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THE EFFECT OF AQUEOUS HERBAL INFUSION IN DRINKING WATER ON BROILER PERFORMANCE AND INTESTINAL MICROFLORA STATUS

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ABSTRACT

An experiment was conducted to evaluate the effect of herbal infused water on the performance of broiler and intestinal microbial population characteristics. Two hundred and eighty (n=280) one day old broiler chicks were randomly divided into seven groups of forty (40) chicks. Each group was further divided into four replicates. Two culinary seeds and a medicinal herb was selected as water supplement additives and 5% w/v infusion was prepared and added @ 20 and 40 ml/L of the drinking water, respectively. The seventh group was treated as control and offered plain water. Isonitrogenous and isocaloric feed was offered to all the treatment groups and water was provided *ad libitum*. The broiler production indices, relative organ weights and microbial counts were observed. It was observed that the performance indices significantly (P<0.05) improved by the infused supplements. No significant (P>0.05) difference in relative weight of the intestine was observed. Supplementation of infused water altered the intestinal microbial counts (P<0.5).

Keywords: broiler performance, herbal infusion, bacterial enumeration.

INTRODUCTION

The use of plants as medicine have been the basis for medical treatments through much of human history, and till date herbs and their products are considered as natural remedies for curing diseases (Alfatemi *et al*., 2014). In recent times, the use of herbal medicine in different forms have been gaining acceptance. Since 2006, post antibiotic ban over the use of antibiotics as feed additives (Windisch *et al*., 2008) by the European Union (EU) resulted in increased disease outbreaks in commercial poultry and this urged the scientific community to find and develop an alternative to antibiotic feed additives without compromising the production parameters. Consequently, extracts from culinary and medicinal herbs are emerging as a potential safer source of treatment and their positive effects have been acknowledged (Denli *et al*., 2004; Wati *et al*., 2015).

Cumin (*Cuminum cyminum*) is an annual plant and the seeds are a rich source of proteins (19.8%), oils (8.07%) and carbohydrate (22%), used for both culinary and therapeutic purposes (Khosravifar *et al*., 2014). The extract from cumin has antioxidant, antimicrobial, digestive enzyme stimulant, gut environment modifier and effects on the flow rate of digesta (De *et al*., 2003). The main constituents of essential oil are β-pinene (16.2876%), Limonene (0.2307%), α-terpinene (5.6551%), p-cymene (15.5464%), 1, 8-cineol (2.2421%,) cumin aldehyde (25.8749%) and cuminyl alcohol (30.023%) (Chaudhry *et al*., 2012).

Achillea wilhelmcii C. Koch (Vernacular name: Zawal) belonging to family Asteraceae is a perennial medicinal herb. It has a wide range of bioactive activities like antacid, antispasmodic (Ali *et al*., 2014) antihyperlipidemia, antihypertensive and antioxidant effects (Niazmand *et al*., 2011; Asgary, 2000). It is a rich source of sesquiterpenes, lactones, flavinoids and monoterpoids (Alftemi *et al*., 2014).

Fennel (*Foeniculum vulgare*) belongs to family Apiaceae. It is commonly used as medicinal plant and aromatic plant as flavoring agent. Different *in vitro* and *in vivo* experiments have convincingly confirmed the ability of *F. vulgare* to exhibit antifungal, antibacterial, antioxidant, antithrombotic and hepatoprotective, cytoprotective diuretic and digestive carminative activities (El-Soud *et al*., 2011; Pradhan *et al*., 2008) .

Different types of studies have been carried out using their essential oils and alcoholic extracts suggesting variable results. However, their use as infusion in broiler chicken is scanty. Therefore, this study was carried out to evaluate the effect of these herbs infused in drinking water on the performance of broiler chicken.

MATERIALS AND METHODS

Experimental layout

Two hundred and eighty (n=280) day old Hubbard broiler mixed sex chicks were randomly divided in seven treatment groups (40 chicks each) with four replicates of ten chicks in each treatment, respectively. Each replicate was taken as an experimental unit. The treatment were (C20) cumin seed infusion at 20 ml/L, (C40) cumin seed infusion at 40 ml/L, (A20) Zawal infusion at 20 ml/L ,(A40) Zawal infusion at 40 ml/L, (F20) Fennel seed infusion at 20 ml/L, (F40) Fennel seed infusion at 40 ml/L, and (C) Control; offered drinking water without herbal infusion.

Feeding and management

For the purpose of this experiment two phase feeding system (Starter and finisher) was chosen. An

isocaloric and isonitrogenous starter and finisher feed was formulated, respectively. The starter diet was given up to day 21 and the finisher diet was fed from day 22 to 42 (Table-1).

Table-1. Composition and calculated analysis of the starter and finisher diet.

Ingredients	**Starter diet** (%)	**Finisher diet** (%)
Corn	52	58
Wheat bran	2.5	2.0
Soybean meal	18	18
Canola meal	5.0	4.0
Cotton seed meal	4.0	3.0
Peas	10	7.6
Corn gluten 60%	3.0	00
Oil	3.1	4.0
Lysine	0.2	0.2
Methionine	0.3	0.3
Vitamin mineral premix[a]	2.0	2.0
Total	100	100
Calculated Analysis		
Metabolizable Energy Kcal/kg	2992	3103
Crude Protein (%)	21	19.1
Ether extract (%)	6.2	6.8
Crude Fiber (%)	4.3	4.1
Lysine (%)	1.1	1.0
Methionine (%)	0.5	0.4
Calcium (%)	1.0	0.8
Phosphorus (%)	0.5	0.4

Vitamin mineral pre mix provides per kg of diet: vitamin A, 9,000 IU; D3, 2,000, IU; E, 18 IU; B1, 1.8 mg; B2, 6.6 mg B2,; Niacin, 10 mg; Pantothenic acid, 30 mg; B6, 3.0 mg; Folic acid, 1 mg; B12, 1.5 mg; K3, 2 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; biotin, 0.14 mg; choline chloride, 500 mg; Mn, 100 mg; Zn, 85 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.

The birds were reared on littered floor pans and rice husk was used as the littering material. 24 h fluorescent lighting was provided. The feeding was controlled by measuring the amount of feed given to the broiler chicken at morning and evening. *Ad- libitum* water was provided in five (5) liter vessels and optimal standard conditions were maintained in the house. Vaccination against Newcastle disease, Infectious Bronchitis and Infectious Bursal disease was carried out as per local recommendations.

Daily feed intake and weekly body weights (per replicate) were observed. From the feed intake, total metabolizable energy (ME) and Total protein intake (TPI) were calculated and used to calculate the energy efficiency ratio (EER) and protein efficiency ratio (PER). The EER was calculated as gram weight gain per 100 kcal ME intake; while the PER was calculated as weight gain in grams divided by protein intake in grams. For body characteristics and visceral organs weights determination, one bird was randomly selected from each replicate of all the treatments on day 42, respectively. The birds were weighed and euthanized by severing the jugular vein. Thereafter the carcass and organ characteristics were determined.

Microbial enumeration

One gram digesta from the intestine (Ileum) was aseptically collected in falcon tubes with 2 ml PBS solution, mixed thoroughly using sterile glass rod to make a volume of 10 ml. For bacterial enumeration, tenfold serial dilution from 10^{-2} to 10^{-8} was prepared and cultured on respective plates. Nutrient agar (OXOID) plates were used for total aerobe and MacConkey agar (OXOID) for coliform bacterial count. The plates were incubated at 37°C for 24 h. Lactic acid bacterial count was observed on MRS agar (OXOID) plates for 48 h of incubation. All the aliquots were used in duplicate and the numbers of colony forming units (CFU) were presented as logCFU/g of digesta (Zhang *et al*., 2003).

Preparation of herbal infusion

The seeds and herbs were purchased from the market and identified by a Botanist. The materials were cleaned using sieve and later rinsed gently to remove any dust particles or any other undesirable object. After drying, it was coarsely grinded and preserved in an air tight container. Herbal infusion at 5% was prepared by weighing 50 g of the respective seeds and herbs and placed in glass vessels, adding one liter of distilled boiling water and capped immediately for a minimum of 6 h to avoid any loss of volatile component and kept at room temperature in the dark; thereafter used as infused water. The prepared infused water was used for maximum of three days and thereafter freshly prepared as required.

Data analysis

The data collected was analyzed using one way analysis of variance (ANOVA) in SPSS-16 software for windows. The means were compared using Duncan's multiple range tests.

RESULTS

The production performance indices for the starter and finisher phases are summarized in Table-2.

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Table-2. Effect of herbal infusion on performance indices in starter and finisher phase (Mean±SE).

Group	ADG		FE		EER		PER	
	0-21d	22-42d	0-21d	22-42d	0-21d	22-42d	0-21d	22-42d
C20	32.66±0.22^{a}	73.23±0.23^{a}	1.38±0.008^{d}	1.89±0.01^{d}	414.09±2.6^{d}	586.86±3.4^{d}	3.44±0.022^{c}	2.78±0.016^{a}
C40	32.91±0.17^{a}	73.59±0.32^{a}	1.39±0.011^{d}	1.89±0.005^{cd}	415.58±3.4^{d}	588.86±1.66^{cd}	3.43±0.029^{ab}	2.77±0.007^{ab}
A20	32.45±0.24^{a}	71.52±0.85^{b}	1.42±0.016^{cd}	1.93±0.015^{bc}	424.85±5.1^{cd}	601.23±4.86^{bc}	3.36±0.039^{cb}	2.71±0.02^{bc}
A40	32.66±0.24^{a}	72.40±0.38^{ab}	1.40±0.015^{d}	1.94±0.015^{b}	420.0±4.77^{d}	604.47±4.9^{b}	3.39±0.038^{ab}	2.69±0.02^{c}
F20	31.45±0.14^{b}	71.07±0.56^{b}	1.46±0.009^{b}	1.97±0.013^{b}	437.16±2.8^{b}	611.12±4.29^{b}	3.26±0.021^{d}	2.67±0.01^{c}
F40	31.73±0.18^{b}	71.44±0.60^{b}	1.45±0.002^{bc}	1.96±0.017^{b}	434.24±0.82^{bc}	608.61±5.51^{b}	3.28±0.006^{cd}	2.68±0.02^{c}
C	30.04±0.20^{c}	68.33±0.44^{c}	1.54±0.009^{a}	2.05±0.015^{a}	461.62±2.71^{a}	636.05±4.93^{a}	3.08±0.018^{e}	2.56±0.02^{d}
Total	31.98±0.19	71.65±0.35	1.43±0.01	1.95±0.01	429.64±3.18	605.31±3.27	3.32±0.024	2.69±0.01

*Means in same column with different superscripts are significantly different (P<0.05)

Initially, the weights of the all treatments were found insignificant. Supplementation of the herbal infused water in drinking water showed improvement in ADG, FE, EER and PER in both starter and finisher phase (P<0.05). The highest ADG observed during the starter phase was noted in C20, C40 and A40 groups followed by F40 (P<0.05) compared to the control and fennel treatment. The supplementation of fennel also exhibited better ADG to the control group. Same trend continued during the finisher phase and the cumin infused water supplemented group had better ADG along with A40 group (P<0.05). Better energy efficiency ratio was observed in treatment groups C20, C40, A20 and A40 respectively, as compared to the control, F20 and F40 group (P<0.05); however there was no significant difference (P>0.05). The highest energy efficiency was observed in treatment groups C20 (414.09) and C40 (415.58) as compared to the C group which consumed 461.62 Kcal/100 g body weight gain (P<0.05). Similarly, the efficiency of the birds to convert diet protein into body mass was also affected by the infused water supplementation (P<0.05). During the finisher phase, 586.86 Kcal/100 g body weight gain was observed in group C20 compared to group C which consumed 636.05 Kcal. Similarly, better utilization of protein shown by group C20 resulted in 8.59% better utilization (P<0.05) of protein provided in the feed compared to group C. While in groups A and F, no significant difference (P>0.05) was observed.

The supplementation of the herbal water treatment groups exhibited better performance in terms of weight gain (P<0.05) during finisher phase and as a whole during the experiment (Table-3).

Table-3. Effect of herbal infused drinking water on the Performance indices day 0-42 (Mean±SE).

Groups	WG	FI	FE	ADG	EER	PER
C20	2223±9.5^{a}	3860±31.96^{b}	1.73±0.01^{d}	52.94±0.226^{a}	533.90±3.08^{d}	2.95±0.017^{a}
C40	2236±5.19^{a}	3895±20.88^{ab}	1.74±0.005^{d}	53.24±0.123^{a}	535.65±1.67^{d}	2.94±0.009^{a}
A20	2183±14.18^{bc}	3879±14.18^{ab}	1.78±0.007^{c}	51.98±0.337^{b}	546.42±2.14^{c}	2.88±0.011^{b}
A40	2206±9.39^{ab}	3927±16.89^{ab}	1.78±0.011^{c}	52.53±0.223^{ab}	547.45±3.63^{c}	2.88±0.019^{b}
F20	2153±11.19^{c}	3906±7.11^{ab}	1.81±0.088^{b}	51.26±0.266^{c}	558.07±2.53^{b}	2.82±0.012^{c}
F40	2166±14.63^{c}	3911±5.32^{ab}	1.80±0.011^{bc}	51.58±0.348^{c}	555.32±3.55^{bc}	2.84±0.017^{bc}
C	2065±11.78^{d}	3917±6.42^{a}	1.89±0.012^{a}	49.1864±0.28^{d}	583.15±3.85^{a}	2.70±0.017^{d}
Total	2176±1086	3899±7.05	1.791±0.01	51.8216±0.258	551.42±3.13	2.86±0.015

*Means in same column with different superscripts are significantly different (P<0.05)

Supplementation of cumin seed infused water at both inclusion rate showed highest weight gain; which is significantly (P<0.05) better than all other groups; however there was no significant difference from treatment group A40 (P<0.05). Supplementation of Zawal and fennel seed infusion also revealed better weight gain performance as compared to the control group (P<0.05). The feed efficiency and ADG was also improved by the herbal infused water as compared to the control group. Supplementation of cumin infused water approximately improved FCR by 13.22% and ADG by 7.62%. Similarly, other treatments showed significantly (P<0.05) improved feed efficiency and ADG from the control group. However, no significant difference between A20, A40,

F20 and F40 was noted. Herbal infusion improved EER and C20 consumed 9.22% and group C40 consumed 8.86% less energy as compared to the control group (P<0.05). In the same way, Madran and fennel had shown better results as compared to the control group.

The effect of herbal infused water supplementation had significant effect on the relative carcass and visceral organ weight (P<0.05). However, no significant difference in the relative weight of intestine and lymphoid organs was observed (P>0.05) in the experiment between infusion (Table-4).

Table-4. Effect of herbal infused drinking water on the relative organ weights on day 42 (Mean±SE).

Treatments	Carcass	Liver	Gizzard	Heart	Spleen	Bursa	Intestine
C20	69.77±0.255^{abc}	2.14±0.02^{b}	2.72±0.022^{b}	0.603±0.012^{abc}	0.2±0.01	0.142±0.015	4.14±0.051
C40	70.40±0.185^{ab}	2.13±0.024^{b}	2.75±0.018^{b}	0.582±0.004^{c}	0.188±0.004	0.14±0.012	4.16±0.043
A20	69.19±0.546^{bc}	2.16±0.032^{ab}	2.81±0.017^{b}	0.595±0.002^{bc}	0.205±0.006	0.15±0.011	4.16±0.025
A40	68.51±0.561^{c}	2.15±0.023^{ab}	2.79±0.031^{b}	0.592±0.011^{bc}	0.202±0.004	0.14±0.017	4.11±0.053
F20	70.96±0.537^{a}	2.24±0.026^{a}	2.76±0.024^{b}	0.620±0.009^{abc}	0.210±0.009	0.155±0.028	4.19±0.057
F40	70.72±0.454^{a}	2.12±0.043^{b}	2.78±0.011^{b}	0.607±0.004^{ab}	0.217±0.004	0.157±0.017	4.23±0.03
C	69.46±0.531^{abc}	2.16±0.017^{ab}	2.94±0.08^{a}	0.625±0.011^{a}	0.225±0.01	0.152±0.017	4.14±0.07
Total	69.86±0.220	2.16±0.011	2.79±0.017	0.602±0.004	0.206±0.003	0.148±0.016	4.16±0.017

*Means in same column with different superscripts are significantly different (P<0.05)

The microbial enumeration of the digesta from Ileum suggests that the infused water had effect on the ileum microbial status. Aerobe and coli form bacterial count was observed to have decreased with the water infused additives (P<0.05). Lowest aerobe bacterial count was noted in C40, while the highest count was observed in treatment group C. There was no significant difference observed between other infusion treatments (P>0.05). The coli form count in the group C was highest, while C40 and A40 had the lowest count, followed by the C20 (P<0.05). The supplementation of the infused water also caused modification in the lactic acid bacteria and the present results suggests that herbal infused water did not support the lactic acid bacteria (Table-5).

Table-5. Effect of herbal infused drinking water on bacterial population in Ileum on day 42 (Mean±SE).

Treatments	Aerobe	Coliform	Lactic acid
C20	4.0±0.2^{b}	5.25±0.32^{c}	6.87±0.23^{a}
C40	3.62±0.31^{b}	5.00±0.20^{c}	6.25±0.14^{abc}
A20	3.87±0.12^{b}	5.50±0.20^{bc}	5.25±0.52^{d}
A40	4.25±0.14^{ab}	5.00±0.20^{c}	6.00±0.20^{d}
F20	4.25±0.14^{ab}	6.25±0.14^{a}	5.50±0.20^{cd}
F40	4.12±0.12^{b}	6.00±0.20^{ab}	5.25±0.25^{d}
C	4.87±0.31^{a}	6.12±0.23^{ab}	6.62±0.12^{ab}
Total	4.14±0.09	5.58±0.12	5.96±0.14

*Means in same column with different superscripts are significantly different (P<0.05)

DISCUSSIONS

Different forms of herbal additives are tested for their efficacy in the broiler production (Onu, 2012; Javed *et al*., 2009; Chaudhry *et al*., 2012; Hashemi *et al*., 2014).The use of herbal infused water additive in the present study improved weight gain and other performance parameters. The results of herbal additive had been inconsistently reported. Authors from different parts of the world reported significant improvement in the performance of broiler supplemented phytogenic feed additive (Al-Kassi and Witwit, 2010; Ertas *et al*., 2005; Wati *et al*., 2015). However, contrary reports suggested no positive effects of herbal additives (Cross, 2007; Sadeghi *et al*., 2012); whereas, Denli *et al*. (2004), Javed *et al*. (2009) and Mohammad and Abbas (2009) reported increased production performance.

In this study, increased feed efficiency and daily weight gain was observed, suggesting better utilization of the nutrients supplied and positive effects of the treatments applied. Goodarzi and Nanekarani, (2014) and Durrani *et*

al. (2007) used onion and mint extract in drinking water and observed increased feed efficiency comparable to antibiotic feed additive.

The enhanced production indices of broilers in this trial show the nutrient sparing effect of the herbs and modification in the gut microbial population characteristics. The mode of action through which the plant and their products exert their effects might be the gut environment modification (Windisch *et al*., 2008). Cumin seeds reported have antimicrobial activity and it increases digestive secretion. Furthermore, cumin is a good source of energy and protein and it contains trace minerals (Kaur and Sharma, 2012). The supply of additional nutrients with other bioactive compounds might have resulted in highest weight gain and feed efficiency. The oil from *A. wilhelmsii* contains higher percentages of Carvacrol (22.49%), Linalool (12%), Cineol (11.42%) and Camphene (8.31%), along with other compounds such as Phenols and flavonoids (Alftemi *et al*., 2014). The camphor and carvacrol is considered safe for the health (Khani and Asgari, 2012) and the presence of other bioactive compounds might have caused the reduction in the microbial count and sparing nutrient. Fennel seeds are reported to have higher percentages of fatty acids and in addition, it contains trans-anethol (16.81%) and estragole (47.2%) in essential oil and these compounds are suggested to have antifungal, antispasmodic and digestive stimulating effects (Mohammad and Abbas, 2009). The presence of bioactive compounds in the gut might have modified the gut environment and resulted in decreased microbial count, sparing nutrients and their availability, hence increased efficiency.

CONCLUSIONS

In conclusion, it can be suggested that aqueous extract of Cumin, Zawal and Fennel improved broiler performance indices and have the potential to be used as water supplement additive, however for a precise dose concentration, further work is needed.

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