



## RESEARCH ARTICLE

### Evaluation of Potential Effects of Locally Isolated *Streptomyces* Species as Growth Promoter in Commercial Broilers

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#### ABSTRACT

In this study, local soil samples were collected for the isolation of *Streptomyces*. Molecular characterization and gene sequencing of 16S rRNA was done for identification of *Streptomyces* species which has been used in this study as a probiotic in feed. To evaluate their potential and efficacy as a probiotic in-Vivo, day old broiler chicks (total 140) were purchased from local hatchery and acclimatized for seven days and then divided in 07 experimental groups having 20 birds in each entitled as Group A, B, C, D, E, F and G, where group A served as control and all other were supplemented with feed having *streptomyces* as a supplement (probiotics). Various species of the streptomyces including *Streptomyces globosporus*, *Streptomyces toxitricin*, *Streptomyces* WSN2, *Streptomyces rochie* and *Streptomyces fimbritus* to group B, C, D and E, respectively. Group G was treated with commercially available growth promoter (Greig® BS). Parameters including physiological (feed intake, weight gain, organ weight) and immunological parameters (carbon clearance assay, Lymphoproliferative response to avian tuberculin) of the birds were evaluated, thoroughly. The data thus obtained was subjected to the M-stat C software for statistical analysis. Significant additive effect on the broiler's performance in all treatment groups those received probiotic was observed as compared to the control in terms of physiological and immunological parameters. The birds supplemented with *Streptomyces globosporus* revealed the better growth and improved feed intake as compared to all other tested species. Hence, it has been concluded that these local isolates of the streptomyces have potential to be promoted as the probiotic in the poultry feed which will be more economical and profitable to the farmers.

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#### INTRODUCTION

Microorganisms are present all around the environment and responsible for many diseases and has been characterized as bacteria, virus, fungi, and some parasites. Mostly microorganisms considered to be the major source of viral and bacterial infections in human as well as in animals, most prominently in poultry birds (Rashad *et al.*, 2015). The secondary metabolites produced from different microorganisms exhibiting antibacterial, antiviral, antiprotozoal, antifungal and antitumor activities are called antibiotics. The antibiotics are most interesting and important group of natural products. Antibiotics are defined as secondary metabolites which facilitate

regulation of different physiological processes including growth, replications and responding actions in prokaryotic and eukaryotic cells in minimum concentrations (Lee *et al.*, 2019). While the living microorganisms including the bacteria and yeast are called probiotics and most commonly used species are *Lactobacillus* and *Bifidobacterium* due to their potential immune-stimulatory and more feed digestibility effects and being used to control diseases in birds and animals (Khater *et al.*, 2020; Bhogju *et al.*, 2021; Coniglio *et al.*, 2023).

In poultry industry, the most important concern is to control the infectious enteric diseases because these diseases result in higher mortality, lower production and posing a serious public health concern due to the

contamination of meat and eggs. To control infectious diseases, different types of antibiotics has been used since long those are responsible for antibiotic resistance in poultry as well as in humans (Trafalska and Grzybowska 2004; Gul and Alsayeqh, 2022; Gul and Alsayeqh, 2023). Another irrational use of antibiotics in poultry industry is as growth promoter alongwith treatment of infectious however, such irrational use of antibiotics has been responsible for the creation of superbugs/antibiotic resistance microbes. Use of antibiotic-growth-promoters in animals affect the human health either directly by antibiotic residues in eggs and meat or indirectly by the spread of antibiotic resistant determinants to human pathogens (Gonzalez and Angeles, 2017; Gul and Alsayeqh, 2022; Coniglio *et al.*, 2023). In addition, consumption of antibiotics as growth promoters also has the bactericidal activity against beneficial bacteria residing in the gut. It has been documented that the frequency of resistant determinants in commensal bacteria is a major cause of development of resistance in pathogenic bacteria (Sarmah *et al.*, 2006; Gul and Alsayeqh, 2023; Coniglio *et al.*, 2023).

*Streptomyces* species including *Streptomyces griseus* and *Streptomyces coelicolor* are being used for the industrial production of antibiotic, however, the most widely used species is *Streptomyces coelicolor* which has been recognized as a workhouse for genetic and a model for antibiotic development and production (Willems *et al.*, 2011). Various methods are being used for the detection and isolation of antibiotics from *Streptomyces* species (Harir *et al.*, 2018). These antibiotics have made significant contribution in the preventive and therapeutic options for infectious diseases, enhancement of productivity in animals, improvement of atmosphere and protection of human health (Nikaido, 2009).

*Streptomyces* species isolated from animal sources can efficiently adapt to the gastric environment of animals. It has been documented that that *Streptomyces* species isolated from chicken faeces have shown resistance at pH 2.3mg/ml pepsin, 1mg/ml pancreatin and 0.3% bile (Latha *et al.* 2015). Due to the spore forming capacity of *Streptomyces* species with high tolerance for bile acids, *streptomyces* are considered promising alternative bacteria for their use as probiotic than non-spore forming bacteria (Das *et al.*, 2010). It has been documented in previous researches that probiotic *Streptomyces* are able to regulate the microflora of aquatic environment and also play an important role in reducing ammonia level and increasing the heterotrophic bacteria in aquaculture (Devaraja *et al.*, 2002). The increased level of nitrite and ammonia in ponds or lakes causes the accumulation of microbes and decomposition of residual feed that adversely affect the health of aquatic organisms (Das *et al.*, 2006, 2010; Aftabuddin *et al.*, 2013).

Keeping in view, global trends in terms of antimicrobial resistance (AMR), there is a dire need to explore the alternatives to antibiotics that may assure the safety and economic viability in poultry production not only in developing countries but also in developed world. Hence the present project was designed to evaluate the beneficial effects of *Streptomyces* species, isolated from local soil samples, as a probiotic on growth and immune system of commercial broilers.

## MATERIALS AND METHODS

### Isolation and identification of streptomyces from soil

**samples:** Soil samples were collected from diverse habitats including rhizosphere of plants and agricultural soil located in Faisalabad, Pakistan. These soil samples were collected from different depths of the earth surface, ranging from layers just below the upper surface to 20cm deep in sterile plastic tubes and were properly labelled. The collected soil samples were dried in oven at 40-50°C for 2 hours, cooled to room temperature and stored at 4°C. After that 1gm of each soil sample was added to a conical flask containing 100 ml of sterile water. All flasks were kept in orbital shaker incubator at 30°C for 1-2 hours. These flasks were kept as source of stock cultures.

**Experimental plan:** For this project, forty-five days experiment trail was conducted at the experimental sheds of Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. Before the arrival of chicks, shed was thoroughly cleaned, disinfected and fumigated to avoid any contamination from the previous flock.

Day-old commercial broiler chicks were purchased from the local hatchery. Recommended requisites for commercial broiler poultry farming were followed and after acclimatization of 7 days, the birds were divided into 07 groups, 20 birds in each. Different *Streptomyces* species along with a commercially available probiotic were supplemented as treatment to these birds and one group was kept as control, that was given basal feed only. As mentioned in the Table 1. Liquid preparations of *Streptomyces* species (@ 1 x 10<sup>9</sup> cfu/ml/g of feed) were mixed with balanced formulated broiler feed, and was offered to the birds *ad libitum*. During this trial, all the birds were properly vaccinated against different infectious diseases as per standard protocol mentioned for broilers in Pakistan.

### Physiological and immunological parameters

**evaluation:** Different physiological parameters were recorded including Morbidity and Mortality, Body weight on weekly basis and feed intake etc. To find out the potential effects of treatments given to these birds on internal organs, 05 birds/group were euthanized humanely, at 15, 30 and 45<sup>th</sup> days of experiment. The absolute organ weight of Liver, kidney, intestines and heart were recorded.

To find out potential beneficial effects on immune system carbon clearance Assay (Sarker *et al.*, 2000), Lymphoproliferative response to avian tuberculin (Akhtar *et al.*, 2008) were recorded as per procedures mentioned and the weight of spleen and thymus were also recorded on 15, 30 and 45<sup>th</sup> days of experiment along with other organs.

**Statistical analysis:** The data thus obtained was subjected to ANOVA. Duncan multiple range tests were used for comparing means of different groups to determine the significant difference. M-STAT-C software package (Michigan State University, East Lansing, MI 48824, USA) was used and level of significance was kept at P≤0.05.

## RESULTS

The results obtained during this trial has been described below:

**Table 1:** Experimental plan

Groups	Number of Birds	Treatments
A	20	With no treatment (Control)
B	20	<i>Streptomyces Globiosporus</i>
C	20	<i>Streptomyces Toxytricin</i>
D	20	<i>Streptomyces WSN2</i>
E	20	<i>Streptomyces rochie</i>
F	20	<i>Streptomyces fimbritus</i>
G	20	Commercially available probiotics

**Table 2:** Absolute weights of immune organs (thymus and spleen) of birds supplemented with *Streptomyces* species.

Experimental Groups	Experimental Days		
	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day
<b>Thymus</b>			
A (Control)	0.75 ± 0.01 <sup>f</sup>	1.04 ± 0.36 <sup>f</sup>	1.50 ± 0.25 <sup>f</sup>
B	1.24 ± 0.03 <sup>a</sup>	2.35 ± 0.78 <sup>a</sup>	3.77 ± 0.28 <sup>a</sup>
C	1.13 ± 0.01 <sup>b</sup>	1.76 ± 0.45 <sup>b</sup>	2.20 ± 0.14 <sup>b</sup>
D	1.08 ± 0.02 <sup>ab</sup>	1.37 ± 0.38 <sup>ab</sup>	1.95 ± 0.74 <sup>ab</sup>
E	0.90 ± 0.01 <sup>bc</sup>	1.45 ± 0.28 <sup>bc</sup>	1.84 ± 0.89 <sup>bc</sup>
F	1.04 ± 0.77 <sup>cd</sup>	1.65 ± 0.95 <sup>cd</sup>	1.78 ± 0.54 <sup>cd</sup>
G	0.90 ± 0.03 <sup>de</sup>	1.29 ± 0.27 <sup>de</sup>	1.14 ± 0.54 <sup>de</sup>
<b>Spleen</b>			
A	0.87 ± 0.05 <sup>f</sup>	1.13 ± 0.05 <sup>f</sup>	1.75 ± 0.11 <sup>f</sup>
B	1.26 ± 0.14 <sup>a</sup>	1.77 ± 0.09 <sup>a</sup>	2.18 ± 0.11 <sup>a</sup>
C	1.17 ± 0.01 <sup>b</sup>	1.61 ± 0.03 <sup>b</sup>	2.01 ± 0.09 <sup>b</sup>
D	1.14 ± 0.02 <sup>ab</sup>	1.53 ± 0.07 <sup>ab</sup>	1.96 ± 0.05 <sup>ab</sup>
E	1.07 ± 0.12 <sup>bc</sup>	1.46 ± 0.06 <sup>bc</sup>	1.85 ± 0.06 <sup>bc</sup>
F	1.04 ± 0.07 <sup>cd</sup>	1.28 ± 0.08 <sup>cd</sup>	1.76 ± 0.05 <sup>cd</sup>
G	0.90 ± 0.03 <sup>de</sup>	1.15 ± 0.07 <sup>de</sup>	1.69 ± 0.03 <sup>de</sup>

**Note:** Values (Means ± SD) having similar alphabets in a column are statistically non-significant ( $p > 0.05$ ): A= Control -, B=*Streptomyces globiosporus*, C= *Streptomyces toxytricin*, D= WSN2, E= *Streptomyces rochie*, F= *Streptomyces fimbritus*, G = Commercially available probiotics

**Morbidity and mortality:** During this trail the birds were acclimatized as per standard protocols and divided into treatments groups after 07 days as mentioned in materials and methods section. After first week, birds in all experimental groups remained healthy and no mortality was recorded. As per daily observations, the birds treated with experimental strains of the *Streptomyces* and commercially available probiotics were more active with good body scoring as compared to the control group A.

**Weekly body weight and feed intake of broiler birds:** In this experimental trail, the body weight of all the groups was not significantly different during the period of acclimatization (1<sup>st</sup> week). A significant increase in the weight gain was observed in all the groups supplemented with different strains of the *Streptomyces* as a probiotic. Highest body weight was recorded in Group B supplanted with *Streptomyces globiosporus* followed by the other treatment groups. The birds supplemented with commercial probiotics (group G) showed comparatively lower weight gain than the other treated groups. However, all the probiotic treated groups gained more body weight as compared to the control (group A) as shown in Fig. 1.

The feed intake of all birds in the experimental groups was not significantly different during the acclimatization (1<sup>st</sup> week) period. However, a non-significant increase in the feed intake by the birds was observed in all the groups supplemented with different strains of the *Streptomyces* as a probiotic on 7, 15 and 21<sup>st</sup> days of experiment as compared to control group A. Feed intake was significantly high in groups supplemented with *Streptomyces* strains at 28, 31 and 38<sup>th</sup> day of age. Feed intake was high in group G as compared to group A, while feed intake in group B,

C, D, E and F was significantly different from control group A as shown in Fig. 2.

**Organ weight: absolute weight of liver, kidney, intestine and heart:** A significant difference in the absolute weight of the liver was seen in the groups supplemented with the different isolates of *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control group A. Highest liver weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Even groups C, D, E and F have higher liver weight as compared to the group G supplemented with commercial probiotic. The same trend was observed on 30<sup>th</sup> and 45<sup>th</sup> day of experiment as shown in Fig. 3.

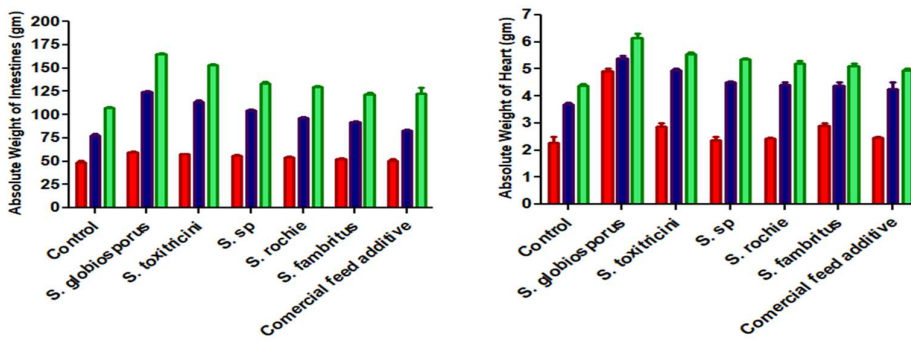
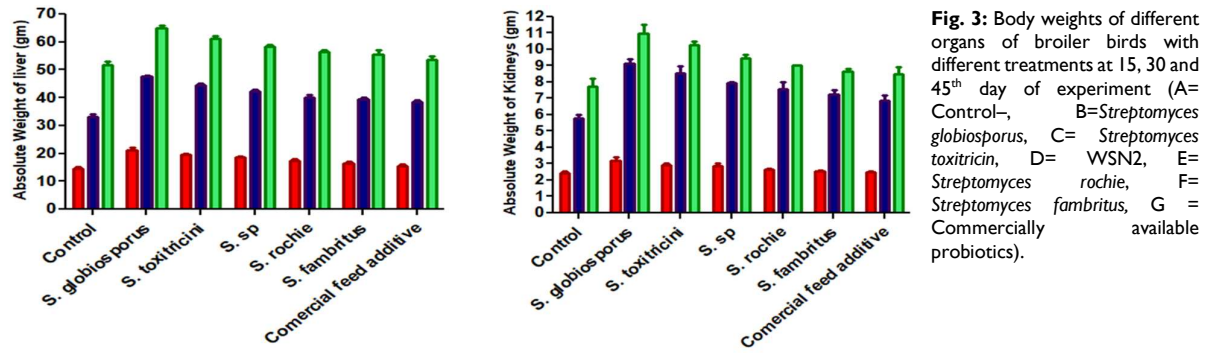
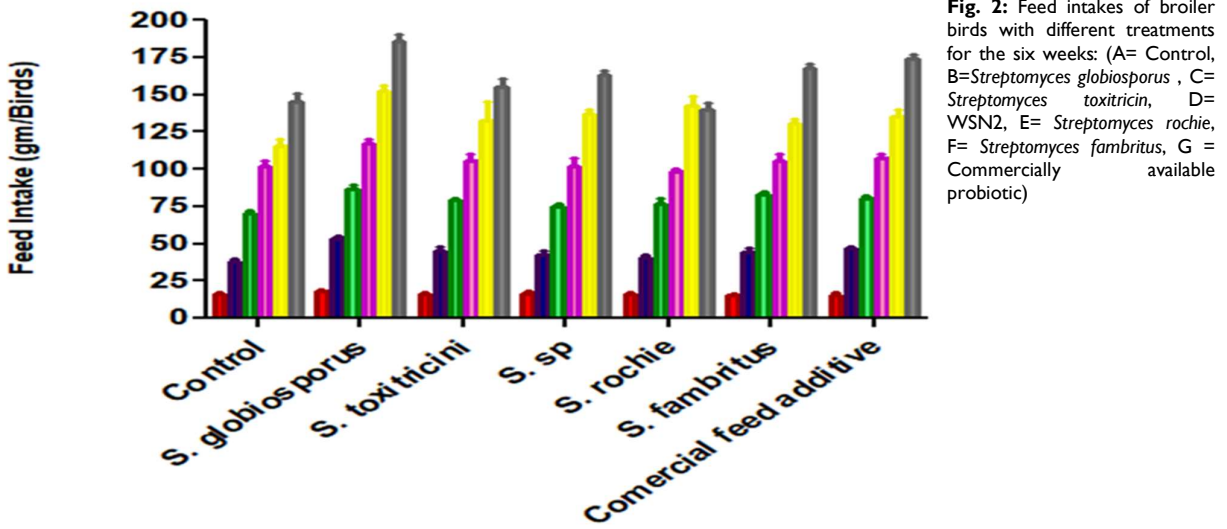
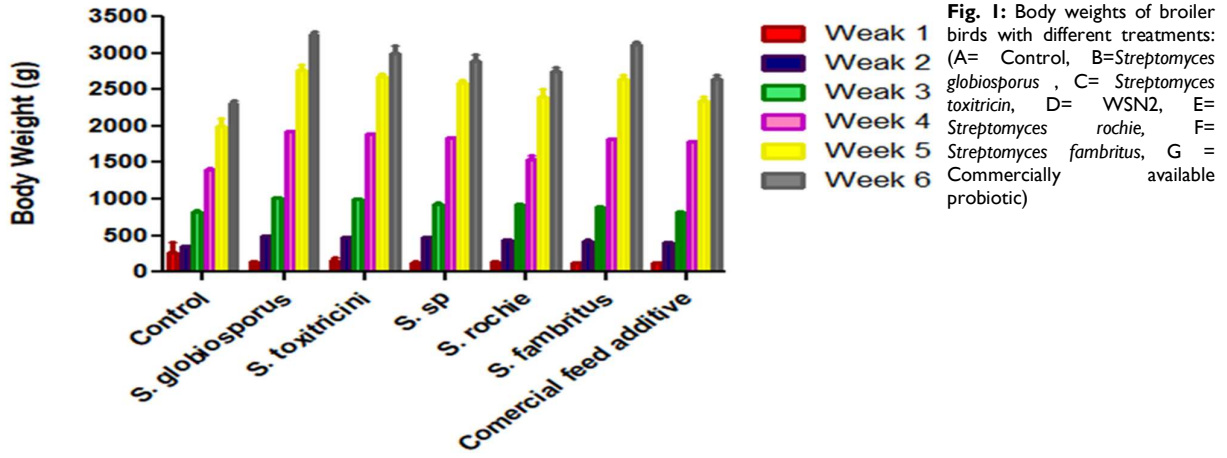
A significant difference in the absolute weight of the of both the kidneys was seen in the groups supplemented with different isolates of *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control group A. Highest kidneys weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Groups C, D, E and F have higher weight as compared to the group G supplemented with commercial probiotic. The similar weight gain trend of kidneys was observed on 30 and 45<sup>th</sup> of experiment as shown in Fig. 3.

A significant difference in the absolute weight of the of intestines was recorded in the groups supplemented with the different isolate of *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control group A. Highest intestinal weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Groups B-F have higher intestinal weight as compared to the group G and similar trend was seen on 30<sup>th</sup> and 45<sup>th</sup> days of experiment as shown in Fig. 3.

Like all other vital organs mentioned above, a significant difference in the absolute weight of the of heart was recorded in the groups supplemented with the different isolates of *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control group A. The highest heart weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Groups B, C, D, E, F have higher heart weight as compared to the group G and similar trend was seen on 30<sup>th</sup> and 45<sup>th</sup> day of experiment as shown in Fig. 3.

**Absolute weight of immune organs (Thymus and Spleen):** A significant difference in the absolute weight of the of thymus was seen in the groups supplemented with the different isolated *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control group A. The highest thymus weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Groups B, C, D, E and F have higher weight as compared to the group G and similar trend was observed on 30<sup>th</sup> and 45<sup>th</sup> days as per experimental data shown in Table 2.

When spleen was observed, a significant difference in the absolute weight was observed in the groups B-E on 15<sup>th</sup> day of the experiment as compared to group A. Comparatively, higher weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. All the groups B-F have higher weight of spleen as compared to the group G and the similar trend was recorded on 30 and 45<sup>th</sup> days of experiment as shown in Table 2.



**Table 3:** Carbon clearance assay and lymphoproliferative response to avian tuberculin in chicks treated with *Streptomyces* species.

Groups	Carbon clearance assay			Lymphoproliferative response (cm)	
	3 mints	15 mints	24 hours	48 hours	
A	138.85 ±11.50 <sup>f</sup>	108.10 ±10.80 <sup>f</sup>	0.543 ±0.03 <sup>c</sup>	0.378 ±0.03 <sup>b</sup>	
B	67.38 ±19.57 <sup>g</sup>	46.67 ±19.88 <sup>g</sup>	0.478 ±0.02 <sup>e</sup>	0.253 ±0.03 <sup>d</sup>	
C	65.27 ±16.14 <sup>e</sup>	76.57 ±18.67 <sup>e</sup>	0.675 ±0.03 <sup>b</sup>	0.422±0.02 <sup>ab</sup>	
D	74.57 ±12.80 <sup>g</sup>	43.51 ±14.17 <sup>g</sup>	0.682 ±0.02 <sup>a</sup>	0.446 ±0.02 <sup>a</sup>	
E	65.60 ±12.30 <sup>c</sup>	49.38 ±10.11 <sup>c</sup>	0.256 ±0.03 <sup>i</sup>	0.245 ±0.03 <sup>e</sup>	
F	79.76 ±12.70 <sup>a</sup>	51.65 ±11a	0.436 ±0.02 <sup>f</sup>	0.287 ±0.01 <sup>d</sup>	
G	64.54 ±10.72 <sup>d</sup>	45.61 ±14.81 <sup>d</sup>	0.532 ±0.02 <sup>d</sup>	0.379 ±0.03 <sup>c</sup>	

**Note:** Values (Means ± SD) having similar alphabets in a column are statistically non-significant ( $p > 0.05$ ): A= Control -, B=*Streptomyces globiosporus*, C= *Streptomyces toxitracin*, D= WSN2, E= *Streptomyces rochie*, F= *Streptomyces fambritus*, G = Commercially available probiotics.

**Carbon clearance assay and lymphoproliferative response to avian tuberculin in treated birds:** In the current study, it was recorded that all the birds treated with *streptomyces* species supplements have better efficiency for the carbon clearance as compared to the Control group A as shown in Table 3. The data indicated that the immune response was better in probiotic supplemented groups both at 3 and 15 mins after injection of India ink. The response shown by experimental groups B to F was also comparable to the commercially available probiotic group (G). Another parameter observed for immune system stimulation was lymphoproliferative response to the avian tuberculin and result obtained has been shown in Table 3. The skin thickness (mm) was significantly increased in group C and D as compared to all other groups at 24 hours and a similar response was recorded at 48 hours.

## DISCUSSION

In Pakistan, poultry industry has major contribution in GDP as a component of livestock and intensive farming has not only contributed towards animal origin protein for the humans as well as a major role in poverty alleviation in terms of employment generation. So it has much potential to enhance the productivity as well safe meat and egg production for the people (Mashkoo *et al.*, 2023; Du *et al.*, 2023). This kind of studies will help to achieve this objective. Briefly, the results obtained has been discussed. The body weight of all the groups was not significantly different during the period of acclimatization (1<sup>st</sup> week). Later on, a significant increase in the body weight was recorded in all the groups supplemented with different strains of the *Streptomyces* as a probiotic. All the groups have significantly higher body weight as compared to the control group (group A) and highest weight was recorded in Group B supplanted with *Streptomyces globiosporus*. These results were in accordance to the Zhang *et al.* (2005), who reported that addition of probiotic in basal feed led to body weight gain in broiler birds. Many other scientists also reported the similar finding about the use of probiotics (bacteria and yeast based) in broiler birds (Mohan *et al.*, 1996; Jin *et al.*, 1996; 1998, Khaksefidi and Rahimi, 2005). Weight gain along with hepatoprotective effects of *Saccharomyces cerevisiae* has been documented by the Hiss and Sauerwein (2003). In terms of FCR, the feed intake of all the groups was not significantly different during the period of acclimatization (1st week). Latterly, a significant increase in the feed intake was observed in all the groups supplemented with different strains of the *Streptomyces* as a probiotic. Published literature also supports these findings that the supplementation of yeast as probiotics results in body weight gain and improved FCR

in broiler birds which is also evident from the current study (Vineetha *et al.*, 2017; He *et al.*, 2019; Mousavi *et al.*, 2018; Zhang *et al.*, 2021; Ebeid *et al.*, 2021).

A significant difference in the absolute weight of the organs including liver, kidney and heart was observed in the groups supplemented with the different isolated *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control. Highest liver weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. The similar trend was observed on 30<sup>th</sup> and 45<sup>th</sup> days of experiment. The previous studies by the Zhang *et al.* (2021) and Ebeid *et al.* (2021) supports the current findings. However, in few studies, it has been mentioned by the Fathi *et al.* (2017) and Ebeid *et al.* (2021), that probiotics supplementation does not always have promising effects due to animal or environmental related factors.

A significant difference in the absolute weight of the of intestines was observed in the groups supplemented with the different isolated *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control. Highest intestinal weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups and similar trend was observed on 30<sup>th</sup> and 45<sup>th</sup>. These positive effects of the probiotic supplementation have been supported by many scientific studies including Gheisari and Kholeghipour (2006), Gao *et al.* (2008) and Ghosh *et al.* (2012). This increase in the weight of the intestine has been attributed to the flourishing of the beneficial microflora in the tract that replaces the pathogens alongwith physiological alterations in absorption surface of intestines. The metabolites produced by the *Streptomyces* are closely related to the bacterial strains like organic acids, oligosaccharides and peptides etc. which results in better protein and vitamins absorption from the intestines. The morphological changes in the intestines are also similar to the other probiotics as described above (Gheisari and Kholeghipour 2006; Gao *et al.*, 2008; Ghosh *et al.*, 2012).

A significant difference in the absolute weight of the immune organs including thymus and spleen was seen in the groups supplemented with the different isolated *Streptomyces* species on 15<sup>th</sup> day of the experiment as compared to the control. The highest thymus weight was recorded in group B supplemented with *Streptomyces globiosporus* followed by other groups. Even these groups B, C, D and E have higher weight as compared to the group supplemented with commercial probiotic (group G). The similar trends in immune parameters were observed on 30<sup>th</sup> and 45<sup>th</sup> day of trail. These findings have been strongly endorsed by the literature that the probiotics supplementation stimulates the immune mechanisms of the poultry through enhanced secretions of immunoglobulins

from primary and secondary lymphoid organs. Mainly IgA, IgG and IgM are in more concentration and among these IgM particularly take part (Haghighi *et al.*, 2006. Tung and Herzenberg, 2007). The immunostimulatory effect has been described differently in literature and the most commonly reported school of thought is that when viable microflora starts to multiply in the intestine, at the same time the pathogenic microflora becomes dead due to competition, hence it starts to secrete antigens, they are absorbed through intestines and boosts immune system (Otutumi *et al.*, 2012).

**Conclusions:** The use of locally isolated species of streptomycetes have significant additive effects on the broiler's performance in all treatment groups as compared to the control in terms of weight gain, feed consumption etc. This supplementation also has not resulted in any kind of negative effects on internal organs including liver, kidney, heart and intestines. The birds supplemented with *Streptomyces globiosporus* revealed the better growth and improve feed consumption as compare to all other tested species. Immunostimulatory effects of these species is also indicative from the no morbidity and mortality during the trail. The Carbon clearance assay is evident of improved phagocytic response through the macrophages. Hence, it has been concluded that these local isolates of the streptomycetes have potential to be promoted as the probiotic in the poultry feed which will be more economical and profitable to the farmers.

**Authors contribution:** BM, MA, SK, SUR and KA, all were active members during this research trial. They conceived the idea, executed the project, performed data analysis and helped in write up of the manuscript.

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