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Supplementation of *Cichorium intybus* roots improved the growth performance, immunity response, gut ecology and morphology of broilers chicken Ross308 strain

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A R T I C L E I N F O

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A B S T R A C T

Gut microbiome plays a key role in maintaining the health of broilers by stimulating the development of immune system, improving the uptake of nutrients and inhibiting several enteric pathogens. “Antibiotic growth promoters (AGPs)” are generally used to inhibit the proliferation of pathogenic gut microbiota. Despite its beneficial characteristics, AGPs could cause severe adverse consequences, one of them is development of antibiotic resistance in bacteria which posed major threat to public health. Whereas, the use of AGP from natural origin could be risk free or low toxicity. A research trial was conducted to investigate the effects of supplementation of various natural prebiotics including inulin and chicory (*Cichorium intybus*) root powder on performance, carcass traits, serum biochemistry, intestinal histology, immune response and gut ecology in broiler chick of Ross308 strain. One-day-old chicks ($n = 200$) were randomly allocated to five dietary treatments. Growth performance, intestinal histomorphology, antibody titer against ND, ileal Lactobacillus count were significantly ($P < 0.05$) improved in treatments groups as compared to control ($P < 0.05$). Total cholesterol levels, serum triglyceride, ileal *E. coli* count, serum albumin, globulin were significantly ($P < 0.05$) reduced in the dietary treatments groups in comparison to control at 42 day of the experiment. A non-significant difference was found in serum Aspartate transaminase (AST), alanine aminotransferase (ALT), Bilirubin and uric acid among all treatment groups ($P > 0.05$) which indicates that no physiological dysfunction was induced in treated birds. In conclusion *Cichorium intybus* root powder and inulin may be considered as risk free alternative to chemically synthesized antibiotic growth promoters.

1. Introduction

Prebiotics are indigestible substances mostly composed of carbohydrates and were being supplemented in the diet as a replacement to antibiotics for maintaining the health and production potential of broilers. Gut microbiome has a key role in maintaining the health of broilers by stimulating the development of immune system, improving the uptake of nutrients and inhibiting several enteric pathogens (Buffie and Pamer, 2013). Antibiotic growth promoters (AGPs) are used to inhibit the proliferation of pathogenic gut microbiota to reduce the enteric infections (Brown et al., 2017) Despite the beneficial characteristics, severe side effects are reported with the use of AGPs, for example development of antibiotic resistance in bacteria, which in turn pose a major public health threat (Oniciuc et al., 2018), Consequently, the use of antibiotics as growth promoters was banned by the European Union, EU in 2005 (Dibner and Richards, 2005) and in China in 2020 (Melaku et al., 2021). In Pakistan, the use of AGPs are not regulated even though a recent legislation has been made to monitor the use of such AGPs in animals’ feeds (Qiu et al., 2024). The AGPs comprises an important proportion of antimicrobial use in animals in Pakistan (Ur Rahman and Mohsin, 2019), Hence there is great urge to search for risk free antibiotic growth promoters as an alternate to conventional AGP for

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the growth of broiler chicken of strain Ross308 (Khan et al., 2014).

Various growth promoters including organic acids, probiotics, prebiotics and enzymes can be supplemented in the diet of broilers (Ayalew et al., 2022). Chicory *Cichorium intybus* roots contain Inulin type fructan that is indigestible, and has a prebiotic property which promote the intestinal health and bird's growth (Gurram et al., 2021). These growth promoters exert beneficial influence on the production of broilers by improving the integrity of intestinal epithelial cells and modifying gut microbiota profile (Yaqoob et al., 2021). Natural prebiotics are chicory, garlic, onion, barley, dandelions and flex seeds and include galacto-oligosaccharides, pectin, fructan, mannan-oligosaccharides and inulin (Sun et al., 2013).

Keeping in view the properties of *C. intybus,* the present study was designed to investigate the effect of inulin and native *C. intybus* root powder as an alternative to AGP.

2. Materials and methods

2.1. Birds husbandry and experimental design

Male broiler chicks of Ross-308 strain, were procured from local chick's supplier. Chicks were individually weighed, and 200 chicks (having equal weight) were randomly allocated to the five dietary treatments of 40 birds each, every treatment was comprising of five replicate of eight chicks each. The broiler chicks were reared on deep littler system. Fresh water was provided *ad-libitum* throughout the experiment period. All experimental chicks were remained under uniform husbandry conditions though out the trail period. The experiment last for 42 days. Broilers were vaccinated against Newcastle disease on day five via intraocular and on day 21 of age via drinking water by using live virus B1 type LaSota strain, Fort Dodge Animal health, USA. Other vaccines administrated were; Infectious Bronchitis (IB) at day seven and Infectious Bursal Disease (IBD) at day 14 and 30.

Experimental layout is given as under:

Groups	Dietary treatments	Supplementation level
T1	Basal diet (supplementary table 1)	No supplementation.
T2	Basal diet + AGP Bacitracin methylene di-salicylate (BMD)	@ 500 g/ton as per of feed as per manufacturer recommendation.
T3	Basal diet + Commercial Inulin Frutafit ®-HD comprises of inulin (FOS), Fructo-oligosaccharide (Sensus, Roosendaal, Netherlands).	@ 1 % of basal diets.
T4	Basal diet + Inulin from Chicory	@ 1 % of basal diets.
T5	Basal diet + Chicory root powder	@ 2.13 %

2.2. Preparation of C. intybus root powder

The seeds of *C. intybus* plant was purchased from local herb shop, were cultivated and grown plants were identified from National Agriculture Research Centre, Islamabad. The roots were washed and dried in hot air oven (40 °C) for 10 h and ground thereafter.

2.3. Extraction of inulin from C. intybus

The inulin from the roots of *C. intybus* were extracted by following the method essentially the same as described by (El-Kholy et al., 2020).

2.4. Growth performance, carcass yield and relative organs weight

Performance parameters including feed intake (FI), body weight gain (BWG) were recorded on weekly basis. Feed conversion ratio (FCR) was determined every week by dividing FI by BWG. At day 42 of the trial, three broiler chicken (Ross 308) from each replicate was randomly selected, weighed and slaughtered by adopting the Halal technique. The carcass yield and relative organ weights were determined.

2.5. Gut morphology

On 42nd day duodenum section were excised, washed with normal saline solution, and transferred to 10 % neutral buffered formalin for fixation for 24 h, The gut morphology including fixation of tissues, dehydration, cleaning, embedding, cutting & staining wre done essentially same as reported by (Gurram et al., 2021).

2.6. Serum biochemistry

Blood from three broiler chicken (Ross308) of each replication was taken, allowed to clot. and stored at −20 °C until use. The serum biochemistry was analysed by using blood chemistry analyser using commercial kits**.**

2.7. Immune response

For immune response antibody titer was measured by performing hemagglutination inhibition test of these serum sample by following the method of (Muraroli et al., 2014).

2.8. Gut ecology

Ten-fold dilution of the ileal digersta was taken in phosphate buffer saline. The count of *Escherichia coli* and *Lactobacillus spp*. was determined by plating of serial ten-fold (10^{-4} to 10^{-8}) dilutions on eosin methylene blue agar and de-man Rogosa sharpe agar, respectively and incubated. Finally, countable colonies were counted and presented as $\log_{10}$ (CFU) colony forming units per gram of the ileum.

2.9. Statistical analysis

Data collected was summarized using MS-excel and analysis was done with the help of one-way ANOVA technique, using SAS software (version 9.1).

3. Results

3.1. Growth parameters

Effects of dietary supplementation of AGP and different prebiotics on growth parameter are presented in Table 1. No significant difference in growth performance was observed among all treatment groups during starter phase ($P > 0.05$). The dietary supplementation of AGP, commercial inulin, chicory inulin and chicory root powder significantly increased FI, higher BWG and improved FCR in comparison to control ($P < 0.05$) during grower and total growth phase. However, there were non-significant differences ($P > 0.05$) observed in growth parameters among AGP and different prebiotics supplemented dietary treatment groups.

3.2. Carcass yield and relative organs weight

Table 2 shows the effects of dietary supplementation AGP and different prebiotics statistically improved dressing carcass yield compared to control T1 ($P < 0.05$). There was no statistical difference in relative organ weight observed among all treatment groups ($P > 0.05$).

3.3. Serum biochemical profile

The Table 3 shows the dietary supplementation of AGP and different prebiotics significantly increase the Serum albumin, globulin and total protein concentration while, significantly reduced cholesterol and triglycerides level as compared to control T1 ($P < 0.05$). There were no significant variations observed regarding serum AST, ALT, uric acid and bilirubin concentrations among dietary treatments supplemented with

Table 1
Effects of dietary supplementation of AGP and different prebiotics on growth parameters[1] in broilers Chicken Ross308 strain.

	Starter phase			Grower phase			Total growth		
Treatments[3]	FI (g)	BWG (g)	FCR	FI (g)	BWG (g)	FCR	FI (g)	BWG (g)	FCR
T1	909.5	703.2	1.292	3107.5[b]	1670.5[b]	1.860[a]	4017.1[b]	2373.7[b]	1.692[a]
T2	908.7	704.5	1.290	3227.5[a]	1780.2[a]	1.812[b]	4136.2[a]	2484.7[a]	1.662[b]
T3	908.2	704.7	1.291	3228.2[a]	1783.2[a]	1.810[b]	4136.5[a]	2488.1[a]	1.661[b]
T4	909.2	705.1	1.290	3228.7[a]	1783.7[a]	1.811[b]	4140.2[a]	2489.3[a]	1.663[b]
T5	911.5	705.5	1.292	3234.1[a]	1785.1[a]	1.813[b]	4143.2[a]	2490.1[a]	1.665[b]
SEM[4]	0.77	5.56	0.003	1.49	0.78	0.003	1.87	1.44	0.002
p-value	0.12	0.48	0.95	0.01	0.01	0.01	0.01	0.01	0.01

[a-b]Means in a column with different alphabets significantly (P < 0.05) differ.
[1]FI = Feed intake, BWG = Body weight gain, FCR = Feed conversion ratio.
[2]Each value represents the mean of 5 replicates.
[3]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = *C. intybus* root powder.
[4]SEM = Standard error mean.

Table 2
Effects of dietary supplementation of AGP and different prebiotics on carcass yield and relative organs weight (Percentage of live weight) in the broiler.

Treatments[2]	Live weight	Carcass yield	Liver	Spleen	Heart	Gizzard	Bursa	Abdominal fat
T1	2372.7[b]	70.61[b]	1.952	0.117	0.520	1.360	0.170	1.275
T2	2483.2[a]	71.95[a]	1.954	0.120	0.523	1.365	0.173	1.277
T3	2484.7[a]	72.03[a]	1.960	0.123	0.521	1.367	0.171	1.280
T4	2485.2[a]	72.06[a]	1.965	0.120	0.520	1.370	0.170	1.282
T5	2485.7[a]	72.13[a]	1.970	0.121	0.522	1.368	0.172	1.284
SEM[4]	0.91	0.47	0.004	0.002	0.002	0.004	0.003	0.004
p-value	0.02	0.01	0.06	0.45	0.44	0.30	0.56	0.67

[a-b]Means in a column with different alphabets significantly (P < 0.05) differ.
[1]Each value is representative of mean of 5 replicates.
[2]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = Chicory root powder.
[3]Dressed weight, heart, liver, gizzard, spleen, bursa and abdominal fat represented as % of live weight.
[4]SEM = Standard error mean.

Table 3
Effects of dietary supplementation of AGP and different prebiotics on Serum biochemical profile.

Parameters	T1	T2	T3	T4	T5	SEM[3]	*P*-Value
Total protein (g/l)	34.37[b]	36.17[a]	36.55[a]	36.47[a]	36.57[a]	0.11	0.03
Albumin (g/l)	12.20[b]	13.10[a]	13.12[a]	13.20[a]	13.25[a]	0.09	0.02
Globulin (g/l)	21.22[b]	23.30[a]	23.50[a]	23.35[a]	23.62[a]	0.10	0.01
ALT (U/L)	62.52	62.72	62.82	62.85	62.97	0.13	0.18
AST (U/L)	239.7	240.1	241.5	241.7	242.2	0.87	0.21
Uric acid (mg/dl)	5.395	5.397	5.40	5.402	5.404	0.02	0.96
Bilirubin (mg/dl)	0.180	0.182	0.175	0.177	0.174	0.04	0.97
Triglycerides (mg/dl)	125.5[a]	108.2[b]	109.7[b]	107.7[b]	107.5[b]	0.88	0.02
Total cholesterol (mg/dl)	167.7[a]	152.7[b]	152.2[b]	151.7[b]	150.2[b]	0.95	0.01

[a-b]Means in a row with different alphabets significantly (P < 0.05) differ.
[1]Each value is representative of mean of 5 replicates.
[2]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = Chicory root powder.
[3]SEM = Standard error mean.

AGP or prebiotics (P > 0.05).

3.4. Gut morphology

The effects of dietary supplementation of antibiotic and prebiotics as growth promoters on gut morphology of broilers is presented in Table 4. Dietary supplementation of AGP and different prebiotics has results in statistically improved villus height (VH) and Villus height crypt depth ratio (VCR), whereas lower crypt depth (CD) compared to control T1 (P < 0.05). However, no statistical differences were observed among the dietary treatments supplemented with either AGP or different prebiotics (P > 0.05).

3.5. Immune response

The impact of dietary supplementation of antibiotic and prebiotics as growth promoters on antibody titer against NDV in the broilers are presented in Table 5. Immune response, Antibody titer against the NDV was significantly improved (P < 0.05) at day 28, 35 and 42 days of age in the broilers fed diets supplemented with either AGP or different prebiotics compared to control T1. The results, however, remain unaffected among dietary treatments supplemented with either AGP or different prebiotics.

3.6. Gut ecology

The dietary supplementation of either AGP or different prebiotics had an increased *Lactobacillus*, whereas decreased *E. coli* count compared to control T1 (P < 0.05). The results however, revealed no significant variations regarding gut ecology (ileal *E.coli* and *lactobacillus* count) among dietary treatment supplemented with either AGP or different prebiotics (P > 0.05) Table 6.

Table 4
Effects of dietary supplementation of AGP and prebiotics on gut (duodenal) morphology in the broilers Ross308 strain[1].

Treatments[2]	Villus height (μm)	Crypt depth (μm)	Villus height crypt depth ratio
T1	872.2[b]	293.2[a]	2.97[b]
T2	1018.5[a]	230.2[b]	4.42[a]
T3	1021.2[a]	231.3[b]	4.41[a]
T4	1023.7[a]	232.5[b]	4.40[a]
T5	1022.7[a]	229.5[b]	4.45[a]
SEM[3]	1.18	0.95	0.16
p-value	0.02	0.03	0.01

[a-b]Means in a column with different alphabets significantly ($P < 0.05$) differ.
[1]Each value is representative of mean of 5 replicates.
[2]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = Chicory root powder.
[3]SEM = Standard error mean.

Table 5
Effects of dietary supplementation of AGP and different prebiotics on immune response, antibody titer against NDV (Reciprocal of log2) in the broilers[1].

Treatment[2]	Day 28	Day 35	Day 42
T1	4.215[b]	5.105[b]	5.885[b]
T2	4.722[a]	5.725[a]	6.220[a]
T3	4.705[a]	5.705[a]	6.235[a]
T4	4.697[a]	5.701[a]	6.252[a]
T5	4.695[a]	5.692[a]	6.257[a]
SEM[3]	0.05	0.03	0.02
p-value	0.01	0.01	0.01

[a-b]Means in a column with different alphabets significantly ($P < 0.05$) differ.
[1]Each value is representative of average of 5 replicates.
[2]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = Chicory root powder.
[3]SEM = Standard error mean.

Table 6
Effects of dietary supplementation of AGP and different prebiotics on gut ecology in the broilers[1].

Treatments[2]	*E. coli* (CFUlog2/g)	*Lactobacillus* (CFUlog2/g)
T1	7.725[a]	6.462[b]
T2	7.325[b]	6.210[c]
T3	7.310[b]	6.910[a]
T4	7.302[b]	6.915[a]
T5	7.305[b]	6.927[a]
SEM[3]	0.04	0.08
p-value	0.01	0.03

[a-c]Means in a column with different alphabets significantly ($P < 0.05$) differ.
[1]Each value is representative of mean of 5 replicates.
[2]T1 = Control, T2 = Antibiotic growth promoter, T3 = Commercial inulin, T4 = Chicory inulin, T5 = Chicory root powder.
[3]SEM = Standard error mean.

4. Discussion

Many studies have reported the better growth performance of the broilers which were fed chicory root powder supplemented diet (Ding et al., 2021, Gurram et al., 2021) and found statistically improved FI and enhanced BWG and better FCR. However,(Liu et al., 2018) and (Rehman et al., 2007) documented no statistical impacts of inulin supplementation on growth performance in the broilers. The improvement might be due to inulin type fructans in the roots of the chicory that act as substrates for growth and multiplication of beneficial microbes in the gut of broilers and thus enhanced beneficial bacteria proliferation. Phytogenic feed additives including chicory root powder exert a positive influence on protein metabolism and enhances the utilization of nutrients, which lead to an increased dressed weight in the broilers (Alagawany et al., 2017).

Supplementing chicory root powder and its inulin in broiler's diet enlarges the surface area of duodenum and jejunum by increasing the number and length of villi (Gurram et al., 2021) and thus the increased surface area is associated with better utilization and absorption of nutrients that resulted in improved broiler production. Dietary supplementation of chicory root powder improves the microbiota balance in the intestinal tract, lowers the endogenous losses of nitrogen and decreases the secretion of mediators of immune system, ultimately leading to an improved dressing percentage in the broilers (Yusrizal and Chen, 2003). Similarly, (Khoobani et al., 2019) reported a non-statistical differences in the liver and heart weight of the broilers fed chicory root powder supplemented and control diets. Significant reduction of in abdominal fat percentage was reported in the broilers fed diet supplemented with prebiotic.

The serum biochemistry of the broilers fed prebiotics supplemented diet in this study were in accordance with the findings of (Xia et al., 2019). Inulin has statistically reduced serum cholesterol and triglyceride levels in the broilers. Prebiotics including inulin increases the excretion of bile acids which in turn, enhances synthesis of bile from cholesterol in the liver. Furthermore, inulin enhances the production of lactic acid, which in turn, causes disintegration and de-conjugation of bile salts. The de-conjugation reduces the absorption and increases the excretion of bile salts from the body, ultimately leading to reduced cholesterol level in the broilers. The improved synthesis of short chain fatty acids, lowers the pH which reduces the solubility of bile salts in the hind gut and promote their excretion from the body (Ashayerizadeh et al., 2009). Moreover, it is reported that Inulin supplementation enhances globulin and total protein concentration in the broilers (Mátéová et al., 2008), which significantly lowers serum cholesterol and triglycerides level in the broilers and we have found same findings in this study.

The gut morphology of the duodenum revealed statistically enhanced VH and CVR, whereas reduced CD in the broilers fed inulin supplemented diet in this study which are in accordance with the published literature (Ding et al., 2021). The fermentation of inulin in the intestinal tract has resulted in the formation of short chain fatty acids, especially butyrate, which indirectly stimulates the repair and development of tissue in small intestine that lead to an improved gut morphology in broilers. (Rehman et al., 2007).

Inulin enhances the production of antibodies by increasing the serum concentration of globulins in the broilers. An Improvement in antibody titer against NDV was reported in the broilers which were fed inulin supplemented diet (Scanes, 2014). An improvement in antibody titer against NDV was also observed in this study.

Statistically higher ileal Lactobacillus population due to supplementation of inulin in broilers was observed in current study. Chicory root powder exhibits excellent antimicrobial properties, which in turn, are responsible for an improved immune response in the broilers. (Guo et al., 2003).

5. Conclusion

Dietary supplementation of prebiotics such as indigenous chicory root powder and its inulin significantly brought the improvement in productive performance, serum biochemical profile, immune response, gut morphology and in ileal *Lactobacillus* count and reduced the ileal *E. coli* count and serum triglycerides and cholesterol levels without bringing significant changes in serum ALT, AST, Bilirubin and uric acid level in all treatment groups which, revealed no physiological dysfunction in all treatment birds. It may be concluded that indigenous chicory inulin and chicory roots powder may be used as an alternative to antibiotic growth promoter in broiler chicken (Ross308).

CRediT authorship contribution statement

Babar Hilal Ahmad Abbasi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nadeem Rashid:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Rana Muhammad Bilal:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Mohammad Ahmad Wadaan:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition. **Muhammad Farooq Khan:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement:

All the data which was generated in this study has been presented however the raw data supporting the conclusions of this article will be made available by the authors on request.

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Institutional review board statement

All authors here by declared that biological trails under this study were in accordance with the standard ethical protocol frame by the (McGlone, 2020), and has been approved and examined by the ethical committee of CASVAB, UoB, Quetta Pakistan. (Ethical approval number: 180-1/Acad dated 17/9/2018).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103314.

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