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Full Length Article

Effects of Threonine Supplementation in Low Protein Diet on Broilers Growth Performance and Biochemical Parameters

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Abstract

The present study was conducted to evaluate the effects of graded levels of threonine (Thr) on growth performance and biochemical parameters in broiler chickens. For this purpose, one-day-old 150 broiler chicks were kept in pens at Poultry Farm of the University of Agriculture, Faisalabad for 5 weeks. Chicks were divided into three treatments including T_1 (control group), T_2 (CP 1% less and Thr 10% extra) and T_3 (CP 2% less and Thr 20% extra). Iso-caloric diets were formulated according to two phases as a starter (1–21 days) and finisher (22–35 days). Treatments were replicated into 5 subgroups having 10 chicks in each. Data collected were analyzed by analysis of variance technique under CRD. Chicks fed diet contained CP 1% less and Thr 10% extra had lower ($P < 0.05$) feed intake, improved ($P < 0.05$) weight gain and FCR than those fed diet containing CP 2% less and Thr 20% extra. Dressing percentage was higher ($P < 0.05$) in chicks fed a control diet. However, higher ($P < 0.05$) breast yield and lower ($P < 0.05$) production cost per kg live weight were recorded in birds fed diet contained CP 1% less and Thr 10% extra than other groups. Different treatments had no effect ($P > 0.05$) on relative organ weight. Chicks fed diets having reduced CP and increased Thr had no effect ($P > 0.05$) on blood parameters and liver enzymes activity. Based on these findings, it can be concluded that lowering CP by 1% and increasing Thr by 10% resulted in better growth performance, breast yield and economics efficiency than lowering CP by 2% and increasing Thr by 20%.

Keywords: Chick feed; Growth Performance; Liver enzymes; Organ weight; Carcass characteristics

Introduction

Chicken meat is a good source of protein, but it also contains vitamins and minerals such as vitamin B. It prevents cataracts and is also used to boost immunity, reduce fatigue, regulate digestion, and strengthen the nervous system. Various breeding programmes and researchers' studies on the genetics of modern broilers can account for this increase in bird body weight gain. Furthermore, high-quality feed formulation and strategies were the primary reasons for raising poultry farming throughout the process (Sadeghi and Tabiedian 2005). In previous years, broiler strains lacked the potential for rapid growth that modern strains have. Factors involved in this type of productivity in poultry farming include high dietary crude protein and amino acid concentrations in their feed (Esonu *et al.* 2006). The higher cost of feedstuff and excretion of nitrogen into the environment is the main concern for nutritionists facing poultry farming. Feed cost is the major hurdle in farming, it required about 65–70% of the total cost. Protein covers 15% of total feed cost (Aggrey *et al.* 2010).

Crude protein (CP) is a vital component of diet presents in high levels as well most costly ingredient. The amount of CP decides the nitrogen excretion by a bird in environment. Corn or sorghum in cereal grains are used for energy purpose and these ingredients have low CP. Further, high price of soybean and canola meal (high CP) limit their use in broilers diet. Recent researches on feed efficiency introduced synthetic amino acids, which may reduce protein usage in a diet regarding the feed and cost. Essential amino acids are those that body cannot synthesis itself and should take from outside sources to fulfill the body requirements (Quadros *et al.* 2009). Broiler's body cannot synthesize Thr, so, it is considered as the third limiting amino acid after lysine and methionine. Addition of Thr in diet had the same effect on the bird's growth as a diet offered with high crude protein (Abdaljaleel *et al.* 2019). Among other amino acids,

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threonine functions as the growth of birds as well used as precursors of L-lysine and serine in the body. It is also used in the synthesis of many proteins that promote gastrointestinal mucus production activity and performed its role in immune responses of broilers. Excessive or imbalanced amount of protein is used to increase the dietary requirement of threonine, which used as a precursor for glycine in uric acid formation (Zhang and Kim 2014). Broilers demand glycine or serine for proper body functioning (Kheiri and Alibeyghi 2017). Threonine metabolism is classified as amino acid metabolism, and it consists of several steps, including protein synthesis and degradation, nitrogen excretion in the form of uric acid in an amino acid corporation and amino acid conversion into fat, energy, glucose, protein, CO_2 and water. It is also involved in the formation of non-protein derivatives (Baker *et al.* 2002). Protein synthesis required the addition of limiting amino acid Thr so alternatively; Thr catabolism covers and participates in many other metabolism processes like (glycine, acetyl CO enzyme –A and pyruvate). Use of limiting amino acids in low protein broilers diet may improve the growth performance and biochemical Parameters. Therefore, the purpose of this research was to evaluate the effects of super dosing of threonine in low protein diet on the growth performance (body weight gain, feed intake, feed conversion ratio, carcass yield), biochemical parameters (total cholesterol, total protein, blood glucose triglycerides, VLDL, HDL and LDL) and for liver enzyme activity (ALT, AST and ALP) and economics efficiency.

Materials and Methods

Trial location

The experimental trial was conducted at R&D house at Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad. The research trial continued for 35 days.

Shed management

The brooder was started in the house 24 h before the arrival of chicks to make sure that the temperature in the brooding area was uniform. Immediately after arrival, the chicks were examined for their physical health and were put in the brooding area. The birds were reared in standard management conditions like floor space, light, ventilation, temperature and humidity. On the daily basis, all drinkers and feeders were washed to avoid contamination and fungus. A matte with limestone powder was used at the entrance of the shed throughout the research trial.

Vaccination schedule

All the experimental birds were treated with vaccination against Newcastle disease + infectious bronchitis at day 3, infectious bursal disease at day 13 and 20 and Newcastle disease at day 25.

Experimental birds

One hundred and fifty-day-old broiler chicks (Hubbard's) were divided into three treatments including T_1 (control group), T_2 (CP 1% less and Thr 10% extra) and T_3 (CP 2% less and Thr 20% extra) (Table 1). Iso-caloric diets were formulated according to two phases as a starter (1–21 days) and finisher (22–35 days) (Table 2 and 3). Treatments were replicated into 5 subgroups having 10 chicks in each. After the arrival of chicks were facilitated with sugar solution (1 kg sugar/5L) for flushing purpose. On day 1st temperature was kept at 95°F and further reduced at 5°F within every week until the 75ºF. Feed and water were provided ad labium 24 h.

Data recording

Growth performance: On arrival, broiler chickens were weighed using a digital weighing balance which uses as an initial weight for the starter phase. On day 22nd, all birds within a pen were weighed again, which use as an initial weight for finisher phase. Body weight of broilers was recorded at the end of each consequent week. Weekly BWG was calculated from the data on body weight of birds. Data were recorded on feed intake and BWG for the determination of birds' efficiency of each replicate on weekly basis. It was calculated as follows:

Feed Intake (g/bird) = [Feed offered (g)-Feed Refusal (g)]/no. of birds

"FCR = Feed Intake (g)/Weight gain (g)"

Carcass characteristics: At the end of the experiment, two birds/pen were randomly selected. Birds were weighed individually and processed to get data on carcass response. After processing feathers detached, evisceration was done in order to obtain carcass weight including internal organs (liver, heart, spleen and gizzard), breast meat weight and thigh meat weight and abdominal fat of the birds were calculated through this relationship:

"Dressing percentage = Carcass weight (g)/Live weight (g) × 100"
"Breast meat yield = Breast meat weight (g)/Live weight (g) × 100"
"Thigh meat yield = Thigh meat weight (g)/Live weight (g) × 100"
"Relative Organs weight = Organ Weight (g)/Live weight (g)× 100"

Biochemical parameters: At the end of the experimental trail, two birds/pen was selected for blood sampling. Blood sampling was done from selected broiler chickens for evaluation of blood glucose and total protein, liver enzyme activity (ALT, AST and ALP) and serum biochemistry parameters (triglycerides, cholesterol, HDL, LDL and VLDL) (Shoaib *et al.* 2021).

Economics

Cost of production per live weight was recorded on the basis of feed cost and live bird weight.

Table 1: Experimental treatments

Treatments	Starter Phase (1-21 days)	Finisher Phase (22-35 days)
T1	CP 21.5% Thr normal	CP 20% Thr normal
T2	CP 1% less (20.5%) + Thr 10%	CP 1% less (19%) + Thr 10%
T3	CP 2% less (19.5%) + Thr 20%	CP 2% less (18%) + Thr 20%

Table 2: Ingredients composition of experimental diets

Ingredients	Starter Diet			Finisher Diet		
	T_1	T_2	T_3	T_1	T_2	T_3
Maize grain	53.00	53.65	55.10	56.15	56.07	56.01
Rice tips	2.00	3.00	3.00	6.10	6.85	8.70
Rice polishing	2.00	2.00	2.00	2.00	2.00	2.00
Soybean meal 44%	27.60	27.60	27.60	23.20	23.20	23.00
Fish Meal	4.00	2.50	1.00	2.40	1.20	0.55
Poultry by-product meal	2.50	1.70	0.90	3.00	1.90	0.55
Canola meal	0.91	0.91	0.91	0	0	0
Molasses	2.00	2.00	2.50	1.50	2.50	2.50
Oil	3.00	3.00	3.00	3.00	3.00	3.00
Lime stone	0.50	0.50	0.50	0.50	0.50	0.50
DCP	1.50	1.90	2.00	1.40	1.80	1.90
L-Lysine sulphate, 55%	0.55	0.67	0.79	0.38	0.50	0.61
DL-Methionine, 99%	0.20	0.22	0.24	0.13	0.15	0.17
L-Threonine, 98%	0.14	0.25	0.36	0.14	0.23	0.41
Nutrimin*	0.05	0.05	0.05	0.05	0.05	0.05
Vitalink**	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00

"Each kg of *Vitalin** supplied: vitamin A 20000 KIU; vitamin D_3 5400 KIU; vitamin E 48000 mg; vitamin K_3 4000 mg; vitamin B_1 4000 mg; vitamin B_2 9000 mg; vitamin B_6 7600 mg; vitamin B_{12} 20 mg; niacin 60000 mg; pantothenic acid 20000 mg; folic acid 1600 mg; biotin 200 mg
Each Kg of *Nutrimin*** supplied: Iron 10000 mg; zinc 120000 mg; manganese 140000 mg; copper 12000 mg; iodine 1800 mg; cobalt 400 mg and selenium 360 mg"

Table 3: Nutrients composition of experimental diets

Nutrients	Starter Diet			Finisher Diet		
	T_1	T_2	T_3	T_1	T_2	T_3
Crude protein	21.5	20.5	19.5	20.00	19.00	18.00
ME (kcal/kg)	3030	3030	3030	3100	3100	3100
Ether extract	6.32	6.12	5.94	6.36	6.12	5.89
Crude fiber	3.27	3.08	2.91	3.11	2.86	2.52
Ash	3.53	3.10	2.71	3.00	2.66	2.32
Dig. Lysine	1.28	1.28	1.28	1.05	1.05	1.05
Dig. Methionine	0.51	0.51	0.51	0.41	0.41	0.41
Dig. Threonine	0.81	0.89	0.97	0.72	0.79	0.95
Calcium	0.99	0.97	0.88	0.88	0.87	0.80
Phosphorus, available	0.46	0.45	0.40	0.40	0.39	0.35

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)

Statistical analysis

Data collected for each treatment group was analyzed through the Analysis of Variance technique under Completely Randomized Design and comparison of means were made by Tukey's test (Steel *et al.* 1997).

Results

Growth performance

Feed intake: During starter phase, feed intake was higher ($P < 0.05$) in birds of T1 (Control) treatment while it was lower ($P < 0.05$) in birds of T2 treatment (1% less CP + 10% higher Thr). During finisher phase, it was not similar ($P > 0.05$) in all treatments. Overall, lower ($P < 0.05$) feed intake was recorded in birds of treatment T2 (1% less CP + 10% higher Thr) than other treatments (Table 4).

Body weight gain: During starter phase, body weight gain was higher ($P < 0.05$) in birds of treatment T1 and T2 (1% less CP + 10% higher Thr), while lower ($P < 0.05$) weight gain was recorded in birds of treatment T3 (2% less CP + 20% higher Thr). During finisher phase, weight gain was higher ($P < 0.05$) in birds of treatment T1 (control) than others. Overall, higher ($P < 0.05$) body weight gain was recorded in birds of treatment T1 and T2 (1% less CP + 10% higher Thr), and lower ($P < 0.05$) body weight was seen in birds of treatment T3 (2% less CP + 20% higher Thr) (Table 5).

Feed conversion ratio: Improved ($P < 0.05$) FCR was recorded in birds of treatment T1 and T2 (1% less CP + 10% higher Thr) and poor ($P < 0.05$) FCR was observed in

Table 4: Feed intake of broilers with diets containing various levels of CP and threonine

Treatments	FI (g/ bird)		
	1–21 days	22–35 days	1–35 days
T_1	1248.20^a	1903.62	3151.82^a
T_2	1230.44^b	1896.90	3127.34^b
T_3	1244.32^{ab}	1905.56	3149.88^a
SEM	4.34	6.68	5.15
P-value	0.032	0.640	0.010

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Table 5: Weight gain of broilers reared on diets containing various levels of CP and threonine

Treatments	WG (g/ bird)		
	1–21 days	22–35 days	1–35 days
T1	839.54^a	1029.24^a	1868.78^a
T_2	836.56^a	1025.70^{ab}	1862.26^a
T_3	803.71^b	1014.59^b	1818.30^b
SEM	2.97	3.12	2.97
P-value	0.0001	0.016	0.0001

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Table 6: Feed conversion ratio of broilers reared on diets containing various levels of CP and threonine

Treatments	Feed conversion ratio		
	1–21 days	22–35 days	1–35 days
T1	1.49^b	1.85^b	1.69^b
T_2	1.47^b	1.85^b	1.68^b
T_3	1.55^a	1.88^a	1.73^a
SEM	0.006	0.007	0.004
P	0.0001	0.016	0.0001

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

birds of treatment T3 (2% less CP + 20% higher Thr) during starter, finisher and overall period (Table 6).

Carcass characteristics

Dressing percentage and chest yield were higher ($P < 0.05$) in birds of treatment T1 (control) and they were lower ($P < 0.05$) in treatment T3 (2% less CP + 20% higher thr). Leg, heart, gizzard, liver, spleen and abdominal fat percentage were not affected ($P > 0.05$) by increasing level of threonine in low protein broilers diet (Table 7).

Biochemical parameters

At the end of experimental trail, two birds/pen was selected for blood sampling. Blood parameters (glucose and total protein), liver enzyme activity (ALT, AST and ALP) and serum biochemistry parameters (triglycerides, cholesterol, HDL, LDL and VLDL) were not affected by increasing level of threonine in low protein broilers diet (Table 8).

Economics efficiency

Cost of production per live weight was lower in birds of T2 (1% less CP + 10% higher Thr) and it was higher in T3 (2% less CP + 20% higher Thr) (Table 9).

Discussion

Super dosing of Thr (10 and 20%) with low protein (1 to 2%) had increased feed intake in broilers. This might be due to that Thr improve the gut health and reduce passage rate resulting in increased feed intake. These results of feed intake were in agreement with Mejia *et al*. (2012) who reported that feed intake was higher when birds fed a diet containing 0.77% threonine during the experimental period day (35–49). Nasr and Kheiri (2011) demonstrated that chicks fed on a diet containing 1.2% Thr had higher feed intake during the overall period day (0–42). Panda *et al.* (2011) reported that feed intake was increased with an elevated level of Thr up to 1.2% during the starter phase day (1–21). Zaghari *et al*. (2011) demonstrated that threonine at 0.8 and 0.9% had higher feed intake than other dietary treatments during the starter phase day (1–21). Sterling *et al*. (2003) recorded results in an increase in feed intake of broilers fed diets with increasing Thr at day (9–18). Results are not in line with the findings of Ahmad *et al.* (2020) who reported that 110 and 120% Thr had lower feed intake. Wijtten *et al*. (2004) concluded that different levels of Thr (0.92, 1.04, 1.17, 1.32 and 1.43%) had no effect on feed intake during the finisher phase day (14–34) in commercial broilers. Vieira *et al*. (2004) concluded that feed intake was similar in birds fed different ratios of Thr and methionine days (14–35).

Super dosing of Thr (10 and 20%) extra with low protein (1 and 2%) less had increased weight gain of broilers. This is because Thr improve the gut health and villus surface area resulting in improved nutrient absorption and body weight gain. The results are similar with Ahmad *et al*. (2020) who reported that Thr (10 and 20%) resulted in a greater growth performance than the control group. Ishii *et al.* (2019) observed that addition on lysine + Thr in broilers diet had increased body weight gain than control diet. Zarrin-Kavyani *et al.* (2018) reported that addition of 110% Thr in broilers diet had higher weight gain than 100 and 120% during grower phase. Wils-Plotz *et al.* (2013) observed that feed intake and weight gain were significantly increased at 0.54% threonine supplementation in those birds, which were not facilitated to that fed 0.17% threonine. The broilers gained 127.8 and 95.4 g more weight at an equal level of feed intake at pre- and post-inoculation. Rezaeipour *et al.* (2012) concluded that the highest FCR and weight gain were found at 7.6 g/kg in birds. Similar results were also obtained by Wijtten *et al*. (2004) observed that BWG was increased in birds fed a high level of threonine (1.45%) during the trial (day 14–34th) in commercial broilers. In contrast, Helal *et al.* (2020) reported that different levels of Thr (100, 150 and

Table 7: Carcass characteristics of broilers reared on diets containing various levels of CP and threonine

Treatments	Carcass characteristics (%)							
	Dressing	Chest	Leg	Relative heart	Relative gizzard	Relative liver	Relative spleen	Relative abdominal fat
T_1	60.39[a]	26.39[a]	22.15	0.52	1.12	2.42	0.16	2.17
T_2	60.37[ab]	26.06[ab]	21.73	0.53	1.15	2.56	0.16	2.19
T_3	58.97[b]	25.19[b]	21.74	0.52	1.15	2.44	0.17	2.24
SEM	0.376	0.246	0.204	0.0310	0.038	0.079	0.006	0.029
P	0.032	0.013	0.289	0.942	0.809	0.443	0.515	0.240

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Table 8: Blood parameters of broilers reared with diets containing various levels of CP and threonine

Treatments	Blood Parameters		Serum biochemistry parameters					Liver enzyme activity		
	Sugar (mg/dL)	Total Protein (g/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T_1	290.80	4.65	99.55	129.28	75.76	39.44	22.08	4.498	165.74	223.00
T_2	302.52	4.58	106.45	126.05	81.59	39.06	21.19	4.448	163.35	229.70
T_3	307.74	4.52	108.19	129.08	80.92	38.47	22.15	4.434	162.39	249.50
SEM	8.17	0.05	6.86	6.47	4.54	4.16	1.41	0.14	2.26	13.90
P	0.356	0.176	0.652	0.925	0.622	0.986	0.868	0.944	0.575	0.403

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

Table 9: Economics of broilers reared with diets containing various levels of CP and threonine

Treatments	Day old bird cost	Total feed cost (per bird)	Miscellaneous	Production cost/bird	Av. body weight (g)	Production cost (per kg)
T_1	25	187.55[a]	20	232.55[a]	1913.86[a]	121.51[b]
T_2	25	184.73[b]	20	229.73[b]	1907.18[a]	120.46[c]
T_3	25	184.93[b]	20	229.93[b]	1863.64[b]	123.38[a]
SEM	-	0.297	-	0.297	2.98	0.22
P	-	0.0001	-	0.0001	0.0001	0.0001

T1 = (Control group)
T_2 = (1% less CP and 10% higher threonine)
T_3 = (2% less CP and 20% higher threonine)
$P > 0.05$ (non-significant), $P < 0.05$ (significant)

200%) had no effect on body weight gain.

Super dosing of Thr (10%) extra with low protein (1%) less had improved FCR. Improved FCR in 10% higher Thr may be associated with improved nitrogen retention. Results are similar with the findings of Ahmad *et al.* (2020) who reported that 110 and 120% Thr had improved FCR. Rasheed *et al.* (2018) reported that birds fed diet containing 10% higher Thr had improved FCR than control and 20% higher Thr. Zarrin-Kavyani *et al*. (2018) reported that addition of 110% Thr in broilers diet had improved FCR than 100 and 120% during starter phase. Ishii *et al.* (2019) observed that addition on lysine + Thr in broilers diet had improved FCR than control diet. Dozier *et al*. (2008) used different Thr levels (0.53, 0.63 and 0.73%) from 42 to 56 days. Highest weight gain and better FCR was recorded in broilers at 0.73 and 0.63% threonine, respectively. Results are not in line with Helal *et al*. (2020) who reported that different levels of Thr (100, 150 and 200%) had no effect on FCR. Zaghari *et al*. (2011) who concluded that the lower FCR ratio was recorded at 0.74% threonine level in the diet. Ayasan *et al*. (2009) demonstrated that at 0.86% Thr level lower the FCR in broilers during the finisher phase (22–42).

Dressing percentage and breast yield of broilers were higher in broilers of group T1 (control) and it was lower in group T3. This might be due to that Thr addition increased the abdominal fat resulting in reduced breast yield. Super dosing of Thr (10 and 20%) with low protein (1 and 2%) had no effects on thigh meat, liver, heart, spleen, gizzard weight and abdominal fat of broilers. Results obtained for carcass yield are agreed with Helal *et al*. (2020) who observed that Thr at 100% had higher dressing percentage, while relative organs weight were not affected by different Thr levels. Nasr and Kheiri (2011) concluded that birds fed a diet containing 1.3 percent Thr had higher carcass weight and lower fat deposition in broilers over the course of a day (0–42). Ghahri *et al*. (2010) discovered that birds fed three different levels of Thr (0.9, 0.10 and 1.1 percent) had a significant dressing percentage, breast, and thigh yield (21–42). The results contradict the findings of Chen *et al*. (2017) who found that adding Thr at 1 g/kg improved spleen weight and Thr at 3 g/kg improved thymus weight.

Blood parameters (glucose and total protein), liver enzyme activity (ALT, AST and ALP) and serum biochemistry parameters (triglycerides, cholesterol, HDL, LDL and VLDL) were not affected by increasing level of threonine in low protein broilers diet. This might due to variation is data leads to non-significant results. Results are in line with the findings of Mehdipour *et al*. (2020) who showed that different levels of Thr had no effect on blood hematology parameters. Helal *et al*. (2020) reported that different levels of Thr (100, 150 and 200%) had no effect on albumin and triglycerides concentration. These results were not supported by widely published work of Al-Hayani (2017) experimented to check the effects of graded levels of threonine (Thr, 300, 600 and 900 mg/kg) on blood parameters in broilers. The concentration of Thr 900 mg/kg showed a significant increase on (ALT, AST, ALP) and on total glucose levels as well.

Conclusion

Based on these findings, it can be concluded that lowering CP by 1% and increasing threonine by 10% improved growth performance, breast yield and economics efficiency than lowering CP by 2% and increasing threonine by 20% without any negative effect of liver enzyme activity and biochemical parameters.

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Author Contribution

FW, AJ and MAJ did experimental work and manuscript writing; MS and AM performed data analysis; KR and NR designed the experiment; MS and FM prepared the manuscript

Conflict of Interest

The authors declare no conflict of interest

Data Availability

Data is available

Ethical Approval

All procedures performed in studies were in accordance with the ethical committee of University of Agriculture, Faisalabad, Pakistan

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