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Abstract

The efficacy of *Cichorium intybus* (chicory) leaf extract was determined on day-old chicks (n=150) that were distributed in 15 replicates (10 birds/replicate). These replicates were allotted to five treatment groups such that each group received three replicates. Group A (positive control) was offered commercial ration supplemented with antibiotic growth promoter (Enracin) and coccidiostat (Salinomycine) along with water without any supplementation. Group E (negative control) was offered water without supplementation of antibiotic and coccidiostat. Whereas, group B, C and D were offered water containing different concentrations of chicory leaves extract at 5, 10 and 15 ml/L, respectively, extracted in distilled water at pH 7. Supplementation of chicory leaf extract in drinking water of birds exhibited a significantly ($P < 0.05$) improved weight gain and efficiency of feed utilization. Aspartate Aminotransferase concentration, antibody titer against Newcastle disease, blood glucose, cholesterol level, total leukocyte count (TLC) and hepatoprotective properties were found to be better in treated groups than those of controls. The feed consumption, Alpha Glutamyltransferase, hemoglobin concentration (Hb), Packed Cell Volume (PCV), heterophils count, lymphocytes count and antibody titer against infectious bursal disease remained unaffected. It was concluded that chicory leaf extract can be used an inexpensive, efficient and safe growth promoter in broiler production.

Keywords: *Cichorium intybus*, broilers, growth promoter, immune modulent, hepatoprotectant.

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Introduction

The antibiotic resistance is becoming an alarming condition throughout the world and the consumers are interesting more on organic food. Rapid advances in science and technology, increase in health care cost, change in food laws, attaining wellness through diet are among the factors fueling interest in functional foods (FDA, 2007). Scientific community are now focusing to finding an alternative and/or new substitutes to antibiotic growth promoters and equally effective against the microbes of gastrointestinal tract by enhancing immunity and feed efficiency. During the last decade poultry industry showed about 138% growth, which revolves around genetic improvement, disease control, high tech poultry production under environmentally controlled housing, processing and value addition (Economic Survey of Pakistan, 2012). The supplementation of antibiotics in diet has been a common practice in poultry feed industry to promote the chicken growth, which usually costs 0.029 US dollars/Kg feed, equivalent to one billion rupees per year (Bhatti, 2011 and Ahmad, 1993). However, there is great concern regarding the development of antibiotic resistance to pathogenic bacteria in birds, hence the European Union has banned the use of antibiotics as growth promoters in animal feed since 2006 (Castanon, 2007).

The herbal plants as natural feed additives are being used in poultry rations to enhance production performance (Abaza *et al.*, 2008). Different parts of these plants such as moringa (Ansari *et al.*, 2008; Ayssiweide *et al.*, 2011), neem (Subripriya and Nagini, 2005) and chicory (Sarwar, 2013) have been used in poultry as growth promoters but with

varying results, and these extracts are also used in drinking water for the same purpose. *Cichorium intybus* (Chicory) is a herbal medicinal plant having mucus and resin as active ingredients. *Cichorium intybus* is known to have antibacterial, anti-inflammatory, digestive, bitter tonic, diuretic, anti-hypercholestermic and laxative properties without presenting any side effects (Kalantari and Rastmanesh, 2009). There is lack of considerable literature where the aforementioned herbal plant has been used as herbal growth promoter in poultry; hence in this study we aimed to use the aqueous leaves extract of this herbal plant as growth promoter, hepatoprotectant and immune modulant in broilers.

Materials and Methods

The research project was conducted at the Poultry Research Centre, University of Agriculture, Faisalabad. Fresh chicory (*Cichorium intybus*) leaves were collected, dried under shade and sliced without washing. Thereafter it was mixed in distilled water at 2 g/100 ml and then extracted at 80°C for 3 hours, according to Amer *et al.*, (Amer *et al.*, 2010). Day-old chicks (n=220) were purchased and reared in groups for seven days as the adaptation period. At 8th day of age, these chicks were individually weighed and 150 chicks of middle weight were selected for experimental purpose. The selected chicks were then distributed in 15 replicates (10 birds/replicate) and were further allotted to five treatment groups (A, B, C, D and E) such that each group received three replicates. Two commercial rations were fed ad libitum to the birds i.e. starter from 0-3 and finisher from 4-5 weeks of age, along with watering and feeding plan, table 1.

Table 1: Watering/treatment plan for the experimental birds.

Group	Treatment
Group A (Positive control)	Fresh water+diet supplemented with Enracin at 100 g/ton, as antibiotic growth promoter and Salinomycin at 500 g/ton as coccidiostat
Group B	Fresh water+Extract of chicory leaves at 5 ml/ liter of water
Group C	Fresh water+Extract of chicory leaves at 10 ml/ liter of water
Group D	Fresh water+Extract of chicory leaves at 15 ml/ liter of water
Group E (Negative control)	Fresh water+diet without addition of antibiotic growth promoter or any coccidiostat

Managerial conditions like ventilation, relative humidity, floor space and light were also kept same during rearing of the birds. Data regarding initial body weight, final body weight and feed intake was recorded to calculate feed utilization efficiency and weight gain, and the water consumption of each replicate was also recorded on daily basis. All birds were vaccinated against infectious bursal and Newcastle disease. Serum samples (2 birds/replicate) were collected 10 days post vaccination *i.e.* 28th and 32nd days of age to check immune response against the respective diseases (MAFF, 1984). At the end of the experiment, the blood samples (5ml each) were collected using anticoagulant from two birds/replicate to determine the packed cell volume and hemoglobin by hematology analyzer (Sysmex KX- 21). Furthermore, the cholesterol and liver enzymes ALT, AST and ALP were also determined from the private laboratory by using kit method with analyzer (Medonic- CA 620) from two serum samples from two birds/replicates, and then statistical analysis was performed (Steel *et al.*, 1997).

Results and Discussion

Table 2: Effect of supplementing different levels of chicory leaf extract in water on body weight, feed consumption, water intake and feed conversion ratio.

Variables	Treatment					S.E
	A (+ve Control)	B (5ml/liter)	C (10ml/liter)	D (15ml/liter)	E (-ve Control)	
Initial body weight (g)	169	169	168	168	167	0.4
Final body weight (g)	1853 ^b	1955 ^b	1997 ^{ab}	2193 ^a	1817 ^b	43.0
Weight gain (g)	1684 ^b	1786 ^b	1830 ^{ab}	2025 ^a	1651 ^b	42.8
Feed consumption (g)	3284	3238	3248	3466	3332	42.8
Feed conversion ratio	1.95 ^b	1.81 ^a	1.77 ^a	1.72 ^a	2.02 ^b	0.034
(feed consume/weight gain)						
Water consumption (ml)	8837	8660	8870	9026	9427	1.1

Values within the same row which have different superscripts are significantly different ($P < 0.05$).

Chicory locally referred as “kasni” belongs to family “Asteraceae”, subfamily “Cichorioideae” of herbs. All parts of chicory *i.e.* Leaves, flowers, seeds and roots have medicinal properties (David and Sears, 2007). In our present study, the leaf extracts of this plant showed growth promoter, hepatoprotectant and immune modulent properties in the broilers, and can be used as an alternative to antibiotic growth promoters in poultry industry.

Effect on Performance

Analysis of variance of data showed significant ($P < 0.05$) effect of chicory leaf extract (0, 5, 10 and 15ml/L) on weight gain and feed conversion ratio (FCR), however, it did not show any significant ($P > 0.05$) effect on feed consumption of broilers. The group D that was offered water supplemented with chicory leaf extract at 15 ml/L, was found to be significantly higher. Whereas, the lowest body weight gain was observed in the birds of treatment group E (negative control). Significant ($P < 0.05$) improvement in FCR of the bird was observed in treatment group D which were offered water supplemented with chicory leaf extract at 15ml/L. The poorest FCR was observed in the birds treatment group E (negative control), table 2.

These findings are consistent with the results of by Liu *et al.*, (2011) and Asia and Gultekin (2012) who have reported a non-significant effect of chicory extract on feed consumption in broiler. However, results of the present study, the findings

of Safamehr *et al.*, (2013) and Behboud *et al.*, (2011) exhibited increased feed consumption in broilers fed diets supplemented with chicory leaf extract. Analysis of variance of the data exhibited significant ($P < 0.05$) effect on FCR of the birds.

Significant ($P<0.05$) improvement in FCR of the bird was observed in treatment group D which were offered water supplemented with chicory leaf extract, @ 15ml/L. The poorest FCR was observed in the birds treatment group E (negative control). Probably improvement in weight may attributed due to better mucosal growth, villus height and width, crypt depth and ratio of villus height to crypt depth, which might have resulted in increased absorption of nutrients, ultimately causing increase in body weight (Awad *et al.*, 2011; Zyl *et al.*, 2010). These findings are compatible with those observed by Safamehr *et al.*, (2013) and Silva *et al.*, (2011) who reported increased body weight gain in broilers offered water containing chicory leaf extract.

Better FCR in birds given water supplemented with chicory leaf extract may probably be due to its complex carbohydrates as oligofructones and oligosaccharides which might have resulted in efficient meat production (Sangoh and Park, 2012). The improvement in digestibility of nutrients and reduction in microbial load in the GIT (Anderson *et*

al., 2000) are other possibilities that might resulted in better growth of birds ultimately leading to better FCR. These findings are in agreement with the results of Silva *et al.*, (2011), however, the findings of Asia and Gultekin (2012) and Liu *et al.*, (2011) were contrary in which they showed that FCR of broilers treated with chicory was not improved. Difference in the results of these studies may be due to the difference in the forms of chicory (water extract vs pulp powder) used in these experiments.

Effect on liver function

Liver enzymes are most sensitive biochemical markers employed in diagnosis of hepatic dysfunction in broiler production (Johnkenedy *et al.*, 2010). Analysis of variance of data did not reveal any significant ($P<0.05$) effect on Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) concentrations, however, Alpha Glutamyltransferase (ALP) concentration was significantly affected by chicory extract, table 3.

Table 3: Effect of supplementing different levels of chicory leaf extract in drinking water of broilers on liver enzymes (ALT, AST and ALP).

Variables	Treatment					S.E
	A	B	C	D	E	
	(+ve Control)	(5ml/L)	(10ml/L)	(15ml/L)	(-ve Control)	
ALT (U/ml)	38.3	30.7	27.7	27.3	31.0	1.57
AST (U/ml)	226.0	204.3	221.3	198.7	202.3	6.41
ALP (U/ml)	2387 ^a	2349 ^{ab}	2197 ^{bc}	2129 ^c	2455 ^a	44.71

Values within the same row which have different superscripts are significantly different ($P<0.05$).

Marzouk *et al.*, (2011) reported that ALT was not significantly affected in broilers treated with chicory leaf extract; however, Noreen (2009) reported a significant effect of supplementing chicory leaf extract on ALT and AST concentration in broilers. On the other hand, findings of Abd El-Mageed (2011); Hassan and Yousef (2010); Mishra and Kamal, (2009) have showed reduction in concentration of AST in rats when fed chicory root powder. Sultan *et al.*, (2009) also reported reduction in the concentration of ALP in rats. It is quite possible that chicory extract might have induced similar effect on liver health of the birds causing

enhancement of endogenous antioxidant defense status, ultimately resulting in reduced ALP concentration.

Effect on Blood Profile

Analysis of variance of data revealed that addition of different levels of chicory leaf extract exhibited a significant ($P<0.05$) effect on blood glucose, total cholesterol level and total leukocyte count (TLC) of birds, table 4, whilst, hemoglobin concentration (Hb), Packed Cell Volume (PCV), heterophils count and lymphocytes count were not affected significantly ($P>0.05$), table 5.

Table 4: Effect of supplementing different levels of chicory leaf extract in water on blood hematological mean values of glucose and cholesterol of broilers.

Variables	Treatment					S.E
	A	B	C	D	E	
	(+ve Control)	(5ml/L)	(10ml/L)	(15ml/L)	(-ve Control)	
Blood Glucose (mg/dl)	160.67 ^a	146.33 ^{ab}	139.00 ^b	115.00 ^c	150.67 ^{ab}	4.48
Total Cholesterol (mg/dl)	153.50 ^a	135.67 ^b	130.83 ^{ab}	117.17 ^c	142.33 ^{ab}	3.67

Values within the same row which have different superscripts are significantly different (P<0.05).

Table 5: Effect of supplementing different levels of chicory leaf extracts in water on blood hematological mean values of Haemogram (Hb and PCV) and Leukogram (TLC and DLC) of broilers.

Variables	Treatment					S.E
	A	B	C	D	E	
	(+ve Control)	(5ml/L)	(10ml/L)	(15ml/L)	(-ve Control)	
Hb (g/dl)	9.07	9.33	9.78	10.58	8.63	0.248
PCV (%)	30.90	30.93	32.00	33.58	27.83	1.77
TLC (per cu. mm)	2510.0 ^b	2911.7 ^{ab}	3716.7 ^{ab}	6783.3 ^a	4533.3 ^{ab}	5.94
Heterophils (%)	42.50	45.17	45.00	61.50	48.17	3.886
Lymphocytes (%)	115.00	109.67	110.00	77.00	103.67	7.773

Values within the same row which have different superscripts are significantly different (P<0.05).

The results showed a significant (P<0.05) decrease in the blood glucose and total cholesterol level in birds of treatment group D which were offered water supplemented with chicory leaf extract at 15 ml/L. The decreased level of blood glucose in birds may be probably due to the presence of inulin in extract that acts as anti-diabetic agent (Sangoh and Park, 2012; Kapoor and Huang, 2006) in broilers. Whereas the decrease in blood cholesterol level might be due to the properties of chicory leaf extract to stimulate lactic acid producing bacteria (Avci *et al.*, 2006) secreting the hydrolase enzyme (Hinton *et al.*, 2000) that in-turn converts bile salts into deconjugated bile acids and ultimately resulted in the reduced serum cholesterol level (Safamehr *et al.*, 2013). These findings are consistent with Velasco *et al.*, (2012),

Behboud *et al.*, (2011) and Rezaei *et al.*, (2010), and also in the agreement with the findings of Sarwar (2013) where supplementation of chicory leaf extract extracted at different pH levels did not show any significant effect on PCV and Hb, values of lymphocytes and heterophils count however had the significant effect on TLC count in broilers.

Immune Response

Analysis of variance of data revealed that addition of different levels of chicory leaf extract in drinking water exhibited significant effect (P<0.05) on ND titer. Birds of treatment group D, showed higher antibody titer. However, immune response against infectious bursal disease (IBD) was not affected by different levels of chicory leaf extract (P>0.05), table 6.

Table 6: Effect of supplementing different levels of chicory leaf extract in drinking water on geometric mean values of the antibody titers against Newcastle disease and Infectious Bursal disease of broilers.

Variables	Treatment					S.E
	A	B	C	D	E	
	(+ve Control)	(5ml/L)	(10ml/L)	(15ml/L)	(-ve Control)	

Titers against ND	66.33 ^b	139.00 ^{ab}	187.33 ^a	199.33 ^a	114.33 ^{ab}	16.912
Titers against IBD	80.33	106.67	112.00	135.67	67.00	11.046

Values within the same row which have different superscripts are significantly different (P<0.05).

The higher level of antibody titer against ND in birds using chicory leaf extract may be due to its antimicrobial effect. Another possible reason might be the increased activity of neutrophils in blood after vaccination, which could play a major role to boost up the immunity (Guo *et al.*, 2003). Similar results have been observed by Huff *et al.*, (2003) and Sarwar (2013) as they reported increased immune-modulatory effect against ND and IBD in broiler. However, contrasted results may be due to the difference in way of supplementation of chicory (comparative vs. alone) in these trials.

Conclusions

Supplementation of different levels of chicory leaf extract as growth promoter revealed better performance in broilers in terms of body weight gain, efficiency of feed utilization, stabilization of serum metabolites with better immune response and hepatoprotective properties, which ultimately increased profit margin.

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