# OBSERVATION ON COMPETITIVE EXCLUSION FOR REDUCING SALMONELLA TYPHIMURIUM INFECTION OF BROILER CHICKS

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***Abstract***

The objective of this work was to study *in vivo* the efficacy of various types of acids and some commercial products such as Acid pack 4 way, Salkile or Fermacto to minimize or inhibit colonization of *S.typhimurium* in broiler chicks. Cecal colonization with *S.typhimurium* was slightly affected by dietary propionic acid or formic acid, but the use of salkile "Bio-Add" was a useful means to protect broilers from *S.typhimurium* colonization. There was no significant difference in cecal pH or body weight gain among acids treated groups of chicks. Acid pack 4 way "Probiotic" caused significant decrease in the colonization rates and more gain body weight. Feramacto treated feeds has little significant effect on rates of colonization, but the mean body weight of chicks was significantly increased. The best overall result was achieved with treatment with both Acid pack 4 way plus lactose. The colonization rates of combined treatment groups were discussed in details.

## INTRODUCTION

Great efforts and attention should be paid to eliminate *Salmonella typhimurium* from poultry farms especially those which apply the modern intensive systems of rearing and management (Barrow and Tucker, 1986).

Competitive exclusion is an approach to prevent infection or colonization of *S.typhimurium*. It produces a protctive barrier against invasion and colonization (Michael *el al.*, 1993). The competitive exclusion concepts have proved effectiveness in several laboratories around the world (Bares *et al.*, 1980 and Nisbet *et al.*, 1992) but the limited number of large scale field trials are still not obvious till now.

Some workers suggested that formic or propionic acids or their mixtures are active in the alimentary tract, particularly in the crops and ceca, before degradation or absorption in the intestinal tract and the acid mixture is likely to be antibacterial both in the crops and in the feed (McHan and Shotts, 1992, Michael *et al.*, 1993 and Ziprin *et al.*, 1993). Thus, it would be useful to test the effect of such acids against *S.typhimurium* for controlling its colonization.

However, few studies have investigated commercial feeds containing a wide variety and combination of antimicrobial and growth promoting feed additives such as acid pack 4 way, Salkile and Fermacto (Snoyenbos *et al.*, 1978 and Hollister *et al.*, 1993).

Thus, the objective of this study was to establish an effective competitive exclusion to prevent *S.typhimurium* colonization in chicks. Accordingly, this work was planned to determine under experimental conditions the use of propionic acid, formic acid or their mixture to control experimental *S.typhimurium* infection in broiler chicken. Also, to evaluate the activity of commercial antisalmonellae products and lactose under Egyptian climatic and storage conditions.

## MATERIALS AND METHODS

### 1. Chicks

A total of 200 (one day old) White Lohman selected chicks were obtained from Elwadi Poultry Company. All chicks, as well as, their ration were examined for the absence of *Salmonellae.*

### 2. *S.typhimurium* strain used

*S.typhimurium* strain was kindly obtained from Animal Health Research Institute, and examined morphologically, culturally, biochemically and serologically for complete confirmation.

### 3. Preparations used for *Salmonella* exclusion

**(a) Lactose α-lactose monohydrate) [Adwic].**

**(b) Commercial Acid Pack 4 way.**

It contains *Streptococcus faecium* fermentation product, dried *Lactobacillus acidophilus*, dried *B.subtilis* beta glycan, *Aspergillus niger*, citric acid, sorbic acid, sodium citrate, sodium chloride, potassium chloride, zinc sulphate, magnesium

sulphate, dextrose, neutral and artificial flavours.

**(c) Formic acid (Misr-Scientific Company)**

**(d) Propionic acid (Aldrich)**

**(e) Salkile (Hercules 2, Calleva Park Aldermaston, Berkshire)**

It contains short chain organic acids (formic and propionic acids).

**(f) Fermacto (pet-Ag Inc, Elgin. Euinosis, USA)**

Consists of commercial Aspergillus, yeast fermentation, corn distillers, dried grains with germ meal and bran dehydrated and dried whey product.

**4. Experimental Design**

All chicks were divided into 10 groups each of 20 chicks. Those of group "1" were considered as a non-challenged control. Chicks of group "2" were used as a challenged control group and fed on normal diet without the addition of any preparation. Chicks of group "3" were given propionic acid in feed at concentration of 1%. Group "4" were given 1% formic acid in feed. Chicks of group "5" were given salkile in their feed. The chicks of group "6" were given Acid Pack "4" way in drinking water at concentration of 1.5%. Group "7" were given Fermacto in their feed. Chicks of group "8" were fed on acid pack 4 in water and lactose (5%) in the feed. The chicks of group "9" were given Fermacto in feed and 2.5% lactose in water. The chicke of group "10"were giwen Fermacto and salkile infeed. ALL chicks except those of unchallenged control group were challenged with $10^8$ C.F.U/ml *S.typhimurium* orally on the 3rd day of hatch. They were kept under obervation twice daily to record their general health conditions and to notice any clinical manifestations and mortalities. On the 10th day of experiment, the weight of chicks from each group was checked and were killed by cervical dislocation. Contents of each cecum was collected aseptically and evaluated for *S.typhimurium* colonization by serial dilution and spreading directly onto brilliant green agar plates, and the number of colonies on each dilution was determined according to Collins and Lyne (1984). Typical *S.typhimurium* colonies were confirmed biochemically and serologically. One gram of cecal content was collected from each chick and examined for pH according to Cruickshank *et al.* (1975).

On the 17th day of experiments, the rest of chicks in each group were killed, and the weight, pH and *S.typhimurium* colony count were determined as mentioned before. The difference in the results was statistically analyzed according to Snedecor and Cochran (1976).

## RESULTS AND DISCUSSION

*S.typhimurium* causes paratyphoid infection in poultry. It is a significant enteric pathogen in human beings and it is the most frequently isolated serovars reported of humans. Contamination of broiler carcasses during processing is increased when *S.typhimurium* is introduced into the processing facility in the intestinal tract and faeces contaminated feet and feathers of market-age broilers. Prevention or *S.typhimurium* colonies in the intestines of chicken may greatly reduce the contamination of meat products and carcasses during processing.

Table 1 illustrates the results of acid inhibition of *S.typhimurium* colonization of the ceca of broiler chicks. The colonization rates were 70%, 90% and 100% among 1% formic acid and *S.typhimurium* challenged control groups, respectively, on 10th day, whereas, similar results were 80%, 90% and 100% among the aforementioned groups, respectively on 17th day of experiment. These findings are nearly in agreement with those obtained by Hinton and Linton (1988) who showed that propionic acid has been used as a feed additive to reduce or eliminate pathogenic bacterial and fungal contamination. Also, McHan and Shotts (1992) found that, when dietary propionic or formic acids were increased up to 1% of the diet, *S.typhimurium* in the ceca was reported to be significantly lowered. Moreover, McHan *et al.* (1992) noted a significant reduction in the cecal *S.typhimurium* due to short chain fatty acids feeding in-vivo. On the contrary, Micheal *et al.* (1993) indicated that, propionic acid in the feed was ineffective in reducing *S.typhimurium* infection in the crops and ceca. The results presented here show that the formic acid or propionic acid is effective in reducing the colonization of *S.typhimurium*. The results of feeding salkile (Group No. 5) which is a mixture of formic and propionic acid showed reduction of 50% and 40% of colonization rates and level of *S.typhimurium* $\log_{10}$ were 1.47 and 2.25 on 10th and 17th days of experiments, respectively. These findings are nearly in agreement with those recorded by Hinton *et al.* (1985) and Iba and Bercheri (1995) who recorded that acid mixture (Bio-Add) was likely to be antisalmonella effect both in the crops or in the feeds.

As shown in Table 1, the effect of acid pack-4 way or Fermacto on *S.typhimurium* and levels of colonization in the intestinal tract of chicks are recorded. The colonization rates were 40% and 90% among Acid pack 4 way group and Fermacto treated group, respectively. The level of *S.typhimurium* in the Acid pack 4 way ranged between $\text{Log}_{10}$ 2.55 and 2.39 on 10th and 17th days,

Table 1. Effecicacy of certain acids, acid pack 4 way, Fermacto and combined treatments to minimize S.typhimurium cecal colonization of broiler chicks.

| Group No. | Treatment | No. of infected chicks/ Total | | % Colonization | | Log $_{10}$ Salmonella g. caecal contents | |
|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B |
| 1 | Unchallenged Control. | 0/0 | 0/10 | 0 | 0 | 0 | 0 |
| 2 | *S.typhimurium* Challenged Control. | 10/10 | 10/10 | 100 | 100 | 6.56 | 6.07 |
| 3 | *S.typhimurium* + (1% propionic acid). | 7/10 | 8/10 | 70 | 80 | 3.61 | 4.49 |
| 4 | *S.typhimurium* + (1% formic acid). | 9/10 | 8/10 | 90 | 80 | 4.36 | 4.77 |
| 5 | *S.typhimurium* + (Salkile "bioadd"formic acid propionic acid). | 5/10 | 4/10 | 50 | 40 | 1.47* | 2.25* |
| 6 | *S.typhimurium* + Acid pack 4 way. | 4/10 | 4/10 | 40 | 40 | 2.55* | 2.39 |
| 7 | *S.typhimurium* + Fermacto. | 9/10 | 8/10 | 90 | 80 | 4.23 | 4.34 |
| 8 | *S.typhimurium* + Acid pack 4 way+ Lactose. | 1/10 | 0/10 | 10 | 0 | 2.00 | 0.00* |
| 9 | *S.typhimurium* + Fermacto + Lactose. | 7/10 | 6/10 | 70 | 60 | 3.46 | 2.76* |
| 10 | *S.typhimurium* + Fermacto + Salkile. | 5/10 | 5/10 | 50 | 50 | 3.20 | 3.88 |

A = Results after $10^{th}$ day from the beginning of the experiment.

B = Results after $17^{th}$ day from the beginning of the experiment.

* = Significant different from the corresponding *S.typhimurium* challenged control ($P<0.001$).

respectively, while, Fermacto group was $\log_{10}$ 4.23 on the 10th day of the experiment. Acid pack 4 way contains *Lactobacillus acidophilus* and *Streptococcus faecium* which are microflora, and when given to chicks causes significant decrease in colonization rates and levels of *S.typhimurium*. These findings nearly coincide with the results obtained by Snoyenbos *et al.* (1978) who observed that, normal microflora has been reported to inhibit colonization of chicks gut by competition for binding sites. The present results agree with those of Blankeenship *et al.* (1993) and Hollister *et al.* (1993). They reported that competitive exclusion cultures encapsulated and itophilized in aliginate beads significantly increased resistance of chicks to *S.typhimurium*.

As shown in Table 1, the effect of combined treatments of Acid pack 4 way plus lactose (Group 8), Feramcto plus lactose (Group 9) and Fermacto plus Salkile (Group 10) on the colonization rates and the number of *S.typhimurium* in the cecal contents of chicks challenged with $10^8$ viable *S.typhimurium* varied between different treated groups. Among groups supplied with Acid pack 4 way plus lactose, the colonization rates were decreased to 10% and 0% on the 10th and 17th days, respectively, while, level of $\log^{10}$ *S.typhimurium* were 2.00 and 0.0 on similar days, respectively. Colonization rates of Fermacto plus lactose treated group and Fermacto plus salkile treated group were 70% and 50% on the 10th day, respectively, and 60% and 50% on the 17th day, respectively. These results agree with those obtained by Nisbet *et al.* (1992) and Koya *et al.* (1993) who explained that *S.typhimurium* colonization of the intestinal epithelium of broiler chicks could be inhibited by using combination of lactose with cecal anaerobic flora.

As shown in Table 2, the results of acid inoculation on cecal pH of broiler chicks are illustrated. There was no significant difference in cecal pH among *S.typhimurium* challenged groups except in 1% formic acid treated group on 17th day of experiment ($P<0.05$). These results agreed with those of Meynell (1963) who indicated that low pH (5.0 - 6.0) was not bacteriostatic in itself, and *S.typhimurium* growth was not directly inhibited by the low pH present in the cecal contents. Also, Michael *et al.* (1993) observed that, providing of dietary propionic acid had no significant effect on crop or cecal pH.

As shown in Table 2, the effects of treatment with Acid pack 4 way or Fermacto on cecal pH of broiler chicks revealed no significant differences if compared with *S.typhimurium* challenged control group as regards to cecal pH. Also, the pH of cecal contents of chicks in groups provided with Acid pack 4 way plus

Table 2. Effet of Acids, Acid Pack 4 Way, Fermacto and combined treatments on cecal pH of broiler chickes chalenged with *S.typhimurium*.

| Group No. | Treatment | Caecal pH | |
|---|---|---|---|
| | | At the 10 th day of experiment | At the 17 th day of experiment |
| 1 | Unchallenged Control. | 5.95±0.03 | 6.01±0.01 |
| 2 | *S.typhimurium* Challenged Control. | 5.89±0.05 | 5.88±0.07 |
| 3 | *S.typhimurium* + (1% propionic acid). | 6.00±0.92 | 6.06±0.90 |
| 4 | *S.typhimurium* + (1% formic acid). | 5.98±0.07 | 5.18±0.13* |
| 5 | *S.typhimurium* + (Salkile "bioadd"formic acid propionic acid). | 5.86±0.07 | 5.75±0.09 |
| 6 | *S.typhimurium* + Acid pack 4 way. | 5.99±0.40 | 5.20±0.69 |
| 7 | *S.typhimurium* + Fermacto. | 5.02±0.15 | 5.10±0.13 |
| 8 | *S.typhimurium* + Acid pack 4 way+ Lactose. | 5.04±0.54 | 5.10±0.49 |
| 9 | *S.typhimurium* + Fermacto + Lactose. | 5.26±0.53 | 5.31±0.47 |
| 10 | *S.typhimurium* + Fermacto + Salkile. | 5.95±0.43 | 6.01±0.43 |

* = Significant different from the corresponding *S.typhimurium* challenged control ($P<0.001$).
No significant difference was recorded in the remaining data.

Table 3. Effect of Acids, Acid pack 4 way or fermacto and some treatment in combination on weight gain of broiler chicks challenged with *S.typhimurium*.

| Group No. | Treatment | Caecal pH | |
|---|---|---|---|
| | | On the 10 th day of experiment | On the 17 th day of experiment |
| 1 | Unchallenged Control. | 80±1.30 | 116±1.74 |
| 2 | *S.typhimurium* Challenged Control. | 77±1.09 | 111±0.84 |
| 3 | *S.typhimurium* + (1% propionic acid). | 75.3±5.07 | 102.5±5.84 |
| 4 | *S.typhimurium* + (1% formic acid). | 78.5±4.56 | 107.14±4.49 |
| 5 | *S.typhimurium* + (Salkile "bioadd"formic acid propionic acid). | 75±1.58 | 110±1.81 |
| 6 | *S.typhimurium* + Acid pack 4 way. | 78±1.14 | 103±1.30 |
| 7 | *S.typhimurium* + Fermacto. | 91±1.38* | 118±0.55* |
| 8 | *S.typhimurium* + Acid pack 4 way+ Lactose. | 90.1.47* | 112±1.30 |
| 9 | *S.typhimurium* + Fermacto + Lactose. | 95±0.53 | 126±1.30** |
| 10 | *S.typhimurium* + Fermacto + Salkile. | 93±0.89** | 121±1.17** |

* Significant difference at P<0.05.
** Significant difference at P<0.001.

lactose, as well as, Fermacto plus lactose and Fermacto plus salkile were 5.04, 5.26 and 5.95 on the 10th day, respectively. There was significant drop in the cecal pH in both groups supplied with lactose than that of the *S.typhimurium* challenged control groups ($P<0.05$).

It could be seen from Table 3, that, there was no relationship between providing dietary acids and weight gain of chicks infected with *S.typhimurium*. Also, the Acid pack 4 way had no significant effect on the mean body weight of broiler chicks ($P<0.001$), whereas, the mean body weight of chicks among Fermacto group ranged from 91 and 118 on the 10th and 17th days, respectively. There was significant increase in body weight among both Fermacto plus lactose group and Fermacto plus salkile group.

Generally, the present results of combined treatments on the number of *S.typhimurium* in the ceca, and the number of cecal culture positive may be explained by the fact that, combined treatement of Acid pack 4 way plus lactose is the treatment which consistently lowered the colonization rate of the organism than did treatments with Fermacto plus lactose or Fermacto plus salkile. The optimal inhibition of Salmonellas growth occurred in the presence of a carbon source such as lactose that is metabolized by the microflora not by Salmonellas.

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# ملاحظات علي خفض عدوي السالمونيلا تيفميوريم في دجاج التسمين باستخدام المقارنة بين المواد المختلفة المستعملة في ذلك

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أن الغرض من هذا البحث هو إجراء دراسة معملية عن مدي تأثير أنواع مختلفة من الأحماض وبعض المنتجات التجارية مثل آسيد باك فور واي وسالكيل أو فيرماكتو للإقلال من تكون مستعمرات ميكروب السالمونيلا تيفميوريم في كتاكيت دجاج التسمين أو منع تكونها تماما. تأثر تكون مستعمرات ميكروب السالمونيلا تيفميوريم في القولون تأثرا بسيطا بواسطة إضافة حامض البروبيونك أو حامض الفورميك الي العلف أو الماء. اما استعمال سالكيل فقد كان الوسيلة العملية لحماية دجاج التسمين من تكون مستعمرات ميكروب السالمونيلا تيفميوريم. لم يحدث في مجموعة كتاكيت التسمين المعالجة بالحمض أي إختلاف ذي أهمية في درجة حموضة القولون أو في حصول الزيادة في الوزن. كما وجد ان الغذاء المعالج بواسطة فرماكتو له تأثير بسيط جدا علي معدلات تكون المستعمرات ولكن إزداد معدل وزن الجسم للكتاكيت بدرجة ملحوظة. وبشكل عام كانت أفضل نتيجة تم الحصول عليها من استعمال كل من أسيد باك فور واي مع اللاكتوز وتم تفصيليا مناقشة معدلات تكون المستعمرات في المجموعات المعالجة بواسطة العلاج المختلط.