Effects of Tannic Acid Added to Diets Containing Low Level of Iron on Performance, Blood Hematology, Iron Status and Fecal Microflora in Weanling Pigs

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ABSTRACT

This study investigated the effects of tannic acid (TA) in the diets for weanling pigs prepared with/without supplemental Fe on performance, hematology, fecal microflora and diarrhea incidence. Limestone and calcium phosphate used in Experiment 1 and 2 were of semi-synthetic and feed-grade quality, respectively; while the trace-mineral premix used in both the experiments was prepared without any added Fe source. In Experiment 1, 108 weaned pigs (Landrace × Yorkshire × Duroc, initially 6.46 ± 1.04 kg BW) were allotted to 3 treatments including control (diet added with $FeSO_4$ and antibiotic), T1 (diet devoid of $FeSO_4$ and antibiotic) and T2 (T1 diet added with 125 mg/kg TA). Each treatment had 4 replicates with 9 pigs in each pen. Feeding of T1 diet had a negative effect on the performance and plasma Fe status of pigs, while addition of TA to T1 diet resulted in performance of pigs comparable to pigs fed the control diet, reduced diarrhea incidence but had a negative influence on the hematological and plasma Fe indices. Additionally, pigs fed T2 diet had fewer ($p<0.05$) total anaerobic bacteria, *Clostridium* spp. and coliforms than pigs fed T1 diet, and greater number of *Bifidobacterium* spp. and *Lactobacillus* spp. in feces when compared with pigs fed control and T1 diets. In Experiment 2, 144 weaned pigs (Landrace × Yorkshire × Duroc, initially 6.00 ± 1.07 kg BW) were allotted to 4 dietary treatments including control (diet added with $FeSO_4$ and antibiotic) and diets devoid of supplemental Fe added with antibiotic (An), TA and both (AnTA). Each treatment had 4 replicates with 9 pigs in each pen. Addition of An, TA or both to diets devoid of supplemental Fe did not have any effect on performance, blood hematology and plasma Fe but resulted in reduced ($p<0.05$) diarrhea incidence and lower ($p<0.05$) fecal coliform population than pigs fed the control diet. These results suggest that TA has a negative influence on blood hematology and plasma Fe status when diets are inadequate in Fe; however, TA reduced diarrhea incidence and might have antimicrobial activity.

(**Key words :** Fecal microflora, Hematological status, Iron, Tannic acid, Weanling pigs)

INTRODUCTION

Tannic acid (TA) is an important gallotannin belonging to the hydrolysable class, which is made up of phenolic acid esters and a polyol, usually glucose (Khanbabaee and van Ree, 2001). A number of antinutritional effects have been attributed to tannins. Previous studies demonstrating the effects of tannins in feedstuffs on animal performance have been carried out by using tannins isolated from feedstuffs or with commercial tannins (Wareham et al., 1993; Marzo et al., 2002). The antinutritional effects of tannins in terms of poor palatability, feed efficiency, growth rate, nutrient digestibility, and increased endogenous losses of minerals and amino acids in simple stomach animals have been extensively reviewed by Jansman (1993).

Tannins have been reported to have bacteriostatic and bacteriocidal properties against wide range of pathogenic bacteria (Chung et al., 1998), including *Escherichia coli*, *Salmonella*, *Proteus* spp., and obligate anaerobes such as *Clostridium* spp. (Sotohy et al., 1995). However, there have been very few attempts demonstrating the *in vivo* reduction of bacteria by tannins.

Diarrhea represents 11% of all postweaning piglet mortality (Alexander, 1994), and enterotoxigenic *Escherichia coli* diarrhea is the most common enteric disease in piglets, accounting for 5% of the 10 million piglets that die annually worldwide (Gyles, 1994). The Fe concentration of most nursery diets is in excess of the NRC (1998) postweaning dietary Fe requirement of 80 mg/kg. This occurs because many feed ingredients have a high Fe concentration, including monocalcium phosphate and limestone

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(Rincker et al., 2005). In our previous study we had noticed supplemental Fe (50 to 250 mg/kg) can linearly increase diarrhea incidence and fecal coliform count in weaned pigs (Lee et al., 2008). All micro-organisms require iron to facilitate basic cellular processes such as respiration and DNA biosynthesis (Rouault, 2004). Tannins are known to have an affinity for binding with Fe and this might reduce Fe absorption and also prevent Fe from being obtained by intestinal microbes thus limiting their growth in intestine and might act as an antidiarrheal agent (Ericsson, 2005).

Thus this study was conducted to evaluate the effect of TA in the diet for weaned pigs which had low Fe (Experiment 1) and sufficient Fe (Experiment 2) contents.

MATERIALS AND METHODS

1. Animals, housing and diets

Before initiating the experiments, piglets were managed according to standard operating procedures and were injected 100 mg of Fe (Fe-dextran) on day 3 and day 7 of age. During the experiment, pigs (Exp. 1: 108, Exp. 2: 144) were housed in partially slotted and concrete floor pens having a pen size of 1.90 m × 2.54 m, that were equipped with a self-feeder and nipple waterer to allow *ad libitum* access to feed and water. The study followed proper ethical standards and was approved by the Animal Care and Use Committee of Kangwon National University.

In Experiment 1, 108 weaned pigs (Landrace × Yorkshire × Duroc, initially 6.46 ± 1.04 kg BW) were allotted to 3 treatments and each treatment had 4 replicates with 9 pigs in each pen. Dietary treatments were control (diet added with $FeSO_4$ and antibiotic: 0.15% apramycin), T1 (diet devoid of $FeSO_4$ and antibiotic) and T2 (T1 diet added with 125 mg/kg TA). The trace mineral premix used in this study was formulated without any Fe source, and semipurified monocalcium phosphate and limestone were used. The experimental feeding was conducted in two phases: phase 1 (d 0 to 14) and phase 2 (d 15 to 28) and lasted for 28 days postweaning. Diets for phase 1 were formulated to contain 3,548 kcal/ kg ME and 19.89% CP and phase 2 diets were formulated to contain 3,550 kcal/ kg ME and 19.00% CP (Table 1). All the diets met or exceeded the nutrient requirements as suggested by NRC (1998).

In Experiment 2, 144 weaned pigs (Landrace × Yorkshire × Duroc, initially 6.00 ± 1.07 kg BW) were allotted to 4 dietary treatments. Each treatment had 4 replicates with 9 pigs in each pen. Dietary treatments were control (diet added with $FeSO_4$ and antibiotic) and diets devoid of supplemental Fe added with antibiotic (An: 0.15% apramycin), tannic acid (TA: 125 mg/kg) and both antibiotic and tannic acid (AnTA).The trace mineral premix used in this study was formulated without any Fe source, and feed grade monocalcium phosphate and limestone were used. The experimental feeding was conducted in two phases: phase 1 (d 0 to 14) and phase 2 (d 15 to 28) and lasted for 28 days postweaning. Diets for phase 1 were formulated to contain 3,527 kcal/ kg ME and 21.80% CP and phase 2 were formulated to contain 3,500 kcal/ kg ME and 21.60% CP (Table 1). All the diets met or exceeded the nutrient requirements as suggested by NRC (1998).

Albumin tannate (50% TA) used as the source of TA and was obtained from Eunjin International Co. Ltd. (Seoul, Republic of Korea) while apramycin was used as an antibiotic.

Table 1. Ingredient and chemical composition of control diet (Exp. 1)[1)]

Ingredients, %	Control diet	
	Phase 1 (d 0 to 14)	Phase 2 (d 15 to 28)
Extruded corn	38.613	51.206
Whey powder	25.000	14.000
Hamlet Protein -300 (55% CP)	23.205	21.787
Sucrose	5.774	3.527
Soybean oil	3.500	3.913
Soybean meal	/	1.000
MCP (semipurified)[2)]	1.500	2.000
Limestone (semipurified)[3)]	0.772	0.767
DL-methionine, 50%	0.521	0.569
L-lysine HCl, 78%	0.279	0.306
Vitamin premix[4)]	0.250	0.250
L-threonine, 99%	0.159	0.235
Trace mineral premix[5)]	0.100	0.100
Vitamin C, 20%	0.050	0.050
Choline chloride, 50%	0.045	0.058
Sweetener	0.020	0.020
Apramycin, 10%	0.150	0.150
Ferrous sulfate, 28%	0.062	0.062
Calculated chemical composition		
ME, kcal/kg	3,548	3,550
CP, %	19.89	19.00
Ca, %	0.81	0.80
Available P, %	0.56	0.59
Fe (analyzed), mg/kg	250 (227)	236 (225)

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic, apramycin), T1 (diet devoid of $FeSO_4$ and antibiotic), T2 (T1 diet added with 125 mg/kg tannic acid).
[2)] Calcium phosphate monobasic monohydrate (95.0%).
[3)] Calcium carbonate (98.0%).
[4)] Supplied per kg diet: 9600 IU vitamin A, 1800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.39 mg folic acid.
[5)] Supplied per kg diet: 10 mg Cu as copper sulfate, 100 mg Zn as zinc sulfate, 30 mg Mn as manganese sulfate, 0.3 mg I as calcium iodate, 0.3 mg Se as sodium selenite.

2. Experimental procedures

In both the experiments, pigs were weighed individually and feed consumption per pen was measured at the end of each phase. Growth performance in terms of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) was calculated for the experimental period. To study the effect of dietary treatments on the incidence of diarrhea, fecal consistency was measured daily using a macroscopic score from 1 to 4 (firm to liquid). Diarrhea was defined as liquid consistency (score 4) over a minimum of 2 consecutive days (Manner and Spieler, 1997). The number of piglets with diarrhea and its duration were recorded. The incidence of diarrhea (%) was calculated as a percentage of the number of affected piglets in each pen during the experimental period divided by the total number of piglets in the respective pens. At the end of the experiments (day 28), fecal samples were randomly collected from each pen, samples were weighed, suspended in sterile phosphate buffer saline (PBS) with cysteine (0.05% wt/vol), and used for enumerating microbial populations.

In both the experiments, blood was collected on day 14 and 28 by jugular venipuncture with 21 gauge, 3.81 cm needle from two pigs of each pen. For the analysis of hematological indices 0.5 ml of collected whole blood was transferred to 500-µl potassium EDTA BD Microtainer tube (BD Microtainer® Franklin Lakes, NJ, USA). For the plasma analysis the whole blood was collected into 10 ml heparinized (143 USP units of sodium heparin per tube) Vacutainer (Becton Dickinson, Co., USA) tubes. These tubes were centrifuged at 2000 × g at 4°C for 20 min for the separation of plasma and stored at −20°C until analyzed for Fe content.

3. Analyses

The Fe concentration in feed and plasma samples was determined by atomic absorption spectrophotometry (Shimadzu, Kyoto, Japan) as described by Miltenburg et al. (1992) and Olson and Hamlin (1969), respectively. All glassware used in the mineral analyses was soaked in 30% nitric acid for at least 12 h and rinsed three times with double-deionized water before use.

The whole blood collected in Microtainer tubes was subjected to analysis of total red blood cell count, total white blood cell count, hemoglobin, and hematocrit by using an autoanalyser (Hemacyte™, Oxford science, Inc., USA). Mean corpuscular hemoglobin concentration (MCHC) was calculated from the hemoglobin and hematocrit values.

Table 2. Ingredient and chemical composition of control diet (Exp. 2)[1)]

Ingredient, %	Control diet	
	Phase 1	Phase 2
Extruded corn	19.948	/
Ground corn	7.930	38.372
Extruded corn-soybean	25.000	5.000
Whey powder	12.550	5.000
Fish meal	8.000	7.790
Hamlet Protein -300 (55% CP)	7.000	/
Full fat soybean	6.000	5.080
Spray dried plasma protein	5.000	5.000
Soybean meal	3.000	20.000
Soybean oil	/	4.150
Wheat flour	/	2.000
Glucose	/	2.000
Sucrose	2.000	2.000
MCP	1.298	1.200
Limestone	0.346	0.827
L-lysine HCl, 78%	0.304	0.200
Vitamin premix[2)]	0.300	0.250
DL-methionine, 50%	0.230	0.087
Tryptophan, 10%	0.200	/
Acidifier	0.200	0.200
Vitamin C, 20%	0.200	0.200
Salt	0.100	0.250
Choline chloride, 50%	0.090	0.090
Trace mineral premix[3)]	0.100	0.100
Apramycin, 10%	0.150	0.150
Ferrous sulfate, 28%	0.054	0.054
Calculated chemical composition		
ME, kcal/kg	3,527	3,500
CP, %	21.80	21.60
Ca, %	0.85	1.00
Av. P, %	0.48	0.45
Lys, %	1.55	1.40
Fe (analyzed), mg/kg	331 (302)	318 (285)

[1)] Dietary treatments were: Control (basal diet added with $FeSO_4$ and antibiotic, apramycin), An (diet containing antibiotic but without $FeSO_4$), TA (diet added with 125 mg/kg tannic acid and devoid of $FeSO_4$ and antibiotic), AnTA (diet added with antibiotic and 125 mg/kg tannic acid but devoid of $FeSO_4$).

[2)] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.39 mg folic acid.

[3)] Supplied per kg diet : 10 mg Cu as copper sulfate, 100 mg Zn as zinc sulfate, 30 mg Mn as manganese sulfate, 0.3 mg I as calcium iodate, 0.3 mg Se as sodium selenite.

The microbiological assay of fecal samples was carried out by the procedure suggested by Torrallardona et al. (2003). One gram of mixed contents was diluted with 9 ml of Buffer-fields phosphate buffer dilution solution, followed by further serial dilutions in Buffer-fields phosphate buffer dilution solution. Duplicate plates were then inoculated with 0.1 ml sample and incubated. The microbial groups that were analyzed were total

anaerobic bacteria (Tryptic soy agar), *Bifidobacterium* spp. (de Man, Rogosa and Sharpe agar), *Lactobacillus* spp. (de Man, Rogosa and Sharpe agar), *Clostridium* spp. (Tryptose sulfite cycloserine agar) and Coliform bacteria (Voilet red bile agar). The anaerobic conditions were generated using an anaerobic jar with a gas generator envelope (GasPak Plus, disposable H_2 and CO_2 generating system with palladium catalyst). The final anaerobic atmosphere consisted of 6.5 to 7.5% CO_2, 25 to 35% H_2, with the balance being N_2.

The data generated in both the experiments was analyzed as a randomized complete block using the general linear model procedure of SAS (SAS Inst., Inc., Cary, NC). Data was analyzed by one way-ANOVA and when significant differences were noticed, means were separated by using Duncan's multiple range tests. Pen was the experimental unit for analysis of all the parameters. The microbial populations were transformed (log) before statistical analysis.

RESULTS

Experiment 1

1. Growth performance and diarrhea incidence

The ADG of pigs during phase 1, phase 2 and for the whole experiment was lower ($p<0.05$) in pigs fed T1 diet when compared with pigs fed control and T2 diets (Table 3). However, the ADFI and FCR did not differ among the dietary treatments. In addition, pigs fed T2 diet had less ($p<0.05$) incidence of diarrhea when compared with pigs fed control and T1 diet.

2. Fecal microflora

In the fecal microbial populations, significant differences ($p<0.05$) were observed among the treatments (Table 4). Pigs fed T2 diet had highest ($p<0.05$) fecal population of *Bifidobacterium* spp. and *Lactobacillus* spp. than T1 and control group. Moreover, the number of total anaerobic bacteria, *Clostridium* spp. and coliforms in the feces of pigs was highest in T1 followed by control and T2 diet.

3. Blood hematology and plasma iron

The red blood cell count, hemoglobin, and hematocrit on d 14, MCHC and white blood cell count on d 14 and 28 were unaffected by dietary treatments (Table 5). However, on d 28, the red blood cell count, hemoglobin and hematocrit values were lower ($p<0.05$) in pigs fed T2 diet when compared with pigs fed T1 and the control diet. Plasma Fe content on d 14 and d 28 was lower ($p<0.05$) in T2 diet, while it was highest in control and medium in T1 diet.

Experiment 2

4. Growth performance and diarrhea incidence

Inclusion of An, TA and both to the diets had no effects on the ADG, ADFI and FCR in pigs (Table 6). However, pigs fed An,

Table 3. Effects of tannic acid added to diets containing low level of iron on the growth performance of weanling pigs (Exp. 1)[1)]

Item	Control	T1	T2	SEM
Phase 1, d 0 to 14				
ADG, g	179[a]	157[b]	177[a]	3.70
ADFI, g	248	233	243	6.01
FCR	1.38	1.49	1.37	0.04
Phase 2, d 15 to 28				
ADG, g	364[a]	327[b]	352[a]	6.31
ADFI, g	565	520	553	10.00
FCR	1.55	1.59	1.57	0.02
Overall, d 0 to 28				
ADG, g	271[a]	242[b]	264[a]	4.72
ADFI, g	406	376	398	6.49
FCR	1.50	1.56	1.51	0.02
Diarrhea incidence[2], %	36.1[a]	37.5[a]	26.4[b]	2.41

[a,b] Means with different superscripts in the same row differ significantly ($p<0.05$).

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic), T1 (diet devoid of $FeSO_4$ and antibiotic), T2 (T1 diet added with 125 mg/kg tannic acid).

[2)] Diarrhea was defined as liquid feces and the diarrhea incidence represents the percentage of the number of affected piglets divided by the total number of piglets in each pen.

Table 4. Effect of tannic acid added to diets containing low level of iron on the fecal microbial populations in weaned pigs (Exp. 1)[1)]

Microbes, log_{10} cfu/g	Control	T1	T2	SEM
Total anaerobic bacteria	8.84[b]	9.22[a]	8.47[c]	0.117
Bifidobacterium spp.	8.95[b]	8.87[b]	9.81[a]	0.154
Lactobacillus spp.	8.89[b]	8.76[b]	9.71[a]	0.150
Clostridium spp.	7.59[b]	8.02[a]	7.50[b]	0.143
Coliforms	7.62[b]	8.15[a]	7.59[b]	0.138

[a,b,c] Means with different superscripts in the same row differ significantly ($p<0.05$).

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic), T1 (diet devoid of $FeSO_4$ and antibiotic), T2 (T1 diet added with 125 mg/kg tannic acid).

Table 5. Effect of tannic acid added to diets containing low level of iron on the hematological and plasma iron status of weaned pigs (Exp. 1)[1)]

Item	Control	T1	T2	SEM
Red blood cell, 10^6/μl				
d 14	7.2	7.2	6.7	0.22
d 28	6.6[a]	7.0[a]	4.7[b]	0.36
Hemoglobin, g/dl				
d 14	11.4	12.5	12.6	0.43
d 28	10.6[a]	10.9[a]	8.7[b]	0.37
Hematocrit, %				
d 14	39.7	45.4	42.2	1.73
d 28	36.1[a]	37.1[a]	29.1[b]	1.39
Mean corpuscular hemoglobin concentration, g/dl				
d 14	28.9	27.6	29.8	0.52
d 28	29.4	29.5	29.9	0.59
White blood cell, 10^3/μl				
d 14	27.2	31.0	24.0	2.00
d 28	25.4	22.4	22.2	1.19
Plasma Fe, μg/dl				
d 14	128[a]	103[ab]	87[b]	6.96
d 28	127[a]	86[b]	70[b]	7.85

[a,b] Means with different superscripts in the same row differ significantly ($p<0.05$).

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic), T1 (diet devoid of $FeSO_4$ and antibiotic), T2 (T1 diet added with 125mg/kg tannic acid).

Table 6. Effect of tannic acid and antibiotic added to diets containing low level of iron on the growth performance of weanling pigs (Exp. 2)[1)]

Item	Control	An	TA	AnTA	SEM
Phase 1, d 0 to 14					
ADG, g	260	276	264	255	5.507
ADFI, g	394	402	381	383	7.862
FCR	1.52	1.46	1.45	1.50	0.040
Phase 2, d 15 to 28					
ADG, g	393	411	403	405	9.482
ADFI, g	624	625	622	611	6.009
FCR	1.59	1.52	1.54	1.51	0.039
Overall, d 0 to 28					
ADG, g	326	343	333	330	3.865
ADFI, g	509	514	501	497	6.242
FCR	1.56	1.50	1.50	1.51	0.026
Diarrhea incidence[2], %	31.9[a]	19.3[b]	14.4[b]	12.5[b]	2.049

[a,b] Means with different superscripts in the same row differ significantly ($p<0.05$).

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and apramycin), An (diet containing antibiotic but without $FeSO_4$), TA (diet added with 125mg/kg tannic acid and devoid of $FeSO_4$ and antibiotic), AnTA (diet added with antibiotic and 125mg/kg tannic acid but devoid of $FeSO_4$).

[2)] Diarrhea was defined as liquid feces and the diarrhea incidence represents the percentage of the number of affected piglets divided by the total number of piglets in each pen.

Table 7. Effect of tannic acid and antibiotic added to diets containing low level of Iron on the fecal microbial population in weanling pigs (Exp. 2)[1)]

Microbes, log_{10} cfu/g	Control	An	TA	AnTA	SEM
Total anaerobic bacteria	9.13	9.08	9.02	9.04	0.051
Bifidobacterium spp.	9.95	9.80	9.76	9.90	0.054
Lactobacillus spp.	9.55	9.59	9.54	9.49	0.056
Clostridium spp.	8.49	8.47	8.44	8.62	0.043
Coliforms	7.33[a]	7.10[b]	6.79[c]	6.94[bc]	0.085

[a,b,c] Means with different superscripts in the same row differ significantly ($p<0.05$).
[1] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic, apramycin), An (diet containing antibiotic but without $FeSO_4$), TA (diet added with 125mg/kg tannic acid and devoid of $FeSO_4$ and antibiotic), AnTA (diet added with antibiotic and 125mg/kg tannic acid but devoid of $FeSO_4$).

Table 8. Effect of tannic acid and antibiotic added to diets containing low level of Iron on the hematological indices and plasma Fe in weanling pigs (Exp. 2)[1)]

Item	Control	An	TA	AnTA	SEM
Red blood cell, 10^6/μl					
d 14	5.7	5.8	5.7	5.6	0.129
d 28	6.6	6.0	6.4	5.9	0.128
Hemoglobin, g/dl					
d 14	12.7	11.8	12.4	11.8	0.289
d 28	10.3	10.2	10.2	9.5	0.149
Hematocrit, %					
d 14	38.9	36.5	39.2	36.5	1.039
d 28	36.4	36.5	35.5	33.5	0.673
Mean corpuscular hemoglobin concentration, g/dl					
d 14	32.7	32.6	31.7	32.3	0.312
d 28	28.2	28.0	28.7	28.4	0.342
White blood cell, 10^3/μl					
d 14	121	124	122	125	3.497
d 28	124	119	124	128	1.696
Plasma Fe					
d 14	121	124	122	125	3.497
d 28	124	119	124	128	1.696

[1)] Dietary treatments: Control (diet added with $FeSO_4$ and antibiotic, apramycin), An (diet containing antibiotic but without $FeSO_4$), TA (diet added with 125mg/kg tannic acid and devoid of $FeSO_4$ and antibiotic), AnTA (diet added with antibiotic and 125mg/kg tannic acid but devoid of $FeSO_4$).

TA and AnTA diets showed less incidence of diarrhea than pigs fed the control diet (19.3, 14.4 and 12.5% vs. 31.9%, Table 6).

5. Fecal microflora

The population of total anaerobic bacteria, *Bifidobacterium* spp., *Clostridium* spp., and *Lactobacillus* spp. was comparable among dietary treatments (Table 7). However, less coliforms were noted in the feces of pigs fed TA, An and AnTA diets when compared with pigs fed the control diet.

6. Blood hematology and plasma iron

The red blood cell count, hemoglobin, hematocrit, MCHC, white blood cell count and plasma Fe values on d 14 and 28 were comparable among dietary treatments (Table 8).

DISCUSSION

The analyzed Fe concentration of the control diet in phase 1 and phase 2 used in Exp. 1 was 227 and 225 mg/kg (Table 1), respectively; whereas, the analyzed Fe concentration of phase 1 and phase 2 treatment diets (T1 and T2) was 91 and 88 mg/kg, respectively (data not shown). The analyzed Fe concentration of the control diet in phase 1 and phase 2 used in Exp. 2 was 302 and 285 mg/kg, respectively; whereas, the analyzed Fe concentration of phase 1 and phase 2 treatment diets (An, TA and AnTA) was 194 and 178 mg/kg, respectively (data not shown). In both the experiments, ferrous sulfate was added to the control diets resulting in higher Fe concentration than other treatment diets.

Moreover, noticeable differences were seen in the Fe concentration of diets used in Exp. 1 and 2, which were due to the use of semisynthetic and feed grade limestone and monocalcium phosphate in Exp. 1 and Exp. 2, respectively. The composition of feed ingredients provided by NRC (1998) suggested that limestone and monocalcium phosphate contained 3500 and 7500 mg Fe/kg. In agreement with our findings, Rincker et al. (2005) had also noticed analyzed Fe values of 189 to 223 mg/kg in the nursery diets that were formulated to contain 50 to 60 mg Fe/kg and they had attributed this high Fe values in the diet to the higher Fe content of feed ingredients including limestone and monocalcium phosphate.

In Exp. 1, poor growth performance was noticed in pigs fed diet devoid of Fe and antibiotic (T1), and addition of TA to such diets was effective in improving the daily gains in pigs. The reason for this effect is not known but may be attributed to lower incidence of diarrhea, and presence of higher numbers of beneficial intestinal microflora in pigs fed TA added diets; moreover, T1 diet was devoid of any added antimicrobial. However, in Exp. 2, no such improvements in performance were noticed in pigs fed diets added with TA or TA in combination with antibiotics, in spite of lower diarrhea incidence in pigs fed An, TA, and AnTA diets. Previous researchers have reported TA to reduce growth performance in rats (Glick and Joslyn, 1970) and chicks (Kubena et al., 2001). Tannins are known to have a bitter or astringent taste which reduces palatability and hence feed intake (Jansman, 1993). In our previous study, pigs were fed with graded levels of TA (0 to 1000 mg/kg diet) and the ADG and ADFI of pigs were reduced when TA was added in the diet at 250 mg/kg and higher levels, but no negative effects were noticed when TA was added at 125 mg/kg diet (Lee, 2007). Thus in the current study the level of 125 mg/kg TA was used.

Weaning piglets are under various stressors. Nevertheless this is also the phase at which microbial fluctuations occur in the intestine which provide an opportunity for pathogenic coliforms and other bacteria for invasions resulting in gastric disturbances and reduced performance (Mathew et al., 1996). Diarrhea in weanling pigs may be either due to indigestion (Nagy et al., 1992) or due to fluctuations in the intestinal microbial populations (Mathew et al., 1996). In both the experiments, inclusion of TA had resulted in lower diarrhea incidence, and this may be attributed to the antimicrobial activity of TA. In Exp. 1, pigs fed TA diets had more beneficial fecal bacteria and lower population of harmful bacteria than pigs fed T1 diet, whereas, in Exp. 2, pigs fed diets devoid of Fe and added with An, TA and AnTA were effective in reducing the fecal coliforms suggesting that TA might have antimicrobial properties.

In line with our findings, Chung et al. (1998) had reported TA can inhibit the growth of all intestinal bacteria except the lactic acid bacteria *Lactobacillus* and *Bifidobacterium*. Inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation, and more importantly Fe binding capacity of TA are the mechanism suggested for the antimicrobial property of TA (Scalbert, 1991; Akiyama et al., 2001). Tannins from green tea were found to be effective in reducing the number of harmful bacteria while increasing the populations of *Lactobacillus* spp. in pigs (Hara et al., 1995), and chickens (Hara, 1997). However, further studies are required for revealing the interaction of intestinal microbes and dietary constituents and its impact on animal performance.

All the values of hematological indices (total erythrocyte count, hemoglobin, hematocrit and mean corpuscular hemoglobin concentration) were in the normal range for pigs during the course of both the experiments. The blood hemoglobin concentration of 8.0 g/dl is considered as borderline anemia, whereas 7.0 g/dl or less is an indicator of anemia (Zimmerman, 1980). In Exp. 1, the pigs fed TA added diet devoid of supplemental Fe had 8.7 g/dl hemoglobin, suggesting that if the feeding period had been extended these pigs might have developed anemia. In addition, the plasma Fe concentration on d 28 was also lower in pigs fed diets devoid of supplemental Fe (T1) and due to addition of TA. In line with our findings, Afsana et al. (2004) had also noticed lower hemoglobin, hematocrit and serum Fe values in rats fed 10 to 25 g TA/kg diets. The lower values of hematological indices and plasma Fe may be attributed to the relationship of TA with Fe.

In contrast with the findings of Exp. 1, in Exp. 2 addition of TA to diets devoid of supplemental Fe did not have any negative influence on the hematological indices as well as plasma Fe status. These findings may be related to the concentration of Fe in the diets used in Exp. 1 and Exp. 2; suggesting that dietary Fe levels during Exp. 2 were sufficient to negate any detrimental effects of TA included in the diets. Additionally, the findings also suggest that there was no negative effect on the performance of pigs fed diets without any supplemental Fe source (as noticed in Exp. 2), when the feed ingredients used while formulating the diets had sufficient Fe concentration.

Thus the findings of our study suggest that TA has a negative influence on blood hematology and plasma Fe status of pigs when diets are inadequate in Fe; however, TA reduced diarrhea incidence and might have antimicrobial activity.

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