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RECYCLING POULTRY WASTE AS FEED FOR CHICKENS AND ITS PROTECTIVE EFFECT ON SALMONELLA TYPHIMURIUM INFECTION.

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SUMMARY

Recycled poultry waste was evaluated for protective effects against Salmonella typhimurium coloization and as growth stimulant supplement in feed ration of chickens. Poultry waste was dried at 60°C for 48 hs. and provided as 5% and 10% of feed ration from day of hatch till 21 days. The chicks were divided into 6 equal groups (1) for control negative, no challenge, no waste (2) no challenge, 5% waste (3) no challenge, 10% waste (4) for control positive, challenge, no waste (5) challenge, 5% waste (6) challenge, 10% waste. Challenge took place at 3 days of age with 0.2 ml of 10^6 c.f.u. S. typhimarium. Twelve days after challenge, ceca, spleen, liver and heart blood were collected from killed chicks and evaluated for S. typhimurium colonization. Growth and general performance of chicks were recorded throughout the experiment. The results indicated that provision of used dried poultry waste in feed ration of newly hatched chicks has dual purpose, it effectively controls S. typhimurium colonization and improves growth response.

INTRODUCTION

Domestic poultry are reported to be the largest single resrvoir of Salmonella and primary cause of human salmonellosis (Williams, 1965). Salmonella contimues to present a food safety and public health concern (St. Louis et al., 1988 and Lee. 1989). Disease and mortality are associaned with oral infection of one day chicks with Salmonela. Salmonella typhimurium is highly virulent in chickens, with a mean lethal dose (LD_{50}) of 2 x 10^3 -< 4x 10^9 c.f.u (Curtiss et al., 1991).

In recent years, researchs have focused on the development of immunoprophylactic measures, microbiological strategies and anti-salmonella feed additives (AbouYossef and Dicuollo 1982; Custafson et al., 1982 and Corrier et al., 1991 b) that will prevent or control intestinal tissue colonization of chicks by invasion of salmonella species (Smith, 1989 and Schneitz et al., 1990).

Colonization resistance of newly hatched broiler chicks to Salmonella species has been increased by early exposure to adult innestinal flora by direct oral inoculation in feed or water (Pivnick et al., 1982; Hinton et al., 1990 and Corrier et al., 1991 a) and indirect by rearing chicks on used poultry litter that contained adult cecal and fecal droppings (Duff et al., 1973 and Corrier et al. 1991 c).

Several investigators have shown that deliberate

introduction of adult enteric microflora orally into newly hatched chicks may inhibit Salmonella colonization (Barnes and Impey, 1980 and Dorn and Krabisch 1981). Snoeyenbos et al. (1978) reported that resistance of young chicks and poults to salomonela expoure was substantially increased by early oral administration of intestinal contents or feces from selected adult chicken. However, Fanelli et al., (1970) and Gustafson and Kobland (1984) reported that the chicks raised on used litter showed an appreciable reduction in susceptibility to Salmonella when compared to control animal on fresh litter.

Nearly 4.5 tons of poultry waste/ 1.000 lb bird live weight are produced annually. Waste disposal is a major problem, increasingly manure causes the problem of environmental pollution (Muller, 1980), Recent tochnology turned to be involved in new methods of handling manure and new uses for it. Recycling manure as livestock feed is the most promising of nonfertilizer uses. Provision of used dried poultry waste in feed ration effectively controls growth and shedding of Campylobacter jejuni (Willis, et al. 1991). Corrier et al., (1993) recorded that addition of 5% used litter to feed ration significantly decreased cecal and organ colonization in chicks infected with S. enteritidis. The preliminary studies in their laboratory indicated that provision of used litter in feed ration effectively controls S. typhimurium colonization in chicks.

The purpose of the present work was to study the protective effects of providing 5% and 10% dried poultry waste in feed ration on cecal and organs colonization of newly hatched chicks by an invasive strain of Salmonella typhimurium. General perfomance of chicks was also concerned.

MATERIAL AND METHODS

Poultry waste

Poultry waste was collected from one year caged layer hens. The hens had been fed on unmedicated corn/soybean meal based ration. The used poultry waste was prepared by drying at 60°C for 48 hrs (Willis et al., 1991) and ground using electric laboratory blender into fine uniform particles. Chemical analysis of dried poultry waste (DPW) showed that it contains 21.0% CP.

Salmonella typhimarium infection:

S. typhimarium was obtained from Dept. of Bacteriology, Mycology, and Immunology, Fac. Vet. Med., Cairo University. The chicks were challenged orally with 0.2 ml of 10^6 c.f.u. S. typhimurium (antibiotic marked) from 18 hrs nutrient broth culture. The infection was done to 3 days old chicks.

Chickens:

One hundred and eighty one day old Lohman chicks were obtained from commercial hatchery. They were tested and proved to be free from S. typhimurium and other salmonella species. The chicks were divided into 6 groups of equal average weight (39± 1.5). Group (1) for control negative, non challenged and received basal diet without any supplement, Groups (2 and 3) non challenged and fed diets suplemented by 5% and 10% DPW respectively. Group (4) for control positive, challenged and received basal diet

without any supplement. Groups (5 and 6), challenged and supplemented by 5% and 10% DPW in their diets respectively. The three used diets were formulated to be approximatly isonitrogenous, isocaloric value and almost similar C/p ratio (Table 1). Water and feed were offered ad-libitum.

Table (1): Composition of the three experimental diets used

Ingredient %	Control (basal)	Basal + 5 % DPW	Basal +10 % DPW
Yellow corn ground	63.3	59.30	55.30
Soybean meal (44 %)	31.0	29.00	27.0
Meat and bone meal	3.0	2.80	2.7
Bone meal	1.6	1.60	1.5
Calcium carbonate	0.38	0.38	0.38
DL. methionine	0.07	0.07	0.07
Salt	0.35	0.35	0.35
Vit. and min mixture*	0.30	0.30	0.30
DPW**	0.00	5.00	10.00
Corn oil	0.00	1.20	2.40
Total	100	100	100
Calculated analysis			
CP %	21.0	20.7	20.5
ME Kcal/kg	2871	2855	2841
Calorie/protein ratio	136.7	137.9	138.5
Calcium %	1.02	1.39	1.74
Phosphorus %	0.49	0.5	0.5
Lysine	1.13	1.1	1.05
Methionine	0.35	0.36	0.38
Methionine & Cystine	0.66	0.68	0.69

* Supplied per Kg of diet: Vit A, 6, 600 IU; Vit D3, 2,200 IU; riboflavin 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 500 mg; Vit B12, .022 mg, Mn, .55 mg; Fe, 50 mg, Cu, 4 mg; Zn, 40 mg
** Contains 21.0 % CP & the rest composition according to El-Boushy and Vander Poel (1994).

Before use, DPW, feed rations litter and containers were determined to be Salmonella cultute negative. Tetrathionate brilliant green (TBG) broth was used incubated for 30 hrs at 48°C before plating on brillant green (BG) agar plates and reincubated at 37°C for 18-24 hrs (Mallinsan and Snoeyebos, 1989).

Parameters for evaluation

Twelve days after Salmonella chalenge 12 chicks from each treatment group were killed by cervical dislocation. The ceca, liver, spleen and heart blood were collect aseptically and evaluated for S. typhimarium colonization. The mortalities were recorded daily throughout the experimental period (21 days). Body gain development was recorded weekly. Overall chicks gain, feed intake and feed conversion were calculated.

At the end of the experiment, blood serum was prepared from each group for further analysis. Serum total protein was estimated by the method of Sermetz (1980). Protein electrophoretic pattern was done using cellulose acetate electrophoresis (Bierer 1964). Quantitation of the different protein fractions was performed by using DCD 16-digital computing densitometer.

Statistical analysis

The obtained results were statistically analyzed using ANOVA and test according to Snedecor (1986).

RESULTS AND DISCUSSION

Organs colonization

Compared with control positive (challenged chicks), the number of chicks that had salmonella culture positive in variable tissues selected for isolation (ceca - liver - spleen - heart blood) decreased in the group which received diet supplemented with DPW (Table 2). These findings indicated that colonization resistance of chicks to S. typhimurium was increased by feeding 5% DPW and the best protection was noticed in chicks fed 10% DPW in their deit as reported by Hintone et al., (1990) Corrier et al., (1991 a) and Corrier et al., (1993).

The probable mechanisms by which the DPW prevents the establishment of an invading Samonella may be amongst the possilbilities of competition of adult intestinal flora present in DPW for limiting carbon source (Freter 1974), or competition for sites of attachment and ultimately crowds out existing microcolonies of pathogen (Snoeyenbos et al., 1979). Another explanation suggested by Barnes et al., (1978) w contributed increasing the colonization resistan due to production of volatile fatty acids anaerobic intestinal flora.

Mortalities

Table (3) showed that the mortality percent for t challenged control group was (33.3%) an reduced to 10.0% by addition of DPW in poultr feed in challenged groups. The obtained mortalit rates confirmed the data of organs colonizatio Using DPW in poultry feed can reduce the letha dose of S. typhimurium recorded by Curtiss et al (1991).

Electrophoretic pattern

Table (4) showed that serum total protein was no affected significantly by any treatment. There were significant increases at $P<0.05$ in B and globulins of control positive group as compared with control negative. Barrrow et al (1990) and Hassan et al (1993) reported that chickens infected orally with S. typhimurium induce humoral immune responses to all infected doses. It is noticed that the immune response of chicks fed DPW was slightly increased. This observation may be due to the competition between intestinal flora and S. typhimurium, resulting in decrease of phagocytosed, processed and presented antigen by B cells as previously reported by Collins (1974).

Chickens performance

Compared with control negative (non challenged chickens), addition of 5% or 10% DPW to poultry feed significantly improved body weight gain of the chickens at the second and third weeks of

experiment (Table 5). Similar finding was reported by Lee and Blair (1973), and Hady (1989), On the other hand, compared with control positive (challenged chickens), use of DPW in poultry diets significanlty imporved body gain at $P< 0.01$ to a level approximate nearly to body gain of control negative. Snoeyenbos et al., (1979) reported that growth rate of the infected chickens was imporved by addition of fecal material from avian source to feed ration.

Feed inatake was not affected except in control positive which showed much reduction. Feed conversion was improved in groups 2,3 which received DPW in their diets and non challenged as reported by Lee and Blair (1973). The generally better growth performance achieved by birds fed DPW might have contributed to the unidentified growth factor reported to be present in poultry excreta (Wehunt et al., 1960).

Table (2): Effect of dried poultry waste on *Salmonella Typhimurium* organs colonization challenged with *S. typhimurium*

Organs / Groups	Ceca		Liver		Spleen		Bile		Heart blood	
	no of +ve control	%	no of +ve control	%	no of +ve control	%	no of +ve control	%	no of +ve control	%
- Control +ve challenged.	8	66	7	58	6	50	6	50	7	58
- Challenged & suppl. with 5% DPW.	5	41	3	25	3	25	5	41	3	25
- Challenged & suppl. with 10% DPW.	3	25	2	16	2	16	2	16	3	25

DPW was provided as 5 % or 10 % of the feed ration. Chicks were challenged orally with *S. typhimurium* at 3 days of age.

Table (3): Effect of dried poultry waste on mortality rates

Groups	(1) Basal control -ve	(2) Basal 5% DPW	(3) Basal 10% DPW	(4) challenged control +ve	(5) challenged 5% DPW	(6) challenged 10% DPW
Number	-	-	-	10	3	3
%	-	-	-	33.3	10.0	10.0

Table (4): Effect of dried poultry waste on serum total protein and electrophoretic pattern.

Groups	Total prot. gm %	Albumin gm %	Globulins			Total globulins	alb / glob ratio
				β			
(1) Control -ve	5.26 +.92	2.81 +.61	1.26 +.09	0.62* +.04	0.57* +.11	2.45	1.15
(2) 5 % DPW	5.14 +.62	2.64 +.64	1.24 +.14	0.66 +.05	0.60 +.11	2.50	1.06
(3) 10 % DPW	4.98 +77	2.51 +46	0.18 +.12	0.62 +7.04	0.65 +.15	2.45	1.03
(4) Callenged control +ve	5.03 +.55	1.98 +.44	1.08 +.08	0.92* +.08	1.05* +.12	3.05	0.65
(5) Callenged 5 % DPW	4.99 +.64	2.17 +.52	1.12 +.07	0.76 +0.07	0.94 +.14	2.82	0.77
(6) Callenged 10 % DPW	5.16 +.62	2.26 +.42	1.15 +.08	0.80 +0.06	0.95 +.14	2.90	0.78

± Standard error
* Means significantly different from control -ve P < 0.05

Table (5): Effect of dried poultry waste on body weight gain and overall performance of experimental chickens.

Groups / weeks	(1) Basal control -ve	(2) Basal 5% DPW	(3) Basal 10% DPW	(4) challenged control +ve	(5) challenged 5% DPW	(6) challenged 10% DPW
initial wt	39 +1.5	39 +1.5	39 +1.5	39 +1.5	39 +1.5	39 +1.5
1	125 +3.4	128 +3.1	126 +2.6	122 +3.5	124 +2.6	122 +3.9
2	215 +5.4	242* +7.3	238* +6.6	163 +5.4	209*** +9.4	211*** +10.0
3	316 +6.1	372** +8.5	381** +9.3	217 +11.6	286*** +12.7	294*** +13.4
Overall performance						
Gain	277	333	342	178	247	255
Feed intake	717	720	725	465	712	710
Feed converson	2.58	2.16	2.11	2.61	2.88	2.78

± Standard error
* Means significantly different from control -ve (P < 0.05).
** Means significantly different from control -ve (P < 0.01)
*** Means significantly different from control -ve (P < 0.01)

The present work attempts to connect between the growth improvement achieved by the use of DPW in challenged chickens (groups 5,6) and the reduction of positive S. typhimurium culture in the same groups. The authors suggested that inclusion of intestinal flora present in the DPW competes pathogens and other undesirable organisms in the bird gut (Snoeyenbos et al., 1979). This competitive exclusion results in favoring intestinal bacterial and controlling microorganisms which compete with the bird for amino acids vitamins or other nutrients that required for maximum growth rate. In addition the volatile fatty acids produced by anaerobic bacteria of the DPW improve the availability of nutrients (Barnes et al., 1978).

From the results obtained one can safely conclude that provision of used DPW in feed ration of newly hatched chicks has dual purpose it effectively controls S. typhimurium colonization and improves growth response.

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