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Comparative Therapeutic Efficacies of Herbal and Synthetic Anthelmintic Drugs Against Gastrointestinal Nematodes in *Gallus gallus domesticus*

ABSTRACT

Parasitic infections in chickens are responsible for significant loss in the economy. This study compared the anthelmintic activity of *Trachyspermum ammi* (ajwain), apple cider vinegar, and synthetic drugs against gastrointestinal nematodes in chickens and assessed their effects on weight gain. 100 naturally infected chickens with *Ascaridia galli* and *Heterakis gallinarum*, and 20 healthy fowls were studied. Infected chickens were divided into four groups for treatment with albendazole (20 mg/kg body weight), levamisole (25 mg/kg body weight), ajwain (3 g/kgbw), Apple cider vinegar (ACV) (5 ml/kg body weight), respectively, and the fifth one remained untreated (–ve control). Healthy chickens served as a positive control. Anthelmintic efficacy was assessed using the fecal egg count reduction percentage (FECR%) on days 31, 39, and 53 post-treatment. Albendazole was found to be most effective against nematodes with FECR% of 75.36% on day 31, 98.58% on days 39, and 99.70% on day 53. On day 31, levamisole and ACV were found to be significantly less effective than albendazole, but their effects were increased from day 39 to day 53. ACV showed FECR 89.45% against *A. galli* and 84.32% against *H. gallinarum* at day 53. ACV showed similar deworming potential at day 53 to levamisole, indicating its strong anthelmintic efficacy. *T. ammi* was found to be more effective in weight gain and *A. galli* deworming. Specimens treated with Albendazole showed 10.04 ± 0.48 g/day weight increase, levamisole 8.64 ± 0.42 g/day, *T. ammi* 8.63 ± 0.39 g/day, and ACV 8.67 ± 0.44 g/day indicating both herbal products act as anthelmintics and are also involved in improving the digestive abilities of fowls.

INTRODUCTION

Poultry industry has become the largest stock of domestic animals in the world in the production of food. It enhances the national economy and ensures a reduction in pressure for mutton and beef supply, food security, and earnings through foreign exchange (Gul & Arshan, 2021). Infections due to parasites are responsible for significant loss in the economy, especially in the free-range and domestic chicken industry (Dube *et al.*, 2010; Jaiswal *et al.*, 2020; Jilo *et al.*, 2022). Many synthetic chemicals like Benzimidazoles (mebendazole and albendazole) and levamisole are mostly used for periodic deworming to get rid from gastrointestinal nematodes (WHO, 2002). Chemical residues can be toxic and develop resistance through prolonged treatments (Brom *et al.*, 2013; Kuhlmann & Fleckenstein, 2017; Roeber *et al.*, 2013). Now there is a need to rely on the non-toxic, easily available, effective, and low-priced compounds against intestinal helminths.

T. ammi, commonly known as “Ajwain,” the most popular pharmaceutical plant, belongs to the family Apiaceae or Umbelliferae.



It is widely distributed across different Asian countries and has been used to treat various disorders, such as hepatitis, diarrhea, bronchitis, arthritis, asthma, and abdominal pain (Apte *et al.*, 2014; Sonar *et al.*, 2016; Dhaiwal *et al.*, 2017). ACV also showed anthelmintic effects (FECR = 88%), almost equal to important and strongest drugs used in sheep (Hayajneh *et al.*, 2019). Due to antibacterial and antioxidant properties of ACV, it is used for many health benefits (Chen *et al.*, 2016; Al-Rousan *et al.*, 2018). *T. ammi* and ACV were used as anthelmintics for deworming sheep and donkeys (Hayajneh *et al.*, 2019; Imani-Baran *et al.*, 2020). *T. ammi* was used as an anticoccidial in chicken (Abbas *et al.*, 2019). To our knowledge, no study is available on the anthelmintic efficacy of *T. ammi* and ACV against gastrointestinal nematodes in *Gallus gallus domesticus*. In the current study, it was hypothesized that *T. ammi* and ACV would reduce nematode egg counts and improve weight gain in naturally infected chickens, with efficacy comparable to levamisole but lower than albendazole.

MATERIALS AND METHODS

Study Animals

Sixty-day-old 120 chickens (local golden breed) with similar characteristics (body weight, body morphology) were procured from a local farmer in the peri-urban area of Faisalabad. From these birds, 100 birds were found to be naturally infected with gastrointestinal nematodes and 20 birds were not infected by helminthiasis analysed through Modified McMaster technique. These birds were divided into 6-groups (20 chickens in each group) i.e. G1, G2, G3, G4, G5 (-ve control) and G6 (+ve control). The sample size (number of chickens) was determined based on practical constraints (convenience sampling technique) at the time of the experiment. Random allocation was performed using a computer-generated random number sequence (Microsoft Excel). Each bird was assigned a unique identification number, and these numbers were randomly mapped to the respective experimental groups. This approach ensured that each bird had an equal probability of assignment, thereby minimizing selection bias and promoting comparable baseline characteristics across groups. Each bird represents an independent biological replicate (experimental unit) throughout the study, and EPG was analyzed at the individual level; there is no

pseudo-replication. The birds in different groups were provided with large surface area for ranging and each group was separated from each other by iron mesh net. Birds were kept in isolation with stocking density of 10 chicken/m² by keeping bedding by rice hulls. Pens were properly cleaned on daily basis during trial to reduce reinfection risks. All precautionary measures regarding sanitation were strictly followed to ensure flock health and safety. All bird groups were provided ad libitum water and standard poultry feed (Feed No. 14, Faisal Feeds). Randomly, fresh droppings were collected early in the morning and transferred to 10% formalin (Wisetmora *et al.*, 2024) containing jars with group details. These fecal samples were subjected to egg per gram (EPG).

Quantitative Analysis of Fecal Samples

Modified McMaster technique using Whitlock Universal (4x0.5mL) slide (Australia) was used for quantitative analysis of fecal samples. Weighed 2 g of feces into a 50 mL mixing jar, then added 2.5 mL of tap water. 45.5 mL of a saturated NaCl solution with a specific gravity of 1.2 was used as the flotation solution. The solution was mixed with a handheld kitchen mixer to break the feces. Suspension was violently stirred and sieved using a sieved-ended pipette to obtain a homogeneous solution. The sample was loaded into the Whitlock chamber. Nematode eggs were counted using 40x magnification (Soulsby, 1982), while the recommended generic dilution for this count was 1.25, and the detection limit was 50 EPG.

$$\text{Egg per gram} = \text{Total number of eggs in chamber} \times 50 \quad (1)$$

The Test Drugs

Two modern broad-spectrum anthelmintics, Albendazole and Levamisole, used in the treatment of parasitic infections, were purchased from the local veterinary medicine market.

The Herbal Products

Two herbal products were used in the current study. The herbal products were ACV and *T. ammi* (Ajwain). ACV was purchased from the local medicine market. Fresh seeds of *T. ammi* were purchased from the local market (Faisalabad, Punjab, Pakistan) 31.4504°N, 73.1350°E. Seeds of *T. ammi* were ground into a powder in an electric grinder and kept in plastic bags at 4 °C until use.



Experimental Designs

All naturally infected (n=100) and healthy (n=20) fowls were transferred to the experimental place on day 1 and acclimatized for 15 days. On days 16 and 23 prior to anthelmintic treatment, fecal samples were collected to calculate the EPG and confirm the increasing trend of parasitic infection (G1-G5), but G6 had no gastrointestinal nematode infection. On the 24th day, groups (G1 to G4) were treated with synthetic drugs and herbal products for three consecutive days (Table 1). On days 31, 39, and 53 after treatments, fecal samples were collected from each group and observed for EPG.

Table 1 – Fowls treated with different anthelmintics.

Groups	Treatments/ Anthelmintics	Rought	Dose
G1	T _{ALB} Albendazole	Orally [†]	20 mg/kg body weight*
G2	T _{LEV} Levamisole	Orally [†]	25 mg/kg body weight*
G3	T _{AJW} Ajwine	Orally ^{††}	3 g/kg body weight**
G4	T _{ACV} Apple cider vinegar	Orally [†]	5 ml/kg body weight***
G5	C _{-ve} Not treated	Naturally infected (un-medicated -ve control group)	
G6	C _{+ve} Not treated	Naturally healthy (un-medicated +ve Control group)	

Dose and treatment ; * Label recommendations, ** Imani-Baran *et al.* (2020), *** Khundoker (2020). Route and mode of administration: mixed into drinking water, ^{††} mixed in feed.

Efficacy Calculation

Anthelmintic activity of synthetic Drugs (albendazole and levamisole) and herbal products (ACV and Ajwine) was calculated based on fecal egg count reduction (FECR) and the percent efficacy was found through equation 2 (Basit *et al.*, 2014). Pre-treatment EPG calculations refer to day 23 only, and FECR% was calculated separately for *A. galli* and *H. gallinarum*.

$$\text{Efficacy} = \frac{\text{Pre-Treatment EPG} - \text{Post-Treatment EPG}}{\text{Pre-Treatment EPG}} \times 100 \quad (2)$$

Statistical Analysis

A two-factor ANOVA (time and treatment) was applied, and mean differences were assessed using Tukey's test at the 5% level of significance. Data were presented as mean $\pm$ SE (standard error). Mean comparisons were conducted for the interaction (time $\times$ treatment), as the interaction was found to be significant in the ANOVA. The Kolmogorov–Smirnov normality test indicated that the data were approximately normally distributed; therefore, no transformation was applied. Statistical analyses were performed using SPSS version 26.0.

RESULTS

Anthelmintic Activity of Modern Drugs and Herbal Products

Two gastrointestinal nematodes, viz. *A. galli* and *H. gallinarum*, were found in experimental chickens. Significant variations were observed in time dependent response of anthelmintic activity of T_{ALB}, T_{LAV}, T_{AJW} and T_{ACV} in egg per gram and fecal egg count reduction (FECR%). Analysis revealed that both albendazole and levamisole significantly ($p < 0.05$) reduced EPG post-treatments, but albendazole demonstrated the highest efficacy by day 53. The herbal treatment ACV also showed a significant reduction ($p < 0.05$), although less effective than synthetic drugs. Herbal treatment T_{AJW} showed a reduction in the EPG, but a non-significant difference was observed in EPG in corresponding days in comparison to other treatments (Table 2).

Table 2 – Efficacy of albendazole, levamisole, *T. ammi*, and apple cider vinegar on egg count (EPG) in *Gallus gallus domesticus* based on the faecal egg count reduction test.

Groups	EPG before treatments		EPG post treatments		
	On day 16	On day 23	On day 31	On day 39	On day 53
G1 T _{ALB}	2,220 $\pm$ 136.6efg	2,750 $\pm$ 231.3cde	690 $\pm$ 117.7jk	40 $\pm$ 18.7k	10 $\pm$ 10.0k
G2 T _{LEV}	2,360 $\pm$ 169.1efg	2,820 $\pm$ 125.1b-e	1,060 $\pm$ 173.5ij	560 $\pm$ 104.2jk	380 $\pm$ 64.4jk
G3 T _{AJW}	2,520 $\pm$ 100.7def	3,120 $\pm$ 60.4a-d	2,210 $\pm$ 155.2efg	1,950 $\pm$ 138.7fgh	1,690 $\pm$ 114.5ghi
G4 T _{ACV}	2,520 $\pm$ 136.6def	2,860 $\pm$ 163.9b-e	1,430 $\pm$ 140.2hi	700 $\pm$ 150.8jk	360 $\pm$ 103.0k
G5 C _{-ve}	2,350 $\pm$ 196.9efg	2,730 $\pm$ 123.1cde	3,230 $\pm$ 76.8abc	3,490 $\pm$ 74.8ab	3,770 $\pm$ 104.4a

Different letters indicate significant differences between interactions (treatment and time) by the Tukey test at the 5% level of significance. Data were presented as mean $\pm$ SE (standard error).

On day 53, the maximum values of FECR% for herbal treatments were 45.83% and 87.20%, respectively, for Ajwine and ACV. Albendazole reached approximately 100% FECR, maintaining its significantly higher efficacy ($p < 0.05$) compared to the other groups by day 39 and 53. Levamisole and apple cider vinegar treatments showed a time-dependent increase in FECR%. The final values of T_{LEV} and T_{ACV} were not significantly different from each other ($p > 0.05$), indicating similar efficacy. However, their performance was significantly lower than that of albendazole. *T. ammi* demonstrated the lowest FECR% values 29.29%, 37.62% and 45.83% by day 31, 39, and 53, respectively, with significantly lower performance compared to the other treatments (Figure 1). At day 53, based on fecal egg count reduction, *A. galli* was found to be significantly susceptible to albendazole, apple cider vinegar, and levamisole. *T. ammi* was also found effective against *A. galli*, but the reduction of parasites in response to *T. ammi* was found to be non-significant compared



to other treatments (Figure 2). Albendazole and ACV were also found to be significantly effective on *H. gallinarum* at day 53. Levamisole and *T. ammi* were not found to be significantly effective against *H. gallinarum* when compared to *A. galli* (Figure 3).

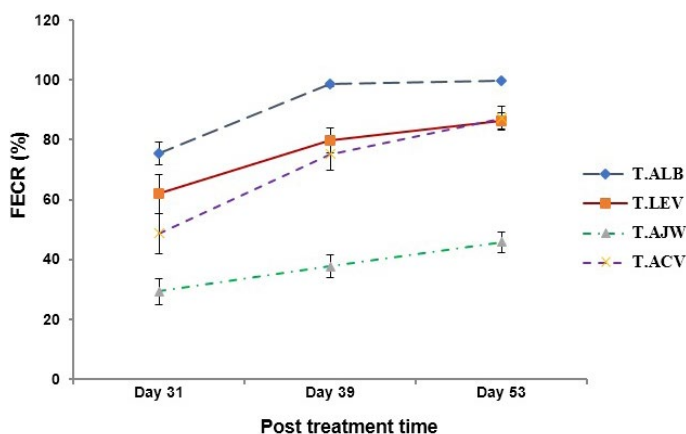


Figure 1 – Total fecal egg count reduction (mean±SEM) for all nematodes across various anthelmintic treatments over time.

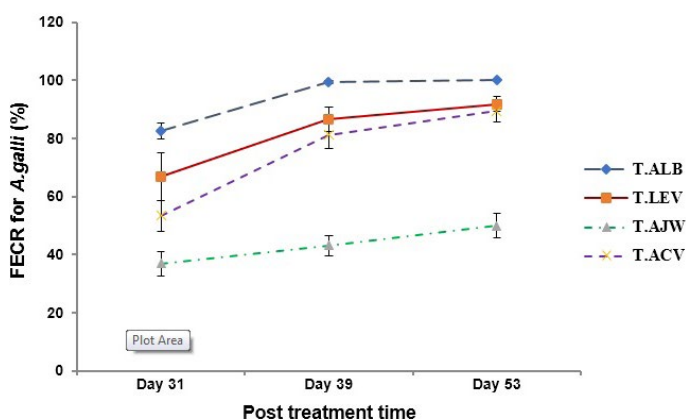


Figure 2 – Effects of various anthelmintics on fecal egg count reduction percent (mean±SEM) of *A. galli* in chicken during the course of time.

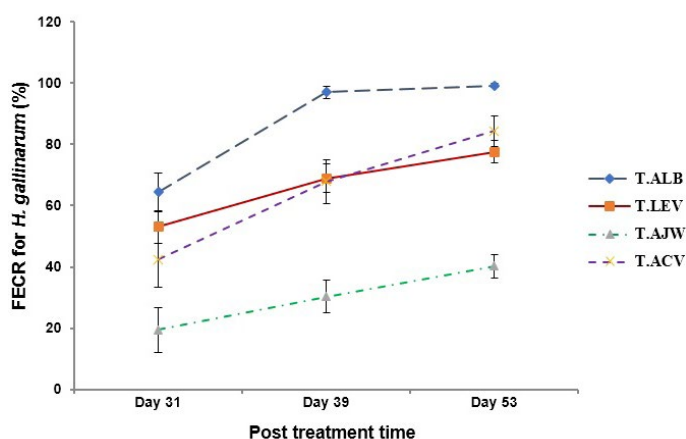


Figure 3 – Effects of various anthelmintics on fecal egg count reduction percent (mean±SEM) of *H. gallinarum* in chicken during the course of time.

Effects of Different Anthelmintic Treatments on the Daily Weight Gain of Chickens

Before treatment (day 23), all of the specimens were gaining weight, but there were non-significant differences ($p>0.05$) in weight gain among any of the groups, including the treated groups, the negative control (G5), and the positive control (G6). Significant differences in daily weight gain emerged after treatment, beginning on day 31 and becoming more pronounced on days 39 and 53. On day 31, weight gain was significantly higher in the positive control group (G6) than in the negative control (G5). However, all values still fell within a moderate range, and multiple groups shared similar findings with mild differences in treatment responses.

By day 39, chickens treated with albendazole and the G6 showed significantly ($p<0.05$) higher weight gains than other groups. In contrast, the G5 group showed minimum weight gain, significantly lower than all treated groups, confirming the negative impact of infection without intervention. G3, G4, and G2 also showed noticeable improvements in weight gain to varying extents. On day 53, the differences were more distinct. The G6 showed the highest weight gain (10.31 ± 0.44 g), significantly ($p<0.05$) outperforming all other groups except G1 (10.04 ± 0.48 g). Both G3 and G4 (8.63 ± 0.39 and 8.67 ± 0.44 , respectively) maintained moderate performance, while G2 also achieved noticeable results (8.64 ± 0.42 g). The G5, however, consistently lagged behind with the lowest gain (5.64 ± 0.23 g), indicating poor growth performance under parasitic infection without treatment. These results demonstrate that all tested anthelmintic treatments had significant positive effects on weight gain, with T_{ALB} showing the highest efficacy (Table 3).

Table 3 – Comparative analysis of weight gain per day (Mean ±SEM) in various treatments with anthelmintics at different times.

Groups	Weight gain/day (g)			
	Before Treatment	After treatment		
	Day 23	Day 31	Day 39	Day 53
G1 T_{ALB}	6.15 ± 0.36 ghi	7.63 ± 0.16 d-i	9.83 ± 0.40 abc	10.04 ± 0.48 ab
G2 T_{LEV}	6.30 ± 0.22 f-i	7.45 ± 0.46 e-i	8.55 ± 0.45 a-e	8.64 ± 0.42 a-e
G3 T_{AJW}	6.28 ± 0.54 f-i	7.30 ± 0.64 e-i	8.03 ± 0.48 c-g	8.63 ± 0.39 a-e
G4 T_{ACV}	6.35 ± 0.27 f-i	7.35 ± 0.18 e-i	8.20 ± 0.25 b-f	8.67 ± 0.44 a-e
G5 C_{-Ve}	6.48 ± 0.38 f-i	6.03 ± 0.27 hi	5.78 ± 0.25 i	5.64 ± 0.23 i
G6 C_{+Ve}	7.40 ± 0.17 e-i	7.88 ± 0.14 c-h	9.50 ± 0.46 a-d	10.31 ± 0.44 a

Different letters indicate significant differences between interactions (treatment and time) by the Tukey test at the 5% level of significance. Data were presented as mean ± SE (standard error).



DISCUSSION

Treatments with synthetic drugs are considered necessary to control and remove the gastrointestinal parasites. In current research, G1 and G2 are treated with albendazole and levamisole, respectively. In chicken of G1, FECR% of albendazole against gastrointestinal nematodes was approximately 100% at day 53. The findings of Tucker *et al.* (2007) and Ashraf *et al.* (2002) were aligned with the current study, which observed the 98.2% and 95.60% efficacy of albendazole, respectively. Albendazole showed 100% and 99.2% FECR% against *A. galli* and *H. gallinarum* in chicken of G1. These findings were similar to Jiang & Li (1984) and Chege *et al.* (2017), who determined 100% efficacy against *A. galli* and *H. gallinarum*. The results of levamisole (86.25% at day 53) in G2 chicken were approximately similar to Zirintunda *et al.* (2025) and Feyera *et al.* (2022), who determined 98.6% and 99.8% efficacy against nematodes of poultry. Levamisole was 91.7% and 77.54% effective at day 53 against *A. galli* and *H. gallinarum*; these findings are corroborated by Feyera *et al.* (2021), who found 96.4% efficacy of levamisole against *A. galli* and Yazwinski *et al.* (2020), who noted 87% and 63% efficacy against *A. galli* and *H. gallinarum*, respectively. The current FECR% of levamisole against *H. gallinarum* was also similar to Soudkolaei *et al.* (2021), who determined efficacy 71.8% in domestic chickens of Iran.

Plants have many such chemicals with antiparasitic properties, such as polyphenols, alkaloids, catechin, and terpenoids. Many herbal dewormers are more effective than synthetic dewormers (Karumari *et al.*, 2014; Ranasinghe *et al.*, 2023). Many studies *in vitro* and *in vivo* showed that *T. ammi* and apple cider vinegar (ACV) have anthelmintic, antibacterial, and anticoccidial activity in different animals (Jabbar *et al.*, 2006; Apte *et al.*, 2014; Hayajneh, 2018; Abbas *et al.*, 2019; Hayajneh *et al.*, 2019; Imani-Baran *et al.*, 2020). There was no study on anthelmintic activity of *T. ammi* and ACV against gastrointestinal nematodes in *Gallus gallus domesticus*. In the current study, *T. ammi* showed FECR 29.28%, 37.62%, and 45.83% against round worms on day 31, 39, and 53 post treatments. number of relevant studies in the literature, the results of G3 are comparable to those of Imani-Baran *et al.* (2020). Who observed the efficacy of *T. ammi* against gastrointestinal nematodes in donkeys ACV showed 87.2% efficacy against gastrointestinal nematodes in G4 chickens. The finding of the current study was very close to the result of Hayajneh *et al.* (2019), who

observed 88% anthelmintic efficacy of ACV in sheep from Jordan.

Many nematodes, such as *A. galli*, *H. gallinarum*, and *Capillaria* species, pierce the mucosal lining, leading to bleeding, and then go back into the intestinal lumen to grow and become mature. This kind of infection has been linked to slower weight gain and lower productivity in chickens (Bessell *et al.*, 2012; Collins *et al.*, 2015). Providing fowls with herbal products or synthetic drugs in their drinking water helped to improve their growth and weight gain. Fowls treated with ACV showed an increase in weight gain per day during the course of time, but a significant difference ($p < 0.005$) was observed on days 39 and 53. Similar observation was found in the study of Allahdo *et al.* (2018) who mixed ACV in drinking water and reported a significant increase in body weight of broilers. At the end of the trial, weight gain per day was highly significant in the chickens of G1 and G6, and a significant increase was observed in the chickens of G2, G3, and G4. There was an increase in weight gain per day in G5, but it was found to be non-significant. The findings of the current work are in line with the study of Katoch *et al.* (2012), who observed the significant difference in live weight gain per day in untreated infected chickens (13.7 g/day) and those treated with anthelmintics (18.0 g/day). A strong association was observed between daily weight gain and EPG in the untreated and treated groups. So, in the present study, treatments with different anthelmintics (G1 to G4) showed improved growth rate. Phiri *et al.* (2007) also found similar findings in free-range chickens in central Zambia. *T. ammi* treatments were not found to be significant against gastrointestinal nematodes but showed significant ($p < 0.05$) weight gain in treated specimens. These findings support the work of Kolbadinejad & Rezaeipour (2020) and Valiollahi *et al.* (2014), who found that *T. ammi* powder improved growth in chickens. This effect might be due to antibacterial and antioxidant properties of these medicinal plants (Al-Rousan *et al.*, 2018; Chen *et al.*, 2016). These findings suggest the use of potential herbal products to improve growth in *Gallus gallus domesticus*.

CONCLUSION

Trachyspermum ammi and apple cider vinegar both showed anthelmintic effects in *Gallus gallus domesticus*. Among synthetic drugs, albendazole



remained the most effective treatment against gastrointestinal nematodes. Levamisole and ACV also produced higher FECR% values and significantly improved weight gain in chickens, indicating that ACV may be considered as a supportive or alternative option in integrated parasite management, particularly where synthetic drugs are limited. *T. ammi* showed moderate anthelmintic activity but contributed to improved growth, suggesting its potential use primarily as a growth-promoting phyto-genic feed additive rather than a stand-alone dewormer.

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ANIMAL ETHICS STATEMENT

This research did not involve any human or wild animal participants. The study did not cause any harm or pain to domestic chickens. Ethical approval for this study was obtained from Ethics Review Committee, Government College University Faisalabad (Ref. No. GCUF/ERC/688). Authors have not experienced any observable adverse effects in chicken in response to repeated dosing with ACV and *T. ammi*. They were found normal in daily activities like morning/evening peak, feeding, social hierarchy, grazing, water consumption etc. and showed no sign of digestive track issues like diarrhea etc. Furthermore, study did not lead to any biosafety or biosecurity issues.

AUTHOR CONTRIBUTIONS

Conceptualization, BH (Bilal Hussain); methodology, MA (Muhammad Adnan); software, MA; validation, BH; formal analysis, MA; investigation, MA and BH; resources, MA and BH; data curation, MA and BH; writing—original draft preparation, MA and AR (Azhar Rafique); writing—review and editing, BH; visualization, BH and AR; supervision, BH; project administration, BH; funding acquisition, MA. All authors have read and agreed to the published version of the manuscript.

FUNDING

None.

DATA AVAILABILITY STATEMENT

The entire dataset supporting the results of this study was published in the article itself.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of the manuscript and the dissemination of the results obtained.

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