different media and multiplied by 1000 µl (i.e 1 g sample).

3-Determination of total Coliform, total viable bacteria and Mold counts of different feed ingredients and finished feeds:

Samples of different feed ingredients including Soya 44%, Yellow corn, DDGs, Fish meal 65%, Corn gluten meal 60%, Bran mash, Rice polish, Poultry offal powder and Bone meal as well as finished feeds (breeder production I mash, breeder

production I pellet, broiler starter mash, broiler finisher pellet, tilapia floating feed 25% and tilapia sinking feed 25%) were subjected to Total viable bacterial count & Total mould and yeast count using aerobic count plate method (table 2 & 3) according to **(13**), as well Total Coliform Count according to **(14).**

4-Identification of bacterial agents:

Twenty five grams of each sample were mixed with 225 ml of buffered peptone water (Oxoid for pre-enrichment. After incubation at 37°C for 24 hours, 0.1 ml were transferred to 10 ml of selective Rappaport-Vassiliadis broth (Oxoid) and were incubated for 24 hours at 42°C. A loopful of broth culture was streaked on xylose lysine desoxycholate agar (XLD, Oxoid), and Hektoen enteric agar (Oxoid Ltd) for isolation of salmonella under aerobic condition at 37°C for 24-48h. All samples were also cultivated on MacCkoney and EMB under aerobic condition at 37°C for 24-48h for isolation of *E-coli* **(15)** & 200 µg/ml Neomycin blood agar medium under anaerobic condition at 37°C for 48h for isolation of Clostridium (**16).** Colonies were inoculated onto microtubes of API 20E strips (bioMe´rieux, France) in accordance with the manufacturers' instructions. The bacteria were identified using the database API LAB Plus version 3.2.2 (bioMe´rieux).

5- Serotyping of the isolates:

Serotyping of *Salmonella* isolates depending on diagnostic polyvalent and monovalent *Salmonella* O and H were obtained SIFIN (Institute fur Immunpruparate und Nuhrmedien Gmbh Berlin, Berliner Allee 417/ 321, D- 13088 Berlin). The serotyping was done according to **(17).**

Serotyping of *E.coli* isolates depending on O and H antigens were conducted in Microbiology Department, Faculty of Veterinary Medicine, Cairo University (SSI Diagnostic Company).

RESULTS AND DISCUSSION

Data in Table (2) show that; yellow corn had the highest total coliform Count (12 x 10^2 CFU/g). Soya revealed highest *Salmonella* species count (74 x 10^2 CFU/g). Poultry offal powder was the only ingredient that revealed presence of *Clostridium* species (20 x10^2 CFU/g) with 30% of isolation. Poultry offal powder also was contaminated with *Salmonella* (6 x 10^4 CFU/g) with 50% isolation with no mycotic existence. Animal products including fish meal were frequently contaminated with enteric bacteria in varying numbers and differences were mainly due to the procedure and handling of fish meal **(18; 19*).***

Salmonella species could be isolated from all examined feed ingredients except

DDGs. **(20)** noted that while laboratory testing of commercially manufactured feeds may produce salmonella negative results, contamination is not uniformly distributed, and the few cells present are often damaged, making detection even more difficult. Beside, salmonella contamination of food products can significantly reduce consumer demand and affect producer profits **(21)**.

The water used for feed processing participated in the microbial input where it harbored highest total bacterial (80 x 10^8), Mycotic (45 x 10^6), *E. coli* count (**42 × 10^5)** and Salmonella (**2 × 10^2)** CFU/g. These results are higher than the acceptable limits of contamination of the products as reported by **(22)** where bacterial count was acceptable at <1,000 (CFU.100 ml-1 or CFU.10 cm-2 or Petri plate) and fungi count limit < 100 (CFU.100 ml-1 or CFU.10 cm-2 or Petri plate). In addition to the directly observed contamination in raw materials and processed products in feed mills, the risks of contamination caused by water is a hidden factor that usually increase the risk. So, the prevention of contaminations in the water supply is the first defense line **(23).**

The results indicate that the % of Salmonella isolation was higher in water samples (100%) followed by rice polish (70%), soya 44% (60%) and poultry offal powder (50%). **(24),** found that 82% of animal-derived ingredients and 37% of plant-derived ingredients were positive for Salmonella.

The % of E coli isolation was higher in water samples (100%), followed by soya 44%, poultry offal powder (70%) and rice polish (50%). E. coli was reported as a common microflora in raw feeding materials and poultry feeds (**25**). However, (**26)** found that corn samples were free from Salmonella and E-coli spp. and he added that E. coli was isolated at rates of 6% from the 15 fish meal samples and 7% from 13 bone meal samples.

Data in Table (3) show that breeder production 1 (pellet) ration had the highest total mycotic count (5 x 10^2 CFU/g). Despite breeder production 1 (mash) didn't reveal high *Salmonella* spp. and *E. coli* count, it still considered as a health risk to poultry. Primary breeder officials recently reported that during the last decade, close to 80% of the *Salmonella* serotypes isolated during routine monitoring of feeds and feed ingredients were the same serotypes found weeks later during the monitoring of breeding flocks and their offspring **(27).**

The isolation rate of E coli and Salmonella are higher in Tilapia Sinking feed (80% & 60%), followed by broiler finisher pellet (70% & 50%) and breeder

production I mash (60% & 40%), respectively. Breeder production (mash) and broiler feeds as well fish sinking feed revealed positive *E.coli* with highest value in tilapia sinking feed. *Escherichia coli* was recovered more frequently from feeds or feed ingredients than *Salmonella* spp where it was recovered from 48.2% of the samples. Nearly one-third of the *Salmonella* isolates came from samples where no *E. coli* was recovered suggesting that the presence of *E. coli* would not be a good screening indicator for *Salmonella* contamination in feed samples (**28).**

E. coli was reported as a common microflora in raw feeding materials and poultry feeds. In addition, animal feed can be contaminated with antimicrobial drug resistant *E. coli (***25).** *Salmonella* species could be isolated from all finished feeds except broiler starter. Salmonella contamination of complete animal feed (finished feed) is common, with studies in the United States and in European countries reporting that Salmonella contamination rates in complete animal feed (finished feed) range from 1.1% to 41.7% (**29; 30**). This result needs effective control measures as recorded by **(12).**

The widespread occurrence of *Salmonella* and *E. coli* in poultry feeds reinforces the need for effective control measures, hygiene in processing as well as handling of feeds. Broiler starter (mash) ration had the highest total coliform count (3×10^3). Also, Broiler finisher (pellet) ration revealed reduced numbers of all microbial counts Vs mash broiler starter ration that had high counts especially for *Salmonella* species, *E. coli*, *Clostridium* species and mycotic contamination. This result is in disagreement with those reported by **(29**) where they confirmed that Salmonella is commonly found in compound feeds, including those that have undergone heat treatment. Moreover, **(31)** reported that finished feeds represented an important source of *Salmonella* contamination in commercial turkey flocks. However, impact of pelleting on *Salmonella* spp in food previously studied by **(32)** who demonstrated that feed moisture and conditioning time play a crucial role determining in the lethality of the pelleting process for bacteria. Also, Chemical treatment of feed may exert its effect before it is consumed, and/or upon ingestion when the feed is moistened by the animal's alimentary secretions and encounters the pH conditions and endogenous acids in the crop, rumen, stomach, and intestines, according to species **(33).** Tilapia sinking feed 25% had the highest *E.coli* and *Salmonella* species count versus all processed rations. Salmonella is believed to be introduced

into fish feed via feed ingredients. In addition, contamination by birds, rodents and insects has been suggested and once Salmonella introduced into the mills, it persists for years as a so-called house strain (**34**).

CONCLUSION

Presence of *Salmonella* strains, E.coli and Mycotic load in raw materials is a very influential factor in order to find *Salmonella* on meal finished feeds. Pelleting broiler finisher ration reduced numbers of all microbial counts Vs mash broiler starter especially for *Salmonella* species, *E. coli*, *Clostridium* species and Mycotic counts. The level of feed microbial contamination plays a key role in order to get a high quality animal production. Having pathogen-free or feeds with safe microbial load has a positive effect over productive parameters and over end products such as meat and eggs. In order to get pathogen-free or feeds with safe microbial load, quality of raw materials and processes must be monitored, well known and controlled.

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Table (1): Number of examined samples of feed ingredients and finished feeds

Samples	No. of samples
Feed ingredients	
Soya	10
Yellow corn 44%	10
DDGs	5
Fish meal 65%	10
Corn gluten meal 60%	5
Bran mash	5
Rice polish	10
Poultry offal	10
Bone meal	5
Water	4
Finished feeds	
Breeder production I (mash)	5
Breeder production I (pellet)	5
Broiler starter (mash)	10
Broiler finisher (pellet)	10
Tilapia floating feed 25%	5
Tilapia sinking feed 25%	5

Table (2) Average bacterial counts of different feed ingredients (CFU/g)

Samples	Average TBC	Average TMC	Average T.Coliform count	Average E-coli count	% of E.coli isolation	Average Salmonella count	% of Salmonella isolation	Clostridium perfringens count
Soya 44%	64 ×105	47 ×103	1.1 ×102	46 ×103	(7/10) (70%)	74×102	(6/10) (60%)	0.00
Yellow corn	56 ×104	5 ×102	12 x102	38 ×102	(6/10) (60%)	2 ×102	(4/10) (40%)	0.00
DDGs	1.5 ×103	4 ×104	5.00	2.2 ×102	(2/5) (40%)	0.00	0.00	0.00
Fish meal 65%	75×104	0.00	9x102	51×102	(2/10) (20%)	2×102	(3/10) (30%)	0.00
Corn gluten meal 60%	1×103	9×102	12.00	1.2×102	(1/5) (20%)	0.8×102	(2/5) (40%)	0.00
Bran mash	14x104	0.00	0.5×102	1×102	(2/5) (40%)	2×103	(2/5) (40%)	0.00
Rice polish	85x107	0.00	1.5×102	8×103	(5/10) (50%)	5×102	(7/10)(70%)	0.00
Poultry offal powder	9x108	0.00	3×102	3×102	(7/10) (70%)	6×104	(5/10) (50%)	20 (3/10) (30%)
Bone meal	35x105	0.00	2×102	2×102	(1/5) (20%)	7×102	(1/5) (20%)	0.00
Water	80x108	45x106		42x105	4/4 (100%)	2x102	(4/4) (100%)	0.00
Total					(37/69) (53.6%)		(34/69) (49.3%)	(3/69) (4.3%)

TBC: Total Bacterial Counts; TMC: Total Mold and Yeast Counts

Table (3) Average bacterial counts of different finished feed (CFU/g)

Samples	TBC	TMC	Total Coliform	*E-coli* count	% of *E-coli* isolation	*Salmonella* Count	% of *Salmonella* isolation	*Clostridium perfringens* count
Breeder production I (mash)	8.2×10^5	0.00	16×10^2	1.5×10^2	(3/5) (60%)	0.4×10^2	(2/5) (40%)	0.00
Breeder production I (pellet)	9×10^5	5×10^2	5×10^2	1×10^2	(1/5) (20%)	1×10^2	(1/5) (20%)	0.00
Broiler starter (mash)	5×10^4	2×10^2	3×10^3	3×10^2	(3/10) (30%)	0.00	0.00	0.00
Broiler finisher (pellet)	1×10^3	0.00	14	2×10^2	(7/10) (70%)	1×10^2	(5/10) (50%)	0.00
Tilapia floating feed 25%	3×10^6	4×10^2	0.00	0.00	0.00	1.7×10^2	(1/5) (20%)	0.00
Tilapia sinking feed 25%	96×10^4	2×10^2	6×10^2	5×10^3	(4/5) (80%)	2.1×10^2	(3/5) (60%)	0.00
Total					(18/35) (51.4%)		(12/35) (34.3%)	0%

TBC: Total Bacterial Counts; TMC: Total Mold and Yeast Counts