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# Effect of Dietary Supplementing Graded Concentrations of Sodium Sulphate on Performance, Carcass Traits, And Antioxidant Variables In Broiler Chicken

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## ABSTRACT

A study was conducted to assess the effect of supplementing graded concentrations of sodium sulphate (NaS) at a constant dietary electrolyte balance (DEB) on performance, carcass traits, immune responses, and antioxidant variables in broiler chicken. A total of 1,800 one-day-old broiler male chicks (Cobb 430) were evenly distributed into 72 pens, with 25 birds per pen (198 x 122 cm). A control diet (CD), based on maize-soybean meal, was prepared in mash form for three different phases: pre-starter (1-14 days), starter (15-28 days), and finisher (29-42 days). Sodium chloride (NaCl) was used as the sole source of supplemental Na and Cl in the CD. Five additional diets were formulated by supplementing sodium sulphate (NaS) at five graded concentrations (1.12, 2.25, 3.37, 4.50, and 5.62g/kg) to the CD. The DEB in pre-starter, starter, and finished diets were 215, 192, and 163 mEq/kg, respectively in all the treatment diets in each phase. The levels of NaCl were adjusted to arrive at a constant DEB among the diets in each phase. Body weight gain (BWG) and feed intake (FI) were not affected due to dietary variations. The feed conversion ratio (FCR) in groups fed with 3.37 or 4.50g/kg NaS was significantly lower compared to those fed 5.62 g/kg NaS. The carcass traits (ready to cook yield, breast meat weight, abdominal fat, liver weight and gizzards weight), immune responses (HI response to ND vaccine and CMI response to phytohaemogglutinin-P), and antioxidant variables (activity of glutathione peroxidase, glutathione reductase), respiration rate (panting), and cloacal temperature were not affected by dietary variation in NaS concentration. Based on the results, it can be concluded that NaS can be included in broiler diets up to 4.50g/kg, at constant dietary electrolyte balance, without any adverse effects on performance.

**KEYWORDS:** Antioxidant status, Broilers, Carcass parameters, Immunity, Production, Sodium sulphate.

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## INTRODUCTION

Sodium and chloride are the major extracellular cation and anion, which play a pivotal role in maintaining the proper balance of body fluids and cell function. Sodium (Na) is essential for several physiological processes, including the regulation of acid-base balance, osmotic pressure, and cell permeability (Olanrewaju et al., 2007). Sodium also facilitates the absorption of monosaccharides and amino acids, which are vital for the utilization of proteins and carbohydrates. Therefore, a deficiency of Na can impair these vital processes (Smith et al., 2000; Gal-Garber et al., 2003). Adequate Na intake has been shown to improve the performance of chicken (Watkins et al., 2005). Sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$) are commonly used as supplemental Na sources in poultry diets. Commercial poultry diets typically contain NaCl to meet the Na and Cl requirements. Inclusion of NaCl as a sole source of Na in the chicken diet may pose a risk of excess Cl intake. The Cl intake may also get complicated due to variation in concentrations of Cl in drinking water. Therefore, an excess intake of Cl can disrupt the balance of chloride ($Cl^-$) and carbonate ions [CO2(3−)], negatively affect the synthesis of calcium carbonate, and result in reduced performance (Mushtaq et al., 2007). Excessive intake of Cl can also result in wet feces, excessive water intake, ascites, edema, and weakened eggshell quality. An estimate reported that about 9 g of water is excreted for every 0.25% increase in dietary NaCl (Smith et al., 2000). Increasing the NaCl in the diet will also increase the Cl concentration in the diet, which may have a negative impact on bird physiology and performance, as indicated. Substituting sulfate for chloride as an anion has been considered particularly beneficial during stress periods that disrupt gut homeostasis and increase electrolyte losses (Hooge et al., 1999; Ahmad et al., 2006; Zdunczyk et al., 2012).

Sodium sulphate (NaS), also known as Glauber's salt, has been explored as chlorine-free Na source for poultry (Ahmad and Sarwar, 2005; Wang et al., 2019). Furthermore, dietary sulphate may contribute to the synthesis of several essential compounds, including methionine, cysteine, taurine, and glutathione, which are crucial for growth and antioxidant properties (Battin and Brumaghim, 2009; Del-Vesco et al., 2014). Furthermore, NaS has been observed to moderately stimulate digestive tract mucosa, enhance gastrointestinal motility, and regulate Na+/K+-ATPase activity, thereby improving digestive function (Gal-Garber et al., 2003).

Studies have demonstrated that dietary NaS at 1 g/kg can effectively replace about 18% of the recommended methionine (Rahimi et al., 2005). It is widely accepted that the sulphate requirements of most animals can generally be fulfilled by sulphate-containing amino acids through the oxidation of these amino acids. Conversely, dietary supplementation of inorganic sulphate may partly spare the dietary requirements for methionine or total sulphur amino acids (TSAA). Therefore, an effort was made in the present study to evaluate the potential advantages of incorporating sodium sulphate (NaS) at graded concentrations on the performance of broiler chicken, while maintaining constant dietary electrolyte balance (DEB) and TSAA levels in the diet.

## MATERIALS AND METHODS

### Diets and treatments

Maize-soybean-meat based control diets (CD) were prepared in the form of mash for three different phases i.e., pre-starter (1-14 d), starter (15-28 d), and finisher (29-42 d) phases (Table 1, 2, and 3, respectively). Feed grade sodium chloride (NaCl) was supplemented as the sole source of supplemental Na and Cl in the CD. Another five diets were prepared by supplementing sodium sulphate (NaS, Prosodium, Garsin, New Delhi, India) at five different concentrations (1.12, 2.25, 3.37, 4.50, and 5.62g/kg) to the CD. The dietary electrolyte balance (DEB) was maintained constant in each phase (about 215, 192, and 163 mEq/kg, respectively in pre-starter, starter, and finisher phases) irrespective of sodium level. Each diet was randomly allotted and fed *ad libitum* to birds in 12 pens (replicates) at the rate of 25 broiler male chickens per pen from d 1 to 42 d of age.

Table 1. Ingredient and nutrient composition (g/kg) of pre-starter (1-14d) diets

| Ingredient | NaSO4, g/kg | | | | | |
|---|---|---|---|---|---|---|
| | CD | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Maize | 565.4 | 565.4 | 566.4 | 566.4 | 566.4 | 566.4 |
| Oil-Veg | 30.9 | 30.9 | 30.9 | 30.9 | 30.9 | 30.9 |
| Soya Doc 45% | 358.3 | 358.3 | 358.3 | 358.3 | 358.3 | 358.3 |
| Salt | 4.70 | 4.30 | 3.90 | 3.50 | 3.10 | 2.70 |
| Dicalcium phosphate | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 |
| LSP-Powder | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 |
| DL-Methionine | 3.643 | 3.643 | 3.643 | 3.643 | 3.643 | 3.643 |
| L-Lysine SO4 | 3.674 | 3.674 | 3.674 | 3.674 | 3.674 | 3.674 |
| L-Threonine | 0.973 | 0.973 | 0.973 | 0.973 | 0.973 | 0.973 |
| L-Tryptophan | 0 | 0 | 0 | 0 | 0 | 0 |
| L- Arginine | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 | 0.465 |
| L-Valine | 0.434 | 0.434 | 0.434 | 0.434 | 0.434 | 0.434 |
| L-Isoleucine | 0.051 | 0.051 | 0.051 | 0.051 | 0.051 | 0.051 |
| Premix[#] | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Na2SO4 | 0 | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Nutrient, g/kg | | | | | | |
| M E (kcal/kg) | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| Protein | 220.0 | 220.0 | 220.0 | 220.0 | 220.0 | 220.0 |
| Dig Lysine | 12.70 | 12.70 | 12.70 | 12.70 | 12.70 | 12.70 |
| Dig. Methionine | 6.65 | 6.65 | 6.65 | 6.65 | 6.65 | 6.65 |
| Dig. Meth + Cyst | 9.60 | 9.60 | 9.60 | 9.60 | 9.60 | 9.60 |
| Calcium | 9.50 | 9.50 | 9.50 | 9.50 | 9.50 | 9.50 |
| Av. Phosphorus | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Sodium | 20.07 | 18.55 | 17.03 | 15.51 | 13.99 | 12.47 |
| Chloride | 32.10 | 29.78 | 27.46 | 25.14 | 22.82 | 20.50 |
| Potassium | 85.07 | 85.07 | 85.07 | 85.07 | 85.07 | 85.07 |
| Sulphur ppm | 2522 | 2634 | 2747 | 2859 | 2972 | 3084 |
| DEB (meq/Kg) | 215 | 215 | 215 | 215 | 215 | 215 |
| Dig. Arginine | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 |
| Dig. Tryptophan | 2.41 | 2.41 | 2.41 | 2.41 | 2.41 | 2.41 |
| Dig.Threonine | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 |
| Dig. Iso-Leucine | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 |
| Dig. Valine | 9.60 | 9.60 | 9.60 | 9.60 | 9.60 | 9.60 |

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase
[##]analysed values

Table 2. Ingredient and nutrient composition (g/kg) of Starter (15-28d) diets

| Ingredient | NaSO4, g/kg | | | | | |
|---|---|---|---|---|---|---|
| | CD | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Maize | 611.9 | 611.9 | 611.9 | 612.9 | 612.9 | 612.9 |
| Oil-Veg | 38.3 | 38.3 | 38.3 | 38.3 | 38.3 | 38.3 |
| Soya Doc 45% | 305.7 | 305.7 | 305.7 | 305.7 | 305.7 | 305.7 |
| Salt | 4.70 | 4.30 | 3.90 | 3.50 | 3.10 | 2.70 |
| Dicalcium phosphate | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 |
| LSP-Powder | 13.6 | 13.6 | 13.6 | 13.6 | 13.6 | 13.6 |
| DL-Methionine | 3.292 | 3.292 | 3.292 | 3.292 | 3.292 | 3.292 |
| L-Lysine SO4 | 4.041 | 4.041 | 4.041 | 4.041 | 4.041 | 4.041 |
| L-Threonine | 0.965 | 0.965 | 0.965 | 0.965 | 0.965 | 0.965 |
| L-Tryptophan | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 | 0.067 |
| L- Arginine | 0.702 | 0.702 | 0.702 | 0.702 | 0.702 | 0.702 |
| L-Valine | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 |
| L-Isoleucine | 0.252 | 0.252 | 0.252 | 0.252 | 0.252 | 0.252 |
| Premix[#] | 4.10 | 4.10 | 4.10 | 4.10 | 4.10 | 4.10 |
| Na2SO4 | 0 | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Nutrient, g/kg | | | | | | |
| M E (kcal/kg) | 3100 | 3100 | 3100 | 3100 | 3100 | 3100 |
| Protein | 200.0 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Dig Lysine | 11.60 | 11.60 | 11.60 | 11.60 | 11.60 | 11.60 |
| Dig. Methionine | 6.08 | 6.08 | 6.08 | 6.08 | 6.08 | 6.08 |
| Dig. Meth + Cyst | 8.80 | 8.80 | 8.80 | 8.80 | 8.80 | 8.80 |
| Calcium | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| Av. Phosphorus | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 |
| Sodium | 20.00 | 18.48 | 16.96 | 15.44 | 13.92 | 12.40 |
| Chloride | 32.13 | 29.81 | 27.49 | 25.17 | 22.85 | 20.53 |
| Potassium | 76.47 | 76.47 | 76.47 | 76.47 | 76.47 | 76.47 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sulphur ppm | 2386 | 2498 | 2611 | 2723 | 2836 | 2948 |
| DEB (meq/Kg) | 192 | 192 | 192 | 192 | 192 | 192 |
| Dig Arginine | 12.30 | 12.30 | 12.30 | 12.30 | 12.30 | 12.30 |
| Dig. Tryptophan | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 |
| Dig .Threonine | 7.80 | 7.80 | 7.80 | 7.80 | 7.80 | 7.80 |
| Dig. Iso-Leucine | 7.80 | 7.80 | 7.80 | 7.80 | 7.80 | 7.80 |
| Dig. Valine | 8.80 | 8.80 | 8.80 | 8.80 | 8.80 | 8.80 |

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase
[##] analysed values

Table 3. Ingredient and nutrient composition (g/kg) of Finisher (29-42d) diets

| Ingredient | NaSO4, g/kg | | | | | |
|---|---|---|---|---|---|---|
| | CD | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Maize | 653.4 | 654.4 | 654.4 | 654.4 | 655.4 | 655.4 |
| Oil-Veg | 43.1 | 43.1 | 43.1 | 43.1 | 43.1 | 43.1 |
| Soya Doc 45% | 263.6 | 263.6 | 263.6 | 263.6 | 263.6 | 263.6 |
| Salt | 4.2 | 3.8 | 3.4 | 3.0 | 2.6 | 2.2 |
| Dicalcium phosphate | 10.7 | 10.7 | 10.7 | 10.7 | 10.7 | 10.7 |
| LSP-Powder | 13.3 | 13.3 | 13.3 | 13.3 | 13.3 | 13.3 |
| DL-Methionine | 2.936 | 2.936 | 2.936 | 2.936 | 2.936 | 2.936 |
| L-Lysine SO4 | 1.532 | 1.532 | 1.532 | 1.532 | 1.532 | 1.532 |
| L-Threonine | 0.804 | 0.804 | 0.804 | 0.804 | 0.804 | 0.804 |
| L-Tryptophan | 0.191 | 0.191 | 0.191 | 0.191 | 0.191 | 0.191 |
| L- Arginine | 0.936 | 0.936 | 0.936 | 0.936 | 0.936 | 0.936 |
| Phytase 5000 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 |
| L-Valine | 0.509 | 0.509 | 0.509 | 0.509 | 0.509 | 0.509 |
| L-Isoleucine | 0.254 | 0.254 | 0.254 | 0.254 | 0.254 | 0.254 |
| Premix[#] | 4.10 | 4.10 | 4.10 | 4.10 | 4.10 | 4.10 |
| Na2SO4 | 0 | 1.12 | 2.25 | 3.37 | 4.50 | 5.62 |
| Nutrient, g/kg | | | | | | |
| M E (kcal / kg) | 3175 | 3175 | 3175 | 3175 | 3175 | 3175 |
| Protein | 182.5 | 182.5 | 182.5 | 182.5 | 182.5 | 182.5 |
| Dig Lysine | 9.20 | 9.20 | 9.20 | 9.20 | 9.20 | 9.20 |
| Dig. Methionine | 5.55 | 5.55 | 5.55 | 5.55 | 5.55 | 5.55 |
| Dig. Meth + Cyst | 8.10 | 8.10 | 8.10 | 8.10 | 8.10 | 8.10 |
| Calcium | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 | 8.50 |
| Av. Phosphorus | 4.20 | 4.20 | 4.20 | 4.20 | 4.20 | 4.20 |
| Sodium | 18.06 | 16.54 | 15.02 | 13.50 | 11.98 | 10.46 |
| Chloride | 33.57 | 31.25 | 28.93 | 26.61 | 24.29 | 21.97 |
| Potassium | 69.72 | 69.72 | 69.72 | 69.72 | 69.72 | 69.72 |
| Sulphur ppm | 1916 | 2028 | 2141 | 2253 | 2366 | 2478 |
| DEB (meq/Kg) | 163 | 163 | 163 | 163 | 163 | 163 |
| Dig Arginine | 11.40 | 11.40 | 11.40 | 11.40 | 11.40 | 11.40 |
| Dig. Tryptophan | 2010 | 2010 | 2010 | 2010 | 2010 | 2010 |
| Dig .Threonine | 7.10 | 7.10 | 7.10 | 7.10 | 7.10 | 7.10 |
| Dig. Iso-Leucine | 7.10 | 7.10 | 7.10 | 7.10 | 7.10 | 7.10 |
| Dig. Valine | 8.10 | 8.10 | 8.10 | 8.10 | 8.10 | 8.10 |

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase
[##] analysed values

**Birds and management**

A total of 1,800 one-day-old broiler male chicks (Cobb 430, Venkateswara Hatcheries Pvt. Ltd., Hyderabad, India) were evenly distributed into 72 pens at the rate of 25 birds each in litter floor pen (198 x 122 cm). The floor of the pens were covered with fresh paddy husk at about 8 cm thickness. The litter was covered with old newspaper to prevent accidental intake of litter material by chicks during the initial 4 days of age, after which the paper was removed. Brooding was done with incandescent bulbs (100 watt/pen) and coal to provide the required temperature (about 35°C during week 1, and gradually reduced to ambient temperature at day 21 and after which the birds were exposed to ambient temperature. The ambient temperature ranged from 24.7±1.35 to 30.4±2.65°C and the humidity were ranged from 32.7±16.01 to 63.5±8.45% at minimum and maximum, respectively. Fluorescent bulbs were used to provide light during nighttime from 4 to 6 weeks of age.

**Performance and slaughter variables**

Body weight and feed intake (FI) were recorded at weekly intervals, and body weight gain (BWG) and feed conversion ratio (FCR) was calculated as FI/BWG. Feed left in the feeder was placed back in the respective feed drum to calculate the average amount of feed consumed by the birds in each pen. All the birds present in each pen were weighed to calculate the average BWG. At the end of the experiment, one bird representing the mean body weight of the respective pen (replicate) was selected to study carcass traits, including ready-to-cook yield (RTC), breast meat weight, thigh weight, liver weight, and abdominal fat content. The relative weight of lymphoid organs (bursa, spleen) was recorded and expressed as g per kg live weight.

**Immune responses**

Immune responses were measured in terms of HI titer to Newcastle disease vaccine and cell-mediated immune (CMI) response to phytohemagglutinin-P (PHA-P) inoculation.

**HI titre against ND vaccine**

The chickens were vaccinated against Newcastle disease (ND) by an ocular route at 5th and 21nd d of age with the Lasota strain (ND Lasota Vac-500, Indovax Pvt., Ltd., Hyderabad, India). The humoral immune response was measured as antibody titre against ND vaccine by collecting blood from the brachial vein on the 35th day of age, which was 14 d post inoculation of the vaccine. For this, 2 mL of blood was collected from one bird per replicate, and the antibody titres in sera against ND virus was measured (Reynolds and Maraqa, 2000) by haemagglutination inhibition test. The antibody titre against the disease was expressed as log2 values. The reciprocal of the highest dilution where there was complete inhibition of agglutination was taken as the titre.

**Cell mediated immune (CMI) response**

The CMI response was assayed by cutaneous basophilic hypersensitivity test *in vivo* by using phytohemagglutinin-P (PHA-P) (TC 226, HiMedia Laboratories Pvt Ltd, Mumbai, India) employing the method as described by Corrier and Deloach (1990). On 35th d of age, one bird from each replicate was selected and the thickness of both right and left wattles was measured by micrometer (p no 7301, Mitutoyo, Japan). A total of 100 µg of PHA-P suspended in 0.10 mL of phosphate buffer saline (PBS) and 0.1 mL of the PBS without PHA-P were injected intradermal into the right and left (acted as a control) wattles, respectively. The thickness of both the wattles was measured at 24 h post-injection. The CMI response was calculated as the difference in thickness between right and left wattles due to PHA-P inoculation, which was expressed in relation to the increased thickness due to PBS alone.

**Anti-oxidant variables**

The activities of antioxidant enzymes like glutathione peroxidase (GSHPx), and glutathione reductase (GSHRx) in blood were measured at the end of the study (42d of age). About 2.0 mL of blood sample from each bird was placed into a centrifuge tube containing citrate buffer (1.5 mL/10 mL blood) for erythrocyte separation and antioxidant enzyme estimation. The blood samples were centrifuged at 500 rpm for 15 min at 4°C to separate the buffy coat (WBC) and form erythrocyte pellet. The erythrocytes were washed three times with PBS (pH 7.4). The packed RBC obtained was mixed with an equal volume of PBS and then diluted as per the requirement with distilled water. The activity of GSHPx and GSHRx was estimated following the method of Paglia and Valentine (1967).

**Panting rate and cloacal temperature**

One bird per pen was selected to count the number of respirations in a minute. The number of respirations was recorded two times in a day i.e. at 1-2 PM and 5-6 PM from all the replicates once in each week. Simultaneously, rectal temperature was measured from the same bird utilizing a digital thermometer.

### Statistical analysis

The performance data were analysed by considering the pen as an experimental unit, and other parameters (carcass traits, immune responses, lymphoid organ weight, panting, and cloacal temperature), the individual bird data were considered as a unit for statistical analysis. The data were statistically analysed by a complete randomized design with One-way Analysis of Variance (ANOVA) (SAS Institute, 1994). The differences among treatment means were compared using Duncan's multiple range test. Further, the response in the dependent variables with change in the concentration of NaS was fitted by the polynomial equation in the form of $Y=a+bx+cx^2$ to know the trend in the dependent variable in relation to the NaS concentration in the diet.

## RESULTS AND DISCUSSION

### Performance

The inclusion of NaS in broiler chicken diets did not significantly influence BWG or FI (P>0.05) during the pre-starter, starter, finisher phases, and overall production period (Table 4 and 5). However, the FCR, expressed as the ratio of FI/BWG, was significantly improved at 2.25 and 3.37 g/kg NaS inclusion compared to 5.62 g/kg during pre-starter phase. In contrast, no significant differences in FCR were observed among broilers fed diets with 0, 1.12, and 4.50 g/kg NaS.

Table 4. Performance of broiler chicken fed graded concentrations of sodium sulphate (NaS)

| NaS | Pre-Starter (1-14 days) | | | Starter (15-28 days) | | |
|---|---|---|---|---|---|---|
| g/kg | BWG, g | FI, g | FI/BWG | BWG, g | FI, g | FI/BWG |
| CD | 474.9 | 504.5 | $1.182^{BC}$ | 996.0 | 1522 | $1.457^{CD}$ |
| 1.12 | 474.1 | 504.7 | $1.185^{AB}$ | 986.1 | 1530 | $1.479^{AB}$ |
| 2.25 | 482.2 | 509.2 | $1.173^{D}$ | 984.7 | 1522 | $1.473^{AB}$ |
| 3.37 | 477.3 | 505.3 | $1.178^{CD}$ | 991.0 | 1525 | $1.468^{BC}$ |
| 4.50 | 476.6 | 505.2 | $1.180^{BCD}$ | 1007 | 1524 | $1.444^{D}$ |
| 5.62 | 469.0 | 500.2 | $1.189^{A}$ | 979.6 | 1527 | $1.486^{A}$ |
| SEM | 1.83 | 2.013 | 0.0011 | 4.43 | 5.87 | 0.0025 |
| P | | | | | | |
| One-way | 0.472 | 0.897 | 0.001 | 0.564 | 0.999 | 0.001 |
| Regression | | | | | | |
| Linear | 0.471 | 0.578 | 0.290 | 0.887 | 0.937 | 0.501 |
| Quadratic | 0.185 | 0.523 | 0.001 | 0.976 | 0.996 | 0.688 |

$^{ABCD}$ means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; BWG body weight gain; FI feed intake; P probability; n: number of replicates; SEM standard error of the mean

Table 5. Performance of broiler chicken fed graded concentrations of sodium sulphate (NaS)

| NaS | Finisher (29-42 days) | | | Cumulative (1-42 days) | | |
|---|---|---|---|---|---|---|
| g/kg | BWG, g | FI, g | FI/BWG | BWG, g | FI, g | FI/BWG |
| CD | 977.2 | 2023 | 2.079 | 2448 | 4049 | $1.654_{BC}$ |
| 1.12 | 945.9 | 1983 | 2.106 | 2406 | 4017 | $1.670_{AB}$ |
| 2.25 | 960.7 | 2007 | 2.093 | 2428 | 4038 | $1.664_{AB}$ |
| 3.37 | 995.5 | 2027 | 2.041 | 2464 | 4057 | $1.647_{C}$ |
| 4.50 | 983.6 | 2020 | 2.060 | 2467 | 4049 | $1.642_{C}$ |
| 5.62 | 939.9 | 1977 | 2.105 | 2389 | 4005 | $1.676^{A}$ |
| SEM | 12.66 | 20.46 | 0.009 | 12.8 | 20.72 | 0.0031 |
| P | | | | | | |
| One-way | 0.787 | 0.972 | 0.235 | 0.406 | 0.977 | 0.001 |
| Regression | | | | | | |
| Linear | 0.882 | 0.819 | 0.765 | 0.764 | 0.800 | 0.878 |
| Quadratic | 0.833 | 0.931 | 0.538 | 0.650 | 0.900 | 0.256 |

$^{ABC}$ means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; BWG body weight gain; FI feed intake; P probability; n: number of replicates; SEM standard error of the mean

Significantly lower FCR was observed at 4.50 g/kg NaS inclusion during the starter phase compared to all other inclusion levels, except the control group. The cumulative FCR was significantly ($P<0.05$) reduced with the level of NaS in diet. Broilers fed diets with 3.37 or 4.50 g/kg NaS demonstrated significantly lower FCR compared to those receiving higher concentrations (5.62 g/kg NaS, Table 5). Similar to the current study, Rahimi et al. (2005) also observed an improvement in the FCR of broiler chickens due to the supplementation of NaS in the diet. However, the improved BWG without affecting the FCR at day 42 was reported by Mushtaq et al. (2014) when the broiler diets were supplemented with NaS at 2.6 g/kg.

The findings of this study differ from those of Sharma et al. (2011), who reported improved body weight with the supplementation of NaS in turkey broiler diets. Similarly, Ali et al. (2019) observed the lowest FI in broilers fed NaS supplemented diets compared to other treatments. In their study, NaS (3 g/kg) was supplemented in combination with tyrosine (0.5g/kg), the additive effect probably might have decreased the FI in Arbor Acers male broilers.

The exact metabolic role of NaS in chicken is not very clear, however, based on the literature, the probable beneficial role of NaS supplementation on chicken performance was discussed. There is a limited recent literature on NaS use in poultry diets, majority of work was conducted during 1970s. The beneficial effects of NaS supplementation on feed efficiency might be due to the methionine-sparing effects of S present in NaS. Similar to this hypothesis, the methionine-sparing effect of NaS on broiler performance was reported in the literature (Ross et al., 1972; Harms, 1972; Hinton and Harms, 1972; Rama Rao et al., 2022). Maintenance of optimum blood sulphate concentration (0.01 µc/ml, higher than those achieved with standard diets) and optimum feather development was attributed (Gordon and Sizer, 1955) to the higher growth rate in chicken fed NaS. Similarly, Almquist (1952) reported that NaS was approximately 40% as effective as amino acid sulphate in promoting growth response. Harms (1972) also found that the peak growth rate of broiler chicks was achieved when a corn-soybean basal diet (containing 4.0 g methionine and 3.9 g/kg cystine) was supplemented with a combination of methionine (1 g/kg) and NaS (1 g/kg). In their studies, an addition of 1.6 g/kg NaS was found as the most effective level for stimulating growth in chicks. Improved nutrient digestion in upper digestive tract with NaS supplementation compared to NaHCO3 was reported by Lawlor et al. (2005). The higher beneficial effects of NaS over NaHCO3 was attributed to lower acid binding capacity (96 mEq H+/kg vs. 110.66 mEq H+/kg) of NaS relative to NaHCO3, which likely contributes to the improved performance of broilers. The beneficial effects of NaS may also be attributed to its potential role in conserving sulphur-containing amino acids by facilitating the direct synthesis of taurine and chondroitin sulphate from NaS, thereby preventing the degradation of sulphur amino acids (Youssef, 2002). Additionally, NaS has been shown to maintain dietary electrolyte balance (DEB) within optimal limits in broiler diets (Jarule et al., 2009). In the current study, the DEB was uniformly maintained among the different treatment groups in each pase, which might explain the lack of negative effects on performance with lower sodium levels than recommended by NRC 1994.

The improvement of FI per BWG indicated that higher levels of sulphates in NaS supplemented diets might have induced appetite in birds since better absorption of sulphates is Na dependent active process or because Na involves in cysteine sparing effect as per the literature (Langridge-Smith et al., 1983; Ahearn and Murer, 1984; Florin et al., 1991). This increased presence of Sulfur might have converted to amino acids, which might have improved the feed efficiency. The amino acid-sparing effect was further demonstrated by Sharma et al. (2012) with turkey broilers by evaluating the impact of adding 4 g/kg NaS to a vegetable-based, fishmeal-free ration.

Improved weight gain and feed efficiency were reported by Plavnik and Bornstein (1978) with increasing levels of NaS in diets containing sub-optimal concentrations of total sulphur amino acids. This could explain the improved feed efficiency observed in the current study with the supplementation of NaS in diets.

In the current study, the feed efficiency reduced significantly in groups fed the highest concentration of NaS (5.62 g/kg). The calculated concentrations of S in those diets (3084, 2948 and 2478 ppm, respectively in pre-starter, starter and finisher diets) were nearer to the harmful level (3000 mg/kg) as suggested by NRC (1994). Trials conducted by the University of Guelph (Canada) to evaluate broiler growth in response to sulphate intake revealed a linear decline in weight gain with increased inorganic sulphate content (1400, 2700, 4000, 5300 mg/kg S at 0.37 and 1.32% Ca levels) in diet and the negative effects were attributed to disruption of anion-cation balance (Pinon et al., 2021).

**Carcass parameters**

Dietary inclusion of NaS in broiler chicken diets did not have any significant effect (P>0.05) on carcass variables such as ready-to-cook yield (RTC), abdominal fat, breast meat, or the weights of lymphoid organs, with the exception of thigh weight (Table 6). The thigh weight showed a progressive increase (P<0.05) with NaS concentration up to 2.25 g/kg and however, further increases in NaS concentration resulted in a reduction in thigh weight. These results are in line with findings of Borgatti et al. (2004) and Musthaq et al. (2013), who reported higher thigh weights in broilers fed graded concentrations of NaS (1.7, 2.6, 3.5, and 4.4 g/kg) in diets.

Table 6. Slaughter parameters (g/kg live weight) of broilers fed graded concentrations of sodium sulphate (NaS)

| NaS | RTC | BW | Abdfat | Liver | Thigh | Bursa | Spleen |
|---|---|---|---|---|---|---|---|
| g/kg | | | | g/kg live weight | | | |
| CD | 782.1 | 257.0 | 14.98 | 18.99 | 214.9$^{BC}$ | 0.494 | 0.899 |
| 1.12 | 778.1 | 259.0 | 17.41 | 18.62 | 214.4$^{BC}$ | 0.315 | 0.905 |
| 2.25 | 790.5 | 269.8 | 15.87 | 19.45 | 238.1$^{A}$ | 0.450 | 0.982 |
| 3.37 | 783.5 | 267.5 | 11.39 | 17.61 | 220.0$^{B}$ | 0.526 | 0.994 |
| 4.50 | 787.3 | 270.1 | 14.65 | 18.13 | 213.1$^{BC}$ | 0.506 | 0.908 |
| 5.62 | 786.1 | 273.2 | 15.21 | 17.62 | 201.1$^{C}$ | 0.373 | 0.916 |
| SEM | 1.874 | 2.653 | 0.631 | 0.365 | 2.679 | 0.029 | 0.031 |
| P | | | | | | | |
| One-way | 0.498 | 0.415 | 0.137 | 0.639 | 0.002 | 0.230 | 0.922 |
| Regression | | | | | | | |
| Linear | 0.292 | 0.038 | 0.377 | 0.174 | 0.098 | 0.945 | 0.867 |
| Quadratic | 0.514 | 0.105 | 0.537 | 0.390 | 0.002 | 0.839 | 0.663 |

$^{ABC}$ means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; RTC ready to cook yield; BW breast weight; Abdfat abdominal fat; P probability; n: number of replicates; SEM standard error of the mean.

Contrarily, Mushtaq et al. (2007) observed reduced breast and leg meat by increasing Na from 2.0 to 3.0 g/kg. These attributes (breast and thigh meat weights) were negatively affected by heat stress conditions provided in the experiment by Mushtaq et al. (2007), therefore it is obvious that more nutrients particularly energy were utilized for increased activities like panting. Whereas, in the present study there is no change in caracass parameters (except thigh muscle weight), which might be due to the fact that the DEB maintained uniform among the groups, and also optimum ambient temperature prevailed during the study period.

In the current study, there is no change in the weights of bursa and spleen. Studies conducted by Raymond and Karunajeewa 1985, Borges et al. (1999) and Borgatti et al. (2004) also did not observe any combination effects of electrolytes (sodium carbonate, potassium carbonate, and ammonium chloride) on the weights of proventriculus, heart, liver, and pancreas.

**Immunity and fitness traits**

Inclusion of NaS in broiler chicken diets did not have a significant impact (P>0.05) on the immune responses (CMI and HI titres against ND, Table 7). Similarly, the inclusion of NaS in the diet did not affect panting rate or cloacal temperature in broilers.

Table 7. Immune responses, panting, cloacal temperature, and serum anti-oxidant variables in broilers fed graded concentrations of sodium sulphate (NaS)

| NaS | ND titres | CMI | Panting | Cloacal temp | GSHPx | GSHRx |
|---|---|---|---|---|---|---|
| g/kg | Log | 2% | no °F | | | u/L |
| CD | 6.70 | 52.50 | 30.92 | 107.1 | 366.3 | 1956 |
| 1.12 | 6.60 | 54.17 | 24.83 | 107.4 | 372.1 | 1737 |
| 2.25 | 6.90 | 63.75 | 26.83 | 106.8 | 381.6 | 1712 |
| 3.37 | 6.70 | 61.50 | 27.92 | 107.2 | 332.3 | 1740 |
| 4.50 | 6.50 | 51.67 | 27.92 | 107.0 | 363.6 | 1637 |
| 5.62 | 6.70 | 56.83 | 27.58 | 107.5 | 392.9 | 1647 |
| SEM | 0.1586 | 2.198 | 0.8201 | 0.1145 | 6.375 | 36.63 |
| P | | | | | | |
| One-way | 0.990 | 0.531 | 0.449 | 0.581 | 0.115 | 0.133 |
| Regression | | | | | | |
| Linear | 0.879 | 0.793 | 0.709 | 0.660 | 0.659 | 0.014 |
| Quadratic | 0.979 | 0.466 | 0.500 | 0.704 | 0.330 | 0.029 |

$^{ABC}$ means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; CMI cell mediated immunity; GSHPx glutathione peroxidase, GSHRx: glutaredoxins; P probability; n: number of replicates; SEM standard error of the mean.

Sulphate undergoes various metabolic processes within living organisms through reduction and oxidation pathways. It is essential for the synthesis of glutathione, which is a component of cysteine and methionine. Sulphate is metabolized into sulphate compounds, which are either assimilated or excreted (Stipanuk, 2004; Nimni et al., 2007; Toledano et al., 2007). As a natural antibiotic, sulphate aids in detoxification by binding with toxins or heavy metals in the liver (Parcell, 2002). However, in the present study, no significant variations were observed in panting rates or cloacal temperatures. Similarly, Ahmad et al. (2006) concluded that maintaining a constant DEB (250 mEq/kg), altering the concentrations of dietary sodium content (through NaHCO3, Na2CO3, and Na2SO4) significantly improved broiler performance during the hot summer months. In the current study, the temperature in the sheds was maintained at optimal comfort levels, allowing the birds' performance to be maximized according to standards, even with lower DEB and sodium (Na) levels than those recommended by the NRC. The National Research Council recommends 2g/kg sodium, chloride, and 3g/kg potassium for the starter phase of broilers, with reduced sodium and chloride levels for the finisher phase. However, under heat stress conditions, these requirements increase, as birds perform better with elevated electrolyte levels while maintaining DEB approximately 250 mEq/kg. Higher electrolyte levels, particularly sodium, effectively promote growth but also lead to increased water consumption, resulting in higher litter moisture during summer (Mushtaq et al., 2013).

In the current study, no variation was observed in the levels of antioxidant enzymes, such as GSHPx and GSHRx, among the different groups of birds fed varying concentrations of NaS. These antioxidant enzymes are vital for protecting cells from oxidative damage by reducing hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively. The lack of change in enzyme levels suggests that the birds were kept within their comfort zone and provided with optimal nutrition. This could be one reason why there is no variation in immunity, organ weights, panting, and temperature among the groups.

## CONCLUSION

Based on the data, it can be concluded that supplementation of sodium sulphate at 3.37 or 4.50 g/kg in broiler diets significantly improves feed efficiency. Additionally, an inclusion level of 2.25 g/kg NaS notably enhances the relative weight of the thigh, demonstrating its beneficial effects on growth performance and carcass characteristics in broiler chicken.

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