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Ameliorative Impact of Some Anti-Stressful Agents in Broilers Exposed to Dexamethasone as a Stress Model

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Abstract

Our study was intended to know the effectiveness of sodium butyrate and betaine as anti-stressful agents when added as a feed additive to boilers exposed to dexamethasone. 96 broilers were divided into four treatments, each having 24 broilers, three replications, and two age groups of 21 and 42 days. The control was G_C, whereas DEX was given at three-day intervals in G_D (Dosage: 1mg/kg B.W S/C) the dosage was evaluated following the preliminary study, then G_S was injected with DEX at three-day intervals and was given sodium butyrate (Dosage: 1.2 g/kg feed), finally, G_B has given DEX injections every three days and betaine (Dosage: 2 g/kg feed). The results revealed that at 21 days of age the G_D that suffered from oxidative stress statically has the highest levels of MDA with an increase in HSP90 in serum, spleen and bursa, and the lowest values of GSH in serum, spleen and bursa, while the feed supplementation with sodium butyrate and betaine gave their effects to cope the oxidative stress in boilers and this reflected in the parameters which implode HSP90 and stress biomarkers that showed statically raising in GSH levels in serum, and bursa except spleen with a decrease in HSP90 and falling in MDA values in serum, spleen and bursa. At 42 days of age observed approximately the same results when compared with control except no statically different of GSH in serum when treated with betaine. We concluded that dietary sodium butyrate and betaine were successfully used as anti-stressful agents.

Keyword: oxidative stress, betaine, HSP90, stress biomarkers.

Introduction

Nowadays, the world's population of chickens has increased by more than fivefold since 1950. Their main products are meat, eggs, and manure for agricultural fertilization, and they're kept and raised in a number of methods. They are therefore considered among the most widely consumed animal source foods worldwide, making them essential for nutrition **(1).** The entire biological response to any stimuli that threaten the creature's homeostasis in the form of physiological and behavioural changes is referred to as "stress," and an attempt to keep them to a bare minimum is crucial for poultry breeding, animal comfort, and increased output **(2).** In an intensive poultry production system, birds are subjected to a variety of stressors that can cause oxidative stress and poor performance resulting from the imbalance between prooxidants and antioxidants, which is known as oxidative stress (in favour of the pro-oxidants) **(3).** And they usually take into account the end product of lipid peroxidation indicator by malondialdehyde (MDA) **(4).**

Dexamethasone (DEX) is a synthetic glucocorticoid that duplicates the actions of the natural steroid cortisol. It has been frequently used to develop a chronic stress model to assess the negative consequences of physiological stress in poultry, as it can alter avian metabolism by inducing oxidative stress via the hypothalamic-pituitary-adrenal axis **(5).** Long-term

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corticosteroid use typically causes mitochondrial accumulation and expansion, as well as mitochondrial dysfunction, which has been linked to DNA damage caused by oxidative stress **(6).**

Antioxidants protect the animal body from the harmful effects of free radicals and hazardous metabolic products and using the feed additives as a growth promoter is a typical nutritional method for improving poultry output **(3).** Butyrate, a popular feed ingredient and a safe alternative to antibiotics have gotten a lot of attention recently because it is odorous and unstable. Nevertheless, because of these disadvantages of butyric acid, sodium butyrate (SB) has been employed as a substitute in poultry production, which is a key energy source for gastrointestinal epithelial cells as well as having antibacterial, anti-inflammatory, and antioxidant properties **(7).**Superoxide dismutase (SOD) and catalase (CAT) are two essential enzymes that protect cells from oxidative damage. Elnesr *et al*. **(8)** found that giving 0.1 percent sodium butyrate supplemented food to 21-day broiler chicks increased blood SOD and CAT levels while decreasing serum MDA levels.

Betaine (N, N, N-trimethylglycine) is a non-toxic amino acid derivative found in a variety of foods including sugar beets, wheat, and spinach, it is produced in the body from choline by the sequential activity of choline oxidase and betaine-aldehyde dehydrogenase in the liver and kidney **(9).** It acts as a methyl donor in the betaine homocysteine methyltransferase enzyme-catalyzed conversion of homocysteine to methionine, therefore, methionine and choline are required for the production/secretion of very-low-density lipoproteins (VLDL) and hepatic beta-oxidation, the failure of which results in lipid deposition, oxidative stress, hepatotoxicity, and inflammation **(10).**

The heat shock protein 90 (HSP90) chaperone machinery is a crucial regulator of proteostasis in eukaryotic cells under both normal and stressful situations which facilitates ATP binding in the middle domain that required for stress tolerance, protein folding, and post-translational regulation of the stability and function of numerous important regulators of cell growth, differentiation, and apoptosis **(11).** Under oxidative stress caused by an increase in reactive oxygen species (ROS) leads to oxidation and aggregation of vital proteins and DNA when dysfunctional oxidized proteins accumulate, inflammatory pathways become activated and the apoptotic cascade is also activated, in this situation the role of HSPs appear to repair the dysfunctional proteins by many ways such as protein sorting and directing for degradation, and refolding damaged proteins .etc. **(12).** Therefore this study was aimed to explain the positive effect of sodium butyrate and betaine as anti-stress and antioxidant agents in broilers exposed to dexamethasone from hatching day to marketing.

Materials and methods

Broilers

To carry out this study, 96 Rose-type broiler chicks were brought from private hatchery and kept in the animal's house/college of veterinary medicine under standard circumstances. Following that, the birds were divided into four groups, each having 24 birds and three replications. Throughout the experiment, water was available at all times, while the basic diet's

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contents and nutrient concentrations were provided following the National Research Council's (NRC) recommendations **(13).** The birds were exposed to natural lighting as well as artificial lighting (60-watt lamps) during the first 7 days of their lives, which was subsequently reduced by 2 hours per week until it was reduced to 16 hours per day at the end of the rearing. The hall was equipped with heaters and air vacuums to control temperature (during hatching, the temperature was 33-34 degrees Celsius, then dropped 1 degree Celsius after the 4th day of age, and then continued to drop 1 degree Celsius every 3 days of bird age) with good ventilation.

Experimental design

96 chicks were randomly divided into four groups (24 birds per group/3 replicate) after three days of acclimatization, each set having three replicates, and two age periods of 21 and 42 days were taken for sampling. The following are the five treatments:
(G_C) represented control (untreated group), (G_D) injected with dexamethasone (DEX) at 3 days interval (Dosage:1mg/kg B.W S/C) The dosage that causes stress was identified following preliminary testing and (G_S) was injected with DEX at 3 days interval and received sodium butyrate (SB) (Dosage:1.2 g/kg feed) **(14)**, finally (G_B) was injected with DEX at 3 days interval and received betaine (B) (Dosage: 2 g/kg feed) **(15)**.

Materials utilized

The dose was made with dexamethasone acquired from (Pioneer company- Slemani) and propylene glycol (Laboratory reagent- India), and the dose was calculated based on DEX solubility as follows: 1 mg dexamethasone dissolved in 1 ml propylene glycol. The ingredients Betaine and Sodium Butyrate were provided by (bio point company- Poland). All of the materials used in the recent experiment were delivered as a 99.9% pure product.

Blood collection

On the day before the slaying, blood samples were taken from the jugular veins of the birds in the above-mentioned groups. The serum was separated and distributed in little volumes in Eppendorf tubes and maintained in the freezer at - 20 C˚ until stress biomarkers were measured, after which the blood samples were centrifuged at 3000 rounds/min for 15 minutes.

Organs samples storage

The birds were slaughtered between the ages of 21 and 42 days for the experiments, and the spleen and bursa were extracted and separated into two portions, each of which was wrapped in aluminum foil and frozen at -20 C˚ until the tests were performed on it.

The parameters

Initially estimated the GSH levels in blood serum were estimated by the modified method used by Burtis & Ashwood (2012) **(16),** and the levels of MDA in blood serum was estimated using the modified (Thiobarbituric acid reaction substance-TBARS) **(17).** While the concentration of glutathione (GSH) in tissues (spleen and bursa) was measured by the modified Ellman method

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(18), and Gilbert *et al.* (1985) **(19),** method was used to measure MDA in the tissues, as well as, chicken heat shock protein 90 by ELISA (Sunlong Biotech Co., Ltd, China).

Statistical analysis

Duncan's multiple range test was used to examine data at the probability level ($P < 0.05$), and a two-way analysis of variance (ANOVA) was used to see if there was a significant difference at the probability level ($P < 0.05$) between groups and between the two periods of birds age **(20).**

Results

1- GSH and MDA in serum:

At 21 days of age, groups treated with G_D reduced GSH levels in serum to the lowest static value compared to G_C and treatment birds ($p < 0.05$), while group G_B indicates a high statically level of GSH to the opposite other groups. Group G_S didn't modify GSH levels with similarity to Gc ($p > 0.05$). At 42 days of bird age, when the groups manually assessed GSH, we discovered that chickens are given G_D, and G_B dropped significance relative to GC and GS, while group G_S had no effect in static value when compared to the G_C.

At 21 days of age, the MDA levels estimation revealed an increase in significance in all treated groups, however, the peak was found in group G_D ($p < 0.05$) when compared to the G_C and resting groups, while GS did not statically different with GB. At 42 days of age, group G_D had a higher static value related to resting treatment, but the other groups were not statically significant ($p > 0.05$) between them and G_C.

All groups related to each other within the 2 periods. GSH test, groups G_C, G_D, and G_S achieved the highest static value within 42 days of age relative to 21 d, while group G_B showed no static change during either period. Analysis of MDA, except for the G_C, all the treated groups compared to each other within 2 periods pointed to altitude values within 21 days of age compared with 42 days of age ($p < 0.05$).

Table (1): efficiency of SB and B on serum GSH and MDA of broilers injected with DEX

Mean ± SE

Treatment	21 days of age		42 days of age	
	GSH serum µmol/L	MDA serum µmol/L	GSH serum µmol/L	MDA serum µmol/L
G_C	4.58 ± 0.37 bB	2.90 ± 0.64 cA	7.78 ± 0.56 aA	3.84 ± 0.51 bA
G_D	2.28 ± 0.38 cB	14.22 ± 0.67 aA	4.74 ± 0.33 bA	7.40 ± 0.59 aB
G_S	3.90 ± 0.40 bB	9.08 ± 0.66 bA	7.50 ± 0.67 aA	5.06 ± 0.46 bB
G_B	6.52 ± 0.55 aA	10.04 ± 0.51 bA	5.26 ± 0.39 bA	5.02 ± 0.53 bB

*Static values with small letters are different significantly at ($p<0.05$) between groups

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2-GSH and MDA in spleen:

At 21 days of age, the treated groups decreased GSH levels in the spleen compared to the G_C ($p < 0.05$), When GD, GS, and GB are statically closely associated with decreasing GSH levels compared to GC. At 42 days of age, the groups were given G_S, and G_B was similar to the G_C ($p > 0.05$), and differ with the injection of G_D into broilers causing significance to fall when compared to the G_C.

At 21 days of age MDA in the spleen, estimation revealed that groups G_S, and G_B had values that were statically similar to G_C ($p > 0.05$), whereas group G_D produced an increase in MDA levels comparative to the G_C. At 42 days of age MDA levels were elevated in G_D as compared to the G_C, however, group G_S and GB were also closely connected significantly to the G_C and G_D.

In both periods when related to each other, the GSH test showed within 42 days of age, the groups G_C, G_D, G_S, and G_B had the highest static values. MDA test, in G_C and G_B, which had the lowest static value at 21 days of age vs 42 d, no groups within either period showed significance.

Table (2): efficiency of SB and B on spleen GSH and MDA of broilers injected with DEX

Mean ± SE

Treatment	21 days of age		42 days of age	
	GSH spleen µMol/L	MDA spleen µMol/g	GSH spleen µMol/L	MDA spleen µMol/g
G_C	**0.33 ± 0.02 aB**	**6.50 ± 0.41 bB**	**0.58 ± 0.02 aA**	**7.86 ± 0.46 bA**
G_D	**0.17 ± 0.008 bB**	**10.34 ± 0.54 aA**	**0.34 ± 0.02 bA**	**9.38 ± 0.46 aA**
G_S	**0.20 ± 0.01 bB**	**6.96 ± 0.58 bA**	**0.53 ± 0.02 aA**	**8.22 ± 0.45 abA**
G_B	**0.19 ± 0.008 bB**	**6.62 ± 0.37 bB**	**0.56 ± 0.02 aA**	**8.32 ± 0.30 abA**

*Static values with small letters are different significantly at ($p<0.05$) between groups
**Static values with capital letters are different significantly at ($p<0.05$) between two periods of age (21 and 42 days).

3-GSH and MDA in bursa:

In the measurement of GSH in bursa at 21 days of age, there was no significance ($p > 0.05$) showed in both G_S and G_B related to control, while G_D noted a decrease in static value compared with G_C. 42 days of age revealed that all treated groups had a lower significance ($p < 0.05$) than G_C, with the smallest value in group G_D. dietary G_S, and G_B was likewise closely connected to each other.

At 21 days of age, the groups were given G_D, G_S, and G_B induced signification increase in MDA levels in the bursa, with group G_D being the highest relative to Gc. At 42 days of age, however,

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the treatment of G_S, and G_B did not affect the values of MDA statically, but administration of G_D did, with a significant elevation competed to the G_C.

The GSH levels showed no significant change in the bursal tissue across groups within both periods, except for G_C in the 1st period showing statically diminished compared with the 2nd period. Furthermore, the MDA estimate revealed reduction significance ($p < 0.05$) at 21 days of age relative to the second period of age, except for group G_S, which didn't create static significance within both periods.

Table (3): efficiency of SB and B on bursa GSH and MDA of broilers injected with DEX

Mean ± SE

Treatment	21 days of age		42 days of age	
	GSH bursa µMol/L	MDA bursa µMol/g	GSH bursa µMol/L	MDA bursa µMol/g
G_C	0.27 ± 0.02 aB	0.32 ± 0.08 cB	0.32 ± 0.02 aA	1.94 ± 0.13 bA
G_D	0.14 ± 0.007 bA	1.64 ± 0.15 aB	0.17 ± 0.01 cA	3.10 ± 0.20 aA
G_S	0.25 ± 0.008 aA	1.28 ± 0.16 abA	0.25 ± 0.01 bA	1.66 ± 0.19 bA
G_B	0.23 ± 0.008 aA	0.96 ± 0.14 bB	0.25 ± 0.01 bA	2.04 ± 0.21 bA

*Static values with small letters are different significantly at ($p<0.05$) between groups
**Static values with capital letters are different significantly at ($p<0.05$) between two periods of age (21 and 42 days).

4- Heat shock protein 90 (HSP90)

When broilers were given G_S, and G_B, the HSP90 values didn't alter statically ($p > 0.05$) compared to the G_C, but when G_D was given, the HSP90 levels increased more than G_C at 21 days of age. After treatment of broilers with G_D, G_S, and G_B to the age of day 42, there was an increase in HSP90 ($p < 0.05$), with the most evident altitude in group G_D among the other groups. The effect of both periods on HSP90 levels, groups G_C, G_S, and G_B increased insignificance ($p < 0.05$), however, the group G_D had the exact opposite significance of groups G_C, G_S, and G_B.

Table (4): efficiency of SB and B on HSP90 of broilers injected with DEX

Mean ± SE

Treatment	21 days of age	42 days of age
	HSP90 ng/ml	HSP90 ng/ml
G_C	10.78 ± 0.24 bA	6.40 ± 0.78 dB
G_D	12.85 ± 0.39 aB	14.63 ± 0.47 aA
G_S	11.58 ± 0.36 bA	11.49 ± 0.68 bA
G_B	10.80 ± 0.35 abA	9.24 ± 0.45 cB

*Static values with small letters are different significantly at ($p<0.05$) between groups

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Discussion

The quantitative and qualitative measurement of biomarkers can be used to monitor stress levels. Thermal stress markings such as heat shock proteins (HSP), immune defense markers such as Acute Phase Proteins (APP), antioxidant enzymes in urine and saliva marking by oxidative stress such as analysis of powerful anti-oxidant system as well as levels of lipid oxidation are all potential stress markers **(21)**.

According to this study after the administration of DEX, the treated broilers suffered from oxidative stress which showed an increase in MDA levels and a decrease in GSH levels in serum and spleen and bursa tissues in both periods, these results are supported by **(22)** which also observed that there is a rise in lipid peroxidation and decrease in the activity of enzymatic oxidant system in hepatic tissue after exposure of broilers to DEX orally with water in dose 20 mg/L from 19 to 41 days of age. While **(23)** also revealed the same results in plasma and hepatic tissues after exposure of laying hens to DEX at 36 weeks of age at a dose of 4 mg/hen/day for 7 days. Following DEX treatment, the body's metabolism alters, resulting in the formation of oxidative stress by lowering mitochondrial levels of anti-oxidant enzymes system and glutathione, creating H2O2 radicals, and increasing lipid peroxidation **(24)**. In a recent study, the supplementation of SB improved the GSH levels and reduced lipid peroxidation in serum, spleen (just at 42 d), and bursa which approved the study **(25)** that observed dietary SB substantially enhanced the activity of antioxidant enzymes in duodenal and jejunal mucosa and reduced MDA concentration in duodenal mucosa after exposure to dietary corticosterone (30 mg/kg of diet) in male broilers from day 7 to day 21 which conclude SB can ameliorate the negative impact on production free radicals by corticosterone. Furthermore, SB supplementation increased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and decreased MDA in the liver, serum, and muscle after birds exposed to heat stress. As a result, it was determined the SB is stable in the presence of heat stress **(26)**. the strategy of butyrate or even its salt to minimizing oxidation is, however, uncertain. Our findings in this study that betaine can act as an antioxidant by the support the antioxidant system inside the body and subsequently reducing the lipid peroxidation and these results were approved by **(27)** which suggested that dietary betaine can promote the raising activities of antioxidant enzymes such as GPx, CAT, and SOD, lowering MDA levels in chest muscles and considering the possibility that betaine's effect is linked with methyl donor properties because its participation in cell membrane adjustment and homocysteine remethylation. While **(28)** It's supposed betaine protects cells from destruction caused by oxidants by replenishing S S-adenosyl methionine by increasing the quantity of substrate needed for the establishment of GSH to protect the cell from free radicals and reactive metabolites.

Negative feedback regulates glucocorticoid hormones via the glucocorticoid receptor (GR), which has a low affinity for corticosteroids but a strong affinity for dexamethasone. Besides

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this stress response, the relationship between transductional signalling and the regulatory sequences of chaperones linked to the GR, GR regulates the stress response. **(29)**. HSP90 is a cytosolic ATP paid molecular mechanism that rearranges the structure of proteins and required a lot of energy to initiate nucleotide modifications **(30).** Our results of this study noticed rising in levels of HSP90 after administration of DEX and we supposed that the reason goes that Hsp90's chaperoning activity might be interrupted by cleavage of proteins generated by H2O2 or other oxidants, implying that oxidative stress causes disrupted the HSP90 function in cells **(31)**. Oxidative stress supresses of glucose metabolism to a decrease in ATP, since the HSP90 activities are required ATP therefore, represents a new strategic plan for inhibiting HSP90 when the body is stressed. **(32).** Betaine, according to recent results, can minimize cleavage, and we propose that it's similar to chaperones to alleviate stressor that effects on protein folding by conserving structure of proteins by strengthening formation of hydrogen bonds in the folded form of aqua proteins. **(33)**, According to some studies, betaine aids in cytoprotection, protein constancy, and signalling pathway regulate proteins in the cell from heat that caused denaturation and limiting HSP inducement. **(34)**, Diamant *et al*. (2001) discovered a boosted role of betaine in protein refolding at a rate of 30–50% with differentiation of proteins by (2.5) fold, confirming that betaine aids in chaperoning. Our findings of the sodium butyrate effects on HSP90 showed a decrease in it, **(36)** observed that dietary SB was favourable for chickens suffered from stress, because of its ability to decline the catabolism and oxidative injury in the body, and according to Zhang et al., observation and our findings of supplementation of SB lowers the HSP90 levels compared with broilers under oxidative stress, we think that SB can depress HSP90 by alleviates the oxidative stress.

Conclusion

We conclude that sodium butyrate and betaine have anti-stressful and anti-oxidant effects, by alleviating the negative impacts of oxidative stress induced experimentally by dexamethasone as a nonspecific stress in the broilers with regular supplementation of sodium butyrate and betaine in their diet.

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