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Panchagavya and *Andrographis paniculata* as Alternatives to Antibiotic Growth Promoters on Haematological, Serum Biochemical Parameters and Immune Status of Broilers

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A biological experiment was conducted to study the effect of panchagavya and *Andrographis paniculata* on haematological, serum biochemical parameters and immune status of broilers with one hundred and eighty commercial, straight run day-old broiler chicks. The chicks were fed basal diet (T_1), basal diet with virginiamycin-20 mg/kg (T_2), basal diet with panchagavya-7.5 g/kg (T_3), basal diet with *A. paniculata*-2.0 g/kg (T_4) probiotics-0.5 g/kg (T_5) and basal diet with mannanoligosaccharide (MOS)-2.0 g/kg (T_6) from 1 to 28 days of starting period and 0.5 g/kg from 29 to 42 days finishing period were maintained for 6 weeks period. The results revealed that the haematological parameters did not vary significantly between treatment groups. The serum total cholesterol level was lower ($P<0.01$) in T_3 and T_5 than virginiamycin and control. The HDL cholesterol was higher ($P<0.05$) in T_5. The serum total protein, albumin, globulin and glucose values were not differed significantly between treatment groups. The serum AST and ALT levels were lower ($P<0.01$) in *A. paniculata* and panchagavya groups compared to other groups. The serum ALP was ($P<0.05$) lower in T_4. Higher HA titre against SRBC ($P<0.01$), HI titre against NDV ($P<0.05$) and QAGPT titre against IBDV were observed in T_3, T_4 and T_5, which indicating immunomodulatory effect of panchagavya and *A. paniculata* in broilers.

Key words: *Andrographis paniculata,* broilers, immune status, panchagavya, serum biochemicals

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Introduction

Antimicrobials have been used as feed supplement for more than 50 years in poultry feed to enhance the growth performance and to prevent diseases in poultry. However, in recent years great concern has arisen about the use of antibiotics as supplement at sub-therapeutic level in poultry feed due to emergence of multiple drug resistant bacteria (Wray and Davies, 2000).

Antibiotics can be replaced by alternatives such as prebiotics, probiotics and botanicals. Recently, Council for Scientific Industrial Research (CSIR), India has identified cow urine distillate for its antimicrobial and antifungal properties. Panchagavya is one such formulation mentioned in Ayurveda, which is prepared with five components derived from cow viz. milk, curd, ghee, urine and dung and can be used as growth promoters in animals (Dhama *et al.,* 2005).

Commercial additives of plant origin like herbs, spices and various plant extracts are also considered to be natural products that consumers would have received an increased attention. *Andrographis pan-*

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iculata is one of such plant having antimicrobial and growth promoting activity and hence may be used as alternative to antibiotics and tonic (Chopra *et al.,* 1992). However, role of these alternatives on health indicating parameters like haematological, serum biochemical characteristics and immunity is not still clear in broilers even though already some works has been carried out in rats and other species. Hence, the present study was carried out to evaluate the effect of panchagavya and *Andrographis paniculata* on haematological, serum biochemical parameters and immune status of broilers.

Materials and Methods

Preparation and Analysis of Panchagavya

The panchagavya samples were prepared by using dung (5 parts), urine (3 parts), milk (2 parts), curd (2 parts) and ghee (1 part) obtained from indigenous cow along with other ingredients viz. sugarcane juice (3 parts), tender coconut juice (3 parts), ripened banana (12 numbers) and toddy (2 parts) as per the methods of Natarajan (2003). The fresh dung was thoroughly mixed with ghee in a wide mouth mud pot and kept for three days. The above mixture was thoroughly mixed once in daily. On the fourth day, other ingredients were added to the mud pot, mixed properly and covered with nylon net to prevent entry of flies into the pot. The pot was placed in shade and mixed thoroughly twice a day for 30 days.

Panchagavya samples were taken and analysed for its chemical and microbial composition. The pH of the samples was assessed using digital pH meter. The total volatile fatty acids (TVFA) content of panchagavya was assessed by using micro-Kjeldahl apparatus. Identification and quantification of individual volatile fatty acids were performed by using gas chromatograph (Chemito 8610 HR, India.).

The panchagavya samples were serially diluted in 10 folds using sterile triple glass distilled water. The selective media used for microbial culture were MacConkey agar (HiMedia Laboratories Pvt Ltd, Mumbai, India.) for coliforms and *Salmonella,* Kanamycine Aesculine Azide agar (HiMedia) for *Streptococci,* Mannitol salt agar (HiMedia) for *Staphylococci,* de Man Rogosa Sharpe (MRS) agar (HiMedia) for *Lactobacilli.* The selective media for the microbial counts of coliforms, *Streptococci* and *Staphylococci* were incubated aerobically at 37℃ for one day. The agar plates for the counts of *Lactobacilli* were incubated in aseptic anaerobic jars at 37℃ for two days. Anaerobic environment was created in the jars using AnaeroGen (HiMedia) sachets. The microbial numbers were expressed as $\log_{10}$ colony forming units per gram ($\log_{10}$ cfu/g) of sample (Smits *et al.,* 1998).

Preparation of Andrographis Paniculata

The whole plants of *A. paniculata* (English: Creat) were collected from the local area after ascertaining their identity. The leaves of *A. paniculata* were collected and shade dried, powdered and kept ready for experimental use.

Animals and Experimental Conditions

One hundred and eighty commercial, straight run day-old broiler chicks belonging to a single hatch were purchased from a local hatchery, wing banded, weighed and randomly allotted into six treatment groups with three replicates of ten chicks each. The chicks were fed basal diet (T_1), basal diet with virginiamycin (Stafac 20-Pfizer Limited, Mumbai, India.)-20 mg/kg (T_2), basal diet with panchagavya-7.5 g/kg (T_3), basal diet with *A. paniculata*-2.0 g/kg (T_4), basal diet with probiotics (Provilacc-each gram contained 5.85×10^9 *Saccharomyces cerevisiae,* 14.04×10^6 *Lactobacillus acidophilus,* 2.34×10^6 *Lactobacillus sporogenes* and 2.34×10^6 Streptococcus faecium)-0.5 g/kg (T_5) and basal diet with mannanoligosaccharide (MOS)-2.0 g/kg from 1 to 28 days starting period and reduced to 0.5 g/kg from 29 to 42 days finishing period (T_6).

The chicks were reared in broiler cages in a gable roofed, open sided house. All the chicks were provided with uniform floor, feeder and waterer space and were reared under standard management conditions throughout the experimental period of six weeks. The experimental diet was formulated according to the standards prescribed in Bureau of Indian Standards (B.I.S, 1992). The broiler starter and finisher diets were fed *ad libitum* to the birds from 1 to 28 and 29 to 42 days of age, respectively.

Measurement of Haematological and Serum Biochemical Characteristics

At the end of the experiment, one male and one female from each replicate, totally six birds per treatment were randomly picked up, blood samples were collected for haematological and serum biochemical characteristics. Blood samples were collected from the birds and immediately assessed for

its packed cell volume, hemoglobin and total leukocyte count as per the methods of Campbell (1995).

Blood samples were allowed to clot and centrifuged at 1500 rpm for 20 min to separate the sera. The sera samples were stored at −20℃ for the analyses of serum glucose, total protein, albumin, cholesterol, HDL-cholesterol, aspartate transaminase (AST), alkaline transaminase (ALT) and alkaline phosphatase (ALP) as per the methods of Gowenlock *et al.* (1988). The serum globulin was calculated by subtracting serum albumin from serum total protein levels.

Immunization and Measurement of Antibody Titres

Whole blood was collected from Mecheri breed of sheep in Alsever's solution (dextrose 5.125 g, sodium citrate 2 g, sodium chloride 1.05 g in 250 m*l* triple glass distilled water). The sheep red blood cells (SRBC) were washed three times in phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to make 25 per cent (v/v) SRBC suspension.

On 28th day of age, one male and one female broiler chick in each replicate totaling six birds per treatment were randomly picked up. Prior to immunization with SRBCs, blood samples were collected from the wing vein of these birds for assessing the general immunity status. Then they were immunized with 0.5 m*l* of 25 per cent SRBC (Kundu *et al.,* 1999) on each thigh muscle. Blood samples were collected 15 days after immunization for assessing haemagglutination (HA) titre using one per cent freshly prepared SRBC suspension.

The test serum (25 μl) was serially diluted to two fold with PBS in microtitre plates. After dilution, 25 μl of one per cent SRBC suspension was added to each well and mixed. The plate was incubated at 37℃ for one hour and HA titre was expressed as the $\log_2$ of the reciprocal of the highest dilution showing 100 per cent agglutination (Siegel and Gross, 1980).

Immunity Against Newcastle Disease Virus

Blood samples were collected randomly from six birds in each treatment group prior to immunization and 15 days after immunization with Newcastle disease vaccine (Ventri biologicals, Pune, India). The separated sera samples were utilized for the HI test (Allan and Gough, 1974) to find out the level of immune titre developed against the Newcastle disease virus (NDV) in different treatments for evaluating the immunostimulation effect of panchagavya and *A. paniculata* in broilers.

Immunity Against Infectious Bursal Disease Virus

Blood samples were collected randomly from six birds in each treatment group prior to immunization and 15 days after immunization with IBD vaccine. The serum samples were utilized for quantifying the antibody level in serum.

Agar 1.2 g in 100 m*l* hypertonic saline (8% sodium chloride) was dissolved and boiled. Sodium azide (0.02%) was added to above boiled solution to prevent fungal contamination, cooled to 50–55 ℃ and then cast approximately 5 m*l* on the clean, grease free slide and allowed for solidification.

The assessment of infectious bursal disease (IBD) antibody level was carried out by quantitative agar gel precipitation test (QAGPT) as per the method described by Wood *et al.* (1979) with slight modification. After solidification, satellite well pattern of six wells surrounding one central well of 5 mm diameter with an inter-space 0.3 cm were punched out. On each slide, two sets of such patterns were punched out and number given to the peripheral wells from 1 to 12. The two central wells were loaded with reference infectious bursal disease virus (IBDV) antigen. The wells 6 and 7 were kept as known negative and known positive control, respectively. The first peripheral well was filled with neat serum. In the remaining wells serial two fold dilutions of the test serum was added from the neat serum. The loaded slides were incubated at 37℃ for 24 to 28 h in a humid chamber. The reciprocal of the highest dilution of serum showing precipitation line was taken as the QAGPT titre of the serum.

Data Analyses

The data collected on various parameters were subjected to statistical analysis as per the methods of Snedecor and Cochran (1989).

Results

Analysis of Panchagavya

The result revealed that the pH of the panchagavya sample was 4.52. The TVFA was 154.87 mmol/litre. The acetate, propionate and butyrate levels in the TVFA of panchagavya were 60.76, 15.66 and 6.40 per cent, respectively. The *Lactobacillus* count was 8.71 $\log_{10}$ cfu/g. Coliforms, *Streptococci* and *Staphylococci* counts were not in detectable range.

Haematological Parameters

The PCV, Hb and total leukocyte count (Table 1)

Table 1. **Mean (±S.E.) haematological, Serum biochemical parameters and hepatic enzymes of broilers as influenced by dietary inclusion of different alternatives to antibiotic growth promoter**

Parameters	T_1-Basal diet	T_2-Basal diet + Virginiamycin	T_3-Basal diet + Panchagavya	T_4-Basal diet + *A. paniculata*	T_5-Basal diet + Probiotics	T_6-Basal diet + MOS
A. Haematological parameters						
PCV (%)	29.70±0.85	31.16±1.15	29.50±1.53	31.33±1.58	31.50±1.77	31.83±0.98
Hb (g/dl)	10.50±0.44	11.16±0.47	11.43±0.55	12.06±0.29	11.20±0.52	12.06±0.38
Total leukocytes ($\times 10^3/\mu l$)	25.34±0.38	24.02±0.38	25.99±0.37	25.32±0.34	24.32±0.20	25.88±0.15
B. Serum biochemical parameters						
Total cholesterol (mg/dl)	180.61^{B}±6.91	184.85^{B} ±6.06	152.58^{A} ±6.64	171.22^{AB}±2.79	153.03^{A}±7.20	175.76^{AB}±7.29
HDL cholesterol (mg/dl)	42.65^{c} ±2.77	43.38^{bc}±3.15	50.98^{ab}±1.59	42.28^{c} ±2.08	51.84^{a} ±3.85	38.61^{c} ±2.83
Total protein (g/dl)	2.77 ±0.17	2.80 ±0.05	2.77 ±0.07	2.75 ±0.14	2.86 ±0.23	2.91 ±0.16
Albumin (g/dl)	0.98 ±0.07	0.85 ±0.03	0.92 ±0.05	0.91 ±0.04	0.99 ±0.05	1.04 ±0.04
Globulin (g/dl)	1.78 ±0.22	1.95 ±0.06	1.85 ±0.07	1.94 ±0.16	1.87 ±0.10	1.86 ±0.08
Glucose (mg/dl)	205.88 ±11.05	191.18 ±10.60	196.08 ±7.98	198.04 ±11.23	181.33 ±11.20	183.33 ±13.01
C. Serum hepatic enzymes (U/l)						
AST	194.77^{B}±3.18	203.04^{B}±1.99	196.98^{B} ±5.93	161.13^{A}±11.89	209.56^{B}±2.60	192.33^{B}±5.79
ALT	56.92^{B}±1.27	54.34^{B}±1.81	48.07^{A} ±3.28	44.46^{A}±2.42	57.00^{B}±1.06	57.57^{B}±1.55
ALP	171.58^{b} ±7.79	172.43^{b} ±5.39	156.37^{ab}±5.77	147.92^{a} ±4.42	170.74^{b} ±3.38	171.58^{b} ±6.33

Value in each cell is the mean of 30 observations except T_2 at 5 and 6 weeks of age, which had only 29 observations.
$^{a-c}$ Means within a row with no common superscript differ significantly ($P<0.05$).
^{A,B} Means within a row with no common superscript differ significantly ($P<0.01$).

did not differ among treatment groups. The PCV, Hb and total leukocyte counts were varied from 29.50 to 31.83 per cent, 10.50 to 12.06 g/dl and 24.02 to 25.99 $\times 10^3$ cells/μl, respectively.

Serum Biochemical Parameters

Serum biochemical parameters are shown in Table 1. The serum total cholesterol level was significantly ($P<0.01$) lower in T_3 and T_5 with values of 152.58 and 153.03 mg/dl, respectively than T_2 (184.85 mg/dl) and T_1 (180.61 mg/dl). No Significant difference was observed between T_3, T_5, T_4, and T_6. The HDL cholesterol was significantly ($P<0.05$) higher in T_5 (51.84 mg/dl) and lower in groups T_6 (38.61 mg/dl), T_4 (42.28 mg/dl) and T_1 (42.65 mg/dl). The serum total protein, albumin, globulin and glucose levels did not show any variation between treatment groups.

The serum AST level was significantly ($P<0.01$) lower in *A. paniculata* (161.13 U/l) as compared to other treatment groups. Higher serum AST value was observed in T_5, which did not differ significantly from T_2, T_3, T_1 and T_6. The mean serum ALT level was lower ($P<0.01$) in T_4 (44.46 U/l) and T_3 (48.07 U/l) as compared to T_5 and T_6, which recorded the higher serum ALT activity of 57.00, and 57.57 U/l, respectively. The serum ALP level was significantly ($P<0.05$) lower in T_4 (147.92 U/l), while T_2, T_1, T_6 and T_5 exhibited the higher activity of 172.43, 171.58, 171.58 and 170.74 U/l, respectively.

Immunity

The antibody titres are shown in Table 2. The mean $\log_2$ HA titre was significantly ($P<0.01$) higher in T_3, T_4, T_5 and T_6 with mean values of 2.99, 2.78, 2.38 and 2.29, respectively as compared to T_2 and T_1 which recorded HA titre of 1.90 and 1.73, respectively. The HI titre against NDV was significantly ($P<0.05$) higher in panchagavya fed group (T_3), which did not differ from probiotic fed group (T_5) than T_2 and T_1 fed group. The QAGPT titre against. IBDV was higher in T_3 (4.00) followed by T_5 (3.66), T_4 (3.33) and T_6 (3.00). The virginiamycin (T_2) and control (T_1) groups recorded the lower titre of 2.00 in each group.

Table 2. **Mean (±S.E.) $\log_2$ HA titre against SRBC, HI titre against NDV and QAGPT titre against IBDV as influenced by dietary inclusion of different alternatives to antibiotic growth promotant in broilers**

Treatment groups	HA titre against SRBC	HI titre against NDV	QAGPT titre against IBDV
T_1-Basal diet	$1.73^{B}\pm0.04$	$2.30^{c}\pm0.02$	2.00 ± 1.15
T_2-Basal diet + Virginiamycin	$1.90^{B}\pm0.05$	$2.32^{c}\pm0.02$	2.00 ± 1.15
T_3-Basal diet + Panchagavya	$2.99^{A}\pm0.05$	$3.66^{a}\pm0.02$	4.00 ± 2.00
T_4-Basal diet + *A. paniculata*	$2.78^{A}\pm0.01$	$2.74^{bc}\pm0.02$	3.33 ± 2.49
T_5-Basal diet + Probiotics	$2.38^{A}\pm0.02$	$3.17^{ab}\pm0.03$	3.66 ± 2.13
T_6-Basal diet + MOS	$2.29^{A}\pm0.02$	$2.84^{bc}\pm0.03$	3.00 ± 1.53

Value in each cell is the mean of six observations.

[a-c] Means within a column with no common superscript differ significantly ($P<0.05$).

[A, B] Means within a column with no common superscript differ significantly ($P<0.01$).

Discussion

The pH of the panchagavya was 4.52 at 30 days of fermentation. The lowered pH may be due to *Lactobacillus* bacteria in panchagavya, which produced more organic acids during fermentation. The difference in pH might be due to variation in composition of ingredients and method of panchagavya preparation. The mean TVFA level of panchagavya was from 154.87 mmol/litre. This might be due to availability of nutrients for the growth of microorganisms, which produced organic acids during fermentation (Dhama *et al.,* 2005) from the components of panchagavya.

The *Lactobacillus* count was 8.71 $\log_{10}$ cfu/g at 30 days of fermentation and this might be due to the presence of curd and milk in panchagavya as a source of *Lactobacillus acidophilus* (Dhama *et al.,* 2005), which lower the pH and favour the growth of beneficial and inhibit the growth of pathogenic microorganisms (Ferd, 1974). The coliforms, *Salmonella, Streptococci* and *Staphylococci* counts were not in the detectable range. This may be accomplished by lowered pH of panchagavya in later stage of fermentation as expressed by Ferd (1974) who reported that the pH level could affect the specific microbial population and most of pathogenic organisms grew at pH of 7 or slightly higher.

The haematological parameters viz. PCV, Hb and total leukocytes count did not differ significantly between groups. Similar report was made by Mohan *et al.* (1996) who observed that PCV did not vary due to supplementation of probiotics in broilers. However, Fulzele *et al.* (2002) observed oral feeding of panchagavya herbal formulation insignificantly increased the WBC in rats compared to control.

The serum total cholesterol was significantly lower in panchagavya and probiotic fed groups compared to virginiamycin fed and control groups. This finding is in agreement with Jin *et al.* (1998) and Kalavathy *et al.* (2003) who reported that addition of probiotics in diet decreased the serum total cholesterol in broilers. However, Santoso *et al.* (1995) and Djouvinov *et al.* (2005) reported that feeding of probiotics did not significantly lower the serum total cholesterol in broilers. Garg *et al.* (2004) also observed that feeding of cow urine to White Leghorn layers significantly increased the serum total cholesterol compared to control layers. Feeding of *A. paniculata* and MOS to broilers did not significantly influence the total serum cholesterol compared to other treatment groups. Dwivedi *et al.* (1987) and Zhang and Tan (2000) also reported that feeding of dried *A. paniculata* leaf powder at different doses did not significantly reduce the serum cholesterol in rabbits and rats, respectively. The HDL cholesterol level was significantly higher in probiotic fed group than virginiamycin and control groups. This is in agreement with findings of De Rodas *et al.* (1996) who found that HDL cholesterol level was significantly increased in broilers supplemented with *L. acidophilus*. However, Kalavathy *et al.* (2003) and Djouvinov *et al.* (2005) did not observe any increase in HDL cholesterol in broilers and mule ducklings, respectively fed probiotics. Nevertheless, *A. paniculata* and MOS in broiler diet were not able to increase HDL cholesterol in this study.

The serum protein level did not differ significantly between treatment groups. Similarly, Djouvinov *et al.* (2005) observed that the feeding of Lactina did

not affect the serum total protein in mule ducklings for a period of 93 days under field conditions. On the contrary, Garg *et al.* (2004) observed a 14.71 per cent increase in serum protein in cow urine supplemented White Leghorn layers. Trivedi and Rawal (1998) also reported an increased serum protein level in BHC treated albino mice supplemented with *A. paniculata.* The serum albumin and globulin level did not vary among groups. The serum glucose level did not differ significantly between the treatment groups. This is in agreement with findings of Dwivedi *et al.* (1987) who did not observe a significant difference in glucose level between *A. paniculata* and control groups. However, Garg *et al.* (2004) found that the serum glucose level increased in White Leghorn layers fed cow urine.

The serum AST level was significantly lower in *A. paniculata* fed group compared to other treatment groups, which proved that the hepatoprotective activity of *A. paniculata* in broilers. This finding is in agreement with earlier works of Dwivedi *et al.* (1987), Trivedi and Rawal (1998) and Bhattacharyya *et al.* (2003) who reported that feeding of *A. paniculata* significantly reduced the serum AST level in rabbit, mice and rats, respectively in induced hepatotoxicity. Achilya *et al.* (2003) also reported that administration of *Panchagavya Ghrita* markedly prevented CCl_4 induced elevation of serum AST in albino rats and the results were comparable to that of silymarin, a standard hepatoprotective allopathic drug. The serum ALT level was significantly lower in T_3 and T_4 as compared to other treatment groups. Similar observation was made by Dwivedi *et al.* (1987), Trivedi and Rawal (1998) and Bhattacharyya *et al.* (2003).

A. paniculata fed groups recorded significantly lower serum ALP activity compared to other treatments except panchagavya fed group. This finding is in accordance with earlier works of Trivedi and Rawal (1998) and Bhattacharyya *et al.* 2003) who reported a reduced ALP level in *A. paniculata* fed group in BHC and CCl_4 induced toxicity in mice and rats, respectively. However, the serum ALP level in panchagavya fed group did not differ significantly from control diet, which is contrary to the report of Achliya *et al.* (2003) who reported a reduced ALP in albino rats fed *Panchagvya Ghrita.* The result indicated that *A. paniculata* and panchagavya had significantly better hepatoprotective activity. Feeding of virginiamycin, probiotics and MOS did not have hepatoprotective effect in broilers.

The mean HA titre against SRBC was significantly higher in all antibiotic alternatives supplemented groups compared to virginiamycin and control groups. This report is well supported that feeding of cow urine significantly improved both humoral and cell mediated immunity in animals (Chauhan and Singhal, 2001 and Dhama *et al.,* 2005). On the other hand, Fulzele *et al.* (2003) reported that feeding of panchagavya herbal formulation did not significantly increase the HA titre in rats. Kabir *et al.* (2004) also found that supplementation of probiotics significantly increased the antibody production in broilers. Panchagavya and probiotics fed group had significantly better HI titre against NDV as compared to virginiamycin and control. Feeding of virginiamycin did not improve the titre against IBDV. However, feeding of antibiotic growth promoter alternatives viz. panchagavya recorded higher titre of 4.00 followed by probiotics (3.66), *A. paniculata* (3.33) and MOS fed (3.00) groups. Similarly, Shashidhara and Devegowda (2003) observed that the antibody response against IBDV was higher in MOS group than control.

In conclusion, dietary supplementation of panchagavya at 7.5 g/kg of broiler feed significantly reduced the serum total cholesterol and increased the HDL-cholesterol levels. Inclusion of both panchagavya and *A. paniculata* (2.0 g/kg) reduced the serum hepatic enzymes, which indicative of hepatoprotective activity. Similary, panchagavya, *A. paniculata* and probiotics improved the immune status of broilers.

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