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Effect of Sodium Formate on Laying Hen Performance, Gastrointestinal Tract pH and Some Blood Components under Heat Stress Conditions

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ABSTRACT

The objective of this study was to investigate response of laying hens to added levels of sodium formate under heat stress conditions. A total number of 96 laying hen aged 53 weeks were randomly divided into four treatment groups of three replicates, 8 hens each during summer season. The first group was served as control and fed the basal diet. While, the other three groups were received the basal diet supplemented with sodium formate at levels of 0.1, 0.2 and 0.3% of diet, respectively. The study duration was 8 weeks (from 53 to 61 WOA). Productive performance, egg quality, pH level of some gastrointestinal tract (GI-tract) segments and some blood components and plasma enzymes were measured. Results showed that egg production and feed conversion ratio were significantly improved by adding sodium formate (87.97 and 2.13%, respectively) with adding, 2% sodium formate . While no significant differences in feed intake and egg weight. Adding sodium formate significantly increased shell thickness (44.3 mm), shell % (11.55%), Haugh units (87.00) also Shell weight per unit surface area (SWUSA) was 99.91 with 0.2% sodium formate. The pH values in different GI-tract segments were insignificantly decreased with supplemental all doses of sodium formate. Furthermore, dietary acidification elevated significantly the concentration of plasma calcium (ca) and phosphorus (p) than the control. A significant reduction in plasma level of total lipids and cholesterol was achieved due to dietary acidification. The liver enzymes-Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) did not significantly changed, in response to addition of acidifiers. The activation of alkaline phosphatase (ALP) was significantly decreased while Lactate Dehydrogenase (LHD) increased with dietary sodium formate supplementation. It is concluded that dietary supplementation with 0.1 or 0.2% sodium formate gave the best result for aged laying hens (53-61 week) in the present study, during the hot summer period (June to Aug.).

Key words: Sodium formate, pH value, blood components, egg quality, laying hen

INTRODUCTION

High environmental temperature considers being one of the most important factors affecting in poultry production. The negative effect of high ambient temperature normally exists on decrease body weight, poor feed conversion ratio and increasing mortality rate. This effect causes significant economic losses, also respiratory alkalosis had been observed in birds exposed to heat stress. (Bottje and Harrison, 1985). Therefore, acidifiers-organic acids or their salts have been used to alleviate negative effects of heat stress and to improve the productive performance by altering acid-base balance.

Organic acids are incorporated to poultry feeds to maintaining the health of the GI-tract of poultry and increase their performances. If applied correctly, organic acids work in poultry, if used correctly not only as a growth promoter but also to control both pathogenic and non-pathogenic. (Naidu, 2000; Wolfenden *et al*., 2007).

The acidifiers are naturally occurring substances used for regulating intestinal pH and microflora's balance, increasing activity of intestinal digestive enzymes in order to increase nutrients digestion, increasing minerals absorption in delight pH condition, increasing palatability and improving usage of minerals for bird (Bonos *et al*., 2010; Seyedeh *et al*., 2012).

Acidifiers in different forms and combination are included in poultry feeds to lower the pH value of the feed and the gut. This effect is exhibited also in digestive tract of poultry (Ogunwole *et al*., 2011). Moreover, It improved growth performance through establishment of low gastro intestinal pH condition by supporting endogenous digestive enzymes and reducing undesired gut micro-organisms (Richards *et al*., 2005; Abdel-Fattah *et al*., 2008), Acidification of diets with weak organic acids such as formic have been reported to improve digestibility of Ca, P and served as substrate in the intermediary metabolism (Veeramani *et al*., 2003).

Organic acids and their salts have shown variable effects on egg production and egg quality parameters. These discrepancies would be related to the source, the amount of organic acids used, location, environmental condition and the composition of the diets (Gama *et al*., 2000). On the other hand, only few data on blood parameters in laying hens supplemented with organic acids are available.

This study was conducted to through light on the effect of different doses of sodium formate on productive performance (egg production and egg quality), pH values of different GI-tract segments, some blood components such as plasma Ca, P, total lipids and cholesterol, enzymes activity (ALT, AST, ALP and LDH), parameters of laying hens under heat stress conditions.

MATERIALS AND METHODS

This study was conducted at Noubaria station- National Research Centre station, during the period from June-August 2011.

Experimental design and measurements: A total number of 96 laying hens aged 53 weeks were used in present study. The birds were individually weighed and distributed into four treatments groups, equal in number and nearly in body weight and average daily egg production.

Three replicates (of 8 hens each) were assigned to each treatment. The birds were allotted to 24 cages (100*60 cm^2) each. Four corn- soybean study diets were used through the study period (53-61 weeks). The first group (T1) was fed on the basal diet without any addition and used as control (Table 1). Groups from the 2nd to the 4th were fed the same basal diet supplemented with sodium formate at levels 0.1, 0.2 and 0.3%, respectively. All groups were maintained under the same environmental and managerial conditions. Water and feed were provided *ad-labitum*.

The study period (53-61 weeks of age) divided to four sub periods each period two weeks. During the study period egg number and egg weight were recorded daily. Egg mass was calculated

Table 1: Composition and proximate analysis of the basal layer diet (g l kg)

Ingredients	Percentage
Yellow corn	63.00
Soybean meal (44%)	16.00
Wheat bran	3.00
Concentrate*	10.00
Limestone	7.00
Bone meal	1.00
Total	100.00
Calculated analysis (%)**	
Crude protein	17.25
Metabolizable energy ME (kcal/kg)	2770.00
Lysine	0.94
Methionine	0.38
Methionine and cystine	0.68
Calcium	3.60
Available P	0.40

*Concentrate contains the following: 45% crude protein (CP), 1.80% crude fiber (CF), 1.63% ether extract (EE), 5.84% Ca, 2.92% available phosphorus (AP), 1.35% methionine, 2.11% methionine and cystine, 2.70% lysine, 1.27% sodium, Metabolisable energy 2656 kcal/kg and each 1 kg of this concentrate contains: Vit A: 100000 IU, Vit D_3: 33000 IU, Vit E: 100 mg, Vit K_3: 25 mg, Vit B_1: 10 mg, Vit B_2: 50 mg, Vit B_6: 15 mg Vit B_{12}: 200 μg Niacin: 400 mg, Pantothenic acid: 100 mg, Folic acid: 10 mg, Choline chloride: 8323 mg, Biotin: 500 μg, Copper: 50 mg, Iodine: 3 mg, Iron: 450 mg, Manganese: 800 mg, Zinc: 600 mg; Selenium: 1 mg, Cobalt: 1 mg, Antioxidant: 7.5 mg, **Calculated based on feed composition tables of NRC (1994)

by multiplying average egg weight by egg number during each period. Feed consumption was recorded and feed conversion was calculated during each period as follows: Feed conversion = total feed intake/total egg mass.

Egg quality measurements: Egg quality traits were measured according to Stino *et al.* (1982) and El-Wardany *et al.* (1994). Egg shell weight was recorded by digital balance to nearest 0.1 g. Egg shell thickness was measured with (membranes) to the nearest mm. Haught units measure according to Haugh (1937). Shell weight per unit surface area (SWUSA) was calculated according to Carter (1975).

Blood parameters: At the end of study period (61 week of age), Gastrointestinal tract (GI-tract) was removed from five birds to measure pH value of its parts. Values of pH in different parts of GI-tract were measured immediately by using a digital pH meter (Al-Natour and Alshawabkeh, 2005).

Blood samples were collected from the five slaughtered birds in heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min and plasma obtained was stored at -20°C in Eppendorf tubes until analysis. Plasma minerals (calcium and phosphorous), total lipids, cholesterol, enzyme activity of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH) were determined calorimetrically by using available commercial kits purchased from Diamond Diagnostics Company.

Statistical analysis: The obtained data were statistically analyzed using the general linear model procedure described in SAS User's Guide (SAS, 2001). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data in Table 2 shows that egg number and egg production (%) increased with adding sodium formate comparing with the control group all over the study period and it was significantly ($p>0.05$) in treatment 3 which received 0.2% sodium formate.

Results also showed that egg mass were significantly increased with treatment 3 in 2nd and 3rd periods and all over the study period. These results are in agreement with Yesilbag and Colpan (2006) who concluded that dietary supplementation with organic acids and their salts could be used in layer hens diets with benefit on egg production and protein metabolism efficiency.

Supplementation with organic acid salts reduced pH of the diets according to the level of organic acids. This effect was interesting because it would allow an increase in protein digestibility

Table 2: Effect of sodium formate on productive performance

	Feed conversion (FC)	Feed intake FI (kg/hen/period)	Egg mass (kg)	Egg weight (g)	Egg production (%)	Egg No. (egg/hen/period)
1st period: 54-55 WOA						
1	2.38	1.754	0.74	66.33	79.46^{b}	11.12^{b}
2	2.24	1.768	0.80	67.46	83.92^{ab}	11.75^{ab}
3	2.16	1.787	0.83	66.86	88.39^{a}	12.37^{a}
4	2.27	1.768	0.78	67.36	83.03^{ab}	11.63^{ab}
SEM	±0.11	±0.020	±0.03	±1.59	±1.62	±0.22
Significance	ns	ns	ns	ns	**	**
2nd period: 56-57 WOA						
1	2.53^{a}	1.890	0.75^{b}	66.66	80.06^{b}	11.21^{b}
2	2.37^{a}	1.880	0.79^{b}	68.27	82.74^{b}	11.58^{b}
3	2.15^{b}	1.871	0.87^{a}	69.20	89.88^{a}	12.58^{a}
4	2.42^{a}	1.876	0.78^{b}	67.16	82.44^{b}	11.54^{b}
SEM	±0.06	±0.030	±0.02	±1.11	±1.18	±0.16
Significance	**	ns	***	ns	***	***
3rd period: 58-59 WOA						
1	2.43^{a}	1.848	0.76^{b}	67.46	80.35^{b}	11.25^{b}
2	2.31^{a}	1.829	0.79^{b}	67.76	83.43^{ab}	11.68^{ab}
3	2.10^{b}	1.787	0.85^{a}	69.30	87.33^{a}	12.22^{a}
4	2.34^{a}	1.868	0.80^{b}	67.53	84.31^{ab}	11.80^{ab}
SEM	±0.06	±0.030	±0.02	±1.65	±1.24	±0.17
Significance	**	ns	**	ns	**	**
4th period: 60-61 WOA						
1	2.35^{a}	1.644	0.70	66.53	80.77^{b}	10.50^{b}
2	2.20^{ab}	1.614	0.73	67.76	82.90^{b}	10.77^{b}
3	2.10^{b}	1.580	0.75	66.53	86.31^{a}	11.22^{a}
4	2.22^{ab}	1.618	0.73	67.43	83.15^{b}	10.81^{b}
SEM	±0.1	±0.090	±0.03	±2.01	±0.82	±0.11
Significance	**	ns	ns	ns	***	***
Overall						
1	2.42^{a}	1.784	0.74^{b}	66.75	80.16^{c}	11.02^{c}
2	2.28^{a}	1.773	0.78^{ab}	67.81	83.25^{b}	11.44^{b}
3	2.13^{b}	1.756	0.82^{a}	68.08	87.97^{a}	12.10^{a}
4	2.31^{a}	1.782	0.77^{b}	67.37	83.23^{b}	11.44^{b}
SEM	±0.05	±0.02	±0.02	±1.01	±0.74	±0.10
Significance	**	ns	**	ns	***	***

ns: Not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, Means within columns with no common superscripts differ significantly, 1: Control, 2: 0.1% sodium formate, 3: 0.2% sodium formate, 4: 0.3% sodium formate, WOA: Weeks of age

by enhancing digestive enzyme activity and the reduction in pathogenic bacteria activity in feed (Langhout, 2000). Soltan (2008) also found that organic acid supplementation significantly increase egg production by about 5.77% comparing with untreated group.

The data of egg weight (Table 2) showed that treatments didn't affect egg weight all over the study period. This is in agreement with Yesilbag and Colpan (2006) and Soltan (2008) who indicated that the addition of different organic acid salts into laying hen diets did not significantly modify the egg weight in laying hens.

Statistical analysis of feed consumption data showed that organic acid supplementation didn't affect feed intake which in agreement with the findings by Yesilbag and Colpan (2006) and Soltan (2008) who indicated that adding organic acid salts to laying hen diets didn't affect feed intake.

In contrast, the results obtained by Samik *et al.* (2007) noticed that use of a single organic acid salt (ammonium formate or calcium propionate) in the broiler diet lowered feed intake when compared with control birds. This difference may be attributed to the type of organic acid salt used in the study.

The data of feed conversion showed that adding sodium formate to laying hen diets improved feed conversion, this improvement was significant with treatment 3 (0.2% sodium formate) in 2nd, 3rd and 4th study periods and allover the study period.

The data are in agreement with Soltan (2008) who found that adding organic acids to laying diets improved feed conversion. The improvement of feed conversion was already demonstrated in broiler and quail chickens (Versteegh and Jongbloed, 1999; Denli *et al*., 2003).

Effect of dietary organic acid supplementation on egg shell quality is shown in Table 3. Statistical analysis of the obtained data indicates that egg shell thickness, shell% and SWUSA were significantly increased with adding sodium formate. This result is in agreement with Soltan (2008) who found improvement in egg shell thicknes in laying hens group fed on basal diet supplemented with organic acids. Shell% and consequently shell quality expressed as shell weight (mg) per square centimeter egg surface area (SWUSA) was significantly increased which may be due to increase of Ca and P utilization. Shell weight per unit surface area (SWUSA) is highly recommended as a factor proportionally well affected by environmental heat stress. The decrease in egg shell quality is normal response in hens exposed to heat stress (Muruni and Harrison, 1991). So adding sodium formate could be recommended as a good solution for alleviating heat stress.

The data of HU indicated that adding sodium formate significantly ($p<0.05$) increased HU. This result disagree with the findings by Yesilbag and Colpan (2006) who found that adding organic acids mixture didn't affect egg quality parameters. On the other hand, Gama *et al.* (2000) reported a slight decline in haugh unit score of hens receiving 0.05% organic acid supplementation.

Table 3: Effect of sodium formate on egg quality traits at the end of the study period

Treatment	SWUSA	Haugh unit	Shell (%)	Shell thickness (mm)
1	86.47^{b}	74.50^{b}	9.80^{b}	38.33^{b}
2	96.46^{a}	83.50^{a}	11.20^{a}	41.50^{a}
3	99.91^{a}	87.00^{a}	11.55^{a}	44.30^{a}
4	95.60^{a}	80.50^{ab}	10.83^{a}	43.00^{a}
SEM	±2.95	±2.82	±0.31	±0.94
Significance	**	**	***	***

** $p<0.01$, *** $p<0.001$, Means within columns with no common superscripts differ significantly, 1: Control, 2: 0.1% sodium formate, 3: 0.2% sodium formate, 4: 0.3% sodium formate, SWUSA: Shell weight per unit surface area

The pH level in specific areas of the gastrointestinal tract (GI-tract) is a factor which establishes a specific microbial population and also affects the digestibility and absorptive value of most nutrients. Most of pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens (Boling-Frankenbach *et al*., 2001; Rahmani and Speer, 2005).

High ambient temperature causes significant economic losses in the broiler industry owing to decreased body weight, poor feed conversion ratio and increasing mortality. Heat-stress leads to panting, decreases the partial pressure of CO_2 in blood and causes respiratory alkalosis. Therefore, sodium formate as acidifier has been used to alleviate negative effects of heat stress and to improve broiler performance by altering acid-base balance (Acikgoz *et al*., 2011).

The effect of sodium formate on pH values of different GI- tract segments are presented in Table 4. The results indicate that all levels of sodium formate supplementation reduced crop, proventriculus, gizzard, duodenum, jejunum, ileum and cecum pH values compared with the control group. However, the differences were not significant. These results are in agreement with the results of Abdel-Fattah *et al*. (2008) and Acikgoz *et al*. (2011) who reported that the pH values in different GI-tract segments were insignificantly decreased with all types of organic acids such as formic acid, acetic acid, citric acid and lactic acid. Similarly, Paul *et al*. (2007) who used different organic acid salts and found no significant difference in the pH values of different segments of the GI-tract. Moreover, Hernandez *et al*. (2006) reported that no effect on intestinal pH with the use of a product containing combination of propionic acid and formic acid. These authors referred this insignificant effect to the strong buffering action of the GI-tract in poultry.

Table 4: Effect of sodium formate on pH values of different gastrointestinal tract

Treatment	Ceca	Ileum	Jejunum	Duodenum	Gizzard	Proventriculus	Crop
1	5.59±0.23	5.07±0.35	4.93±0.23	4.61±0.18	4.30±0.22	4.82±0.15	4.61±0.50
2	5.57±0.14	5.05±0.28	4.76±0.28	4.56±0.19	4.19±0.28	4.78±0.22	4.51±0.06
3	5.49±0.06	5.02±0.41	4.71±0.17	4.53±0.16	4.14±0.25	4.73±0.17	4.45±0.15
4	5.21±0.08	4.98±0.15	4.68±0.048	4.45±0.19	4.07±0.11	4.65±0.43	4.36±0.28
Significance	ns	ns	ns	ns	ns	ns	ns

ns: Non-significant. 1: Control, 2: 0.1% sodium formate, 3: 0.2% sodium formate, 4: 0.3% sodium formate

The effect of sodium formate on some blood constituents are shown in Table 5. The data indicated that sodium formate increased plasma Ca and P concentrations significantly compared with control group. The increase of Ca and P levels in plasma produced by addition of sodium formate may be attributed to the lowering of GI-tract pH by using sodium formate (acidifier), which increases the absorption of such minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus due to provision of organic acids was approved by Boling-Frankenbach *et al*. (2001) and Abdel-Fattah *et al*. (2008).

Table 5: Effect of sodium formate on some blood constituents

Treatment	Cholesterol (mg dL^{-1})	Total lipid (mg dL^{-1})	P (mg dL^{-1})	Ca (mg dL^{-1})
1	393.19 ± 3.16^{a}	883.93 ± 1.840^{a}	6.65 ± 0.080^{c}	14.78 ± 0.05^{c}
2	303.05 ± 9.17^{b}	721.06 ± 10.68^{b}	7.23 ± 0.060^{b}	16.90 ± 0.02^{b}
3	274.14 ± 4.98^{c}	648.63 ± 8.720^{c}	7.66a±0.08[a]	17.27 ± 0.15^{b}
4	255.07 ± 3.01^{c}	610.41 ± 5.760^{d}	7.85 ± 0.100^{a}	18.42 ± 0.16^{a}
Significance	**	**	**	**

**$p\leq0.0001$, ns: Non-significant. Means within columns with no common superscripts differ significantly, 1: Control, 2: 0.1% sodium formate, 3: 0.2% sodium formate, 4: 0.3% sodium formate

Table 6: Effect of sodium formate on plasma enzymes activity

Treatment	LDH (IU L^{-1})	ALP (IU L^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
1	1824.52±3.42^{d}	2711.84±7.36^{a}	103.01±0.81	17.50±0.36
2	1873.60±1.97^{c}	2611.75±5.49^{b}	103.50±0.98	17.25±1.11
3	1907.93±9.54^{b}	2517.47±8.65^{c}	102.49±1.37	18.06±1.13
4	2185.07±7.51^{a}	2505.07±3.64^{c}	102.89±0.69	17.47±0.52
Significance	**	**	ns	ns

**p≤0.0001, ns: Non-significant, Means within columns with no common superscripts differ significantly, 1: Control, 2: 0.1% sodium formate, 3: 0.2% sodium formate, 4: 0.3% sodium formate, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase

Also, Abdo (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier. In this respect, Li *et al.* (1998) found that the acidic anion has been shown to complex with Ca and P which results in an improved digestibility of these minerals.

As shown in Table 5, the total lipids are significantly decreased by increasing the level of sodium formate in the diet compared with the control group. Additionally, the lowest level (610.41 mg dL^{-1}) was recorded for high level of sodium formate (0.3%). A similar trend was almost observed for plasma concentration of cholesterol (255.07 mg dL^{-1}). These results are in agreement with El-Kerdawy (1996), Abdo (2004), Abdel-Fattah *et al.* (2008) and Nourmohammadi *et al.* (2010), they reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. The beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence on decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of anions (Young and Foegeding, 1993).

The effect of sodium formate on plasma enzymes activity (ALT, AST, ALP and LDH) is shown in Table 6. The data indicate that sodium formate had no significant effects on liver enzymes activity (ALT and AST). These results are in agreement with El-Kerdawy (1996) and Abdel-Fattah *et al.* (2008) who reported that broiler chicks could tolerate the addition of organic acids up to 3% without any deleterious effects on kidney and liver function. It is well known that Aspartate aminotransferase (AST) is not specific for hepatocellular damage but is highly sensitive in detecting liver damage. Alanine aminotranseferase (ALT) is found in hepatocyte cytosol as well as in muscles and other tissues of bird. ALT has poor specificity for liver disease and the clinical relevance of an increased ALT value is decreased (Harr, 2002). Furthermore, sodium formate was significantly decreased the activity of Alkaline Phosphatase (ALP) compared with the control group. Also it increases the activity of Lactate Dehydrogenase (LDH). It appears that as plasma P concentration increased (as observed in our study), the plasma level of ALP decreased.

LDH is an important enzyme in the anaerobic metabolism of glucose for the generation of ATP. The activity of this enzyme is primarily responsible for explosive anaerobic athletic activity. The reaction that the enzyme catalyzes is the interconversion of pyruvate and lactate. Under anaerobic conditions, pyruvate is converted to lactate, with the concomitant conversion of NADH to NAD^{+}. The regeneration of the NAD^{+} permits continued metabolic flux down the glycolytic pathway. This flow continues until the energy demands cease or until the cell and blood levels of lactate become intolerably high.

LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show

false-positively high levels of LDH due to erythrocyte damage. It can also be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH peak at 3-4 days and remain elevated for up to 10 days.

LDH An enzyme that catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells. Many different types of cells in the body contain this enzyme. There are five LDH isoenzymes in birds; each occurring in several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone and red blood cells that are found to decrease LDH activity which could be related to liver disease progresses (Nourmohammadi *et al*., 2010). In this concern the best results could be achieved by 0.1 or 0.2% sodium formate addition.

CONCLUSION

It can be said that the addition of sodium formate at level of 0.2% to laying hens diet can help improving the productive performance and enhancing the internal environment of the gastrointestinal tract without deleterious effects on the physiological status of hens.

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