

## SOME STUDIES ON SALMONELLA CONTROL IN CHICKENS: PROBIOTIC, PREBIOTIC AND VACCINATION

Hegazy, A.M<sup>1</sup>, Nasra, Awadin<sup>2</sup>, and Soliman, A. H<sup>1</sup>.

Animal Health Research Institute, Kafr El-Sheikh Regional lab.<sup>1</sup>.

Animal Production Research Institute Kafr El-Sheikh<sup>2</sup>

### ABSTRACT

*This study was designed to evaluate some biological methods used in either controlling or prophylaxis against salmonella. Eight groups are treated as follow, four groups serve as control (control negative, probiotic control, prebiotic control and vaccine control), the 2nd four groups are the same but challenged at 21 day-old with 1ml over night broth culture of S. enteritidis. The three treated groups were capable to reduce the harmful effect of salmonella infection like colonization and shedding of S. enteritidis, performance under experimental infection, some kidney function and liver enzymes but with varying degree.*

### INTRODUCTION

Poultry and poultry products are implicated as the cause of the prevalence paratyphoid infection in human being (*Chambers, et al., 1998 and Hang'ombe, et al., 1999*).

Feed additives were used for the control of salmonellae including antibiotics, prebiotic, probiotics, symbiotic, organic acid and volatile fatty acids (*Immerseel, et al., 2002*). In the modern intensive poultry production, newly hatched chicks have little chance to contact with their mother. thereby normal microflora is slow to colonize in the intestine (*Fuller, 1989*).

On the other hand, several experiments have demonstrated that the prevention of Salmonella colonization in chickens can be achieved by feeding Prebiotic (*Patterson and Burkholder, 2003*) or probiotics (*Mead, 2000*).

Prebiotics are, non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. The use of prebiotics against Salmonella colonization can be efficient in young chicks but might be unsuccessful in adult hens, *Corrier, et al., (1993), Gibson and Roberfroid (1995)*.

Many intestinal pathogens like type 1 fimbriated E.coli, salmonellae and campylobacter utilize oligosaccharide receptor sites in the gut. Once established, they can then cause gastroenteritis through invasive and/or toxin forming properties. One extrapolation of the prebiotic concept is to simulate such receptor sites in the gut lumen. Hence, the pathogen can not binding at the host mucosal interface (*Gibson, et al. 2005*).

The anti-infectious effect of probiotic has been reported previously, and one mechanism may be the non-specific stimulation of immunity. The increase of local IgA levels resulting from ingestion of the probiotic may contribute to enhancement of the mucosal resistance against GIT infections (*Fukushima, et al. 1998*).

Also, many lactobacilli and bifidobacteria species are able to excrete natural antibiotics, which can have a broad spectrum activity. Other mechanisms include an improved immune stimulation, competition for nutrients and blocking of pathogen adhesion sites in the gut. Many probiotics have been shown to reduce colonization and shedding of salmonella and campylobacter in poultry (*Morishita, et al 1997 & Johannsen, et al. 2004*).

Moreover, vaccination is an alternative control measurement for the disease and some diseases have been eliminated by use of the vaccines. Live attenuated salmonellae vaccine are protective, and are candidate vaccines against invasive salmonella infections in man and animals (*Carlos, 1991*). The use of vaccines against salmonella is still a problem because bacterin (killed vaccine) induces poor immunity and the live attenuated vaccine may restore its virulence, shedding and emerging of vertical transmission.

This work was planned to evaluate the effect and compare between prebiotic, probiotic and vaccine in prevention and control salmonella infection in local breed chicks.

## MATERIAL & METHODS

### A-Experimental design:

Two hundred and ten one - day -old local breed chicks were divided into 8 groups (G) 25 each by ranking methods. Ten birds were sacrificed and 25 fecal swabs to prove salmonella free.

Group	Treatment	Remarks
1	control negative plain diet	
2	probiotic control( <b>Biogen</b> ®)	from 18 <sup>th</sup> day of age till the end
3	Prebiotic control( <b>Admix</b> ®)	from 18 <sup>th</sup> day of age till the end
4	vaccine control	at the 1 <sup>st</sup> week of age
5	S.enteritidis positive control	challenged at 21 day of age
6	probiotic + S.enteritidis	challenged at 21 day of age
7	Prebiotic+ S.enteritidis	challenged at 21 day of age
8	vaccine +S.enteritidis	challenged at 21 day of age

- All groups were kept under observation until 11 week of age.
- feed are offered ad lib till 3<sup>rd</sup> week of age, then the feed are offered with restricted system
- Five birds are sacrificed every 2 week, for serum collection and isolation from internal organs.
- Feed consumption and weight are recorded bi-weekly.
- All chicks were vaccinated against Newcastle disease, at 7<sup>th</sup> and 22<sup>nd</sup> day of age with Hitchener B1 and Lasota strain vaccine, respectively and IBD vaccine at 14<sup>th</sup> day .

#### **B-Probiotic ( Biogen)®**

- Allicin 0.247 µmol/g
- B.subtilis Natto 6x10<sup>7</sup>/g
- Hydrolytic enzyme 3690 u/g

#### **C- Prebiotic (Admix) ®**

- -Sodium butyrate

#### **D-Vaccination of bird with S.enteritidis.**

Preparation of the killed vaccine followed mainly the technique described by (Timms, et al., 1990) and the time of vaccination 2 week before challenge.

**E- Measurement of blood chemistry:** Biochemical parameters were assayed calorimetrically by using of commercial diagnostic kits of total protein (Weichselbaum, 1946), albumin (Doumas, 1971), aspartate ami-notransferase (AST) and alanine aminotransferase (ALT) (Retiman and Francke, 1957). Creatinine (Husdan and Rapūport, 1968), uric acid (Arliss and Entwistle, 1981).

**F- Statistical analysis:** using the General Linear Model for analysis of variance (*SAS, 1990*). Duncan's multiple range test (*Duncan, 1955*) was used for test the significance ( $p < 0.05$ ) of differences among means.

**G- S.enteritidis strain** used in artificial infection was supplied by Dr *A.M. Hegazy* (Animal Health Research Institute, Kafr El-Sheikh Regional lab.)

## RESULT & DISCUSSION

Symptoms appeared at 5 days post infection. as depression, low feed intake and whitish diarrhoea in salmonella infected control group, while the symptoms was mild in treated groups but it was milder in vaccinated –salmonella infected group. Mortality was 4 % in salmonella control group and the deaths are restricted to the 1<sup>st</sup> week post infection; the low mortality rate or absence in other groups may be attributed to the treatment applied or genetic factors related to the native breed used in experimental infection or the fact that the paratyphoid bacteria are not host specific and produce mortality only in young chicks.

Infection of chickens with salmonella involve three stages, first , intestinal colonization where the shedding occur (*Muir et al., 1998*), second. invasion beyond gastrointestinal tract can lead to multiplication of the organisms in macrophage – phagocyte system of liver , spleen and other organs (*Barrow et al., 1987*), third , extensive bacteraemia which may cause high mortality specially in young birds. In the present work S.enteritidis was capable to colonize different organs (Table,1) with different rates, intestine 46.6%, liver 45% , spleen 40% and gallbladder 25% (*Barrow, et al. 1987*), but it is clear that the rate of colonization was affected as the addition of either probiotic or prebiotic protect

organs to be colonized with the same rate as in salmonella infected group, and the reduction in colonization rate was higher in probiotic than prebiotic in the liver 25% vs.15% respectively ; while in the intestine the reduction in shedding rate were 20% vs. 13.3% for probiotic and prebiotic, respectively and this could be supported by the findings of (Fuller 1989; Line et al.,1998 and Tollba et al.,2007) as they reported that probiotics, prebiotic or both, suppressed the counts of pathogenic intestinal bacteria where severe decreases in counts of E.coli,S.pullorum and Clostridium perfringens of duodenum, jejunum, ileum and caecum.

The colonization rate in the spleen was 15% for each of probiotic and prebiotic vs.40% in salmonella control group. No differences in the reduction of colonization rate between probiotic and prebiotic in each of spleen and gall bladder. The overall result may supported by the finding of (Corrier et al., 1995; Tellez, et al., 2001 and Ellakany, et al., 2004) and disagree with the results reported by (Soerjadi, et al., 1981 and Weinack, et al., 1985).

The effect of vaccination on colonization and shedding was evident as the colonization rate were 45%, 40%, 25%, and 46.6% in each of liver, spleen, gallbladder and intestine in salmonella infected group, respectively. This rate of colonization has been greatly changed in vaccinated challenged group, 10%, 5%, 5% and 20%, respectively. So, we can state that vaccination does not prevent either shedding or colonization but it reduces both (Gast, et al., 1992; Barbour, et al., 1993 and Hegazy, 2002).

The body gain showed variable significant along the experimental period (Table, 2), but totally the obvious significant difference were encountered in the following occasion, Salmonella infected group vs. all

control groups; Salmonella infected group vs. treated challenged groups a long the experiment. From the previously mentioned level of significance we can conclude that ; probiotics improved body weight 665 gm vs. 638 gm, in probiotic control and control group respectively (*Ellakany, et al., 2004 and Tollba, et al., 2007*); all the treatments applied (probiotic, prebiotic and vaccination) protect the severe body weight loss due to S.enteritidis infection in comparison to S.enteritidis infected control group (*Hegazy, 2002*). Also, it is evident that vaccine does not enhance body gain (648gm) like probiotic (665gm) but it is efficient in protection the weight loss (573gm), than probiotic (522gm).

Feed conversion rate in the different groups are presented in (Table3), it is clear that the significance between groups more or less are fixed with few exceptions along the experimental period but with the mean of feed conversion, it is noticed. that S.enteritidis infection significantly increase FCR 4.6 vs., 3.13, 2.86, 3.15 and 2.96 in control, probiotic control, prebiotic control and vaccine control, respectively. All the infected and treated groups showed protection for FCR, but the best protection was offered by vaccination followed by probiotic and prebiotics. Other differences could be traced but not significant like that between control and probiotic control group. Our result in performance could be supported by the findings of (*Hegazy, 2002 .; Ellakany, et al., 2004., and Tollba, et al., 2007*).

Changes that happened in the blood biochemical values is a mirror of the changes occurred in the tissues and organs as a result of bacterial infection, although histopathological picture was not done, yet, salmonella infection had been proved to cause histopathological changes by many investigator (*Bayoumi, et al., 1979 and Hegazy, 1991*) and in turn these changes are reflected on the biochemical picture.

Albumin is considered the large fraction of the total protein, chronic renal or hepatic diseases; malnutrition and malabsorption cause hypoproteinemia (*Embert, 1986*). Liver is affected greatly due to infection or sepsis which in turn affects its function (*Kokosharov, et al., 1997*), who reported degenerative changes in the liver to which attribute the decreased protein synthesis. In the present work, there were significant differences between each of all control groups vs. *S. enteritidis* infected group; and treated challenged groups vs. *S. enteritidis* infected group; as *S. enteritidis* infection leads to severe decrease in total protein (Table, 4); in the same time, the treatment applied showed protection from salmonella harmful effect with no significant difference between them. Among the control group, there was significant difference between probiotic treated group and other control group. Decrease in the protein level may be attributed to liver lesion and damage in the glomerular filtration barrier may result in the presence of plasma proteins in the urine; in addition, inflammation of the renal parenchyma or epithelial damage of the tubules may cause loss of protein to the urine, (*Relford, 1996 and Freitas, 2007*).

The pattern of significance in albumin (Table, 4) was the same as total protein with some differences, which may be traced between the following: significance difference between each of probiotic vs. prebiotic and vaccinated control groups. 2.94 gm vs. 2.3gm & 2.55gm, respectively (*El-Hommosany & Gihan, 2007 and Freitas, 2007*). Also, proteins as well as albumin increased in groups treated with probiotics and prebiotics (*Abd El-Azeem, et al., 2001; El-Hommosany and Gihan, 2007 and Tollba, et al., 2007*).



Liver enzymes AST & ALT were increased under the effect of each of probiotic, prebiotic and salmonella (*Freitas, 2007*) significantly in comparison to control negative group. In treated challenged groups the effect of probiotic, prebiotic and salmonella was expected to be a summation of the effect of each one of them with the effect of salmonella but, it was not happened as in treated groups the values of AST & ALT was less than that recorded in salmonella control group (Table, 4), this may be disagree with the following statements: ALT and AST enzymes were not affected due to probiotic and prebiotic treatments and may record low level than the control (*Abd El-Azeem, et al., 2001; and El-Hommosany and Gihan, 2007*).

Uric acid had no significant difference between any of the control groups and also, between treated and challenged groups, while significances were traced between each of control groups vs. *S. enteritidis* infected group and treated challenged groups and *S. enteritidis* infected one, these mean that, the applied treatments prevent harmful effect of *S. enteritidis* infection (Table, 4); while the creatinine level among control groups were significant between control negative, vaccine control vs. probiotic, prebiotic control. Significant differences were detected between vaccine challenged group and each of salmonella infected, probiotic challenge and prebiotic challenged groups. The following reviews may determine some points of agreement and discrepancies, on *S. gallinarum* there was an increase in the activity of aspartate-aminotransferase (AST) in birds five days post-inoculation as compared to the mean value in birds of the same group (*Freitas, N., 2007*). Salmonella infection in Kafrelsheikh Vet. Med. J. Vol. 6 No. 2 (2008)

chicks leads to greater increase in the values of blood constituents which reflecting the symptoms of impaired liver and kidney functions (such as cholesterol, triglyceride, AST, ALT, and uric acid (*El-Hommosany & Gihan, 2007*); Uric acid and creatinine were not affected due to probiotics and, prebiotics treatments and may record low level than the control (*Abd El-Azeem, et al., 2001; El-Hommosany and Gihan, 2007 and Tollba, et al., 2007*).

It is concluded that the applied treatments (prebiotic, probiotic and vaccine) showed positive effect on the measured parameters which appear in much instances significantly, and in low instances their effects were negative. But generally all applied treatments decreased significantly the drastic effect of the infection on growth, feed conversion, and liver and kidney functions.

**Table (1):** Colonization of *S. enteritidis* and rate of shedding as judged by intestinal colonization.

Groups	Salmonella (S)		Probiotic+(S)		Prebiotic+(S)		Vaccination+(S)	
	+/tot.	%	+/tot.	%	+/tot.	%	+/tot.	%
Liver	9/20	45	4/20	20	6/20	30	2/20	10
Spleen	8/20	40	3/20	15	3/20	15	1/20	5
G.bladder	5/20	25	4/20	20	5/20	25	1/20	5
Intestine	14/30	46.6	8/30	26.7	10/30	33.3	6/30	20
total	36/90	40	19/90	21.1	24/90	26.6	10/90	11.1

**Table (2):** Average body weight gain in different groups in gm.

Groups Period pi	Control negative	Probiotic cont.	Prebiotic cont	Vaccinatio n control	Salmon. (S)	Probiotic+ (S)	prebiotic.+ (S)	Vaccinatio n+ (S)
2 wks.	171 <sup>bcf</sup>	175 <sup>bf</sup>	157 <sup>cd</sup>	168 <sup>fd</sup>	98 <sup>a</sup>	141 <sup>cb</sup>	130 <sup>b</sup>	153 <sup>dc</sup>
4 wks	161 <sup>cd</sup>	177 <sup>bf</sup>	166 <sup>fdc</sup>	157 <sup>d</sup>	106 <sup>a</sup>	126 <sup>b</sup>	125 <sup>b</sup>	141 <sup>c</sup>
6 wks	150 <sup>dc</sup>	161 <sup>cd</sup>	149 <sup>dc</sup>	164 <sup>d</sup>	116 <sup>a</sup>	123 <sup>ba</sup>	119 <sup>a</sup>	137 <sup>cb</sup>
8 wks.	156 <sup>cc</sup>	152 <sup>dc</sup>	141 <sup>cb</sup>	159 <sup>fd</sup>	115 <sup>a</sup>	132 <sup>b</sup>	129 <sup>ab</sup>	142 <sup>cb</sup>
Total	638 <sup>c</sup>	665 <sup>c</sup>	613 <sup>de</sup>	648 <sup>c</sup>	435 <sup>a</sup>	522 <sup>bc</sup>	503 <sup>b</sup>	573 <sup>cd</sup>
R.A.B.G 100 %	100	104.23	96.08	101.6	68.2	82	78.84	89.8
Mean of gain/period	159.5 <sup>e</sup>	166.3 <sup>c</sup>	153.3 <sup>dc</sup>	162 <sup>e</sup>	108.8 <sup>a</sup>	130.5 <sup>bc</sup>	125.8 <sup>b</sup>	143.3 <sup>cd</sup>

R.A.B.G= relative average body gain.

\*there is no significant difference between items carrying the same letter.

**Table (3):** Average feed conversion ratio in different groups.

Period group	Control	Probiotic cont.	Prebiotic cont.	Vaccination control	Salmonella (S)	Probiotic +(S)	Prebiotic +(S)	Vaccination +(S)
2weeks	2.46 <sup>ab</sup>	2.15 <sup>a</sup>	2.25 <sup>a</sup>	2.5 <sup>ab</sup>	3.29 <sup>b</sup>	2.78 <sup>ab</sup>	3 <sup>ab</sup>	2.58 <sup>ab</sup>
4 weeks	2.95 <sup>a,b</sup>	2.49 <sup>a</sup>	3.18 <sup>ab</sup>	2.81 <sup>a</sup>	4.28 <sup>c</sup>	3.63 <sup>bc</sup>	3.48 <sup>bc</sup>	3.63 <sup>ab</sup>
6 weeks	3.29 <sup>a</sup>	3.18 <sup>a</sup>	3.53 <sup>ab</sup>	2.84 <sup>a</sup>	5.33 <sup>c</sup>	3.72 <sup>ab</sup>	4.48 <sup>bc</sup>	3.96 <sup>b</sup>
8 weeks	3.83 <sup>a</sup>	3.61 <sup>a</sup>	3.64 <sup>a</sup>	3.7 <sup>a</sup>	5.31 <sup>c</sup>	4.51 <sup>abc</sup>	4.88 <sup>bc</sup>	3.91 <sup>a</sup>
Mean =	3.13 <sup>ab</sup>	2.86 <sup>a</sup>	3.15 <sup>ab</sup>	2.96 <sup>a</sup>	4.6 <sup>c</sup>	3.66 <sup>ab</sup>	3.96 <sup>bc</sup>	3.52 <sup>a</sup>
S.E	= 0.29	= 0.33	= 0.32	= 0.28	= 0.49	= 0.38	= 0.43	= 0.32

\*there is no significant difference between items carrying the same letter in the same row

**Table (4):** blood chemistry in different group.

Groups	Control -	Probiotic control	Prebiotic control	Vaccine control	Salmonella	Probiotic +(S)	Prebiotic +(S)	Vaccine -(S)
Item	Mean ±S.E	Mean ±S.E	Mean ±S.E	Mean ± S.E	Mean ±S.E	Mean ± S.E	Mean ±S.E	Mean ± S.E
T. protein g dl	4.9 <sup>a</sup> ±0.2	5.4 <sup>d</sup> ±0.32	4.9 <sup>a</sup> ±0.13	4.86 <sup>bc</sup> ±0.06	3.2 <sup>a</sup> ±0.09	4.37 <sup>b</sup> ±0.12	3.96 <sup>b</sup> ±0.23	4.2 <sup>a</sup> ±0.21
Albumin g dl	2.79 <sup>bc</sup> ±0.04	2.94 <sup>b</sup> ±0.08	2.3 <sup>bc</sup> ±0.16	2.55 <sup>cd</sup> ±0.14	1.7 <sup>a</sup> ±0.03	2.44 <sup>a</sup> ±0.1	1.99 <sup>ab</sup> ±0.16	2.28 <sup>b</sup> ±0.08
ALT U.L	41 <sup>a</sup> ±1.1	52 <sup>d</sup> ±1.9	59 <sup>bc</sup> ±2.3	42 <sup>ab</sup> ±3.6	65 <sup>cd</sup> ±3.9	59 <sup>bc</sup> ±1.3	69 <sup>d</sup> ±1.1	48.5 <sup>bc</sup> ±2.8
AST U.L	10 <sup>a</sup> ±0.4	14.6 <sup>bc</sup> ±0.5	16.6 <sup>cd</sup> ±1	9 <sup>a</sup> ±0.3	26 <sup>c</sup> ±0.9	17 <sup>cdm</sup> ±1	20.6 <sup>cd</sup> ±0.8	14 <sup>ab</sup> ±0.8
Unc acid	4.36 <sup>a</sup> ±0.05	4.64 <sup>a</sup> ±0.17	4.6 <sup>a</sup> ±0.09	4.42 <sup>a</sup> ±0.05	6.75 <sup>a</sup> ±0.23	5.85 <sup>b</sup> ±0.17	5.4 <sup>b</sup> ±0.11	5.8 <sup>c</sup> ±0.32
Creatinme	0.79 <sup>a</sup> ±0.02	0.98 <sup>b</sup> ±0.04	1.12 <sup>c</sup> ±0.06	0.78 <sup>a</sup> ±0.03	1.38 <sup>d</sup> ±0.04	1.28 <sup>d</sup> ±0.05	1.3 <sup>d</sup> ±0.03	0.85 <sup>a</sup> ±0.02

\*there is no significant difference between items carrying the same letter in the same row

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## بعض الدراسات عن طرق المقاومة لعدوى ميكروب السالمونيلا في الدجاج

عبد الجليل محمد محمد حجازي<sup>\*</sup>، نصره بدير عوضين<sup>\*\*</sup>، حمزة عبد المنعم على سليمان<sup>\*</sup>

<sup>\*</sup>معهد بحوث صحة الحيوان - (معمل كفر الشيخ)

<sup>\*\*</sup>معهد الإنتاج الحيواني (محطة بحوث الإنتاج الحيواني بسخا)

أجرى هذا البحث لتقييم بعض الطرق البيولوجية في الوقاية والسيطرة على عدوى ميكروب السالمونيلا في الدجاج. وقد تم عمل ثماني مجاميع تم معالجتها كالتالي:- المجموعة الأولى ضابطة الثانية ضابطة للبروبيوتيك الثالثة ضابطة للبروبيوتك والرابعة ضابطة للتحصين الميت للسالمونيلا أنتريبتيدس والخامسة ضابطة لعدوى السالمونيلا السادسة (بروبيوتيك + سالمونيلا) المجموعة السابعة (بروبيوتك + سالمونيلا) والمجموعة الثامنة (تحصين + سالمونيلا). تم دراسة العدوى الصناعية وتقييمها في المجموعات المختلفة من حيث أداء الطيور ومدى تواجد السالمونيلا في الأعضاء الداخلية وتقيم بعض الوظائف الحيوية للكبد والكلى. وتم تحليل النتائج إحصائياً.