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Dept. of Animal Hygiene
Fac. of Vet. Med., Assiut University
Head of Dept. Prof. Dr. A. H. Hafez.

SURVIVAL OF *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* IN POULTRY ENVIRONMENT UNDER DIFFERENT THERMAL CONDITIONS

(With 4 Tables and 4 Figures)

By
M. M. AHMED* and *S.A. SOTOHY

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مـدى بقاء ميكروب السـالمونيلا تيفيميوريم والإشـريشـيا كـولى فى بيئـة الـدواجـن عند درجـات حـرارية مـخـتـلفـة

مصطفى أحمد ، سطوحى أحمد

أجريت هذة الدراسة بغرض الوقوف على مـدى بقـاء وحيويـة كل من ميكروب السالمونيلا تيفميوريم والإشريشيا كولى داخل بيئة الدواجن والتى شملت عينات علائق بادى وناهى وكذلك عينات فرشة دواجن حديثة وقديمة هذا بالإضافة إلى عينات تربة زراعية. وقد أوضحت نتائج الدراسة بـأن متوسط المحتوى المائى للعينات تراوح بين٢٣, ١١ إلى٢٨,٧٤ ٪ وذلك عند بدء التجربه لكل من درجتي حراره التحضين ٣٨ ، ٢٥ مْ بينما تناقص هذا المتوسط فى نهاية التجربة إلـى ٣,٠٢ – ٨,٠٣ ٪ وكذلك ٣,١٩ – ٦,٥٦ ٪ عند درجة حرارة ٣٨ ، ٢٥مْ على التوالى. وقد لوحـظ أن فترة بقـاء كـل مـن ميكروب السالمونيلا تيفيميوريم والإشريشيا كولى قد زادت لفترة زمنية أكبر فى بيئة التربة عنها بالقياس لباقى البيئات الأخرى والتى بلغت ٤٢ ، ٣٩ أسبوعاً عند درجة ٢٥ مْ وأيضاً ١٦ ، ١٤ أسبوعاً عند ٣٨ مْ لكل من الميكروبيـن تحت الإختبار على التوالى . كما تبين أيضاً أن بقاء ميكروب السالمونيلا تيفيميوريم فى كل من عـلا نـق الدواجن البادى والناهى تراوحت لفترات زمنية بين ١٠ ، ١٧ أسبوعاً عند درجـة حـرارة ٣٨ ، ٢٥مْ. بينما سجل ميكروب الإشريشيا كولى فترة بقاء بلغت ١١ ، ١٠ أسبوعاً لكل من عليقـة البادى و عليقـة الناهى عند درجة حرارة ٣٨ مْ على التوالى بينما زادت هذة الفترة إلى ١٨ أسبوعاً لكل من نوعى العليقة على السواء عند٢٥ مْ . كما أظهرت النتائج أيضاً بأن فترة بقاء هذة الميكروبات قد تقلصت نسبياً فى كـل من فرشة الطيور الحديثة والقديمة وذلك بالمقارنة لبقائها فى كل من علائق الدواجن والتربة حيث سجل ميكروب السالمونيلا تيفيميوريم فترة بقاء تراوحـت بين ١١ ، ١٢ أسبوعاً عنـد ٣٨ مْ وكذلك ١٣ ، ١٤ عند ٢٥ مْ كما أن ميكروب الإشريشيا كولى تأرجحت فترة بقانة فى فرشة الدواجن بين ١٢ ، ١٣ أسبوعاً عند ٣٨ مْ وكذلك ١٤ ، ١٥ أسبوعاً عند درجـة ٢٥ مْ . وقد لوحظ بصفـة عامـة من خـلال نتـائج هـذة الدراسة زيادة فترة بقاء ميكروب السالمونيلا تيفيميوريم فى علائق الدواجن عنها فى حالـة الفرشـة وذلـك عند درجة ٢٥ مْ . هذا بالإضافة إلى زيادة فترة بقاء كل من ميكروب السالمونيلا تيفيميوريم والإشريشيا

كولى نسبياً فى فرشة الدواجن الحديثة عنها فى حالة الفرشة القديمة وقد تم مناقشة بعض الإحتيطات الواجب توافرها للمنع أو التقليل من تلوث بيئة الدواجن .

SUMMARY

The survival period of *Salmonella typhimurium* and *Escherichia coli* (NTCC1106) were evaluated in a variety of poultry environment including rations (starter and finisher), litter (new and old) as well as agricultural soil. The obtained results revealed that, the moisture contents of the experimental media were fluctuated between 11.23 to 28.74% at starting of the experiment. At the end of the experiment, it was recorded that the moisture content was reduced to 3.02 - 8.03% and 3.19 - 6.56% at 38 and 25°C, respectively. On the other hand, it was detected that both tested pathogens, *Salmonella typhimurium* and *Escherichia coli* were survived more longer in soil than in poultry feeds and litter. In this respect it was found that, both the organisms *S. Typhimurium* and *E.coli* were survived for 42 and 39 weeks at 25°C while they were survived for 16 and 14 weeks at 38 °C,respectively. The longevity of tested *Salmonella typhimurium* in starter and finisher poultry rations was 10 and 17 weeks at 38 and 25 °C, while survival of *E.coli* reached 11 and 10 weeks at 38 °C in poultry feed and litters, respectively. Moreover, its survival was prolonged to 18 weeks at 25 °C for both starter and finisher poultry rations. Furthermore the obtained results demonstrated that, longevity of the tested microbes were relatively short in both new and old poultry litter. However *S. typhimurium* showed persistence between 11 and 12 weeks at 38 °C and from 13 to 14 weeks at 25 °C, while *E.coli* survival was fluctuated between 12 to 13 at 38 °C and 14 to 15 weeks at 25 °C. In general, it was concluded that survival of *S.typhimurium* was longer in poultry rations than poultry litter at 25 °C. With the same manner the longevity of both microbes was relatively prolonged in new poultry litter than the old one. Hygienic measures for prevention or even minimizing contamination of poultry environment were discussed.

Key words: *Survival, Survival, S. typhimurium, E. coli, poultry environment, Different temperaturer*

INTRODUCTION

Salmonellae are widely distributed in nature and the salmonella infection remain one of the principle zoontic diseases for animal and man.

The ability of salmonellae to remain viable in the environment contribute to their persistence in poultry flocks and their transmission and spread. The literatures on their ability to live under various conditions are limited (Williams & Benson,1978). In recent years, there has been an increased awareness of the environmental application on the survival and viability of microorganisms. Mair & Ross (1960) showed that salmonellae have been survived in or on soil for periods of up to 300 days, especially in the faecal pats that remained intact. Smyser et al. (1963) found that *S. Typhimurium* survived about 7 or 8 days in feedstuffs contaminated with fewer than 10 organisms/g at 20 - 25 °C,but survived longer in poultry feed stored at 4° C. Furthermore Smyser et al. (1966) recovered salmonellae for 18 months from feed stored at 20 - 25°C and for 7 months from litter collected from contaminated poultry houses. Snoeyenbose et al. (1967) found that salmonellae in dry feed reached relatively stable numbers in a short time and persist viable for 11 weeks in new litter and 2 weeks in old litter. Brownell et al.(1969) failed to isolate salmonellae from deep poultry litter that taken on 39^{th} and 51^{st} days after the last experimentally infected birds were removed. Furthermore, Fanelli et al. (1970) detected that salmonellae could not persist as long in built - up poultry litter as in fresh litter. Williams & Benson (1978) revealed that *S. typhimurium* survived for 18 months at 11° C in both of poultry feed and litter, while at 25 °C the organism persisted for 16 months in feeds and 18 months in litter. Furthermore, at 38 °Cthe organism survived for 40 days in poultry feed and only 13 days in litter. They concluded that the organism died rapidly at the highest temperature. Moreover, Shikaleev (1980) found that both *S. typhimurium* and enteropathogenic strains of *E. coli* could survive for 9 and 5 months in meat and bone - meal , respectively. Davies and Wray (1996) found that salmonellae survived at least 26 months in artificially contaminated poultry feed and at least for one year in an empty broiler house that had naturally contaminated.

On the other hand Donsel et al. (1967) recorded 90% reduction of *E. coli* count in soil at periods of 3.3 and 13.4 days in both of summer and winter, respectively. While Roy et al. (1955) showed that cycle of *E. coli* infection could be broken by leaving the poultry houses empty for four weeks. Moreover, Devanas et al. (1986) compared the survival of *E. coli* strains carrying plasmides in non sterile soil, and recorded that the cell numbers for the tested strains were declined after 24 days. As early as 1935 it was shown that the death rate of *E. coli* increased as the relative humidity near saturation point (Wells,1935). Moreover, *E. coli* K - 12 was shown to survive better at low humidities and temperatures than at high humidities

when sprayed into an atmosphere of nitrogen (Cox,1968). However, survival of the organism was reduced at low humidities in an atmosphere of oxygen. Generally the survival is dependent on the species of organism (Cox,1966 and Marthi et al,1990). Furthermore, Carlson & Snoeyenbos (1970) concluded that the population die - off of salmonellae in poultry feed was directly proportional to elevation in moisture level except at moisture levels that allowed growth.

the present work was conducted to elucidate the survival of both *Salmonella Typhimurium* and *Escherichia coli* in poultry feeds, litter and soil at 38 ° C and 25° C under controlled laboratory conditions.

MATERIALS and METHODS

1- Inoculated materials:

The used materials in which survival of the selected microorganisms were tested included poultry ration (starter & Finisher) , poultry litter (new & old) and agricultural soil (about 1 Kg of each), obtained from the poultry farm, Faculty of Agriculture. The materials were thoroughly chopped and sterilized by autoclaving at 121^{0}C for 20 min. The prepared materials were kept sealed in sterile glass containers (jars).

2- Determination of moisture contents:

The moisture percentages of each inoculated material (ration , litter and soil) were measured twice, both at starting and end of each experiment according to the official methods adopted by Association of Official Agriculture Chemists (1970).

3- Cultural strains used:

Two reference strains including *Salmonella typhimurium* and *Esherichia coli* (NTCC 1106) were used in our study. The strains were submitted from Institute of Animal Hygiene , Berlin ,Germany. The cultures were kept at 4 ^{0}C as stock slants in screw - cap tubes and subcultured every one month.

4- Bacterial culture and suspension:

The organism under test was firstly streaked on nutrient agar plates (Difco) and incubated at 38 ^{0}C for 24 h., then the bacterial growth was harvested by scraping the plates with a sterilized spatula to minimize the amount of the nutrient carryover. The cells were thoroughly suspended in sterile saline (0.85% Na Cl solution,w/v). The final concentration was 3.1 x 10^{8} and 2.6 x. 10^{9}/ml for *Salmonella typhimurium* and *Esherichia coli*, respectively.

5- Inoculation and counting procedures:

The technique was carried out as that adopted by Willims & Benson (1978) where 8 ml of the prepared bacterial suspension was inoculated into 100 g of each sterilized material (ration, litter and soil) and thoroughly mixed in a glass screw - capped mixer on a Waring blender, and was allowed to mix at high speed for 20 seconds. A sterile spatula was used to scrape the feed, litter and soil back into middle of the mixture. The blender was then turned on for another 20 seconds, after which the sides were scraped for a second time. After a final blending, the contents of the mixer were placed in a sterile glass jar. This process was repeated until 700 g of each inoculated material has been prepared and placed into sealed sterilized jar. Four jars of each material were prepared, two of them were inoculated with *S. typhimurium* and the others with *E. coli.* Then all inoculated jars were matched into 2 groups for each organism, one group was incubated at 38^0 C and the other one was incubated at 25^0C. Uncovered Petri dishes contain sterile water were placed inside the incubators to maintain the relative humidity levels and to overcome the excessive dryness of the incubated materials. The viable count of the inoculated materials (count/g) was achieved at regular intervals daily/weekly cultures from the incubated jars by standard plate counts (SPC) according to Cruickshank et al.(1980). The colony forming units (CFU/g) was conducted regularly till the end of each experiment which was noted by absence of growth. The counting techniques were undertaken using sterile MacConkey's agar and Bismuth sulphite agar (Oxoid) for *S. typhimurium*, while MacConkey's agar and Eosin methylene blue agar (Oxoid) were used for *E. coli.*

RESULTS

The obtained results were summerized in tables 1, 2, 3 & 4 and figures 1, 2, 3 & 4.

DISCUSSION

Several indices have been used to express the survival of bacteria. Most literatures have been determined as the period during which the organism could be isolated, while in others, as the time required to kill 90% of the bacteria (Vaccaro et al.1950). It was recorded that, a high percentage of mortality usually occurs within the first few days, but some cells survive for long periods and in some circumstances multiplication may occur (Orlob,

1956 and Andre et al., 1967). However, the survival of pathogenic microorganism in the environment is in itself only a potential disease hazard, and to induce disease the organism must be virulent and present in sufficient numbers (Wray and Mrcvs,1975). In the present investigation, the obtained results showed that, the mean moisture contents of the inoculated materials (rations, litter and soil) were fluctuated between 11.23 to 28.74% with a total mean value of 22.74 ± 3.39% at starting of the experiments for both 38 °C and 25 °C. The moisture content of the inoculated materials were decreased drastically by the time. In this respect it was found that, it was reduced to 3.02-8.03 with a mean value of 5.99 ± 0.99% after the end of the experiment at 38 °C while it was reduced to 3.19 - 6.56% with a total mean value of 5.47 ± 0.69% at 25 °C after end of the experiment for each organism (Table 1). Liu et al. (1969) could detect a close correlation between the water activity, holding temperature and salmonella survival and multiplication in meat and bone meal. Moreover, Carlson and Snoeyenbos (1970) found that, the population die-off of salmonella in poultry feeds was directly proportional to elevation in moisture level except at moisture levels that allowed growth.

On the other hand, McFeters and Stuart (1972) reported that, the microorganism survival below 15°C was inversely related to temperature, but above 15°C the temperature was critical. They added that, low temperature favours bacterial survival independently on the amount of organic matter present. It was found that, *S. Typhimurium* and *E. Coli* (NTCC 1106) could persist for a fairly long time in the agricultural soil at both of 38 °C and 25 °C (Table 2) where *S. typhimurium* and *E. Coli* were survived for 42 and 39 weeks at 25 °C, respectively while the survival was reduced to 16 and 14 weeks for both organisms at 38 °C. These results were coincided with that achieved by Chao et al. (1988), who noticed a slowly decreased of bacterial population in soil throughout their test period.

In poultry rations, the tested *S.typhimurium* was survived for 10 weeks at 38 °C and 17 weeks at 25 °C in both starter and finisher poultry rations. However, *E.coli* longevity was varied from 10 weeks in finisher to 11 weeks in starter at 38 °C, but the survival was prolonged for 18 weeks at 25 °C for both inoculated rations (Table 2).

Bacterial population of the tested organisms in poultry litter (new and old) showed more or less rapid decline than that obtained in case of poultry rations and agricultural soil (tables 3 & 4) and (figures 1, 2, 3 & 4). Moreover, survival of *S. typhimurium* was 11 and 12 weeks (at 38 °C) while it only persists for 13 and 14 weeks (at 25 °C) in the new and old litter,

respectively. On the other hand, survival of *E.coli* was fluctuated from 12 to 13 weeks at 38°C and from 14 to 15 weeks at 25 °C in both poultry litters, respectively. It could be detected from table (2) that, both *S. typhimurium* and *E.coli* were persist longer in poultry feeds than in poultry litter at 25 °C. Moreover, both tested organisms were survived longer in new poultry litter than old one.These results were in agreement with that recorded by Tucker, (1967) and Williams and Benson,(1987). Generally, no consistent patterns were noted to indicate that either the poultry feeds or litter favoured the survival of tested organisms.

Although, the factors affecting survival of some microorganisms are poorly understood, but the physiological stress response to the physical and chemical conditions of the inoculated materials was a rapid increase in sublethal injury. These factors are concomitant with an initial period of constant viability which was mainly seen during the first few days of exposure. Moreover, a period of relatively linear decline in viability followed that inversely related to temperature changes (Figures 1, 2, 3 & 4).

Presence and survival of pathogenic microorganisms in poultry environment specially salmonellae and *E. coli* constitute a continuing problem. These microbes are not only cause disease in birds and animals consuming the contaminated feeds, but also they may ultimately cause gastroenteritis and haemorrhagic colitis in people who either handle or consume meat derived from these concerned birds and animals.

Necessary measures are needed to control the poultry environment that include, control of dust, periodic removal of feed accumulations, moreover a control program for insects, rodents and wild birds is considered. In addition, it is essential to keep ingredients, finished feed and litter dry to prevent microbial growth since salmonellae, for example are capable of multiplying in feed if the water activity is 0.97 or higher (Hinton and Mead,1992).

CONCLUSION

The survival of pathogenic microorganisms in poultry environment in itself is only a potential disease hazard and to rise disease, the organism must be virulent and present in sufficient numbers.

It is reasonable to assume that a long survival period of pathogenic microorganisms in the environment will result in an increase the exposure risk of their infection to poultry, animal and man.

In general, it could be concluded that the lower ambient temperature 25 °C was dramatically increase ability of the tested organisms to survive in the inoculated media more than that obtained at 38 °C.

Finally, one of the important consideration in the present concern is that, the field samples of poultry feeds and litter should be routinely maintained at low temperatures to favour the survival of its herbouring pathogenic microorganisms, before their bacteriological examinations and such samples should never be allowed to stand for extended periods at high temperatures.

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REFERENCES

Andre, D.A., Weiser, H.H. and Malaney, G.W.(1967): Survival of bacterial enteric pathogens in farm pond. J.Am.Water Works Ass.Vol.59:503-508.

AOAC (1970): Official methods of analysis. Association of Official Agriculture Chemists.11th, edn., Washington,D.C.

Brownell, J.R.; Sandler, W.W. and Fanelli, M.J. (1969): Factors influencing the intestinal infection of chickens with Salmonella typhimurium. Avian Dis.Vol.13:804-816.

Carlson, V.L. and Snoeyenbos, G.H. (1970): Effect of moisture on salmonella populations in animal feeds. Poultry Sci. Vol. 49: 717-725.

Chao, W.L.; Ding, R.J. and Chen, R.S. (1988): Survival of *Yersinia enterocolitica* in the environment. Can. J.Microbiol. Vol.34:753-756.

Cox, C.S. (1966): The survival of *E.coli* sprayed into air and into nitrogen from distilled water and from solutions of protecting agents. J, Gen. Microbiology, Vol.44:15-31.

Cox, C.S. (1968): The aerosol survival and cause of death of *E.coli* K12. J. Gen. Microbiology,Vol.54:169-175.

Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H. (1980): Medical Microbiology. 12th Edition,Vol.11, reprinted Churchill Livingstone and Robert Stevenson Edinbrgh, EHI 3 AF.

Davies, R.H. and Wray, C. (1996): Persistence of *Salmonella enteritidis* in poultry units and poultry food. Br. Poult. Sci., Vol.37(3): 589-596.

Devanas, M.A.; Rafaeli, E.D. and Stotsky, G. (1986): Survival of plasmid-containing strains of *E.coli* in soil curr. Microbiol.13: 269-277.

Donsel, D.J.; Geldreich, E.E. and Clark, N.A. (1967): Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. Appl.Microbiol.Vol.15:1362-1370.

Duff, R.H.; Ross, J.G. and Brown, D.D. (1973): Influence of litter on *Salmonella Typhimurium* infection in poultry. Avian path.Vol.2:263-268.

Fanelli, M.J.; Sadler, W.W. and Brownell, J.R. (1970): Preliminary studies on persistence of salmonellae in poultry litter. Avian Dis.Vol.14:131-141.

Hinton, M. and Meat, G.C. (1992): Bacterial pathogens in animal feed and their control. World's Poultry Science Journal, Vol.48:(3),72-73.

Liu, T.S.; Snoeyenbos, G.H. and Carlson, V.L.(1969): The effect of moisture and storage temperature on a Salmonella senftenberg775W population in meat and bone meal. Poultry sci.Vol.48:1628-1633.

Mair, N.S. and Ross, A.I. (1960): Survival of *Salmonella Typhimurium* in the soil.Vol.19:39-41.

Marthi, B.; Fieland, V.P.; Walter, M. and Seidler, R.J. (1990): Survival of bacteria during Aerosolization. Applied and Environ. Microbiology Vol. 56: No 11, 3463-3467.

McFeters, G.A. and Stuart, D.G. (1972): Survival of coliform in natural waters: Field and laboratory studies with membrane-filter chambers. Appl. Microbiol. Vol. 24: 805-811.

Orlob, G.T. (1956): Viability of sewage bacteria in sea water. Sewage Ind. Wastes, Vol. 28: 1147-1167.

Roy, J.H.; Palmer, J.; Shillam, K.W.; Ingram, P.L. and Wood, P.C. (1955): The relationship between the period of time that a calfhouse has been occupied and the incidence of scouring and mortality in young calves. Br. J. Nutr. Vol. 9: 11-20.

Shikaleev, Y.N. (1980): Survival of salmonella and *Escherichia coli* in meat-and-bone meal. Veterinariya, Moscow, USSR, No. 9: 18-19.

Smyser, C.F.; Adinarayanan, N.; Van-Rockel,H. and Snoeyenbos, G.H. (1966): Field and laboratory observation on Salmonella heidelberg infection in three chicken breeding flocks. Avian Dis. Vol.10:314-329.

Smyser, C.F.; Racharz, J. and Van-Rockel, H. (1963): Detection of *Salmonella typhimurium* from artificially contaminated poultry feed and animal by-products. Avian Dis.Vol. 7: 428-434.

Snoeyenbos, G.H.; Carlson, V.L.; Mekie, B.A. and Smyser, C.F. (1967): An epidemiological study of salmonellosis of chickens. Avian dis. Vol.11: 653-667.

Tucker, J.F. (1967): Survival of Salmonellae in built-up litter for housing of rearing and laying fowls. Brit.Vet. J. Vol. 123: 92-103.

Vaccaro, R.F.; Briggs, M.P.; Carey, C.L. and Ketchum, B.H. (1950): Viability of Escherichia coli in sea water. Am. J. Publ. Hlth, Vol. 40: 1257-1266.

Wells, W.F. (1935): Airborne infection and sanitary air control. J. Ind. Hyg. Toxicol. Vol. 17: 253.

Willims, J.E. and Benson, S.T.(1978): Survival of *Salmonella Typhimurium* in poultry feed and litter at three temperatures. Avian diseases Vol.22:NO4, 742-747.

Wray, C. and Mrcvs, S.(1975): Survival and spread of pathogenic bacteria of veterinary importance within the environment. The Veterinary Bulletin, Vol. 45: No 8: 543-550.

Table (1): Mean moisture content (%) in the media used.

Experimental materials	At starting of the experiment		After complete disappearance of the tested organism	
	At 38 °C	At 25 °C	At 38 °C	At 25 °C
Poultry ration (Starter)	28.71	28.74	8.03	6.09
Poultry ration (Finisher)	24.66	24.63	7.50	6.56
Poultry litter (new)	26.28	26.25	5.33	5.17
Poultry litter (old)	22.82	22.86	6.06	6.32
Agricultural soil	11.25	11.23	3.02	3.19
Total mean value %	22.74± 3.39	22.74± 3.39	5.99± o.99	5.47± 0.69

Table (2): Survival of both *S. typhimurium* and *E. coli* in weeks.

Inoculated media	*S. typhimurium*		*E. coli*	
	At 38 °C	At 25 °C	At 38 °C	At 25 °C
Poultry ration (Starter)	10	17	11	18
Poultry ration (Finisher)	10	17	10	18
Poultry litter (new)	11	14	13	15
Poultry litter (old)	12	13	12	14
Agricultural soil	16	42	14	39

Table (3): Mean viable count of *S. typhimurium*/g in different media used in weeks.

Time/W	Inoculated materials									
	P.R. (Starter)		P.R. (Finisher)		P.L. (New)		P.L. (old)		A. Soil	
	38 °C	25°C	38 °C	25°C	38 °C	25°C	38 °C	25°C	38 °C	25°C
1st	$1.5x10^7$	$3.2x10^6$	$5.8x10^6$	$2.1x10^6$	$2.5x10^6$	$2.4x10^6$	$2.4x10^6$	$1.7x10^6$	$1.6x10^7$	$7.2x10^6$
2nd	$6.3x10^5$	$2.5x10^6$	$7.3x10^5$	$7.3x10^5$	$4.3x10^4$	$5.2x10^5$	$1.1x10^4$	$9.2x10^5$	$4.3x10^6$	$5.6x10^6$
3rd	$3.2x10^5$	$2.1x10^6$	$5.1x10^4$	$6.2x10^5$	$2.4x10^4$	$4.7x10^5$	$3.2x10^4$	$8.3x10^5$	$3.7x10^5$	$4.8x10^5$
4th	$5.1x10^4$	$1.8x10^6$	$4.3x10^4$	$5.9x10^5$	$9.6x10^3$	$2.8x10^5$	$1.7x10^4$	$7.2x10^5$	$1.3x10^5$	$3.7x10^5$
5th	$4.2x10^4$	$5.3x10^5$	$3.7x10^4$	$5.3x10^5$	$7.1x10^3$	$2.5x10^5$	$8.3x10^3$	$5.1x10^5$	$9.2x10^4$	$3.3x10^5$
6th	$7.2x10^3$	$4.1x10^5$	$6.7x10^3$	$4.8x10^5$	$5.2x10^3$	$2.6x10^5$	$5.4x10^3$	$4.2x10^5$	$5.5x10^4$	$2.5x10^5$
7th	$2.5x10^3$	$32.x10^5$	$5.4x10^3$	$4.6x10^5$	$4.1x10^3$	$3.0x10^5$	$3.8x10^3$	$2.7x10^5$	$3.3x10^4$	$1.7x10^5$
8th	$1.6x10^2$	$3.0x10^5$	$2.7x10^2$	$3.2x10^4$	$2.2x10^2$	$2.6x10^5$	$2.2x10^3$	$6.3x10^4$	$2.7x10^4$	$8.3x10^4$
9th	$8.0x10$	$3.3x10^4$	$1.8x10^2$	$2.1x10^4$	$1.8x10^2$	$5.3x10^4$	$1.6x10^2$	$5.5x10^4$	$1.8x10^4$	$6.1x10^4$
10th	0	$2.7x10^4$	0	$1.7x10^4$	$7.1x10$	$3.2x10^4$	$1.1x10^2$	$1.9x10^4$	$3.9x10^3$	$2.3x10^4$
11th	0	$1.8x10^4$	0	$5.2x10^3$	0	$2.1x10^3$	$3.2x10$	$3.3x10^3$	$3.0x10^3$	$2.3x10^4$
12th		$1.1x10^4$		$1.7x10^3$	0	$6.2x10^3$	0	$1.6x10$	$5.2x10^3$	$6.7x10^3$
13th		$3.7x10^3$		$1.2x10^3$		$6.0x10^3$	0	0	$2.7x10^3$	$4.6x10^3$
14th		$3.5x10^3$		$1.0x10^3$		0		0	$8.0x10$	$3.8x10^2$
15th		$7.0x10^2$		$1.0x10^3$		0			$3.0x10$	$3.4x10^3$
16th		$3.0x10$		$6.0x10$					0	$3.1x10^2$
17th		0		0					0	$2.8x10^2$
18th		0		0						$1.9x10^2$
19th										$1.6x10^3$
20th										$1.4x10^3$
22nd										$1.4x10^3$
24th										$1.2x10^3$
26th										$6.0x10$
28th										$7.0x10$
30th										$9.0x10$
32nd										$5.0x10$
34th										$7.0x10$
36th										$8.0x10$
38th										$5.0x10$
40th										$3.0x10$
42nd										0
44th										0

P.R= Poultry ration, P.L.= Poultry litter, A.= Agriculture, W=Weeks

Table (4): Mean viable count of *E. coli* /g in different media used in weeks.

Time/W	Inoculated materials									
	P.R. (Starter)		P.R. (Finisher)		P.L. (New)		P.L. (old)		A. Soil	
	38 °C	25°C	38 °C	25°C	38 °C	25°C	38 °C	25°C	38 °C	25°C
1st	$8.7x10^6$	$4.3x10^7$	$3.1x10^7$	$2.9x10^7$	$4.0x10^7$	$3.7x10^7$	$4.8x10^7$	$4.5x10^7$	$3.5x10^7$	$3.2x10^7$
2nd	$6.2x10^6$	$2.1x10^7$	$2.4x10^7$	$2.3x10^7$	$3.1x10^7$	$2.8x10^7$	$3.2x10^5$	$3.0x10^7$	$2.3x10^7$	$2.9x10^7$
3rd	$3.1x10^3$	$1.0x10^7$	$2.4x10^7$	$4.5x10^7$	$2.9x10^6$	$1.7x10^7$	$8.1x10^4$	$1.2x10^7$	$1.8x10^6$	$5.1x10^5$
4th	$1.4x10^6$	$6.6x10^7$	$2.0x10^7$	$6.2x10^7$	$2.6x10^6$	$1.4x^{107}$	$7.7x10^4$	$5.6x10^6$	$8.2x10^5$	$4.4x10^6$
5th	$1.4x10^6$	$5.1x10^7$	$1.7x10^6$	$4.2x10^7$	$2.6x10^6$	$1.6x10^7$	$5.1x10^4$	$4.8x10^6$	$6.2x10^5$	$4.2x10^6$
6th	$1.0x10^6$	$4.7x10^6$	$1.4x10^5$	$1.8x10^7$	$2.4x10^6$	$1.5x10^7$	$3.8x10^4$	$3.3x10^6$	$5.7x10^5$	$3.7x10^5$
7th	$9.8x10^5$	$1.4x10^6$	$1.4x10^4$	$6.8x10^6$	$2.3x10^6$	$1.4x10^7$	$1.8x10^4$	$1.1x10^6$	$3.7x10^5$	$9.4x10^4$
8th	$8.3x10^5$	$2.3x10^5$	$1.2x10^3$	$5.2x10^5$	$1.1x10^5$	$2.2x10^5$	$1.1x10^3$	$3.5x10^4$	$3.0x10^5$	$7.3x10^4$
9th	$4.1x10^3$	$1.1x10^5$	4.0x10	$3.7x10^4$	$3.3x10^4$	$1.6x10^4$	$2.6x10^3$	$2.2x10^3$	$2.1x10^5$	$2.9x10^4$
10th	$2.3x10^2$	$2.8x10^4$	0	$3.2x10^3$	$1.8x10^3$	$1.4x10^3$	$2.0x10^2$	$1.2x10^2$	$3.3x10^3$	$1.3x10^4$
11th	0	$1.6x10^4$	0	$2.9x10^3$	$1.1x10^2$	$1.1x10^3$	1.4x10	$1.0x10^2$	$2.6x10^2$	$6.2x10^3$
12th	0	$4.8x10^3$		$2.2x10^3$	$1.1x10^2$	$2.3x10^2$	0	1.8x10	$1.5x10^2$	$5.1x10^3$
13th		$3.1x10^3$		$1.7x10^3$	0	$1.1x10^2$	0	1.2x10	1.9x10	$1.4x10^3$
14th		$2.5x10^3$		$1.4x10^3$	0	3.2x10		0	0	$1.0x10^3$
15th		$1.8x10^3$		$1.1x10^3$		0		0	0	$6.3x10^2$
16th		$1.2x10^3$		$2.8x10^3$		0				$5.7x10^2$
17th		1.8x10		1.2x10						$5.1x10^2$
18th		0		0						$4.4x10^2$
19th		0		0						$3.6x10^2$
20th										$1.7x10^2$
22nd										$2.1x10^2$
24th										$1.3x10^2$
26th										$1.3x10^2$
28th										$1.0x10^2$
30th										8.0x10
32nd										6.0x10
34th										8.0x10
36th										5.0x10
38th										6.0x10
40th										0
42nd										0

P.R= Poultry ration, P.L.= Poultry litter, A = Agriculture, W=Weeks

Fig. (1): Survival of S. typhimurium at 38 C in weeks

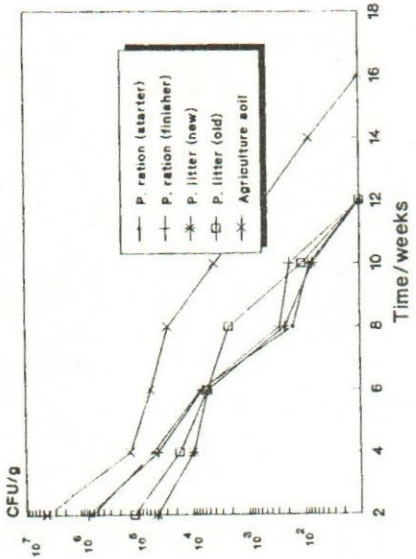

Fig. (2): survival of S. typhimurium at 25 C. in weeks

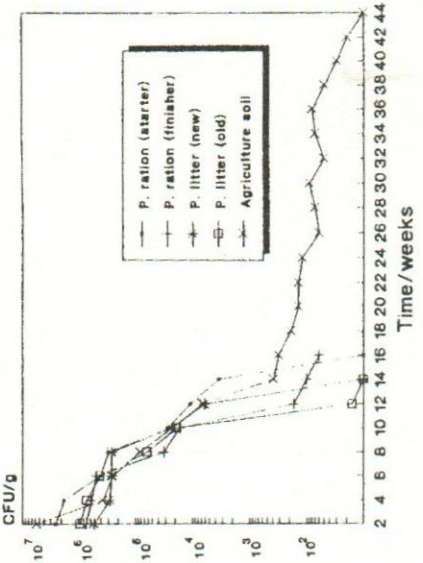

Fig. (3): Survival of E. coli at 38 C in weeks

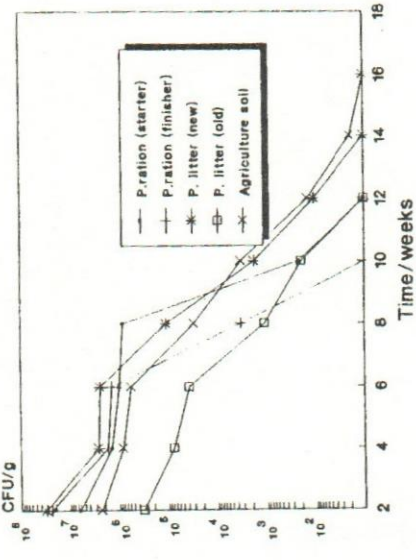

Fig. (4): Survival of E.coli at 25C in weeks