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Original Article

Studies on Pharmacological Screening of Some Medicinal Plants for their Antimicrobial and Feed Additives

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ABSTRACT

Antibiotics such as avoparcin, bacitracin, lincomycin, penicillin-G -procaine, chlortetracycline and virginiamycin promote growth because of an effect on the microflorain the gastrointestinal tract. Throughout the world, the use of these antibiotics as dietary growth promoters in poultry diets differ dramatically. Sweden now allows no use of antibiotics for growth promotion purposes whereas the USA uses a wide range of antibiotics.Antibiotic growth promoters (AGP) have made a tremendous contribution to the profitability of the poultry industry. However, as a consequence of the increasing concern about the potential public health problems because of antibiotic resistant strains of bacteria, poultry nutritionists are being challenged to develop an alternative for AGP. If herbal alternative to AGP can be found, poultry nutritionists could formulate a ration that would meet the needs of the commercial broiler industry without using AGP. This study showed that herbal extracts, particularly a cinaamon extract, when added in the broiler diet, may have a similar effect as that of AGP. This study also showed that adding thyme in broiler diets may decrease body weight significantly compared to the diet with AGP, while not affecting the feed consumption. This result is helpful for further research on reducing body weight in the obese subjects.

Keywords: Avoparcin, bacitracin, lincomycin, cinaamon extract.

1. INTRODUCTION

Antimicrobial resistance in zoonotic enteropathogens including Salmonella, Escherichia coli (E. coli), and Enterococci in food animals is of special concern to human health because these bacteria are likely to transfer from the food chain to. As a consequence, the European Commission banned for commonly used feed antibiotics monensin sodium, salinomycin sodium, avilamycin, flavophospholipol. To minimize this resistance, different agencies including the Centers for Disease Control & Prevention (CDC), Atlanta,

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USA are in favor of banning these feed antibiotics in the USA[1].

The phasing out of antibiotic growth promoters (AGP) will affect the poultry and animal industry at large. To minimize the loss in growth, there is a need to find alternatives to AGP. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immunostimulant and other management practices[2].

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial. More recently, medicinal plant extracts were developed and proposed for use in food as natural. However, little or no work has been done on the effects of plant extracts on body weight and performance in poultry. The present study was conducted to determine the effect of different medicinal plant (herbs) extracts in broiler diets as a possible alternative to antibiotic feed additives[3].

2. MATERIAL AND METHODS

Selection of medicinal plants for this study

Seven medicinal plants including Zinziberofficinale rhizomes (Ginger), Cinnamomum cassia bark (Cinnamon), Piper nigrum fruits (Black Pepper), Curcuma longa rhizomes (Turmeric), Thymus vulagaris leaves (Thyme), Laurusnobilis leaves (Bay leaf), Syzgiumaromaticumfruits (Clove), were utilized in this studies. These plants have previously been reported to have antibacterial activity against different bacterial strains[4].

Preparation of Extracts

Grinding of the selected plant materials

After drying at 370C for 24 h the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to sunlight was avoided to prevent the loss of active components[5].

Extraction of selected plant material powder by maceration method

One liter of an 80 % ethanol extraction fluid was mixed with 200 g of powdered plant material. The mixtures were kept for 2-5days in tightly sealed vessels at room temperature at 220C, protected from sunlight, and mixed several times daily with a sterile glass rod. This mixture is filtered through muslin cloth and the residue, if necessary, adjusted to the required concentration (500 ml of 80% ethanol for the residue of 200 g of powdered plant material) with the extraction fluid for further extraction. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible[6].

The extracted liquid was subjected to rota-evaporatoration or water bath evaporation (Precision shaking water bath, model #25) to remove the ethanol. Either method is good depends on the quantity of extraction fluid, for more quantity, water bath evaporation is used. To concentrate the larger quantity of aliquote, water-bath evaporation was used. Rota evaporation was used[7] to concentrate the smaller quantity of extract. A 250 ml aliquot of extracted liquid was subjected to rota-evaporatoration for 3-4 h. The water bath temperature was adjusted to 700 C. The semisolid extract produced was kept in the deep freezer at -800C overnight and then subjected to freeze drying for 24 hrs at -600C at 200 millitorr vacuum.

For water bath evaporation, 3000 ml of liquid extract material was placed into a 3500 ml beaker. It was subjected to water bath evaporation at 700C temperature for 7-10 hrs daily for 2-3 days until a semisolid state of extracted liquid was obtained. Continuous evaporation was not done to avoid the charring of the extract constituents. The approximate volume of semisolid liquid by that time was 200-300 ml. The level of the water in the water bath was adjusted to 1/4 of the beaker height while shaker speed was adjusted to 27 to 32 oscillations per minute. The semisolid extract produced was frozen at - 800C and then freeze dried to completely remove ethanol and water from the extract at -600C at 200 millitorr vacuum. Extract from this method was then weighed and stored at 220C in desiccators until further use.

In vitro antibacterial studies

Antimicrobial susceptibility studies

Inhibition of microbial growth was tested by using the paper disc agar diffusion method (Kirby-Bauer Method; Drago et al., 1999) (Appendix-B), while the MIC was determined by the dilution (both micro and macro) method (de Paiva et al., 2003) (Appendix-C). Standard aseptic microbiological methods were followed throughout this antibacterial study[8].

Microorganisms

ATCC strains of, E. faecium and E. faecium, S. typhimurium (Microbiology teaching culture collection, Department of Biology, Virginia Tech, USA), were obtained. In addition, clinical isolates of E. coli[9].

Disc diffusion method for antibacterial activity

This method was used to assay the plant extracts for antimicrobial activity. The procedure, as explained in detail in Appendix B was followed. In brief, the test quantity of specific extract as shown in table 3 was dissolved in either distilled water or tween-80, depending upon the solubility of the extract. In order to detect potential antimicrobial activity in the plant extracts, paper discs (diameter 12 mm) were soaked in an extract solution containing different concentration as mentioned in table 3. The plant species and type of extract tested are shown in Table 1, while the bacteria are listed in Table 2. Entire surface of agar plate was inoculated with the culture of bacteria. The paper discs soaked in each of the test solutions containing different extract solutions at varying concentrations, as well as the standard drug solution (an antibiotic which is used as a feed additive) and the control-blank (sterile water discs or sterile tween 80 discs) were placed separately in each quarter of the plate under aseptic conditions. Multiple plates were (four replications) done for each of the extract was done. The plates were then maintained at room temperature for 2 h

allowing for diffusion of the solution. All plates were then incubated at 37^0C for 24 h and the zones of inhibition were subsequently measured in mm[10].

3. RESULTS AND DISCUSSION

Table 1: Antibacterial effect of different concentrations of medicinal plant extracts on common poultry pathogens

Medicinal plants extracts	Extract dilution	Corresponding effects on microorganism			
		E .coli	*S. thyphimurium*	*E. faecalis*	*E. faecium*
Zingiberofficinale	2 gm/2ml	+	+	+	+
	2 gm/3ml	-	+	-	-
	2 gm/4ml	-	-	+	-
	2 gm/5ml	-	-	-	-
Curcuma longa	2 gm/2ml	+	+	+	+
	2 gm/3ml	-	-	+	+
	2 gm/4ml	-	-	-	-
	2 gm/5ml	-	-	-	-
Piper nigrum	2 gm/2ml	+	+	+	+
	2 gm/3ml	-	-	-	-
	2 gm/4ml	-	-	-	-
	2 gm/5ml	-	-	-	-
Cinnamomum cassia	1 gm/1ml	+ [2]	+	+	-
	1 gm/3ml	+	+	+	+
	1 gm/4ml	+	+	+	+
	1 gm/5ml	+	+	-	-
	1 gm/6ml	-	-	-	-
Laurusnobilis	1 gm/3ml	+	+	-	+
	1 gm/4ml	+	-	-	-
	1 gm/5ml	-	-	-	-
	1 gm/6ml	-	-	-	-
Syzgiumaromaticum	1 gm/3ml	+	+	+	+
	1 gm/4ml	-	+	+	-
	1 gm/5ml	-	+	-	-
	1 gm/6ml	-	-	-	-
Thymus vulgaris	0.5 gm/3ml	+	+	+	+
	0.5 gm/4ml	_	+	+	+
	0.5 gm/5ml	+	-	+	-
	0.5 gm/6ml	-	-	+	-

Table 2: Antibacterial activity of specific concentration of medicinal plant extract compare to control by disc diffusion method

		Antibacterial activity			
Medicinal Plants	Concentration/disk	*E.coli*	*S. typhimurium*	*E.faecalis*	*E. faeciu m*
Z. officinale	127 mg	Negative	Negative	Negative	Negative
C. longa	135mg	Negative	Negative	Negative	Negative
C. cassia	129mg	20.75 ± 0.144	20.73 ± 0.144	20.75 ± 0.204	Negative
S. aromaticum	75.6 mg	Negative	Negative	Negative	Negative
P. nigrum	130 mg	Negative	Negative	Negative	Negative
L. nobilis	86.5 mg	Negative	Negative	Negative	Negative
T. vulgaris	65.75 mg	24.74	Negative	22.8	39.75
Tween-80	-	Negative	Negative	Negative	Negative
Distilled Water	-	Negative	Negative	Negative	Negative

Table 3: Minimum inhibitory concentration (MIC) of different extracts and bacitracin by dilution method

Test material	Bacteria	MIC mg/ml
Cinnamomum cassia	*E. coli*	30.43 mg/ml
Cinnamomum cassia	*S. typhmuriumi*	29.27 mg/ml
Cinnamomum cassia	*E. faecalis*	33.16 mg/ml
Cinnamomum cassia	*E. faecium*	ND
Thymus vulgaris	*E. coli*	57.28 mg/ml
Thymus vulgaris	*S. typhimurium*	97.57 mg/ml
Thymus vulgaris	*E. faecalis*	47.65 mg/ml
Thymus vulgaris	*E. faecium*	67.46 mg/ml
Bacitracin	*E. coli*	874 µg/ml
Bacitracin	*E. faecium*	957 µg//ml
Bacitracin	*E. faecium*	869 µg//ml
Bacitracin	*S. typhimurium*	994 µg//ml

There was no antibacterial activity in extracts of *C. longa*, *Z. officinale*, *P. nigrum*, *L. nobilis*, or *S. aromaticum* against the tested pathogens at the specific dose. Our results are contradictory with some researchers who reported antibacterial activity of above plants against gram positive and gram negative bacteria. This variation may be because of the dose used in this study, the method of extraction of medicinal plants, the method of antibacterial study, the genetic variation of plant, age of the plant or the environment. The addition of sub-therapeutic levels of antibiotics to broiler feed causes an increase in weight[11]. The plant extracts used in the present study which showed antibacterial activity *in vitro* did not results in any significant increase in body weight gain compared to the positive or negative control. The results, however, were encouraging compared to the negative control for the HCE, since the means of the HCE are higher than the NC. Furthermore, TE was found to decrease body weight significantly.

At the conclusion of 21 d feeding trial, the high level of cinnamon (HCE) did not results in any significant change in body weight gain compare to the PC treatment. This result is encouraging since the means of 0-21d data are for NC is lower (non-significant) than the means for HCE. Although non-significant, there was a trend towards increased body weight in birds fed with the HCE diets compare to NC diet. There are no other published reports on this effect[12].

There was a dose-dependent effect of LCE and HCE on increasing the body weight gain at 7-14 days (P=0.02). This result suggests the need for further research on the effect of cinnamon as a possible feed additive to replace antibiotics in broiler diets.There was conflicting evidence of the

relationship between antibacterial activity of thyme extract (TE) *in vitro* and its ability to increase body weight gain when provided in the diet for 21 d in broilers. Thyme had antibacterial activity *in vitro*, however, when added to the broiler diet, body weight gain decreased significantly during the 21 d feeding trial ($P < 0.02$). These results are contradictory since addition of antibacterial compounds to broiler diets generally increases the body weight gain. Thyme extract may possess active compounds that produce antibacterial activity *in vitro*, but it may also possess an active compound responsible for reducing the body weight *in vivo*[13]. The thyme though produced antibacterial activity *in vitro* but when given in diet, might be losing its antibacterial activity because of action of different enzymes while the process of its digestion and absorption[14].

The decrease in the body weight induced by thyme may have implications with regards to obesity. A significant reduction in weight gain was observed at 0-7 d ($P < 0.003$) and 7-14 d ($P < 0.05$) but not at 14-21 d period ($P > 0.05$). This may indicate that adding thyme in the diet from 14-21 d is not as effective in reducing body weight as it was from 0-7 and 0-21 d. The results of thyme in this study provide a strong basis for further research in obese subjects to reduce body weight. The reduction in body weight induced by thyme was observed without a change in feed consumption[15].

Feed consumption was not affected by the LCE or HCE compare to the PC treatment at 14 or 21 d ($P > 0.05$). Periodic feed consumption was also not affected at 7-14 or 14-21 d ($P > 0.05$). This suggests that the CE did not cause a feed aversion. Also, feed consumption was not affected by LTE or HTE at 7, 14, or 21 d. This also suggests that the thyme extract did not affect the bird©s perception of taste of the diets.

Feed efficiency was found to be affected when the diet was supplied with LCE compared to the PC, NC or HCE ($P = 0.03$). These results suggest that there is dose-dependent variation in the feed efficiency of HCE and LCE. Increasing the dose of cinnamon increased feed efficiency. This finding is important basis for the dose-dependent studies of cinnamon to find an alternative to AGP since improved feed efficiency will decrease the cost of production. Since HCE showed better feed efficiency than LCE, increasing dose of HCE may increase feed efficiency. However, we did not find any scientific reports to support these views.

4. CONCLUSION

Antibiotic growth promoters (AGP) have made a tremendous contribution to the profitability of the poultry industry. However, as a consequence of the increasing concern about the potential public health problems because of antibiotic resistant strains of bacteria, poultry nutritionists are being challenged to develop an alternative for AGP. If herbal alternative to AGP can be found, poultry nutritionists could formulate a ration that would meet the needs of the commercial broiler industry without using AGP. This study showed that herbal extracts, particularly a cinaamon extract, when added in the broiler diet, may have a similar effect as that of AGP. This study also showed that adding thyme in broiler diets may decrease body weight significantly compared to the diet with AGP, while not affecting the feed consumption. This result is helpful for further research on reducing body weight in the obese subjects.

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