Effect of organic acids on body weight, serum total protein, total cholesterol, glucose and cecal colonization of *Salmonella* spp. of broilers

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Abstract

The effect of organic acids mixture which represent a commercial product of Galliacid^{TM} on the body weight, serum total protein, total cholesterol, glucose, and *Salmonella* colonization of cecal contents were studied.140 of one day old male broiler chicks (Hubbard) were assigned at random to two dietary treatments: a basal diet (control) and a basal diet with 600gm/ton of Galliacid which mixed with diet at 21 days of age.The supplementation of Galliacid in broiler diet improved the body weight gain significantly ($P \leq 0.05$), from 35and 43 days of age.The Galliacids did not affects the level of total protein at 28 days comparing to the control but cause elevation in the total protein levels at 35 days and reduce serum total cholesterol at 28, 35 days of age ($P \leq 0.05$).However, there were no significant differences in glucose level at 28, 35 days of age between control and organic acids fed broilers.*Salmonella* isolation rate was differe but no significantly while *Salmonella* count from cecal content showed that it was significantly decreased ($P \leq 0.05$) in treated group comparing to the control.

Introduction

Organic acids efficacy is defined as the ability to inhibit (mold, bacteria and yeast) with broad spectrum, the minimum inhibition concentration (MIC) of organic acids has been established as a standard to determine the effectiveness against different micro-organism (1).Organic acids and salts(2,3),have been studied as potential carcass disinfectants because they exhibit good bactericidal activity and are generally regarded as safe (GRAS)food additives (4).Organic acids exert their antimicrobial action in feed and in GI tract of the animal (5), and metabolism (1), following dietary intake organic acids are only recovered from proximal part of GI tract (5), Several studies have been shown the addition of organic acids to the diets of broilers leads to lowering pathogenic bacteria in the chicken intestine (5,6,7).In view of the severe restriction or total ban on the use of antibiotics as growth promoters in the poultry production and worldwide separation of many bacterial diseases like salmonellosis, colibacillosis, clostridial dis., probiotic containing lactic acids bacteria alone or with other flora or organic acids or combination of them have been suggested as an alternative to antibiotics (6, 8, 9). Propionic acid is a volatile fatty acids(VFA) has been utilized as poultry feed additive or exploited as a bacterial fermentation product in the crop or ceca to take advantage of its antimicrobial action. (10,11), dietary propionic acid may be used as energy source by chicks or metabolized in to derivatives for assimilation in to body components, dietary propionic acid can be transformed in the digestive tract of the chick to other VFA such as acetic,butyric,isobutyric,isovaleric acids, dietary propionic acid coupled with second *Salmonella* control approach i.e. the competitive exclusion cultures might prove efficacious in managing *Salmonella* colonization in the poultry (11) it is possible that the commercial acidifiers provided an extra energy source for aerobic bacterial growth (12).A study on the metabolism of dietary added propionic acid reveals that only little if any dietary propionc acid reaches the lower digestive tract and the ceca(11), and may be other organic acids undergo same end due to metabolism and absorption(13), propionic acid in broilers

the inclusion of 0.4% and 0.8% (Luprosil-NC)commercial products of propionic acid decrease the number of coli forms, *E. coli* and *Salmonella* in the small intestine with out effect on the intestinal PH (8)and propionic acid and it's salts are very effective against moulds in poultry feed (14),Fumaric and Lactic acids according to Waldroup et al (1995) (15) did not offer protection from caecal *Salmonella* colonization or carcass contamination, Formic acid/propionic acid blend the information are very little according to Waldroup etal(1995) (15) ,but it cause reduction in cecal PH and did not offer a reliable protection regarding the cecal colonization with *S. typhimurium* , while Thompson, and Hinton(1997),Hinton and Linton (1988) and Berchieri and Barrow(1996) (5, 6, 7),showed that formic acid alone or in a combination of formic acid with propionic acid (Bio-Add)at concentration of 0.6% were effective with respect to the prevention of infection with *S. kedougou*,*S.gallinarum* and *S.enteritidis*, other organic acids like Citric acid, results concerning the inclusion of citric acid in broiler rations are very limited, Citric acid is not reliable with respect to the prevention of *Salmonella* colonization of the caeca (15) ,Sorbic acid is unsaturated fatty acids its mold ,bacteria, yeast inhibitor ,it may incorporated in feed as potassium sorbate (16),combination of anaerobic cecal microflora and acetic acid reduced the amount of the *S. enteritidis* in feces, as well as cecal PH ,but not *S. typhimurium*, (17), these former results seems to be vary a lot there for it needs more study ,may be the reason for this is depend upon the *Salmonella* serotypes and the challenge dose or the number of *Salmonellas* in the cecum , or the effects of organic acids.in poultry may also depend on the composition of the diet and its buffering capacity is well known recently (18),the literatures concerning with the effect of organic acids on serum biochemistry are very rare.There for the present study was undertaken to investigate the effect of Galliacid^{TM} (Commercial products of organic acids) on, body weight, serum total protein, total cholesterol, and glucose, considering the *Salmonella* carriage status or colonization.

Material and Methods

1-Animals and Diets:

A total of 140 one day old commercial male broiler chicks (Hubbard) are used, the birds were weighed before beginning of treatment and assigned at random to two isolators of 70 birds each, and the chicks were kept under controlled conditions and the experiment was carried out for 43 days.Birds feeding were provided *ad libitum*. the mash feed was beginning daily at 07:00 o'clock a.m ,the birds were divided in tow groups randomly ,the dietary treatments were basal diet (control) and basal diet plus 0.6% of (Galliacids)which contents the following {Fumaric acid 25%, Ca.propionate + Ca.formate + Pot.sorbate 25% , hydrogenated veg.. oil 50%}.The Galliacid were mixed weekly diet and stored until used, the basal diet it meet the nutrient requirements for grower and finisher its composition is shown in Table (1, 2).

Table(1) Composition of broiler feed grower

Material	Amount/kg
Soybean	250
Protein	100
Corn	250
Wheat	400
Ca	4
Salt	2

Diet analysis

Feed components	Protein %	Meta. Energy /Kcalorie
Soybean	110	557.5
Protein	50	260
Corn	21.5	857.5
Wheat	52	1300
Total	23.35	2975

Table(2) Composition of broiler feed finisher

Material	Amount/kg
Soybean	200
Protein	100
Corn	400
Wheat	300
Ca	4
Salt	2

Diet analysis

Feed components	Protein %	Meta. Energy /Kcalorie
Soybean	88	446
Protein	50	260
Corn	34.4	1372
Wheat	39	975
Total	21.14	3053

2-Sampling procedures:

A/Determination of weight

At 28, 35, 43, days of age samples of birds from each group were randomly selected and weighed.

B/ Collection of blood sample

The blood samples were collected from wing vein in non heparinized sterilized glass tubes for serum separation, the collected serum was transferred in to vials

C/Assay of serum biochemistry (Analytical procedures)

Blood samples were centrifuged at 2500/5min and serum samples were stored in a deep freezer for further analysis.Serum samples were analyzed for serum total protein, total cholesterol, glucose by spectrophotometer (PD-303, APEL, Japan) with enzymatic kits for each parameter which include the following kits.

1-Total protein (Atlas Medical, Cambridge) with 546 nm wave length

2-Cholesterol SL (CHOD-PAP) Giesse Diagnostic Snc, Italy. with 510nm wave length.

3-Glucose SL (GOD-POD) Giesse Diagnostic Snc, Italy. with 500nm wave length.

D/*Salmonella* isolation

Cecal contents were collected at 35 days of age and each sample transferred to broth tubes(selenite broth) , then mixed well until homogenate then serial dilution made up after that all diluted tubes incubated at 41C^{o} for 18 hr, positive samples was plated on to the bismuth sulfite agar/brilliant green agar plates were incubated overnight at 37C^{o},and *salmonella* colony forming units were determined, the identity of presumptive *Salmonella* colonies was confirmed biochemically and serologically, the numbers of *Salmonella* colony forming

units per gram in each group were transformed to $\log_{10}$ and then means were calculated .

E/Statistical analysis

All data presented in this study were subjected to - CRD desigen (19), and t-test (20) for MPN (most probable number) of *Salmonella*.

Results and Discussion

The effects of organic acids on body weight and serum total protein of broiler chickens are summarized in tables. (3, 4, 5) There was a significant effect of dietary treatment on the body weight and serum total protein.Initially, from 28 days of age there is a numerically difference between the treated group and control, from 35 days of age there was a significant gained more weight ($P \leq 0.05$) than control, same difference at 43 days of age i.e. consistent increments in body weight gains, this match with reports by other researchers (12, 21, 22, 23) ,the effect on total protein level it seems to be higher in treated group with significant difference than control group at 35 days but not at the 28 days.The difference in body weight and total protein are obviously clear, the high weight gain and the elevation in the level of serum total protein in treated group may be due to enhancement of digestion of nutrients through the stimulation of the secretion of digestive enzymes, or the acidification of chickens gut may have positive effect through increase the activity of pepsinogen, thus improved protein digestion and utilization and better amino acids digestibility and absorption by anther word decrease the endogenous amino acid loss.Anther explenation is a very important objective of dietary acidification is the inhibition of intestinal bacteria competing with host for available nutrients and a reduction of possibly toxic bacterial metabolites,(e.g. ammonia and amines), which could cause intestinal epithelial cells damage ,thus negatively affecting the absorption, or organic acids improve microbial ecology balance may contribute in this effect through enhancing the digestion of some nutrients, furthermore, the growth inhibition of potential pathogen bacteria and zoonotic bacteria e.g. *E. coli* and *Salmonella* by organic acids, in the feed and GI tract are of benefit with respect to animal health. (5,6,7,11,24), this refers to that the organic acids mixture have positive effect on the general health status of broiler chickens, thus improving weight gain of the chickens, we suggest more, since that the organic acids is mold inhibitor it will enhance body weight through that mold can reduce the nutritive value of feed (grains), or organic acids as feed additives inhibit growth of many hazardous toxin producing molds which cause loss of appetite and consequently the loss of weight and decline in total protein level. Anther factor may contribute is that the difference of body weight and the elevation in the level of serum total protein may be due to that the organic acids enhance the ecology of microflora in the GI tract especially the cecum flora through enhancement the growth of the microflora ,this microflora had the ability to convert the retrogradely uric acid which carried to the cecum from the coprodeum to amino acids(25,26),since that the cecum is also important in amino acids absorption(27),and has greater ability to transport amino acids than sugar (26), this point may contribute with enhancement of body weight and the level of serum total protein. As Chaveerach ,et al., (12) reported that the esophagus ,crop ,small intestine and ceca ,the total number of degenerated epithelial cells in the treated group with acidified water did not differ from control, there for we assume that the acidification of feed it is not affect the gut epithelial cells, and well established that the intestinal epithelial cells utilize propionate as a source of energy therefore it can hypothesized that propionic acid enhance nutrients absorption, thus it will contribute

in the improving the body weight , and the level of total protein.The effects on, total cholesterol, and glucose in broilers fed with or with out organic acids at 28, 35 days are shown in Table (4,5).Serum total cholesterol concentration was decreased in broilers supplemented with organic acids compared with the control birds at 28,35 days of age, this demonstrate of the hypocholesterlaemic effect of organic acids. The hypocho-lesterolaemic effect may be results from enhancing Lactic acid bacteria growth in the low PH in the intestine which are able to grow at relatively low PH, which means that they are more resistant to organic acids than other bacteria e.g. *E coli* ,an explanation for this may be that gram-positive bacteria have a high intracellular potassium concentration, which provides a counteraction for the acid anions (28) , this lactic acid bacteria to produce bile salt hydrolase (BSH) in the intestine the enzyme responsible for bile salt deconjugation (29), deconjugated bile acids are less soluble at low PH and less absorbed in the intestine and are more likely to be excreted in the faeces (29), and to maintain bile salt homeostasis is more bile acids need to be synthesized and this in turn will reduce cholesterol in the body pool as cholesterol is the precursor for bile acids., second suggested reason is the higher lipid catabolism i.e. low lipid absorption.As shown in the Table (4, 5) there is no differences between comparable groups at 28, 35 days of age of glucose levels, the reason for these may be due to the poultry lack specific indigenous enzymes necessary to hydrolyzed these indigestible components such as wheat carbohydrate and soybean oligosaccharides (raffinose and stachyose) i.e. the organic acids has no effect on the glucose level.The effects on *Salmonella* isolation rate & it's numbers in caecal content in broilers fed with Galliacid as shown in Table (6), its obviously clear that the isolation rate numerically lower than control and *Salmonella* numbers were differ significantly ($P \leq 0.05$) in broilers supple-mented with organic acids compared to the control birds at 35 days of age.As mentioned previously , organic acids exert their antimicrobial action both in the feed and in the GI tract of the animal(5), in poultry, *Salmonella* enters the GI tract via the crop, the environment of the crop with respect to microbial composition and PH seems to be very important in relation to the resistance to pathogens, high amount of *Lactobacilli* and low PH in the crop have shown to decrease the occurrence of *Salmonella* in the crop (25).also the antimicrobial effect of dietary organic acids in chickens is believed to take mainly place in the upper part of the digestive tract(27), as reported by Hume,etal., (11), a dietary added propionic acid reveals that only little if any dietary propionc acid reaches the lower digestive tract considering the absorption and metabolism, we can suggest anther factor which is the buffering capacity of the intestine. This effect on the *Salmonella* at the low detectable level it seems to be to the effect of the organic acids on *Salmonella* which control microorganisms by intervention in the energy metabolism by blocking the enzymes pyruvatedecarboxylase and influencing DNA synthesis (21)or through penetration the cell wall of pathogens in un dissociated form and become dissociated in the cytoplasm releasing H+ ions decreasing the internal PH of bacteria leading to the cell death, i.e. the effect is due to the organic acids can penetrate in to cells and alter cytoplasmic PH, hence the cellular metabolism is interrupted ,thus inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to release protons leading to an intracellular accumulation of acid anions this accumulation depends on the PH gradient across the membrane.(23, 30). Our results is similar to that reported by (6),despite that the number of *Salmonella* /gm of cecal content in untreated group was also low this refer to that *Salmonellas* are self limiting by

such means of the development of the microbial ecology of the chickens intestine with aging competition the *Salmonella* organisms for surviving through many mechanisms (31,32,33,34,35) in this study, because *Salmonella* colonization was commensal (i.e. there is no challenge dose)and may be not pathogenic, the term colonization was favored over infection. The effect of organic acids on *Salmonella* colonization is lowering the isolation rate from fecal material as reported by (17) and the number of organism/gm of cecal content as shown in this research ,this result are analogue to that reported by (6,7,8).The results from this study indicated that organic acids have positive effect on body weight, and have hyperproteinaemic effect through increasing the level of total protein in serum and hypocholesteroaemic effect on the host through decreasing the level of total cholesterol in serum and have no influence on glucose levels and cause decreasing the isolation rate and the number of *Salmonella* in the cecal contents.

Table (3) Represents the means of the weight at the experiment

Group	Weight/28days gm	Weight/35days gm	Weight/43days gm
A	906^{a} ±18.1*	1187^{a} ±15.9	1780^{a} ±25.3
B	859^{a} ±17.8*	1112^{b} ±34.8	1668^{b} ±50.6

A:treated group, B:control, results with in one column indicated by different superscripts differ (P≤0.05),*St. Error.

Table(4) Represents the serum biochemistry values at 28 days old

Group	Total protein g/dl	Total cholesterol Mg/dl	Glucose Mg/dl
A	4.46^{a} ±0.250*	160.8^{a} ±16.53	79.8^{a} ±8.18
B	4.36^{a} ±0.103*	299^{b} ±17.99	84^{a} ±9.58

A: treated group, B: control, results with in one column indicated by different superscripts differ (P≤0.05), * St. Error.

Table(5) Represents the serum biochemistry values at 35 days old

Group	Total protein g/dl	Total cholesterol Mg/dl	Glucose Mg/dl
A	5.58^{a} ±0.760*	246.7^{a} ±12.44	78^{a} ±16.05
B	3.31^{b} ±0.261*	306.8^{b} ±20.57	85.7^{a} ±11.7

A: treated, B: control, results with in one column indicated by different superscripts differ (P≤0.05), * St. Error.

Table(6)Represents the isolation rate of *Salmonella* and MPN $\log_{10}$/gm of cecal content

Group	No. of positive samples	%	MPN log_{10}/gm cecal content (means)
A	4/12	33	$0.55^{a} \pm 0.262^{*}$
B	6/12	50	$1.6^{b} \pm 0.336^{*}$

A: treated and B: control, results with different superscripts differ ($P \leq 0.05$), *St. Error.

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تأثير الأحماض العضوية على وزن الجسم ومستوى البروتين الكلي والكولسترول الكلي والكلوكوز في المصل واستيطان جراثيم السالمونيلا للاعورين في افراخ اللحم

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الخلاصة

تمت في هذه البحث دراسة تأثير خليط من الأحماض العضوية والمتمثلة بمركب الغالاسيد التجاري علـى وزن الجسـم وفحوصات بايوكيمياوية مصلية تضمنت فحص مستوى البروتين الكلي و مستوى الكولسترول الكلي ومستوى الكلوكوز وتـم أيضا دراسة مدى تأثير هذا الخليط على استيطان جراثيم السالمونيلا في الأعورين. في هذا البحث تم تربية ٤٠ افرخـة لحـم نوع هبرد (ذكور) وزعت بشكل عشوائي إلى مجموعتين بأعداد متساوية، مجموعة السيطرة تـم تعليفهـا عليقـه أساسـية ومجموعة المعاملة والتي تضمنت استعمال ٦٠٠ غرام من مزيج الأحماض العضوية ابتدءا من عمر ٢١ يوم مـع كـل طـن علف من العليقة الأساسية نفسها المستخدمة لمجموعة السيطرة.إن نتائج هذا البحث أظهرت وجود فروق معنويـة علـى وزن الأفراخ للمجموعة المعاملة بمركب الغالاسيد بعمر ٣٥ يوم و ٤٣ يوم.كما لم يؤدي استعمال مزيج الأحماض العضـوية إلـى زيادة في نسبة البروتين الكلية بعمر٢٨ ولكن بعمر ٣٥ يوم كان يوجد فرق معنوي مقارنة بمجموعة السيطرة،كما أدى إلـى انخفاض في نسبة الكولسترول الكلي بعمر ٢٨ و ٣٥ يوم من عمر الطيور ،بينما لم يسجل أي تأثير على مستوى الكلوكـوز بعمر ٢٨،٣٥ يوم..كانت نسبة عزل السالمونيلا اقل من مجموعة السيطرة لكن بفارق غير معنوي وتم عد السالمونيلا / غـم من محتويات الأعورين للمجموعتين وكانت النتيجة تبين وجود فارق معنوي في إعـداد السـالمونيلا للمجموعـة المعاملـة مقارنة بمجموعة السيطرة .