BioScientific Review (BSR)
Volume 6 Issue 3, 2024
ISSN$_{(P)}$: 2663-4198, ISSN$_{(E)}$: 2663-4201
Homepage: https://journals.umt.edu.pk/index.php/bsr

Article QR

Title: **Supplemental Effects of Sodium Gluconate (SG) on Growth Promotion, Organ Development, and Selected Serum Blood Metabolites in Broiler Chickens**

Author (s): Hira Nawaz[1], Inam Ullah[2], Abdul Hameed[3], Obaid Ullah[4], Rahat Ullah Khan[5,6], Rahman Ullah[7], Muhammad Adil[8], Qurat ul Ain[9], Aamir Iqbal[10], Baseer Ahmad[11], Mirwaise Khan[12]

Affiliation (s):
[1]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Poultry Husbandry, AH&VS, Sindh Agriculture University, Hyderabad, Pakistan
[3]Directorate of Poultry Research Institute, Rawalpindi, Pakistan
[4]Department of Veterinary Clinical Sciences, University of Poonch, Rawalakot, Pakistan
[5]Institute of Microbiology, Chinese Academy of Sciences, China
[6]University of Chinese Academy of Sciences, Beijing, China
[7]The University of Agriculture, Dera Ismail Khan, Pakistan
[8]University of Agriculture, Faisalabad, Pakistan
[9]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[10]Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan
[11]Department of Animal and Poultry Production, University of Poonch, Rawalakot, Pakistan
[12]Chinese Academy of Agricultural Sciences Harbin Veterinary Institute, Harbin, China

DOI: https://doi.org/10.32350/bsr.63.03
History: Received: April 24, 2024, Revised: June 14, 2024, Accepted: June 20, 2024, Published: July 25, 2024
Citation: Nawaz H, Ullah I, Hameed A, et al. Supplemental effects of Sodium Gluconate (SG) on growth promotion, organ development, and selected serum blood metabolites in broiler chickens. *BioSci Rev.* 2024;6(3):49-64. https://doi.org/10.32350/bsr.63.03
Copyright: © The Authors
Licensing: This article is open access and is distributed under the terms of Creative Commons Attribution 4.0 International License

Conflict of Interest: Author(s) declared no conflict of interest

A publication of
The Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Supplemental Effects of Sodium Gluconate (SG) on Growth Promotion, Organ Development, and Selected Serum Blood Metabolites in Broiler Chickens

Hira Nawaz[1], Inam Ullah[2], Abdul Hameed[3], Obaid Ullah[4], Rahat Ullah Khan[5,6], Rahman Ullah[7], Muhammad Adil[8], Qurat ul Ain[9], Aamir Iqbal[10], Baseer Ahmad[11*], Mirwaise Khan[12*]

[1]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

[2]Poultry Husbandry, AH&VS, Sindh Agriculture University, Pakistan

[3]Directorate of Poultry Research Institute, Rawalpindi, Pakistan

[4]Department of Veterinary Clinical Sciences, Faculty of Veterinary and Animal Sciences, University of Poonch Rawalakot, Azad Kashmir, Pakistan

[5]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Center for Influenza Research and Early-warning (CASCIRE), CAS-TWAS Center of Excellence for Emerging Infectious Diseases (CEEID), Chinese Academy of Sciences, Beijing, China

[6]College of Life Science, University of Chinese Academy of Sciences, Beijing, China

[7]Faculty of Veterinary and Animal Sciences, The University of Agriculture, Dera Ismail Khan, Pakistan

[8]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

[9]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

[10]Department of Animal and Poultry Production, University of Poonch, Rawalakot, Azad Kashmir, Pakistan

[11]Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan

[12]State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

ABSTRACT

Background. The poultry sector is crucial in addressing nutritional deficiencies since it provides essential nutrients and proteins. To achieve optimal chicken production, it is important to understand how the gut microbiota functions, as it affects immunity, digestion, and pathogen control. This study examines the effects of Sodium Gluconate (SG) as a

*Corresponding Author: dr.baseerahmadkhan@gmail.com; drmirwaise2321@gmail.com

growth promoter and investigates its impact on growth promotion, organ development, and selected serum blood metabolites.

Methodology. A total of one hundred (100) one-day-old broiler chicks were used in this investigation. The chicks were procured from a hatchery and housed at an experimental farm at the University of Veterinary and Animal Sciences, Department of Physiology, Lahore, Pakistan. The chicks were divided into four (04) groups of twenty-five (25) birds each. Then, each group was split up into four (04) duplicates. Four SG treatments (control, SG3.5%, SG4.5%, and SG5.5%) were made, combined with ration, and fed to the birds.

Results. In the first week, feed conversion ratio (FCR) showed substantial impacts, with the SG 3.5% group showing a significantly greater FCR than the control. Similarly, in week 5, the SG 5.5% group showed a considerable higher FCR compared to the control and SG 4.5% groups. The addition of SG did not change the weights of the viscera or the length of the small intestine. When the SG 5.5% supplemented birds were compared to SG 3.5% supplemented birds, the only item that showed a significant increase ($p < 0.05$) was the caecum length. Except for uric acid and cholesterol, all of the chosen blood metabolites remained unaffected by the dietary SG addition. In contrast to the control and SG 3.5% groups, the cholesterol concentration was lower in the SG 4.5% and SG 5.5% groups. Additionally, the SG 3.5% group had higher uric acid ($p < 0.05$) than the SG 4.5% and SG 5.5% groups.

Conclusion. The results support sustainable poultry production methods by offering insightful observations about the effectiveness of SG as a growth enhancer and its effects on broiler health indices.

Keywords: additives, antibiotics, metabolites, microbiota, poultry, resistance, Sodium Gluconate (SG)

Highlights

- The findings demonstrated that in the first week, the FCR of SG 3.5% group was much higher than the control group.
- Similarly, in week 5, the FCR of SG 5.5% group was significantly higher than that of the control and SG 4.5% groups.
- The addition of SG did not affect the length of the small intestine or the weight of the viscera.
- The SG dietary supplementation of did not affect most of the selected blood metabolites, except for uric acid and cholesterol.

1. INTRODUCTION

Poultry is a major sector of livestock and is a particularly important source of protein for the growing population of the nation [1]. The increase in demand for poultry meat and eggs, globally as well as locally, makes this sector vulnerable to future challenges [2–4]. The gut health of poultry is dependent upon physiological, physical, and microbiological factors that keep the birds' health in a homeostatic condition [5]. The diversified and very complex microbial intestinal population in poultry helps in the digestion process,

pathogenic bacteria elimination, and in developing the immunity of the host [6]. The gastrointestinal tract (GIT) microbiota can ferment the indigestible feed to essential amino acids and volatile fatty acids. In livestock and poultry industries, the use of antibiotics in sub-therapeutic doses contributes significantly to animal health, welfare, and overall production [7]. It has been observed that feeding sub-therapeutic concentrations of antibiotics to poultry has beneficial effects on the gut microbial ecology which promotes the growth rate of poultry [8]. A study also revealed that sub-therapeutic doses of some antibiotics have antioxidant, as well as immune and inflammatory mediated responses, on the gut tissues of chicken [9].

Contrary to the growth-promoting effects of low-concentration antibiotics, it was found that antibiotics as growth promoters can cause resistance in bacteria. This can directly or indirectly be transmitted to the human population through environmental contamination, causing illness and mortality [10]. In this regard, various feed supplements such as organic acids, prebiotics, probiotics, plants, oils, and nanoparticles have been tested with varying degrees of success. Non-antibiotic growth promoters function as antimicrobials, anti-inflammatory, and anti-stress and have shown positive effects on FCR, feed intake, digestion and immunity, as well as minerals and vitamin absorption [11, 12]. Plant-origin feed additives are called photogenic and include oils, herbs, and botanical plants, such as ginger, curcumin, and pepper that work as immune boosters, anti-oxidation agents, and anti-microbial agents [13]. The other feed additives are probiotics which include yeast species, bacteria or spores of bacilli, and prebiotics (oligosaccharide nature) that cannot be digested by host enzymes, although beneficial micro-flora in the host gut can digest them to meet their nutritional demands [13, 14]. Nanoparticles have been studied as growth promoters in poultry birds with mixed beneficial and harmful effects, depending on their concentration and size [15, 16].

Sodium gluconate (SG) is a salt of gluconic acid which has prebiotic properties. It produces butyrate in the large intestine and can be a potential alternative source of antibiotic growth promoters. Numerous studies have revealed that gluconic acid and its salt (SG) enhance body growth and feed conversion efficiency in animals. The inclusion of SG (20gms/kg) and phytase (1000 U/kg) in the feed improves feed intake and causes average gain in the body weight of broilers [17]. Similarly, SG and phytase enhance magnesium, zinc, calcium, and phosphorus retention and mineralization of bones in chickens [18, 19]. SG shows significant effects on intestinal villus morphometry and volatile fatty acids in the duodenum and jejunum [20]. The role of antibiotics in enhancing the growth of animals remains obscure. Although, they are seemingly more associated with symbiotic microflora of the colon. Some antibiotics are not absorbed totally in the small intestine; rather, they encounter gut microbiota in the large bowl which reduces the competition for nutrients and lessens the number of toxic metabolites in the large intestine [21, 22].

The growth-promoting effects of bacitracin and virginiamycin were studied in broilers. They significantly increased the body weight, feed conversion ratio (FCR), and height of villus after 7 weeks of age [23]. More recent research has revealed that bacitracin antibiotic at a dose rate of 55mg/kg with mash starter ration changes the structure of intestinal symbiotic

bacteria, promoting the growth of broilers by 11.08% on the 14th day and 20.13% on the 28th day of trial, as compared with the control group [24]. However, despite the effective role of antibiotics in growth stimulation, promoting FCR, and reducing health issues in animals, the issue of antibiotic resistance against zoonotic microbes was identified in many studies [25].

SG is a fermentation product of gluconic acid oxidation, has chelating and prebiotic properties, and has been used as an alternative to antibiotics. It improves the production of short-chain fatty acids, as well as increases FCR and body weight upon inclusion in feed as an additive in broiler chickens. Along with phytase 1000U/kg, SG 20gms/kg as a feed additive has proved to be a useful growth promoter in broilers [17]. Similarly, feed supplemented with SG at 2% along with 750U/kg microbial phytase significantly improves mineral retention and mineral deposition in chicken bones [18]. The morphometry of the duodenum and jejunum, as well as the concentration of propionate and total short-chain fatty acids and lactate, is boosted by the supplementation of sodium gluconate, potassium diformate, and mannan oligosaccharides in the feed [26, 27]. Gluconic acid is an organic acid. Hence, it can be metabolized in the gizzard. Consequently, its salt can reach the small intestine where it has progressive effects. The gluconic acid (0.5g/kg feed supplement) and quercetin promote the production of butyrate which quickens the apoptosis process and hence, anti-tumor properties in the large intestine [17, 28]. It is evident from the above discussion that SG has multi-faceted beneficial effects on intestinal morphology, micro-flora of GIT, and also prevents the colonization of pathogenic microbes in the alimentary tract. So, this may be a better alternative to antibiotics used for enhancing the growth of broiler chickens.

This study investigated the effects of SG on body weight, viscera weights, FCR, and blood metabolites including serum albumin, glucose, total protein, ALT, AST, triglycerides, cholesterol, and uric acid in broiler chicken. It was designed to use SG as a feed supplement for promoting the growth of broiler chicken. SG is a salt of gluconic acid, which has prebiotic effects and growth-promoting properties and may be used as a substitute for antibiotics. For this purpose, four (04) concentrations of SG were prepared including control, SG 3.5%, SG 4.5%, and SG 5.5%, and supplemented in the feed of chicks. Their overall body weight was measured in every trial to check any beneficial effects of supplements on their overall growth patterns using different testing concentrations and control groups.

2. MATERIALS AND METHODS

2.1. Research Area, Study Animals, and Source of Samples

In the current study, a total of 100 one-day-old broiler chicks were purchased from a hatchery and kept at an experimental farm at the Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The chicks were divided into four (4) groups with 25 birds in each group. Further, each group was divided into four (4) replicates. Afterwards, four (04) treatments of SG designated as control, SG 3.5%, SG 4.5%, and SG 5.5% were prepared, mixed with ration, and fed to birds according to [23]. The grouping was as follows:

Group 1. This group was the control group. The birds in this group were not supplemented with SG for 35 days.

Group 2. The birds in this group were supplemented with SG treatment designated as SG 3.5% for 35 days.

Group 3. The birds in this group were supplemented with SG treatment designated as SG 4.5% for 35 days.

Group 4. The birds in this group were supplemented with SG treatment designated as SG 5.5% for 35 days.

2.2. Standard Protocols Regarding Management

Ad-libitum feed and water were provided to birds throughout the experimental duration and the standard vaccination protocol was also followed. The temperature provided to birds during the first week was 35ºC, while the relative humidity was 65±5%. All the birds were vaccinated for Newcastle disease on day 4 and then on day 21. The vaccine for Gumboro disease was given on day 7 and then on day 24 at the experimental farm. Mortality and health-related issues were also observed daily. The total length of the trial was 35 days. At the end of the trial, 8 birds from each one of 4 groups (control, SG 3.5%, SG 4.5%, and SG 5.5%) or 2 birds from each group replicates (A, B, C, D replicates/group) were selected randomly and slaughtered.

2.3. Growth Performance

Body weight of one-day-old broiler chicks at the experimental farm of the Physiology Department was measured before feeding by using digital weighing balance. Subsequently, it was noted on a weekly basis for 35 days. To calculate the weekly weight gain, initial weights were subtracted from final weights. Moreover, to calculate the average weekly weight gain, the gained weight was divided by the total number of birds.

2.4. Feed Conversion Ratio (FCR)

Feed intake by each group was recorded daily. Keeping in view the feed consumed, FCR was calculated by dividing the total amount of feed intake with average weight gain. The average weekly feed intake was also calculated from the recorded data on a weekly basis.

2.5. Samples Collection and Processing

On the 36th day, two (02) birds from each replicate were randomly selected and slaughtered. The viscera were also collected to measure their weight and length. Blood was collected in test tubes, kept for four (04) hours at room temperature, and centrifuged at 4000 rpm for 20 min. Serum was collected from the top of the centrifuge tubes and stored at -40°C for biochemical tests.

2.6. Organ Weights

Internal organs including the heart, liver, pancreas, gizzard, proventriculus, bursa, intestines, and caeca were weighed by using digital weighing balance. Further, the length of the caecum and small intestine was measured with the measuring tape for each treatment group.

2.7. Selected Serum Blood Metabolites

Serum was collected after the end of the experiment in an aseptic process for the biochemical analysis of each group. Biochemical tests were performed for serum glucose, cholesterol, albumin, total protein, triglycerides, and uric acid. Liver enzymes, such as aspartate aminotransferase and alanine aminotransferase were also assessed. For preparing serological profiles, commercial kits were used. Further, a spectrophotometer was used for reading reactions. Serum samples were thawed before proceeding with biochemical tests.

2.7.1. Albumin Assay. DiaSys Albumin FS (REF:1 0220 99 10 021) kit was used for albumin estimation. Albumin is an important binding and transport protein for various substances in plasma and the main contributor to plasma osmotic pressure. In the presence of bromocresol green at a slightly acidic pH, serum albumin produces a color change of the indicator from yellowish green to greenish blue. For testing, 2µl of each sample was loaded into a 96-well microplate and 2µl of distilled water was added as blank. Afterwards, 200µl of reagent was added into all wells including the blank, mixed, and incubated wells for approximately 10 min. Then, absorbance was read at 546nm wavelength against reagent blank within 60 min using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA). Concentration was calculated by using the respective formula.

2.7.2. Total Protein Assay. DiaSys Total Protein FS (REF:1 2311 99 10 021) kit was used for the estimation of total protein. Proteins form a violet-blue color complex with copper ions in an alkaline solution. For testing, 4µl of each sample was loaded in a 96-well microplate and 4µl of distilled water was added as blank. Afterwards, 200µl of Reagent 1 was added in all wells and mixed and absorbance A1 was read after 1-5 min at 20-25ºC/37ºC at a wavelength of 546nm. Then, 50µl of Reagent 2 was added in all wells, mixed, and incubated for 5 minutes at 20-25ºC/37ºC. Absorbance A2 was read at a wavelength of 546nm using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA).

2.7.3. Cholesterol Assay. DiaSys Cholesterol FS (REF:1 1300 99 10 021) kit was used for the estimation of cholesterol. For determining cholesterol after enzymatic hydrolysis and oxidation, the colorimetric indicator is quinonimine. It is generated from 4-aminoantipyrine and phenol by using hydrogen peroxide under the catalytic action of peroxidase. For testing, 20µl of each sample was added to the Eppendorf tube and 50µl of a precipitation reagent was added to it as well. It was incubated for 15 min at room temperature and then centrifuged for 20 min at 2500g. Within 2 hours of centrifugation, 10µl of clear supernatant was transferred to the reaction solution for determining cholesterol. Afterwards, 10µl of each sample was loaded into a 96-well microplate and 10µl of the standard was added as well. Further, 100µl of cholesterol reagent was added to all wells including the blank, mixed, and incubated wells for 10 min at room temperature or 5 min at 37ºC. Absorbance was observed at a wavelength of 546nm against the reagent blank value within 45 min using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA). Concentration was calculated by the respective formula.

2.7.4. Triglycerides Assay. DiaSys Triglycerides FS (REF:15710 99 10 021) kit was used for the estimation of triglycerides. Triglycerides are esters of cholesterol with three fatty acids and are the most abundant naturally occurring lipids. Triglycerides are determined after enzymatic splitting with lipoprotein lipase. The indicator is quinonimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase. For testing, 2µl of each sample was loaded into a 96-well microplate and 2µl of distilled water was added as blank. Afterwards, 2µl of the standard was added as well. Then, reagent was added, mixed, and incubated for 20 minutes at 20-25ºC or 10 minutes at

37ºC. Absorbance was read at a wavelength of 546nm against the blank within 60 min using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA). Concentration was calculated by using the respective formula.

2.7.5. Uric Acid. DiaSys Uric Acid FS TBHBA (REF:1 3021 99 10 021) kit was used for the estimation of uric acid. Uric acid is oxidized to allantoin by uricase. The generated hydrogen peroxide reacts with 4-amino antipyrine which reacts with 4-amino antipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid to quinonimine. For testing, 4µl of each sample was loaded into a 96-well microplate and 4µl of distilled water was added as blank. Afterwards, 4µl of the standard was added as well. Then, 200µl of Reagent 1 was added to all wells including blank, mixed, and incubated for 5 min. Furthermore, Reagent 2 of 50µl was added, mixed, and incubated for 30 min at 20-25ºC or 10 min at 37ºC. Absorbance was read at a wavelength of 546nm against the reagent blank within 60 min using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA). Concentration was calculated by using the respective formula.

2.7.6. Aspartate Aminotransferase (ASAT). DiaSys ASAT (GOT) FS (IFCC mod.) (REF: 1 2601 99 10 021) kit was used for the estimation of ASAT. Aspartate aminotransferase is the most important representative of a group of enzymes, that is, aminotransferases or transaminases which catalyze the conversion of alpha-keto acids into amino acids by transferring amino groups. The addition of pyridoxal-5-phosphate (P-5-P), recommended by IFCC stabilizes the activity of transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P. For testing, 10µl of each sample was added into a 96-well microplate. Afterwards, 100µl of Reagent 1 was loaded into all wells, mixed, and incubated for 5 min. Then, 25µl of Reagent 2 was added to all wells and similarly mixed. Absorbance was read at a wavelength of 365nm after 1 min and a stopwatch was started at that point in time for further reading. Absorbance was read again after 1, 2, and 3 mins, thereafter using an Epoch microplate spectrophotometer (Biotechnology Medical Services MA 02052, USA).

2.8. Statistical Analysis

The obtained data were assessed statistically by using the analysis of variance (ANOVA) and presented as means ± SEM. Group differences were compared by using post hoc Tukey's test with a significance level of $p < 0.05$.

3. RESULTS

3.1. Effects of Sodium Gluconate (SG) on Growth Performance

Results were obtained for growth parameters, such as body weight gain, FCR, feed intake, organ weight and length, and selected serum metabolites by using standard procedures or kits. The data were recorded and statistically evaluated, means were compared by one-way analysis of variance (ANOVA), and significant results were further analyzed by using Tukey's post hoc test.

3.2. Body Weight Response to SG Supplementation

Table 1 presents the mean body weight of both the control and SG supplemented groups obtained using ANOVA. The results show that in week 4, growth of control group chicks was significantly higher than SG 4.5% supplemented group. Moreover, in week 5, growth of control group chicks was statistically higher than

both SG 4.5% and SG 5.5% supplemented groups.

Table 1. Effects of Different SG Concentrations on Body Weight of Broilers

Body Weight (G)	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
W1	153.50±3.07	150.00±4.18	152.75±1.70	152.25±2.66	0.862
W2	456.00±34.22	463.00±40.48	408.50±27.82	435.25±38.01	0.704
W3	866.50±14.08	827.50±45.91	848.25±27.45	833.50±30.33	0.822
W4	1141.75±17.8^{a}	1018.50±37.34^{ab}	992.00±24.74^{b}	1031.75±47.41^{ab}	0.040
W5	1564.25±58.6^{a}	1401.75±28.26^{ab}	1320.25±62.3^{b}	1305.00±48.88^{b}	0.014

Data are presented as means ± SEM. $^{a-b}$ Superscripts show a significant group difference at $p < 0.05$.

Table 2. Effects of Different SG Concentrations on Body Weight Gain of Broilers

Body Weight Gain (G)	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
W1	107.75±2.66	117.25±7.44	108.25±2.10	106.50±2.22	0.303
W2	303.00±32.36	294.75±35.73	245.75±30.65	283.00±37.26	0.658
W3	410.50±24.56	364.75±45.33	440.25±36.96	398.00±60.63	0.684
W4	275.00±10.4^{a}	190.75±13.26^{ab}	143.50±32.23^{b}	198.00±33.89^{ab}	0.020
W5	422.50±48.7	391.67±21.06	328.50±63.93	273.50±15.39	0.137

Data are presented as means ± SEM. $^{a-b}$ Superscripts show a significant group difference at $p < 0.05$.

3.3. Body Weight Gain in Response to SG Addition in Feed

Table 2 presents the mean body weight of both the control and supplemented SG groups obtained using ANOVA. The results show that in week 4, the growth of control group chicks was significantly higher as compared to SG 4.5% treated group. Hence, overall body weight gain was not affected by any of the SG treatment groups as compared to the control group.

3.4. Effects of SG on Feed Intake in Broilers

Data on feed intake was collected as grams of feed intake per week for both the control and SG supplemented groups. The collected data were evaluated by using one-way ANOVA and presented as means ± SEM, as shown in Table 3. The results show that there were no statistical differences ($p < 0.05$) between the control and SG supplemented groups in any week of trial.

Table 3. Effects of Different SG Concentrations on Feed Intake in Broilers

Feed Intake (G)	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
W1	155.25±4.89	170.75±6.94	164.75±3.35	163.25±5.66	0.287
W2	366.00±10.67	423.25±19.42	392.00±12.41	373.75±18.30	0.097
W3	568.25±18.11	545.25±26.52	552.50±13.38	545.00±14.98	0.803

Feed Intake (G)	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
W4	844.50±15.52	869.75±38.33	771.00±16.11	773.25±38.76	0.079
W5	690±3.07	707.21±37.27	759.27±9.86	781.4±7.71	0.167

Data are presented as means ± SEM.

Table 4. Effects of SG Inclusion on FCR in Broilers

FCR	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
W1	1.44±0.02[b]	1.64±0.02[a]	1.52±0.01[ab]	1.54±0.07[ab]	0.028
W2	1.25±0.13	1.50±0.19	1.67±0.21	1.38±0.14	0.390
W3	1.40±0.10	1.55±0.16	1.28±0.11	1.47±0.22	0.664
W4	3.08±0.07	4.62±0.30	6.37±1.57	4.24±0.72	0.118
W5	1.68±0.14[b]	1.75±0.21[b]	2.50±0.33[ab]	2.88±0.14[a]	0.007

Data are presented as mean ± SEM. [a-b] Superscripts show a significant group difference at $p < 0.05$.

3.5. Effects of SG on FCR

The FCR data for SG was analyzed by using ANOVA, as shown in Table 4. The data revealed a significantly higher FCR in week 1 in SG 3.5% supplemented group in comparison to the control, SG 4.5%, and SG 5.5% supplemented groups. Further, the lowest FCR ($p < 0.05$) was manifested in the control group. Later, in week 5, a highly significant FCR was found in SG 5.5% supplemented group, as compared to all other groups. Thus, SG improved FCR at concentrations of 3.5% and 5.5% in week 1 and week 5, respectively.

3.6. Effects of Different SG Concentrations on Viscera Weights and Intestinal Lengths of Broilers

Organ's weight of all 4 groups, namely control, SG 3.5%, SG 4.5%, and SG 5.5% were measured. Further, the lengths of the intestines and caeca were also measured. All data were statistically analyzed through one-way ANOVA. Using Tukey's post hoc test, significant mean group differences were calculated, as presented in Table 5. The statistical results show that the weights of the liver, heart, gizzard, proventriculus, spleen, pancreas, small intestine, and caecum remained unaffected by any concentration of SG. Only caecum length was increased ($p < 0.05$) in the birds supplemented with 5.5% SG, as compared with 3.5% SG supplemented birds. The length of the small intestine remained unaffected ($p < 0.05$) with SG supplementation.

3.7. Blood Metabolites Status in Response to SG Supplementation

At the end of the trial for SG as dietary supplementation, serum was collected (after slaughtering the birds) and tested for blood glucose, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, triglycerides, cholesterol, and uric acid by using commercial kits. A significantly higher value for cholesterol was observed in control and SG 3.5% supplemented groups than in SG 4.5% and SG 5.5% groups. Uric acid was also higher ($p < 0.05$) in SG 3.5% as compared to both SG 4.5% and 5.5% groups. All the remaining blood metabolites remained unaffected ($p < 0.05$) by the dietary SG supplementation.

Table 5. Effects of Different SG Concentrations on Viscera Weights and Intestinal Lengths of Broilers

Organs Weight G)	Control	3.5% SG	4.5% SG	5.5% SG	*p*-value
Liver	41.63±2.10	37.47±1.99	37.13±2.44	40.55±1.24	0.308
Heart	10.01±0.67	10.14±0.87	9.94±0.51	10.65±0.40	0.856
Gizzard	35.43±1.28	32.81±1.41	33.24±0.89	34.28±1.52	0.496
Proventriculus	7.18±0.32	6.80±0.26	7.59±0.34	7.74±0.27	0.132
Spleen	1.72±0.22	1.21±0 .08	1.37±0.07	1.33±0.07	0.056
Bursa	2.98±0.31	2.61±0.24	3.34±0.32	2.84±0.53	0.553
Pancreas	4.23±0.24	3.59±0.22	3.95±0.22	4.03±0.26	0.301
Small intestine	48.52±1.87	44.67±1.59	45.71±2.44	44.16±2.30	0.465
Caecum	2.44±0.18	2.33±0.10	2.09±0.18	2.57±0.19	0.243
Caecal tonsil	0.59±0.06	0.51±0.03	0.59±0.07	0.56±0.03	0.634
Organs Length (cms)					
Small intestine	67.69±1.40	67.73±2.28	64.83±1.80	64.75±1.38	0.443
Caecum	5.70±0.24^{ab}	5.34±0.17^{b}	6.09±0.23^{ab}	6.43±0.25^{a}	0.012

Data are presented as means ± SEM. $^{a-b}$ Superscripts show a significant group difference at $p < 0.05$.

Table 6. Effects of Different SG Concentrations on Selected Serum Metabolites of Broilers

Blood Biochemistry	Control	SG 3.5%	SG 4.5%	SG 5.5%	*p*-value
Glucose (mg/dL)	162.73±6.64	176.56±8.66	171.76±6.87	168.00±10.62	0.691
Total Protein (g/dL)	2.91±0.44	3.33±0.13	3.83±0.51	3.43±0.17	0.352
Albumin (g/dL)	2.31±0.11	2.46±0.14	2.34±0.08	2.48±0.07	0.572
ALT (U/L)	7.84±4.47	11.59±6.76	5.42±3.51	2.88±1.06	0.563
AST (U/L)	20.48±I.40	17.87±0.96	16.88±1.87	17.50±1.03	0.277
Triglycerides (mg/dL)	95.14±2.93	95.63±10.27	161.31±51.46	138.39±33.28	0.361
Cholesterol (mg/dL)	98.73±10.54^{a}	99.91±4.67^{a}	77.86±2.70^{b}	79.27±3.82^{b}	0.023
Uric acid (mg/dL)	4.26±0.29^{ab}	5.06±0.82^{a}	3.48±0.22^{b}	3.34±0.17^{b}	0.048

Data are presented as mean ± SEM. $^{a-b}$ Superscripts show a significant group difference at $p < 0.05$.

4. DISCUSSION

The poultry gut is home to varied microbiota including bacteria, protozoa, and fungi which live in symbiotic association within the host gut. This microbiota keeps the gut healthy in homeostasis due to certain microbiological, physiological, and physical factors. The complex microbial intestinal population has a beneficial role in boosting the immune system, improving digestion, and eliminating pathogenic bacteria in the host. Any imbalance in this homeostatic condition may lead to poor feed intake, stunted growth, or mortality of birds [29]. Sub-therapeutic antibiotic supplementation has been used with significantly beneficial effects on the gut microbial population, immunological responses, antioxidant effects, and growth of the host [9].

In the current study, a total of 100, one-day-old, broilers were divided into four (04) groups. An ad-libitum basal diet was given to Group 1 without SG supplementation. While, groups 2, 3, and 4 were given ad-libitum basal diet with SG supplementation at 3.5%, 4.5%, and 5.5% mixed with ration, respectively. Birds were weighed on the day of arrival at the experimental farm and before feeding by using digital weighing balance and then on a weekly basis for 35 days. FCR was calculated based on average weekly feed intake divided by average weight gain in grams. On the 36th day, two (02) birds from each replicate were randomly selected and slaughtered and their organs (heart, liver, pancreas, spleen, gizzard, proventriculus, small intestine, and caecum) were collected for weight and length measurements. It was determined that the concentrations of SG did not improve body weight ($p < 0.05$) in broilers. Hence, overall body weight was not affected by any treatment group as compared to the control group. The results are similar to [30] where no significant body weight gain in any treatment groups (0.0%, 0.1%, 0.2%, 0.3%, 0.4%) was noticed in broilers from 3 to 6 weeks of age. The current study revealed that in week 1 of the trial, a significantly higher FCR was observed in SG 3.5% group in comparison to control. Later, in week 5, a significant FCR ($p < 0.05$) was determined in SG 5.5% group as compared to the control and SG 4.5% groups. Similarly, another study [31] reported that SG 3.35% supplementation significantly improved FCR.

Organs are measured and weighed to ascertain any deviation from normal growth. The statistical results showed that the weight of the liver, heart, gizzard, proventriculus, spleen, pancreas, small intestine, and caecum remained unaffected by any concentration of SG. Only the caecum length increased ($p < 0.05$) in birds supplemented with SG 5.5%, as compared with SG 3.5% supplemented birds. A similar study [32] concluded that there was no significant increase in the weight of broilers' heart, liver, and gizzard in response to organic acid supplementation. In the current study, the length of the small intestine remained unaffected ($p < 0.05$) with SG supplementation.

The serum is collected and analyzed through various available kits to assess the health status of animals. For this purpose, blood metabolites such as glucose, cholesterol, triglycerides, total protein, albumin, AST, ALT, and uric acid were assessed in this research. In this trial, a significantly higher value of cholesterol was observed in the control and SG 3.5% groups, than in SG 4.5% and 5.5% groups. On the contrary, cholesterol was lowered by SG 4.5% and 5.5% supplementation. Uric acid was also higher ($p < 0.05$) in SG 3.5% group as compared to both SG 4.5% and 5.5% groups. All the remaining blood

metabolites, that is, glucose, total protein, albumin, AST, ALT, and triglycerides remained unaffected *($p < 0.05$)* by the dietary SG supplementation. A study [33] reported an increase in total protein as organic acids were supplemented in a feed of Cobb straight-run commercial broilers, which is contradictory to the results obtained about total serum protein. These results revealed that supplementing SG in broiler ration in concentrations of 3.5%, 4.5%, and 5.5% didn't enhance the growth parameters of chickens except that the FCR increased at a concentration of 5.5%, whereas cholesterol decreased at the same concentration. On the other hand, uric acid increased at 3.5% concentration. This trial established that SG has no significant effect in promoting the overall growth of broilers, although it does show some beneficial effects in terms of FCR and cholesterol reduction. More extensive study is suggested to investigate the effects of SG at different concentrations, feed formulation, and other experimental animals. Broilers are raised for meat and require growth promoters for optimal development. Hence, it is hypothesized that SG supplementation can improve growth performance in broilers.

4.1. Conclusion

In the current study, no significant effects were observed on body weight, body weight gain, feed intake, and FCR with SG in the diet during all the weeks, except in the 1st week where a significantly higher FCR was observed in SG 3.5% group, in comparison to the control. Similarly, a significant FCR was determined in week 5 in SG 5.5% group, as compared to the control and SG 4.5% groups. In the current study, viscera weights remained unaffected by SG supplementation and the small intestine length was also not affected. Only the caecum length increased ($p < 0.05$) in the birds supplemented with SG 5.5% in comparison with SG 3.5% supplemented birds. Moreover, all selected blood metabolites remained unaffected by the dietary SG supplementation, except cholesterol and uric acid. Both in SG 4.5% and SG 5.5% groups, cholesterol concentration was lesser in comparison to control and SG 3.5% groups. Uric acid was also higher ($p < 0.05$) in SG 3.5% as compared to both SG 4.5% and 5.5% groups.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

ETHICAL APPROVAL

The approval of all methods used in this research was given by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan Vide letter no. DR/523 Dated: 23-08-2022.

REFERENCES

1. Akhtar P, Farooq U, Khan MS. Poultry production status, issues, and future prospect. In: Khan IA, Khan MS, eds. *Developing Sustainable Agriculture in Pakistan*. CRC Press, 2018:525–41.

2. Khan M, Maqsood R, Rashid HB, et al. Seroprevalence of avian influenza H9N2 virus in backyard poultry of district Zhob Pakistan. *Pak J Zool.* 2022:56(3):1141–1146.

https://dx.doi.org/10.17582/journal.pjz/20220706050740

3. Khan M, Chaudhry M, Fatima Z, et al. Effect of avian influenza H9N2 subtype virus infection on backyard poultry production. *Sci Lett*. 2021;9(1):19–23. https://doi.org/10.47262/SL/9.1.132021005

4. Chaudhry M, Webby R, Swayne D, et al. Avian influenza at animal-human interface: One-health challenge in live poultry retail stalls of Chakwal, Pakistan. *Influenza Other Respir Viruses*. 2020;14(3):257–265. https://doi.org/10.1111/irv.12718

5. Polansky O, Sekelova Z, Faldynova M, Sebkova A, Sisak F, Rychlik I. Important metabolic pathways and biological processes expressed by chicken cecal microbiota. *Appl Environ Microbiol*. 2016;82(5):1569–1576. https://doi.org/10.1128/AEM.03473-15

6. Shang Y, Kumar S, Oakley B, Kim WK. Chicken gut microbiota: importance and detection technology. *Front Veter Sci*. 2018;5:e254. https://doi.org/10.3389/fvets.2018.00254

7. Broom LJ. The sub-inhibitory theory for antibiotic growth promoters. *Poult Sci*. 2017;96(9):3104–3108. https://doi.org/10. 3382/ps/pex114

8. Costa MC, Bessegatto JA, Alfieri AA, Weese JS, Filho JA, Oba A. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. *PLoS One*. 2017;12(2):e0171642. https://doi.org/10.1371/journal.pone.0171642

9. Kabploy K, Bunyapraphatsara N, Morales NP, Paraksa N. Effect of antibiotic growth promoters on anti-oxidative and anti-inflammatory activities in broiler chickens. *Thai J Veter Med*. 2016;46(1):89–95. https://doi.org/10.56808/2985-1130.2722

10. Rahman MRT, Fliss I, Biron E. Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. *Antibiotics*. 2022;11(6):e766. https://doi.org/10.3390/antibiotics11060766

11. Sheiha AM, Abdelnour SA, Abd El-Hack ME, et al. Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. *Animals*. 2020;10(3):e430. https://doi.org/10.3390 /ani10030430

12. Moreira LM, Sousa LS, Guamán CAG, et al. Effects of cold stress on physiologic metabolism in the initial phase and performance of broiler rearing. *J Therm Biol*. 2024;119:e103773. https://doi.org/10.1016/j.jtherbio.2023.103773

13. Abdelnour S, El-Saadony M, Saghir S, et al. Mitigating negative impacts of heat stress in growing rabbits via dietary prodigiosin supplementation. *Livest Sci*. 2020;240:e104220. https://doi.org/10.1016/j.livsci.2020.104220

14. Pirgozliev V, Rose SP, Ivanova S. Feed additives in poultry nutrition. *Bulgar J Agricul Sci*. 2019;25(1):8–11.

15. Joshua PP, Valli C, Balakrishnan V. Effect of in ovo supplementation of nano forms of zinc, copper, and

selenium on post-hatch performance of broiler chicken. *Veter World*. 2016;9(3):287–294. https://doi.org/10.14202%2Fvetworld.2016.287-294

16. Sharif M, Rahman MA, Ahmed B, Abbas RZ, Hassan FU. Copper nanoparticles as growth promoter, antioxidant and anti-bacterial agents in poultry nutrition: prospects and future implications. *Biol Trace Elem Res*. 2021;199(10):3825–3836. https://doi.org/10.1007/s12011-020-02485-1

17. Guo Y, Shi Y, Li F, Chen J, Zhen C, Hao Z. Effects of sodium gluconate and phytase on performance and bone characteristics in broiler chickers. *Anim Feed Sci Technol*. 2009;150(3-4):270–282. https://doi.org/10.1016/j.anifeedsci.2008.09.006

18. Günal M. The effects of sodium gluconate and microbial phytase on performance and mineral utilisation in broiler chicks. *Anim Prod Sci*. 2013;53(4):316–321. https://doi.org/10.1071/AN12221

19. Jacob RH, Afify AS, Shanab SM, Shalaby EA. Chelated amino acids: biomass sources, preparation, properties, and biological activities. *Biomass Conv Bioref*. 2024;14(3):2907–2921. https://doi.org/ 10.1007/s13399-022-02333-3

20. Shahir MH, Moradi S, Afsarian O, Esmaeilipour O. Effects of cereal type, enzyme and sodium butyrate addition on growth performance, carcass traits and intestinal morphology of broilers. *Braz J Poult Sci*. 2013;15:181–199. https://doi.org/10.1590/S1516-635X2013000300003

21. Anderson DB, McCracken VJ, Aminov RI, et al. *Gut Microbiology and Growth-Promoting Antibiotics In Swine*. Pig News and Information; 1999

22. Sampath V, Gao S, Park JH, Kim IH. Exogenous phytase improves growth performance, nutrient retention, tibia mineralization, and breast meat quality in ross-308 broilers. *Agriculture*. 2023;13(10):e1906. https://doi.org/10.3390/agriculture13101906

23. Miles R, Butcher G, Henry P, Littell R. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poul Sci*. 2006;85(3):476–485. https://doi.org/10.1093/ps/85.3.476

24. Li J, Xiao Y, Fan Q, et al. Dietary bacitracin methylene disalicylate improves growth performance by mediating the gut microbiota in broilers. *Antibiotics*. 2022;11(6):e818. https://doi.org/10.3390/antibiotics11060818

25. Braykov NP, Eisenberg JN, Grossman M, et al. Antibiotic resistance in animal and environmental samples associated with small-scale poultry farming in northwestern Ecuador. *Msphere*. 2016;1(1): e00021-15. https://doi.org/10.1128/msphere.00021-15

26. Junqueira OM, Duarte KF, Garcia EA, Sgavioli S, Praes MF, Molino AD. Effect of sodium gluconate on performance, carcass characteristics, and intestinal morphometry of broilers from 22 to 42 days of age. *Acta Sci Anim Sci*. 2012;34:35–40. https://doi.org/10.4025/actascianimsci.v34i1.11211

27. Liang Z, Yan Y, Zhang W, et al. Review of glucose oxidase as a feed additive: production, engineering, applications, growth-promoting mechanisms, and outlook. *Crit Rev Biotechnol*. 2023;43(5):698–715. https://doi.org/10. 1080/07388551.2022.2057275

28. Yegani M, Korver D. Factors affecting intestinal health in poultry. *Poult Sci*. 2008;87(10):2052–2063. https://doi.org/ 10.3382/ps.2008-00091

29. Dowarah R. The role of poultry meat and eggs in human nutrition. *Poult Line*. 2013:31–33.

30. Den Besten G, Van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*. 2013;54(9):2325–2340. https://doi.org/10. 1194/jlr.R036012

31. Rafacz-Livingston K, Parsons C, Jungk R. The effects of various organic acids on phytate phosphorus utilization in chicks. *Poult Sci*. 2005;84(9):1356–1362. https://doi.org/10.1093/ps/84.9.1356

32. Kamal AM, Ragaa NM. Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. *Nature Sci*. 2014;12(2):38–45.

33. Adil S, Banday M, Bhat G, Qureshi S, Wani S. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. *Livest Res Rural Develop*. 2011;23(1):1–8.