

## EVALUATION OF USING SOME ORGANIC ACIDS AGAINST SALMONELLA INFECTION IN BROILER CHICKENS EXPERIMENTALLY

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### ABSTRACT

A total 110 one-day-old chicks were used in this study to investigate antibacterial effects of organic acid (formic and propionic acid) either alone or together against *Salmonella pullorum* (*Sal. pullorum*). 10 chicks were slaughtered and examined bacteriologically to prove that chicks free from *salmonella*, the remainder 100 broiler chicks were divided into 5 groups (20 chicks/each), 1<sup>st</sup> group healthy chicks (negative control), At 14<sup>th</sup> day of age broilers in group 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> experimentally infected with *Sal. pullorum*, 2<sup>nd</sup> group infected chicks (positive control), 3<sup>rd</sup> group chicks received 0.1 ml formic acid /liter drinking water from 1<sup>st</sup> to 35<sup>th</sup> day of age and infected with *Sal. pullorum*, 4<sup>th</sup> group chicks received 0.1 ml propionic acid/liter drinking water from 1<sup>st</sup> to 35<sup>th</sup> day of age and infected with *Sal. pullorum*. 5<sup>th</sup> group received formic acid and propionic by same dose, period and route of infection with *Sal. pullorum*. At 1<sup>st</sup> day post supplementation 5 chicks from each group were slaughtered and two blood samples were taken for estimation of hematobiochemical parameters. Cloacal swabs were collected for salmonella reisolation. Infected broilers showed clinical **signs** represented by anorexia, closed eyes diarrhea, dehydration, mortality rate 30% and reduction in body weight gain, RBCs, Hb, PCV%, MCHC, lymphocytes total protein, albumin, CAT and SOD beside significant increase in FCR, MCV, MCH, WBCs, heterophil, monocyte globulin, ALT, AST, ALP, uric acid creatinine and MDA, associated with insignificant decrease in eosinophil and basophil. Broilers received formic acid or propionic acid either alone or together for 35 days and infected with *Sal. pullorum* showed no clinical signs, insignificant decrease in RBCs, Hb, PCV%, MCHC, lymphocyte eosinophil, T. protein, albumin, A/G ratio, CAT, SOD and insignificant increase in weight gain, MCV, MCH, WBCs, heterophil, basophil, monocyte globulin, ALT, AST, ALP, **uric** acid, creatinine and MDA, improved FCR, reduced mortality rate to 5% but both acids together induced zero mortality beside reduction in re-isolation of *Sal. pullorum*. It could be concluded that, **formic** and propionic acid play an important role in control of salmonellosis in broiler and act as growth promoters beside improved hematobiochemical parameters so we recommend using formic acid and propionic acid all over fattening period.

**Keyword:** *Sal. Pullorum*-formic - propionic-hematobiochemical – broiler chickens

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## INTRODUCTION

Chicken meat is considered one of the most desirable meats all over the world (Karine, 2002). Control of broiler diseases leading to increase poultry production (Hassan *et al.*, 2010). *Salmonella* in poultry inducing many diseases (pullorum, typhoid & paratyphoid) (Tatiane *et al.*, 2013). *Salmonella* is a bacterial pathogen causing enteritis and high mortality (Kee *et al.*, 2015). *Salmonella* is transmitted by vertical and horizontal (Eriksson *et al.*, 2018). Pullorum disease is a septicemic disease in poultry mostly infects young chicks (Marwa *et al.*, 2021).

Antibiotic growth promoters induce antibiotic resistant strains of bacteria, which has compelled the researchers to use other non-therapeutic alternatives like organic acids as feed additives in poultry production (Gunal *et al.*, 2006). Organic acids are used as growth promoters and stimulate growth performance in poultry (Dibner, 2004). Organic acids have antimicrobial benefits (Hajati, 2018). Organic acids to date have focused on food safety aspects due to lowering incidence of foodborne pathogens in poultry and other livestock (Oakley *et al.*, 2014). Organic acids continue to receive considerable attention as feed additives for animal and poultry production and poultry because organic acids inhibit growth of bacteria (Pande and Akoh 2010). It has an antimicrobial action in the gastrointestinal tract of animals (Nour *et al.*, 2011) and lowering pathogenic bacteria in the intestine (Artur *et al.*, 2020). Formic acid induces reduction in *Sal* spp. in feed and potentially in the gastrointestinal tract (Ricke *et al.*, 2020).

The present study was carried out to evaluate the efficacy of formic and probiotic acid either alone or together for control of salmonellosis in broiler chickens, with regard to its effects in some blood constituents in broiler chickens.

## MATERIALS AND METHODS

### Experimental chicks:

About 110 one-day-old chicks, the average body weight 45.22- 48.10 gm body weight were used in this study. Ten chicks were slaughtered and examined bacteriologically to prove chicks free from *salmonella*. Chicks reared under hygienic conditions, fed balanced starter fresh ration free from any medications and given water ad-libitum.

### *Salmonella pullorum* titration

At 14<sup>th</sup> day of age 80 broilers were infected by *Sal pullorum* ( $1 \times 10^4$  CFU via crop) (Corrier *et al.*, 1990) (*Sal pullorum* obtained from Fac. of Vet. Med Zag Univ).

### Experimental design:

At 14 day of age, 100 broilers were divided into 5 groups (20/ each), 1<sup>st</sup> group healthy chicks (negative control), 2<sup>nd</sup> group infected chicks with *Sal. pullorum*. (positive control), 3<sup>rd</sup> group chicks received 0.1 ml formic acid/liter drinking water from 1<sup>st</sup> to 35<sup>th</sup> day of age and at 14 day of age infected with *Sal. pullorum*, 4<sup>th</sup> group chicks received 0.1 ml propionic acid / liter drinking water from 1<sup>st</sup> to 35<sup>th</sup> day of age and at 14 day of age infected with *Sal. pullorum*, 5<sup>th</sup> group received formic and propionic acid together by same dose for each type, period and infected with *Sal. pullorum*.

**Body weight** Chicks were individually weighed at 14<sup>th</sup> day of age and at 36<sup>th</sup> day of age) for determination of body performance.

Blood samples two blood samples were collected from 5 chicks from each group at 1<sup>st</sup> day post supplementation. 1<sup>st</sup> sample was taken in test tube containing EDTA for estimation of blood picture (Jain, 1986). 2<sup>nd</sup> sample was taken for obtaining serum for estimation of AST and ALT (Reitman and Frankel 1957), ALP (John 1982), T. protein (Doumas *et al.*, 1981) albumin (Doumas, 1971), uric acid (Trinder, 1969) Creatinine

(Bartels 1971) SOD (Nishikimi *et al.*, 1972), CAT (Sinha, 1972), MDA (Nielsen *et al.*, 1997)

### Salmonella reisolation

Cloacal swabs were taken from all chicks under aseptic condition, inoculated into Selenite F broth, then incubated at 37°C for 24h. in Mac Conkey's agar media incubated at 37°C for 24-48h. Positive plates give pale colony (Waltman *et al.*, 1991). Suspected colony was identified morphologically and biochemically (Cheesbrough, 1985).

**Statistical analysis** was performed using analysis of variance (ANOVA). Duncan's Multiple Range (Duncan, 1955)

## RESULTS

Infected broilers with *Sal. pullorum* showed clinical signs represented by anorexia, closed eyes, diarrhea, dehydration, mortality rate

30%, reduction in body weight gain, RBCs, Hb, PCV% MCHC, lymphocytes, total protein, albumin, CAT, SOD and significant increase in FCR, MCV, MCH, WBCs, heterophil monocyte globulin, ALT, AST, ALP, uric acid ceatinine, MDA beside insignificant decrease in eosinophil and basophil (Tables 1-5).

healthy broilers received formic acid or propionic acid either alone or together for 35 days and infected with *Sal. pullorum* showed no clinical signs, insignificant decrease in RBCs, Hb, PCV%, MCHC, lymphocyte eosinophil, T. protein, albumin, A/G ratio, CAT, SOD and insignificant increase in weight gain, MCV, MCH, WBCs, heterophil, basophil, monocyte globulin, ALT AST, ALP, uric acid, creatinine and MDA, improved FCR, reduced mortality rate to 5% but both acids together induced zero mortality beside reduction in reisolation of *sal. pullorum* (Table 1-5).

**Table 1:** Effect of salmonellosis on mortality rate and reisolated *salmonella* of chicks.

| Parameters<br>Groups | total<br>number | Mortality rate |    | Reisolated <i>Salmonella</i> at 1 <sup>st</sup> day post<br>supplementation |
|----------------------|-----------------|----------------|----|---|
|                      |                 | No             | %  |   |
| Gp (1)               | 20              | 0              | 0  | 0   |
| Gp (2)               | 20              | 6              | 30 | 14/14   |
| Gp (3)               | 20              | 1              | 5  | 4/19  |
| Gp (4)               | 20              | 1              | 5  | 4/19  |
| Gp (5)               | 20              | 0              | 0  | 1/20  |

**Table 2:** Effect of organic acid and *Sal Pullorum* on body performance of broiler (n= 5).

| Parameters<br>Group | IW(14 <sup>th</sup> day of<br>age) (gm) | FBW (36 <sup>th</sup> day of<br>age) (gm) | Weight gain<br>(gm) | FC      | FCR  |
|---------------------|---|---|---------------------|---------|------|
| Gp (1)              | 582.31±1.64a                            | 2190.09±4.27a                             | 1607.78±5.16a       | 2017.03 | 1.26 |
| Gp (2)              | 577.89±1.84a                            | 1998.58±9.49b                             | 1420.69±6.89b       | 1899.40 | 1.34 |
| Gp (3)              | 581.44±1.88a                            | 2178.76±9.87a                             | 1597.32±8.63a       | 2036.64 | 1.28 |
| Gp (4)              | 591.06±1.79a                            | 2178.43±9.48a                             | 1587.37±8.30a       | 2014.54 | 1.27 |
| Gp (5)              | 584.18±1.63a                            | 2181.55±9.72a                             | 1597.37±8.86a       | 1969.21 | 1.23 |

Initial weight=IW final body weight=FBW feed consumption= FC feed conversion rate=FCR

Means with different superscripts of same column indicate significant difference at P< 0.05

**Table 3:** Effect of organic acid and *Sal. pullorum* on blood picture of broiler (N=5).

| Group                                     |                                  | Gp(1)       | Gp(2)       | Gp(3)       | Gp(4)       | Gp(5)       |
|---|----------------------------------|-------------|-------------|-------------|-------------|-------------|
| RBCs                                      | 10 <sup>6</sup> /mm <sup>3</sup> | 4.17±0.21a  | 3.12±0.18b  | 4.05±0.21a  | 4.18±0.30a  | 4.09±0.33a  |
| Hb  | gm/dl                            | 13.14±0.3a  | 9.78±0.64b  | 12.89±0.28a | 12.94±0.32a | 13.05±0.41a |
| PCV                                       | (%)                              | 28.21±0.24  | 25.97±0.6b  | 27.96±0.42a | 27.97±0.36a | 28.02±0.27a |
| Blood indices                             | MCV(fL)                          | 67.21±1.43b | 83.24±1.42a | 69.04±1.54b | 68.84±1.55b | 68.63±1.9b  |
|   | MCH(pg)                          | 31.14±1.65a | 31.35±1.93  | 31.82±1.46a | 31.82±1.46a | 31.91±1.43a |
|   | MCHC(gL)                         | 6758±0.76   | 37.66±0.76c | 46.10±0.76b | 46.40±1.16b | 46.57±0.76b |
| WBCs X10 <sup>3</sup> /μl<br>Differential | Total WBCs                       | 12.19±0.65b | 14.08±0.3a  | 12.64±0.27b | 12.92±0.32b | 12.88±0.3b  |
|   | Heterophil                       | 3.23±0.21b  | 5.83±0.42a  | 3.94±0.69b  | 3.68±0.38b  | 3.61±0.55b  |
|   | Lymphocyte                       | 5.11±0.25a  | 3.84±0.87b  | 4.69±0.83a  | 4.60±0.55a  | 4.64±0.60a  |
|   | Esinophil                        | 1.42±0.05a  | 1.32±0.09a  | 1.40±0.08a  | 1.41±0.09a  | 1.41±0.08a  |
|   | Basophil                         | 1.12±0.08a  | 1.05±0.16a  | 1.09±0.13a  | 1.13±0.19a  | 1.11±0.18a  |
|   | Monocyte                         | 1.31±0.08b  | 2.04±0.12a  | 1.53±0.11b  | 1.91±0.12b  | 1.84±0.12b  |

Means with different superscripts of the same column indicate significant difference at P < 0.05

**Table 4:** Effect of organic acid and *Sal. pullorum* on liver function of broiler (N =5).

| Group                   |           | Gp(1)       | Gp(2)       | Gp(3)       | Gp(4)       | Gp(5)       |
|-------------------------|-----------|-------------|-------------|-------------|-------------|-------------|
| Protein profile (gm/dl) | T.Protein | 5.73±0.27a  | 4.93±0.17b  | 5.61±0.21a  | 5.70±0.17a  | 5.69±0.19a  |
|                         | Albumin   | 3.03±0.17a  | 2.08±0.30b  | 2.91±0.16a  | 2.96±0.18a  | 2.96±0.18a  |
|                         | Globulin  | 2.70±0.11a  | 2.85±0.12b  | 2.70±0.14a  | 2.74±0.13a  | 2.74±0.14a  |
|                         | A/G ratio | 1.12±0.19a  | 0.73±0.08b  | 1.12±0.14a  | 1.09±0.17a  | 1.08±0.19a  |
| Liver enzymes (U/L)     | AST       | 47.71±0.48a | 49.88±0.28b | 48.14±0.21a | 48.06±0.16a | 47.85±0.4a  |
|                         | ALT       | 36.12±0.59a | 38.51±0.26b | 37.01±0.41a | 36.71±0.32a | 36.62±0.2a  |
|                         | ALP       | 33.42±0.53a | 36.31±0.64b | 34.36±0.44a | 33.71±0.21a | 343.7±0.23a |

Means with different superscripts of the same column indicate significant difference at P < 0.05

**Table 5:** Effect of organic acid and *Sal. pullorum* on MDA, SOD & CAT of broiler (N =5).

| Group                |           | Gp(1)       | Gp(2)       | Gp(3)       | Gp(4)       | Gp(5)       |
|----------------------|-----------|-------------|-------------|-------------|-------------|-------------|
| kidney (mg/dL)       | Uric acid | 5.57±0.33a  | 7.02±0.36b  | 5.76±0.41a  | 5.79±0.32a  | 5.66±0.31a  |
|                      | Creatinin | 1.53±0.15a  | 2.09±0.13b  | 1.62±0.19a  | 1.63±0.22a  | 1.65±0.25a  |
| MDA                  | (mmol/ml) | 20.52±0.89b | 29.17±1.12a | 23.19±0.68b | 23.08±0.89b | 21.32±0.76b |
| Antioxidant t (U/mL) | CAT       | 56.17±1.62a | 46.43±1.23b | 52.28±1.08a | 51.97±1.15a | 53.21±1.05a |
|                      | SOD       | 179.17±1.8a | 165.38±1.3b | 175.05±1.2a | 174.32±1.4a | 176.19±1.2a |

Means with different superscripts of the same column indicate significant difference at P < 0.05

## DISCUSSION

In the present study, the main clinical signs appeared on broiler chickens infected with *Sal pullorum* were ruffled feather, emaciation, dropy wings, anoxia, diarrhea, dehydration, decreased body weight and mortality rate was 30 % beside reduction in body weight gain and increase FCR (table 1 and 2). Reduction in body weight due to deleterious effect of *Sal pullorum* in intestinal

tract (Shivaprasad, 2000). Typical clinical signs and reduction in body performance of Salmonellosis were recorded by Eslam (2000) in broilers and Garcia *et al.* (2010) in layers infected with *Sal pullorum*. Broilers suffering from ruffled feathers, dullness, droppings, huddled together, white diarrhea, loss of appetite and mortality rate 24.4% (Gemechu and Abdisa 2021).

Our obtained results revealed that infected broilers received formic acid or propionic acid either alone or together from 1<sup>st</sup> to 35<sup>th</sup> day of age showed no clinical signs with significant increase in weight gain, improved FCR and reduced mortality rate to 5% but combination of both acids induced zero mortality beside reduction in reisolation of *Sal pullorum* (table 1 and 2). Similar results were agreed with (Al shawabkeh and Tabbaa 2002) reported that broilers received propionic acid improved body performance and reduced intestinal *Salmonella* colonization. Formic acid decreased salmonella in intestinal tract in broilers (Bourassa *et al.*, 2018 and Ricke *et al.*, 2020). Dietary formic acid and propionic acid lowered *Sal. pullorum* in intestine and reduced mortality rate (Al-Tarazi and Alshawabkeh, 2003). Reduced shedding of *salmonella* in poultry received organic acid may be due to penetration of organic acids bacterial cell wall and disrupt the normal physiology of bacteria (Dhawale, 2005). Same results were reported by Cengiz *et al.* (2012) stated that broilers received formic acid-propionic acid mixture not isolate *Salmonella* from caecal contents. Organic acids reduced pH and dissociation capacity of their carboxyl groups so gut environment is acidic and prevent growth pathogenic bacteria (Ganguly, 2013). Also, Marin *et al.* (2014) stated that organic acids improve feeding efficiency and reduce mortality due to the reduced toxin excretion by bacteria and colonization of pathogens on intestine. Broilers received organic acids in diets decreased *salmonella* in intestine and improved body performance (Ghazvinian *et al.*, 2019).

Hematological parameters in broilers suffering from salmonellosis revealed significant decrease in RBCs, Hb, PCV%, MCHC, lymphocyte and increase in MCV, MCH, WBCs, heterophil, monocyte beside insignificant decrease in eosinophil and basophil (table 3). Change in blood picture may be due to bacterial toxins cause intravascular destruction of RBCs in body leading to haemolysis with breakdown of

hemoglobin (Karaivanov, 1984). Leukocytosis in infected broiler may be due to inflammatory response in intestinal tract (Kaneko, 1989). Also, Shand ah *et al.* (2013) stated that salmonellosis induced reduction in RBCs, Hb, PCV% and increase in WBCs. Our results were in agreement with El Sayed *et al.* (2014) in broilers suffering from salmonellosis. Salmonellosis induced acute anaemia, leukocytosis, heterophilia, lymphopenia (Ahmed and Mahmoud 2014).

Our results revealed that, infected broilers received formic acid or propionic acid either alone or together from 1<sup>st</sup> to 35<sup>th</sup> day of age showed insignificant decrease in RBCs Hb PCV%, MCHC, lymphocyte, esinophil beside insignificant increase in MCV, MCH, WBCs, heterophil, basophil and monocyte (table 3) Similar result was observed by Talebi *et al.* (2005) stated that organic acid induced increase in RBCs, Hb, PCV % and WBCs count. Organic acid induced leukocytosis in broilers (Sabry *et al.*, 2016).

Salmonellosis in broilers revealed significant decrease in serum total protein, albumin, A/G ratio beside increase in globulin, ALT, AST, ALP (table 4). Reduction in total protein and albumin in infected chicks with *Sal Pullorum* may be due to a state of anorexia and male absorption of nutrients from inflamed intestine leading to inability of liver to synthesis albumin (Kaneko, 1980). Decrease in serum albumin beside increase AST, ALT and ALP in broiler infected with *Sal. pullorum* may be due to destruction liver cells by bacterial toxin (Macpherson, 1986). *Salmonella* toxins induce liver cell damage and liberation of liver enzymes lead to increase in liver enzymes (Doxy, 1983). Elevation in globulin may be due to antigenic stimulation of infectious agent (Azza *et al.*, 2012). Also Ahmed and Mahmoud (2014) stated salmenlosis in broilers induce significant reduction in total protein and albumin beside increase in globulin, ALT, AST and ALP. *Sal pullorum* induced significant decrease in serum total protein, albumin and A/G ratio associated with increase on serum globulin (Belih *et al.*,

2017). Salmonellosis induces decrease in liver enzyme and increase in globulin, ALT, AST, ALP (Belih *et al.*, 2017).

Broilers received formic acid or propionic acid either alone or together from 1<sup>st</sup> to 35<sup>th</sup> day of age and at 14<sup>th</sup> day of age artificially infected with *sal. pullorum* showed insignificant decrease in total protein, albumin, A/G ratio and insignificant increase in globulin, AST, ALT, ALP (table 4). Organic acids reduced *salmonella* colonization in intestine and improved absorption of nutrient beside improvement of protein picture and liver enzymes (Coax *et al.*, 1994), Also, Van Immerseel *et al.* (2002) stated that organic acids are used in poultry farms to control salmonellosis beside improved protein picture and liver enzymes. Increase in protein profile in broiler fed organic acid may be due to improvement in intestinal environment leads to an improve digestion and absorption of nutrients with increase amino acids and protein (Samanta *et al.*, 2010). Organic acids induced insignificant increase in AST ALT, ALP, albumin and protein of broilers (Adil *et al.*, 2010). Same results were reported by Ezzat *et al.* (2015) in broilers received formic acid. Broilers suffering from salmonellosis and received organic acids showed insignificant decrease in total protein, albumin and insignificant increase in globulin, AST ALT and ALP (Jing *et al.*, 2019).

Infected broilers with *Sal. Pullorum* showed significant increase in uric acid, ceatinine, MDA beside significant decrease in CAT and SOD (table 5). Elevation in uric acid and creatinine in broilers suffering from salmonellosis due to kidney damage by *salmonella* toxins (Doxy, 1983). *Salmonellosis* induced increase oxidative stress leading to increase in uric acid, ceatinine and MDA beside decrease in CAT and SOD (Mine, 2009). In addition, Ismail *et al.* (2013) mentioned that oxidation induced increase serum uric acid, ceatinine, MDA beside significant decrease in CAT and SOD.

Salmonellosis induces increase in uric acid and creatinine in broiler (Rauber *et al.*, 2014). Salmonellosis in broilers induces marked increase in creatinine and uric acid (Ahmed and Mahmoud, 2014). Our finding agreed with Belih *et al.* (2017) in broilers suffering from salmonellosis. *Salmonella* infection leads to a significant increase of serum MDA and decreased SOD and CAT (Sokoudjou *et al.*, 2019).

Broilers received formic and propionic acid either alone or together from 1<sup>st</sup> to 35<sup>th</sup> day of age and at 14<sup>th</sup> day of age infected with *Sal. pullorum* showed insignificant increase in Uric acid and creatinine, MDA beside insignificant decrease in CAT and SOD (table 5). Organic acids in diet reduced *salmonllae* in hens intestine leading insignificant increase in uric acid and creatinine (Thompson and Hinton 1997). Our data coincide with results of Abdel-Fattah *et al.* (2008) stated that organic acid induced insignificant increase in uric acid and creatinine in broilers. Organic acids induced insignificant increase in uric acid and creatinine of broilers (Adil *et al.*, 2010). These results agreed with those stated by Abudabos and Al-Mufarrej (2014) stated that organic acids reduced oxidative stress in broilers infected with *Salmonella* improving serum uric acid and creatinine, MDA, SOD and CAT. Organic acid had positive influence on *Salmonella* and reduced oxidation stress beside improve serum antioxidant enzymes and MDA (Alaeldein *et al.*, 2017). Propionic acid induces decrease in MDA and increase in CAT and SOD (Huda *et al.*, 2020).

Finally, it could be concluded that, formic and probionic acid play an important role in control salmonellosis in broilers and act as growth promoter beside improved liver and kidney function so we recommend using formic acid and propionic acid alleover fattening period due to its low side effect and improved feed conversion rate and boy weight.

## REFERENCES

- Abdel-Fattah, S.; El-Sanhoury, M. and Abdel-Azeem, F. (2008):* Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *Inter. J. Poult. Sci.*, 7(3) 215-222
- Abudabos, A. and Al-Mufarrej, S. (2014):* Effects of organic acid on antioxidant capacity and immune responses of broilers challenged orally with *Salmonella enterica* subsp. *enterica* Typhimurium. *South Afr. J. of Animal Sci.* 44 (4)41-49
- Adil, S.; Bandy, T.; Bhat, G.; Mir, M. and Rehman, M. (2010):* Effect of supplementation of organic acids on performance, intestinal morphology, and serum biochemistry of broiler chicken. *Vet. Med. Inter. J.* 67(6): 1-7.
- Ahmed, F. and Mahmoud, S. (2014):* Alterations of Blood Components in Broiler Chicks Experimentally Infected with *Salmonella Gallinarum*. *Global Vet.* 13 (5)787-793,
- Alaeldein, M.; Abdullah, H.; Youusif, M. and Rifat, U. (2017):* effect of organic acid and *Bacillus subtilis* alone or together on growth traits, biochemical and antioxidant status in broilers exposed to *Sal. typhimurium* challenge during starter phase. *J. Appl Animal Res.* 45 (1)41-50
- Alshawabkeh, K. and Tabbaa, M. (2002):* dietary propionic acid to limit *Sal gallinarum* colonization in broiler chicks. *Asian-Aust. J. Anim. Sci.* 15(2): 243-246.
- Al-Tarazi, Y. and Alshawabkeh, K. (2003):* Effect of dietary formic and propionic acids on *salmonella pullorum* shedding and mortality in layer chicks after experimental infection. *Asian-Australasian J of Animal Sci*;16 (1): 77-82
- Artur, A.; Marcin, O. and Tomasz, M. (2020):* Antibacterial Activity Flavonoids and Organic Acids Widely Distributed in Plants *J. Clin. Med.* 9(1): 109.
- Azza, H.; Kamel, H.; Walaa, M. and Amira, H. (2012):* Effect of bactoCell® and probiotic food supplements on growth performance, hematological, biochemical and humoral immune response of broiler chickens. *World Applied Sci J*; 18 (3): 305-316.
- Bartels, H. (1971):* Determination of serum Creatinine *Clin. Chem. Acte* 32-81
- Belih, S.; EL-Hadad, F.; Amen, E. and Basiony, R. (2016):* Influence of sodium butyrate on *salmonella* infection in broilers. *Benha Vet. Med. J.* 31(2) 21-32.
- Bourassa, V.; Wilson, M.; Ritz, B.; Kiepper, K. and Buhr, R. (2018):* Evaluation of the addition of organic acids in the feed and/or water for broilers and the subsequent recovery of *Salmonella Typhimurium* from litter and ceca *Poultry Sci* 97(1) 64-73.
- Cengiz, O.; Koksall, B.; Tatli, O.; Sevim, O.; Avci, H.; Epikmen, T.; Beyaz, D.; Buyukyoruk, S.; Boyacioglu, M.; Uner, A. and Onol, A. (2012):* Influence of dietary organic acid blend supplementation and interaction with delayed feed access after hatch on broiler growth performance and intestinal health. *Vet. Med.* 57,515-552
- Cheesbrough, M. (1985):* Medical Laboratory Manual for Tropical Countries *Micr.* 2 40-44
- Corrier, D.; Hinton, J.; Beier, R. and DeLoach, J. (1990):* Effect of lactose on cecal pH and *Sal typhi* colonization of broiler chicks. *Avian Dis* 34: 17-25
- Cox, N.; Petacchi, F. and Shotts, E. (1994):* Effect of butyric or lactic acid on the In vivo colonization of *Salmonella typhimurium* *J. Appl. Poul. Res.* 3 15-18
- Dibner, J. (2004):* Organic acids: Can they replace antibiotic growth promoters? *Feed Int.* 25, 14-16.

- Dhawale, A. (2005):* Better egg shell quality with a gut acidifier. *Poult Int*, (44)18-21.
- Doumas, B. (1971):* Determination of albumin. *Clin. Chem Acta* (22)41-42.
- Doumas, B.; Cartor, R.; Peers, T. and Schaffier, R. (1981):* A candidate reference method for determination of total protein in serum *Clin.Chem*27, 1642
- Doxy, D. (1983):* Clinical pathology and diagnostic 2<sup>nd</sup> Ed. Baillier London 56-60.
- Duncan, D. (1955):* Multiple ranges and multiple "F" test. *Biometrics*, 11: 10.
- El Sayed, M.; Allam, H.; Shawky, N.; El Rashidy, R. and Abdalla, S. (2014):* effect of *salmonella pullorum* in broilers and its treat with doxycycline. *Zag. Vet J.* 42(3)45-56
- Eriksson, H.; Söderlund, L.; Ernholm, L.; Melin R. and Jansson, D. (2018):* Diagnostics, epidemiological observations and genomic subtyping in an outbreak of pullorum disease in non-commercial chickens. *Vet. Micro*, 217:247
- Eslam, E. (2000):* some pharmacological studies on florfenicol and its interaction with streptomycin in chickens. *BVSc. Fac. Vet. Med. Zag. Uni*
- Ezzat, H.; Shawky, N.; Hasan, A. and El Sayed, M. (2015):* Pathological and biochemical studies on effect of formic acid in broilers. *Zag. Vet. J.* 43(3)34-43
- Ganguly, S. (2013):* Potential non-antibiotic growth promoting dietary supplements for animal nutrition: A Review. *J. of Appl. Pharma. Sci*, 3 (7)74-78.
- Garcia, K.; Santana, A.; Freitas, O.; Berchieri, A. and Fagliari, J. (2010):* Experimental infection of layers using a *Salmonella enterica*: blood serum component and histopathological changes. *Brazilian J. of Vet. Pathol*, 3(2): 11-17
- Gemechu, B. and Abdisa F. (2021):* Pullorum Disease and Fowl Typhoid in Poultry: A Review. *British J. of Poultry Sci.* 10 (3): 48-56
- Ghazvinian, K.; authors and Tufarelli, D. (2019):* Effects of various levels of organic acids and virginiamycin on performance, blood parameters, immunoglobulins and microbial population of broiler chicks. *South African J. of Animal Sci.* 48(5)
- Gunal, G.; Yayli, O. and Sulak, O. (2006):* effect of organic acid on performance and intestinal microflora of broilers. *Inter J. of Poultry Sci* 5(2)149-155.
- Hajati, H. (2018):* Application of organic acids in poultry nutrition. *Int J Avian & Wildlife Biol.*; 3(4): 324–329.
- Hassan, H.; Mohamed, M. and Youssef, A. (2010):* Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. *Asian-Aus J. Animal Sci.* 23(10): 1348–1353
- Huda, S.; Hanan, M.; Abdelkadr, E.; Musarat, A.; Iman, S. and Ramesa, S. (2020):* Antioxidant and hepatorenal protective effects of bee pollen fractions against propionic acid induced autistic feature in rats. *Food Sci. Nutr.* 8(9)14–27.
- Ismail, I.; Al-Busadah, K. and El-Bahr, S. (2013):* oxidative stress biomarkers and biochemical profile in broilers fed zinc bacitracin and ascorbic acid under hot climate. *American J. of Biochemistry and Molecular Biology*, 3: 202-214.
- Jain, N. (1986):* Schalm's Vet. Haematology, 4<sup>th</sup>Ed, Lea Fibiger Philadelphia, USA
- Jing, W.; Dong, D.; Haijun, Z.; Yuan, W. and Guang, Q. (2019):* organic acids modulate systemic metabolic perturbation caused by salmonella pullorum challenge in early-stage broilers. *Avian Phyio* 23(2) 342-349.
- John, D. (1982):* determination of alkaline phosphatase 9<sup>th</sup> Ed. 580-581.
- Kaneko, J. (1980):* clinical Biochemistry of domestic animals. 4<sup>th</sup> ed., pp. 365-



390. Academic press, Inc., New York, Lonodn. Tokyo.
- Kaneko, J. (1989):* Clinical biochemistry of domestic animals. 4th ed Academic Press, Inc., New York, London, 365 - 339.
- Karaivanov (1984):* Biochemical tests for identifying *Pasteurella multocida*. *Vet. Med. Nauki*, 21(9): 38- 44.
- Karine, P.; Florence, H.; Eric, J. and Gilles, T. (2002):* Improvements required for detection of *Sal Pullorum*. *Can J. Vet. Res.*; 66 (3) 51-57.
- Kee, E.; Hooi, A.; Syakima, S. and Lee, L. (2015):* *Salmonella*: review on pathogenesis, epidemiology and antibiotic resistance, *Frontier in Life Sci.* 8: 284-293.
- Macpherson, I. (1986):* Immunosuppressive drugs and chemical toxic factors in commercial poultry. *Clin. Veterinaria*, 109 (1): 47-54.
- Marin, E.; Vazquez, A. and Mendz, A. (2014):* Effect of organic acid on growth performance, blood constituents and immune response of broilers. *J. Poultry Sci.* 51(2)14-20.
- Marwa, F.; Amr, A.; Jan, P. and Josef, K. (2021):* mitigating the spread and translocation of *Salmonella enteritidis* in experimentally Infected broilers under the influence of different flooring housing systems and feed particle sizes. *Microorganisms* 9(1)87-93
- Mine, E. (2009):* effect of enrofloxacin on antioxidant enzyme activities of broilers infected with *salmonella enteritidis*. *J of New World Sci Acad.* 4 (2)24-28.
- Nielsen, F.; Mikkelsen, B. and Grandjean, P. (1997):* Malondialdehyde as biomarker for oxidative stress. Reference interval and effects of life style factors. *Clin. Chem.* 43(7)19
- Nishikimi, M.; Appaji, N. and Yagi, K. (1972):* occurrence of superoxide in reaction of reduced phenazine methosulfate and molecular oxygen. *Bioc Biop Res Com*; 46(2)49-54.
- Nour, V.; Trandafir, I. and Ionica, M. (2011):* Effect of acidification on blood parameters and performance of chickens. *J. Anim. Vet. Adv.*, 9: 92-107.
- Oakley, B.; Seal, B. and Cox, N. (2014):* changes in chicken cecal microbeome during 42 days growth are independent of organic acid feed additives. *BMC Vet. Res.* 27(10) 282
- Pande, G. and Akoh, C. (2010):* organic acids antioxidant phenolic content and charactized lipid georgiagrown underutilized fruit crop. *Food Chem* 120:67-75.
- Rauber, R.; Mallmann, A.; Miranda, D.; Giacomini, L. and Nasciment, O. (2014):* Interference of *Salmonella typhimurium* lipopolysaccharide on perform-ance and biological parameters of chickens. *Rev. Bras. Cienc. Avic* 16 (1)24-36.
- Reitman, S. and Frankel, S. (1957):* Calorimetric determination of transamin-ases activity *Am. J. Clin. Path.* 28: 56
- Ricke, S.; Dittope, D. and Richardson, K. (2020):* Formic acid as an antimicrobial for Poultry production: A Review. *Front Vet. Sci.* 3; 7: 563
- Sabry, M.; Mohammed, M.; Atallah, S. and Ramadan, K. (2016):* Effect of probiotics, prebiotics, synbiotics, organic acids and enzymes on broilers' immunity in relation to the economic performance. *Benha Vet. Med. J.*, 30 (2)34-44.
- Samanta, S.; Sudipto, H. and Tapan, K. (2010):* Efficacy of organic acid and bacitracin as growth promoters in broilers: effects on performance. *Vet. Med. Inter.* 2(1) 201-210
- Shah, S.; Kamil, S.; Darzi, M.; Mir, M. and Bhat, S. (2013):* Haematological and some biochemical changes in experimental fowl typhoid infection in broiler chickens. *Comparative Clinical Pathology. Academic Journal*, 22(1): 83
- Shivaprasad, H. (2000):* pullorum disease. *Rev. Sci. tech. Int. Epiz* 19 (2) 45-54.

- Sinha, K. (1972):* Colorimetric assay of catalase. *Anal Biochem*; 47: 89-94.
- Sokoudjou, J.; Kodjio, N. and Gatsing, D. (2019):* Antisalmonellal and antioxidant potential of hydroethanolic extract of *Canarium schwainfurthii* Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks. *Asian Pac J Trop* 9:474-83.
- Talebi, A.; Asri-Rezaei, S. and Sahraei, R. (2005):* Studies on Haematological Values of Broiler Strains (Ross, Cobb, Arboracres and Aria). *Inter. J. Poult. Sci.* 4 (8): 573-579.
- Tatiane, M.; Elisabeth, G. and Cíntia, S. (2013):* Liver function in broiler infected with *Sal Typhi* treated with organic acid. *Italian J. of Ani. Sci*12 (3) 15-26.
- Trinder, P. (1969):* Calorimetric estimation of uric acid. *Ann. Clin.Bioch.* 6: 24.
- Thompson, JL. and Hinton, M. (1997):* Antibacterial activity of formic and propionic acids in diet of hens on *salmonellas*. *British Poultry Sci.*, 38(1) 59-65.
- Van Immerseel, F.; Cauwerts, K.; Devriese, L. and Ducatelle, R. (2002):* Feed additives to control *Salmonella* in poultry *World's Poult. Sci. J.* 58, 51-53.
- Waltman, W.; Horne, A. and Dicksom, T. (1991):* Use of delayed secondary enrichment for isolated *Sal* in poultry. *Avian Dis.*, 35(1): 88-92.

### التقييم المعملى لاستخدام بعض الأحماض العضوية فى مقاومة عدوى السالمونيلا فى دواجن التسمين

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كان الهدف من هذه الدراسة هو معرفة تأثير حمضى البريبونك والفورمك منفردين أو معا على السالمونيلا وعلى وزن الجسم وبعض وظائف الدم الكيمياء-حيوية. تم استخدام ١١٠ كتكوت عمر يوم تم ذبح ١٠ منها وأخذ مسحات للفحص البكتريولوجي للتأكد من خلو الكتاكيت من الإصابة بالسالمونيلا. باقى الكتاكيت (١٠٠ كتكوت) تم تقسيمهم الى ٥ مجموعات (٢٠ / بكل منها). الأولى تركت بدون إصابة أو علاجات (ضابطة سالبة)، عند اليوم ١٤ من العمر الكتاكيت بالمجموعات ٢, ٣, ٤, ٥. تم عمل عدوى اصطناعية بالسالمونيلا بلورم, المجموعة الثانية مصابة ولم تعالج (مجموعة موجبة), المجموعة الثالثة تم إعطائها ٠,١ ملي لتر من حمض الفورمك/ لتر من اليوم الأول من العمر حتى اليوم ٣٥ من العمر وعند اليوم ١٤ تم عمل عدوى اصطناعية بالسالمونيلا بلورم, المجموعة الرابعة تم إعطائها ٠,١ ملي من حمض البريبونك/ لتر ماء من اليوم الأول من العمر حتى اليوم ٣٥ من العمر وعند اليوم ١٤ تم عمل عدوى اصطناعية بالسالمونيلا بلورم أما المجموعة الخامسة تم إعطائها حمض البريبونك والفورمك معا بنفس الجرعة والمدة وعند اليوم ١٤ تم عمل عدوى اصطناعية بالسالمونيلا بلورم. عند اليوم الأول من نهايه الإمداد تم ذبح ٥ كتاكيت من كل مجموعة وتم أخذ عينتين دم لتعيين بعض وظائف الدم الكيمياء-حيوية. وتم تجميع مسحات من المجمع لإعادة عزل السالمونيلا.

الإصابة بالسالمونيلا فى الكتاكيت أدت إلى ظهور أعراض تتمثل فى: عدم الأكل، قفل العيون، الإسهال، الجفاف، ونقص فى الوزن ونسبة الوفيات ٣٠% ونقص معنوي فى وزن الجسم المكتسب، عدد كرات الدم الحمراء، تركيز الهيموجلوبين، حجم خلايا الدم المرصوصة، MCHC، الخلايا الليمفاوية، البروتين الكلي، الزلال، SOD, CAT, بجانب زيادة معنوية فى معدل التحويل الغذائى، MCV, MCH, WBCs، الخلايا المتعادلة، الخلايا الملتهمة الكبيرة، الجلوبيولين، ALT-AST-ALP، حمض البوليك والكرياتينين MDA ونقص غير معنوي فى الخلايا القاعدية والحمضية.

الكتاكيت التى تم إعطائها حمض البريبونك والفورمك سواء منفردين أو معا من اليوم الأول حتى اليوم ٣٥ من العمر وعند اليوم ١٤ تم عمل عدوى اصطناعية بالسالمونيلا بلورم أدت الى عدم ظهور أعراض مرضية ونقص غير معنوي فى RBCs, Hb, PCV%, MCHC, الخلايا الليمفاوية والخلايا الحمضية البروتين الكلي، الزلال A/G, SOD, CAT ratio وزيادة غير معنوية فى وزن الجسم المكتسب MCV, MCH, WBCs، الخلايا المتعادلة الخلايا القاعدية الخلايا الملتهمة الكبيرة، الجلوبيولين، انزيمات الكبد، حمض اليوريك والكرياتينين، MDA وتحسن فى معدل التحويل الغذائى ونسبة الوفيات ٥%. ولكن البريبونك والفورمك معا أديا إلى عدم وجود نسبة وفيات كما قلل عدد السالمونيلا المعزولة.

يتبين لنا أن حمضى البريبونك والفورمك لهم دور فى مقاومة السالمونيلا ويعملان كمحفز للنمو وأديا إلى تحسين وظائف الكبد والكلى. لذلك نوصى باستخدام حمضى البريبونك والفورمك طوال مدة تربية كتاكيت التسمين نظرا لتأثيره المهم على وزن الجسم ومعامل التحويل الغذائى ولقله إثارة الضارة