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DETECTION OF SALMONELLAE IN POULTRY FEEDS BY ELISA AND THEIR CONTROL BY BIO-ADD.

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SUMMARY : Recovery of Salmonella from poultry feeds was low (2.00 %). The isolated serovars included *S. typhimurium* *S. rissen*, *S. blockley*, *S. newport*, *S. kottbus* and *S. virchow*. There was an agreement or good correlation between ELISA and culture method for diagnosis of Salmonella in poultry feeds. Addition of Bio-Add to feeds contaminated with *S. typhimurium* resulted in the inhibition of its growth.

INTRODUCTION

No doubt that salmonellae are one of the most important causative agents which infect poultry population and cause great economic losses or constitute a hazard to public health either directly through handling of infected feeds or poultry or indirectly through their consumption.

Williams (1981) considered feeds as an important source for transmission of salmonellae to poultry.Presence of salmonellae in small numbers in these feeds is undesirable and in many countries render them unfit for poultry consumption. Accordingly, different methods for treatment of such feeds against salmonellae were suggested.

Traditional methods for isolation of salmonellae from poultry feeds are considered both time and labour

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consuming. In recent years, methods have been developed to shorten the time necessary for the assay, sensitive and allow for the resuscitation and growth initiation of injuried cells. One of these methods is the ELISA in which enzyme labeled antibodies have been used to detect specific microbial antigens in these feeds.

The present work included incidence of salmonellae in poultry feeds, use of ELISA for their detection in experimentally contaminated ones and addition of Bio-Add presumed to inhibit Salmonella growth in such feeds.

MATERIAL AND METHODS

The following materials were used:

Poultry feeds: A total of 800 samples were obtained from different imported sources. They contained 340 samples from concentrates (200 ration conc., 75 broiler conc, and 65 layer conc.), 260 from animal origin (90 bone meal, 60 meat and bone meal, 60 meat meal and 50 blood meal), 75 from poultry origin (40 poultry meal and 35 poultry residue), 75 fish meal and 50 corn.

Bio-Add: 68 % formic acid, 20 % propionic acid and 12 % water, B.P. Chemicals, London.

Contamination of poultry feeds with *S. typhimurium* and effects of Bio-Add:

Three types of poultry feeds, namely, meat and bone meal, layer conc. and broiler conc. were studied. Each type was collected in 2 Macarteny bottles (10 grams each), then autoclaved for 15 minutes. The bottles were contaminated with 4×10^8 cells/gm *S. typhimurium* and incubated at 37°C for 2 hrs. Then Bio-Add (0.006 ml/gm) was added to one of the two identical ones.

All bottles were incubated at 37°C for 12 hrs. A loopful was taken after 12 and 24 hrs., then daily for 10

days, streaked on S.S. agar and examined bacteriologically.

Examination of these contaminated feeds by ELISA was carried out by preparation of *S. typhimurium* antigen according to Todd et al. (1987). Meanwhile, enzyme linked immuno-sorbent assay was performed on the basis of Emsuriler Rose et al. (1984).

RESULTS AND DISCUSSION

The demand for poultry feeds has increased tremendously in the last years as a result of expansion of poultry industry. One of the main problems in this industry is the presence of salmonellae which are transmitted to poultry farms through many ways particularly via feed-stuffs (Allerd et al. , 1967 and Williams, 1981).

Importance of Salmonella micro-organisms in poultry farms arises from their direct pathogenicity to the chickens and those which escape the infection or recover from it become commonly carriers. Accordingly, they are of public health importance as they can infect human beings either during handling contaminated poultry feeds or slaughtered poultry or through consumption of infected or contaminated improperly cooked chickens or their products.

In this work, incidence of Salmonella in concentrates, feeds of animal origin, poultry origin and fish meal was low with little variation between them which is due to their conditioning either by steaming as reported by Cox et al. (1986) who stated that salmonellae were present in about 50.00 % of feed samples before conditioning and in only about 4.00 % of all samples after conditioning and pelleting or by addition of Salmonella inhibitors as reported by Hinton and Linton (1988) about the reduction in the isolation rate of *S. kedougou* from artificially contaminated feed containing BPO 12 (mixture of 0.50 to 0.68 % formic acid and propionic acid).

Table 1 : Rate of Salmonella recovery from poultry feed-stuffs.

Feed-stuffs	Number of examined samples	Recovery rate	
		Number	Percentage
Concentrates :	340	5	1.47 %
1. Ration conc.	200	3	1.50 %
2. Broiler conc.	75	1	1.33 %
3. Layer conc.	65	1	1.54 %
Animal origin :	260	3	1.15 %
1. Bone meal	90	0	0.00 %
2. Meat and bone meal	60	2	3.33 %
3. Meat meal	60	1	1.67 %
4. Blood meal	50	0	0.00 %
Poultry origin :	75	4	5.33 %
1. Poultry meal	40	2	5.00 %
2. Poultry residue	35	2	5.71 %
Fish meal	75	3	4.00 %
Corn	50	1	2.00 %
Total	800	16	2.00 %

This low incidence was similar to reports of Vander Schaaf et al. (1962) who recovered Salmonella in 1.6 % of whole meat meal, Patterson (1969) who isolated Salmonella from 7.00 % of meat and bone meals, Patterson (1972) who reported that one out of 30 imported fish meals and 17 out of 242 meat and bone meals yielded salmonellae. Also, Skovgard and Nielsen (1972) found that 0.30 % of resterilized imported meat and bone meals were contaminated with Salmonella; Rudy (1986) isolated Salmonella from 7.50 % feed samples and Kaloyanov et al. (1987) found fewer feed samples to be contaminated with Salmonella (2.50 %).

Serovars of Salmonella isolated from poultry feeds included *S. typhimurium* which was the predominant one and was recovered from the various feed-stuffs. However, Jacobs et al. (1963) accounted *S. typhimurium* for 10-14 % of isolated salmonellae from fish meal; Abdel-Hamid et al. (1985) recovered 6 isolates of this serovar from 37 imported broiler fish meal and Bezrukavaya et al. (1989) isolated it from 11 out of 36 batches of meat and bone meal.

Other serovars were also reported, namely *S. rissen* and *S. blockley* from poultry concentrates. On the other hand, *S. newport* and *S. kottbus* were isolated from feeds of poultry origin. These last two serovars were also recorded by Velandapilla and Tonev (1964) from a table poultry processing plant and bone meal respectively. At the same time *S. virchow* was recovered from fish meal.

ELISA is one of the methods used for detection of Salmonella in poultry feeds. The comparison between ELISA and culture method exhibited that both could detect *S. typhimurium* in all examined samples from experimentally contaminated feeds during 10 days post-contamination. This means that there was an agreement between them as reported by Krysiniski and Heimsch (1977).Also, Swaminathan and Aryes (1980) recorded a good correlation between immuno-enzyme assay and the conventional culture procedure while Emsuriler-rose et al. (1984)

Table 2 : Serovars of Salmonella isolated from poultry feed-stuffs.

Feed-stuffs	Salmonella serovars	Number of isolates
Concentrates	S. typhimurium	3
	S. rissen	1
	S. blockley	1
Animal origin	S. typhimurium	3
Poultry origin	S. newport	2
	S. typhimurium	1
	S. kottbus	1
Fish meal	S. typhimurium	2
	S. virchow	1
Corn	S. typhimurium	1

and Todd et al. (1987) reported agreements of 100.00% and 82.00 % respectively between ELISA and culture method for detection of Salmonella in feedstuffs. However, ELISA was unable to identify the serovars of Salmonella.

ELISA diagnosed *S. typhimurium* in 27.30 %, 27.30 % and 18.20 % of experimentally contaminated and Bio-Add treated samples of meat and bone meal, layer concentrate and broiler concentrate respectively in contrast to culture method which could not detect this serovar in any sample. This may be due to the sensitivity of ELISA as reported by Minnich et al. (1982) thus detecting smaller numbers of the organism or due to the ability of the assay to demonstrate dead organisms. The negative ELISA after 3 days from the addition of Bio-Add is however of great interest and needs further studies to explain the reason.

Addition of Bio-Add to the experimentally contaminated poultry feeds with *S. typhimurium* greatly inhibited its growth that means it is preferable to use such chemicals in these feeds as reported by Hinton et al. (1985) who could not isolate Salmonella from any sampling of birds fed diet containing 0.50 % or 0.75 % of Bio-Add. They also recorded that addition of organic acids such as formic acid, firstly, provided the manufacturers of animal feeds with relatively few technical problems and the treatment of all feed fed to table poultry is possible. Secondly, the action of formic acid probably persists and will remain effective against subsequent re-contamination with Salmonella and thirdly, the treated diets can be fed right up to the time of slaughter since there is no withdrawal period for organic acids. On the other hand, addition of chemical disinfectants to the feed must be non-toxic agents at the concentrations used and remain undegraded in the feed until it is consumed as reported by Hinton and Linton (1988).

Table 3 : Evaluation of ELISA in comparison to culture method for detection of S. typhimurium in experimentally contaminated poultry feeds with and without addition of Bio-Add for 10 days post-contamination.

Bio-Add	Number of examined samples	Poultry feeds					
		Meat and bone meal		Layer concentrate		Broiler concentrate	
		Culture	ELISA	Culture	ELISA	Culture	ELISA
		No.+ %	No.+ %	No.+ %	No.+ %	No.+ %	No.+ %
Added	11	0 0.0	3 27.3	0 0.0	3 27.3	0 0.0	2 18.2
Not added	11	11 100.0	11 100.0	11 100.0	11 100.0	11 100.0	11 100.0
Total	22	11 50.0	14 63.6	11 50.0	14 63.6	11 50.0	13 59.1

No.+ : Number of positive samples.

% : Percentage of positive.

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