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Effect of herbal residues on gut pathogens in cross-bred pigs

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Abstract

In a completely randomized design, 5 experimental diets (T_1 to T_5) were fed to 5 groups of animals with 6 animals (35 kg ± 1.3 body wt.) in each group. The diets were supplemented with or without herbal residues (turmeric, amla, ginger) and enzyme cocktail (xylanase, β-glucanase, cellulase and phytase). Thus the five diets were a standard diet (T_1), economic diet with enzyme cocktail but without herbal residue (T_2), T_2 with turmeric residue (T_3), T_2 with amla residue (T_4) and T_2 with ginger residue (T_5). After assessing the sensitivity of the individual herbal residue to inhibit the bacterial growth, Ginger residue exhibited the maximum ($P<0.05$) inhibition of Escherichia coli, Staphylococcus aureus and Salmonella typhimurium than amla while the inhibition of turmeric was comparable with ginger and amla. It was shown that the maximum inhibition of all pathogenic bacteria was at 2 per cent level. The total viable count (CFU/gm) was significantly higher ($P<0.01$) in T_1 or T_2 than in T_3, T_4 or T_5 fed pigs. Feeding diets containing herbal residues (T_3 to T_5) reduced ($P<0.01$) the Coliform, Staphylococci and Salmonella count. It can be concluded that herbal residues when included in the pig diets at 2% level were able to inhibit the growth of pathogens in the gut and thereby reducing the competition by the microbes for the nutrients leading to a better utilization and performance.

Introduction

Non-starch polysaccharides (NSP) are some important anti-nutritive components in plant based feed stuffs. Exogenous enzymes can hydrolyze these NSP into smaller units that can be utilized by pigs (Partridge and Bedford, 2000). Similarly phosphorus from plants is of low bio-availability to swine and poultry as a result of phytate, the principal form of phosphorus storage in plants, being relatively indigestible by non-ruminants (NRC, 1998). Exogenous supplementation of feeds with phytase has demonstrated the ability to increase phosphorus bio-availability and thus growth rates in pigs by cleavage of phosphorus molecule from phytase. Since four decades, concern about antibiotic resistance has increased worldwide (Cromwell, 2002). The ban on some Antibiotic Growth Promoters lead to think on phytogenic feed additives which include herbs and their residues, essential oils, botanicals, extracts etc. The mode of action of plant active substances include improvement of endogenous enzyme secretions, stimulation of the appetite, improvement of the digestibility and absorption of nutrients, promote proliferation of beneficial bacteria like Lactobacillus spp. in the gut. Hence the present experiment was planned with the objective of studying the effect of exogenous enzymes on pig performance either with or without herbal residues and their role in gut pathogen inhibition in finishers.

Materials and methods

Five experimental diets (table 1) were formulated as per NRC (1998) requirements and were evaluated

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during finisher (35-70 kg) phases. The dietary treatments were as shown below

T_1 = Standard diet without enzyme cocktail or herbal residues

T_2 = Economic diet with enzyme cocktail but without herbal residues

T_3= T_2 + *turmeric* residue

T_4= T_2 + *amla* residue

T_5 = T_2 + *ginger* residue

All the herbal residues were diluted in Diethyl ether. Dilutions were made from 0.2% to 2.0%. All concentrations of the herbal residues were prepared a day prior to use and stored at 4^0C.

Table 1: Ingredient and chemical composition (%) of treatment diets

Ingredient	T_1	T_2	T_3	T_4	T_5
Maize	42.00	20.00	20.00	20.00	20.00
Soybean meal	14.00	8.00	8.00	8.00	8.00
Sunflower Cake	-	12.00	12.00	12.00	12.00
Deioled Rice bran	41.50	57.50	55.50	55.50	55.50
Mineral mixture #	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Turmeric residue	0.00	0.00	2.00	0.00	0.00
Amla residue	0.00	0.00	0.00	2.00	0.00
Ginger residue	0.00	0.00	0.00	0.00	2.00
	100	**100**	**100**	**100**	**100**
Lysine (%)	0.41	0.44	0.44	0.44	0.44
Methionine (%)	0.01	0.60	0.60	0.60	0.60
AB_2D_3	0.02	0.02	0.02	0.02	0.02
Biovital	0.02	0.02	0.02	0.02	0.02
Enzyme cocktail (xylanase 3500, β-glucanase 2500, cellulase 1250 and phytase 3000 Units / Kg)	-	+	+	+	+
Cost per 100 Kg (Rs.)	1320	1266	1266	1266	1266

contained, Ca 32%; P 6%; Mn 0.27%; Zn 0.26%; Cu 100 ppm; Fe 1000 ppm, Iodine 0.01%; Fluorine (max.) 0.03%

Test to determine the antimicrobial activity

The disc diffusion method was used to determine the antimicrobial activity of the herbal residues. The volume of 0.1ml (approximately 10^9 cells / ml) of the tested microorganisms grown in liquid growth media at 37^0C was inoculated on Muller – Hinton growth media and then spread on the entire surface of the Petri dish using a sterile swab. Then sterile paper discs (Whatman 1.6 mm) with 30 µl absorbed extracts of herbal residues were placed on to the Muller – Hinton agar by pressing gently. The plates were incubated at 35 $\pm$ 1^0C for 48 hours. After the incubation period the inhibition zones around the paper discs were measured in millimeters. The sensitivity of the individual herbal residue was classified by the diameter of the inhibition zone as per the procedure of Moreira *et al.,* (2005).

Thirty entire male finisher pigs (35.41±0.65 kg) were made into five groups of 6 animals each and were fed with the diets T_1 to T_5, respectively till they attain a body weight of about 70 kg. In the present study, T_1(without herbal residues and enzymes) and T_2 (with enzymes) act as control. At the end of the finisher phase, all the 6 animals in each group were slaughtered to study the gut pathogenic bacteria. A portion of large intestine between caecum and colon of 25cm length was ligated on both the sides it was cut behind the ligation on both the sides with a sterilized knife. The collected intestine piece was placed in a sterilized beaker and kept at refrigeration temperature (4 ± 1^0C)

Results and Discussion

The Minimum Inhibitory Concentration (MIC) for different concentrations of all residues on the growth of pathogenic bacteria was different at different levels. Turmeric residue (table 2) was effective in preventing the growth of *Escherichia coli* at 0.8, 1, 1.5 and 2 percent levels; *Staphylococcus aureus* at

0.4,0.8,1,1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.4, 0.8,1, 1.5 and 2 per cent levels ; *Bacillus cereus* at 0.8,1,1.5 and 2 % levels; *Campylobacter jejuni* at 0.2, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 0.6, 1, 1.5 and 2 per cent levels; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1, 1.5 and 2 per cent levels. The table shows that the maximum inhibition of all bacterial pathogens was at 2 per cent level.

Table 2: Inhibitory effect of turmeric residue on the pathogenic bacterial growth

Pathogenic bacteria	Concentrations of turmeric residue						
	0.20%	0.40%	0.60%	0.80%	1%	1.50%	2%
Escherichia coli	--	--	--	+	+	+	++
Staphylococcus aureus	--	+	--	+	+	+	++
Salmonella typhimurium	--	+	--	+	+	+	+
Bacillus cereus	--	--	--	+	+	+	++
Campylobacter jejuni	+	--	--	--	-	+	++
Listeria monocytogenes	--	--	+	--	+	++	++
Streptococcus pyogenes	--	--	+	--	+	+	++
Methicillin resistant *Staphylococcus aureus*	--	--	--	--	+	+	++

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

Amla residue (Table 3) was effective in preventing the growth of *Escherichia coli* at 0.8, 1, and 2 per cent levels; *Staphylococcus aureus* at 0.8, 1, 1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.8, 1, 1.5 and 2 percent levels; *Bacillus cereus* at 1,1.5 and 2 % levels; *Campylobacter jejuni* at 1, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 1, 1.5 and 2 per cent levels ; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1 and 2 percent levels. The table shows that the maximum inhibition of all pathogenic bacteria was at 2 per cent level.

Table 3: Inhibitory effect of amla residue on the pathogenic bacterial growth

Pathogenic bacteria	Concentrations of amla residue						
	0.20%	0.40%	0.60%	0.80%	1%	1.50%	2%
Escherichia coli	--	--	--	+	+	--	+
Staphylococcus aureus	--	--	--	+	+	+	+
Salmonella typhimurium	--	--	--	+	+	+	+
Bacillus cereus	--	--	--	--	+	+	++
Campylobacter jejuni	--	--	--	--	+	+	+
Listeria monocytogenes	--	--	--	--	+	++	+
Streptococcus pyogenes	--	--	+	--	+	+	++
Methicillin resistant *Staphylococcus aureus*	--	--	--	--	+	--	++

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

The ginger residue (table 4) has shown the maximum inhibition at 2 per cent level for all pathogens. It was effective in preventing the growth of *Escherichia coli* at 0.8, 1, and 2 per cent levels; *Staphylococcus aureus* at 0.4, 0.8, 1, 1.5 and 2 per cent levels ; *Salmonella typhimurium* at 0.4, 0.8, 1, 1.5 and 2 per cent levels; *Bacillus cereus* at 0.8, 1,1.5 and 2 % levels; *Campylobacter jejuni* at 0.2, 1.5 and 2 per cent levels; *Listeria monocytogenes* at 0.6, 1, 1.5 and 2 per cent levels ; *Streptococcus pyrogenes* at 0.6, 1, 1.5 and 2 per cent levels; Methicillin resistant *Staphylococcus aureus* at 1, 1.5 and 2 per cent levels. The table shows that the maximum inhibition of all pathogenic bacteria was at 2 percent level.

The maximum inhibition for all the pathogenic bacteria was observed at 2% with all the herbal residues. Hence the herbal residues were included at 2% in the experimental diets T_3 to T_5.

Table 4: Inhibitory effect of ginger residue on the pathogenic bacterial growth

Pathogenic bacteria	Concentrations of ginger residue						
	0.20%	0.40%	0.60%	0.80%	1%	1.50%	2%
Escherichia coli	--	--	--	+	+	+	++
Staphylococcus aureus	--	+	--	+	+	+	++
Salmonella typhimurium	--	+	--	+	+	+	+
Bacillus cereus	--	--	--	+	+	+	++
Campylobacter jejuni	+	--	--	--	-	+	++
Listeria monocytogenes	--	--	+	--	+	++	+++
Streptococcus pyogenes	--	--	+	--	+	+	+++
Methicillin resistant *Staphylococcus aureus*	--	--	--	--	+	+	+++

+ : 08-09 mm; ++ : 10-13 mm; +++ : 14-16 mm

Antibacterial activity (inhibition zones) exhibited by herbal residues

Ginger (Table 5) exhibited the maximum (P<0.05) inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* than amla while the inhibition of turmeric was comparable with ginger and amla. The values (mm) were 26.00, 30.67 and 24.33 (ginger); 21.00, 25.00 and 22.00 (turmeric) and 18.00, 19.33 and 13.33 (amla) for *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* respectively. The inhibition (mm) of *Bacillus cereus* was maximum (P<0.05) with ginger residue (18.00) than with amla (14.00) or turmeric (12.00). The inhibition of *Campylobacter jejuni* was comparable among three residues and the values (mm) were 13.00 for all the three residues. Ginger exhibited maximum (P<0.05) inhibition of *Listeria monocytogenes* and *Streptococcus pyogenes* than amla while the effect of turmeric was comparable to amla and ginger and the values (mm) were 16.67, 12.00 (amla); 21.00, 18.00 (turmeric) and 25.00, 20.33 (ginger) for *Listeria monocytogenes* and *Streptococcus pyogenes* respectively. The inhibition zone (mm) for *Methicillin resistant Staphylococcus aureus* was maximum (P<0.01) with ginger (22.67) than with turmeric (18.66) or amla (13.00).

Table 5: Antibacterial activity (Inhibition zone in mm) of herbal residues (*in vitro*)

Herbal residues	*Escherichia coli**	*Staphylococcus aureus**	*Salmonella typhimurium**	*Bacillus cereus**	*Campylobacter jejuni*	*Listeria monocytogenes**	*Streptococcus pyogenes**	*Methicillin resistant Staphylococcus aureus***
Amla	$18.00^{b} \pm 1.15$	$19.33^{b} \pm 0.33$	$13.33^{b} \pm 0.33$	$14.00^{b} \pm 1.15$	13.00 ± 0.58	$16.67^{b} \pm 2.33$	$12.00^{b} \pm 0.00$	$13.00^{c} \pm 0.57$
Turmeric	$21.00^{ab} \pm 2.31$	$25.00^{ab} \pm 2.88$	$22.00^{ab} \pm 3.46$	$12.00^{b} \pm 1.15$	13.00 ± 0.58	$21.00^{ab} \pm 1.73$	$18.00^{ab} \pm 2.31$	$18.66^{b} \pm 0.68$
Ginger	$26.00^{a} \pm 1.15$	$30.67^{a} \pm 3.48$	$24.33^{a} \pm 3.48$	$18.00^{a} \pm 1.15$	13.00 ± 1.15	$25.00^{a} \pm 1.15$	$20.33^{a} \pm 4.09$	$22.67^{a} \pm 1.21$

^{abc} values in a column not sharing common superscripts differ significantly ** (P<0.01) * (P<0.05)

The anti bacterial activity of herbal residues was judged by measuring the length of the inhibition zone formed by each residue for each pathogenic bacterium. Except for *Campylobacter jejuni*, amla showed least antibacterial activity and ginger showed the maximum (P<0.05) activity. Ginger residue was effective in preventing the growth of pathogens in the gut followed by turmeric and amla.

Effect of dietary treatments on pathogenic bacteria of large intestinal contents

The total viable count (CFU / gm) was significantly (table 6) higher (P<0.01) in T_1 or T_2 than in T_3, T_4 or T_5 fed pigs and the values were 143.08 (T_1), 109.17 (T_2), 29.58(T_3), 67.33(T_4) and 19.75(T_5). Feeding diets containing herbal residues (T_3 to T_5) reduced (P<0.01) the Coliform, *Staphylococci* and *Salmonella* and the values (CFU / gm) were 68.58, 77.50, 26.83, 52.33 and 14.00 (Coliforms); 60.67, 59.50, 27.83, 36.58 and 10.08 (*Staphylococcus)* and 46.25, 54.67, 29.42, 34.50 and 13.08 (*Salmonella*) in pigs fed T_1 to T_5, respectively.

Table 6: Effect of dietary treatments on pathogenic bacteria (cfu / g) of large intestinal contents

	T_1	T_2	T_3	T_4	T_5
Total viable count * *	$143.08^{a} \pm 10.64$	$109.17^{b} \pm 5.70$	$29.58^{d} \pm 1.69$	$67.33^{c} \pm 2.77$	$19.75^{d} \pm 0.60$
Coliforms * *	$68.58^{b} \pm 3.42$	$77.50^{a} \pm 3.05$	$26.83^{d} \pm 0.94$	$52.33^{c} \pm 1.45$	$14.00^{e} \pm 1.13$
Staphylococcus * *	$60.67^{a} \pm 3.49$	$59.50^{a} \pm 1.73$	$27.83^{c} \pm 0.99$	$36.58^{b} \pm 1.05$	$10.08^{d} \pm 1.19$
Salmonella * *	$46.25^{b} \pm 1.20$	$54.67^{a} \pm 2.37$	$29.42^{d} \pm 0.94$	$34.50^{c} \pm 1.14$	$13.08^{e} \pm 0.89$

^{abcd} Values in a row not bearing common superscripts differ significantly ** (P<0.01)

Similar to the present results (Table 6), earlier reports also indicated antimicrobial effects of plants extracts (Newbold *et al*., 2004). The antimicrobial property was attributed to the hydrophobicity of plant extracts which facilitates their union to the bacterial surface inducing unstabilization (Tsuchiya *et al.,* 1996; Zhang and Lewis, 1997) or the inactivation of different molecules of the bacteria such as enzymes or receptors through their union to the specific site (Sharon and Ofek, 1986; Ya *et al*., 1988; Stern *et al.,* 1996).

It was reported that phytogenic feed additives have a strong antibacterial and to some extent antifungal properties. They inhibit the growth of *Escherichia coli*, *Proteus sp*, *Staphylococci*, *Streptococci* and *Salmonella* (Aruoma *et al.,* 1996; Benencia and Courreges, 2000; Garcia *et al.,* 2003) which otherwise compete with the host for nutrients.

Conclusion

Herbal residues when included in the pig diets at 2% level were able to inhibit the growth of pathogens in the gut and thereby reducing the competition by the microbes for the nutrients leading to a better utilization and performance.

References

1. Aruoma, O. I., Spencer, J. P,. Rossi, R., Aeschbach, R., Khan, A., Mahmood, N., Munoz, A., Murcia, A., Butler, J and Halliwell, B. An evaluation of the anti-oxidant and anti-viral action of extracts of rosemary and Provencal herbs. *Food Chemistry and Toxicology.*1996; 34: 449-456.
2. Benencia, F and Courreges M.C. *In vitro* and *in vivo* activity of eugenol on human herpesvirus. *Phytotherapy Research,* 2000; 14: 495-500.
3. Cromwell, G L. Why and how antibiotics are used in swine production. *Animal Biotechnology*, 2002; 13(1): 7-27.
4. Garcia, C. C., Talarico, L., Almeida, N., Colombres, S., Duschatzky, C and Damonte, E. B. Virucidal activity of essential oils from aromatic plants of San Luis, *Argentina. Phytotherapy Research*, 2003; 17: 1073-1075.
5. Moreira, R., Ponce, A. G., Carlos, E., Del valle and Sarai Roura. (2005) Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanched spinach and minced cooked beef. *Journal of Food Processing and Preservation,* 2005; 31(4**):** 379-391.
6. National Research Council 1998 *Nutrient Requirements of Swine*, 10th Ed. National Academy Press, Washington DC.
7. Newbold, C. J., McIntosh, F. M., Williams, P., Losa, R and Wallance, R J. Effects of a specific blend of essentials oil compounds on rumen fermentation. *Animal Feed Science Technology,* 2004; 114: 105-112.
8. Sharon, N and Ofek, I. Mannose specific bacterial surface lectins. In: Microbial lectins and agglutinins (Mirelman, D., Ed.),1986; pp. 55-82. John Wiley & sons, Inc., New York.
9. Stern, J. L., Hagerman, P. D., Steinberg, P. D., and Mason, P. K., Phlorotannin-protein interactions. *Journal of Chemical Ecology,*1986; 22: 1887-1899.
10. Tsuchiya, H., Sato, M., Miyazzaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T and Iinuma, M. (1996) Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. *Journal of Ethnopharmacology,*1996; 50: 27-34
11. Ya, C., Gaffney, S. H., Lilley, T. H. and Haslam, E. Carbohydrate-polyphenol complexation. In: Chemistry and significance of condensed tannins. (Hemingway, R.W & Karchesy, J.J., eds.), 1988; p. 553. Plenum press, New York, N.Y.
12. Zhang, Y and Lewis, K. Fabatins: new antimicrobial plant peptides. *FEMS Microbiology Letters,* 1997; 149: 59-64.