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Natural Additives Influence the Performance and Humoral Immunity of Broilers

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Abstract: Animal nutrition as a scientific field is based on many basic sciences, such as chemistry, biochemistry, physiology and even microbiology. In this relation broiler production is one of the economic ways for protein supply. Regarding more than 32 antimicrobial compounds which is used in poultry production without any prescriptions and entering these compounds in human nutrition is considered more seriously. Recent advances in this field suggest using pre- and pro-biotics are more beneficial than antibiotics and have fewer disadvantages. This study was conducted to compare the effects of using an organic acid (citric acid) and natural additive (APC) on broiler performance and its relation with gut circumstances. 960 sexed Ross 308 chicken in a completely randomized design test in 6 groups with 4 replicates received corn-soybean meal based diet (control, group C) supplemented with 2% citric acid (group B) or 0.2% APC (group A). Body weight (BW), feed conversion ratio (FCR), duodenum and jejunum pH, ileum microflora, gut histological changes and serum gamma globulin were evaluated on days 21 and 42. Analyzed results and compared means by Duncan's range test showed that microflora content of the intestine is dynamically changed by adding these compounds and decreasing the pH of intestine significantly ($p<0.05$) affects broiler performance and other parameters by acting on microbial population of digestive system.

Key words: APC, citric acid, broiler, gut microflora, immunity

Introduction

Nowadays food safety is more seriously considered than before. On the other hand economy of food production is also a factor which is not ignorable. In applied physiology nutrition and growth are two closely related and complementary subjects which are considered with each other and applying different strategies in this relation have considerably improved animal production. Antibiotics are normally used in poultry for therapeutic or prophylactic purposes and even growth promoters to improve performance, as today's around 32 antibiotics are used in poultry production (Jones and Ricket, 2003). All these antimicrobial compounds improve the gut conditions by affecting on microflora content of the digestive system, which make the nutrients more available for the host than microorganisms which live in this area. Gut conditions, as the main part of the body and responsible for digestion and absorption, are the subject of many researches. Digestive system of all species including avian has a dynamic property which regulates itself depending on the physiological requirements and present circumstances. This dynamic situation of the gastrointestinal tract (GIT) is dependent to many factors and the intestinal pH as well, particularly in chicken which seems different from other species (Farner, 1942). Relatively acidic pH of the avian GIT is also dependent to some factors such as, health of the chicken, kind of nutrients and more important, microflora content of the GIT. Correlation between the pH and microflora content, and microflora and nutrient are mutual (Sarra *et al*., 1985). The pH level in specific areas of the GIT is a factor which establishes a specific microbial population, and also affects the digestibility and absorptive value of most nutrients. Most of the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens (Ferd, 1974). In addition, it has been suggested that lowering the pH by organic acids improves nutrient absorption (Boling *et al*., 2001).

The history of using antibiotics in poultry ration began 60 yeas ago (Moore *et al*., 1946), and today there are several antibiotics which are allowed to be used in poultry production (Jones and Ricket, 2003) as growth promoters. All of them decrease microbial load in the GIT and improve weight gain and feed conversion ratio because they make more nutrients available to the host. Using antibiotics to establish a beneficial condition in the GIT has some disadvantages as well. These days antibiotic resistance (Bates *et al*., 1994; Roy *et al*., 2002) and entering the chemical drugs to human food chain are seriously considered. Several ways have been suggested as strategies to limit the antibiotic usage. Addition of prebiotics, probiotics (Fuller, 1989) and some organic acids (Chaveerach *et al*., 2004) to the poultry ration are few of these solutions. The present study was conducted to evaluate the effects of citric acid and a

Table 1: Nutrient content of the ration over three periods of trial. A (APC group), B (citric acid group) and C (control group). All groups had two levels of Ca and P (NRC recommended and 35% less than NRC level)

Nutrient (%)	0-14 day			15-28 day			+29 day		
	A	B	C	A	B	C	A	B	C
APC	0.2	-	-	0.2	-	-	0.2	-	-
Corn	50.70	48.90	47.40	50.66	48.86	47.90	54.82	53.02	52.15
Soya	41.69	41.69	42.69	39.69	39.69	39.53	35.71	35.71	35.89
Poultry Fat	4.59	4.59	5.55	6.71	6.71	8.19	6.68	6.68	7.95
Methionine	0.28	0.28	0.29	0.33	0.33	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.11	0.19	0.19	0.04	0.09	0.09	0.08
DCP	0.99	0.99	2.04	0.64	0.64	2.09	0.68	0.68	1.83
Limestone	0.48	0.48	1.06	0.68	0.68	1.08	0.68	0.68	0.96
Salt	0.47	0.47	0.37	0.40	0.40	0.37	0.34	0.34	0.34
Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Citric acid	-	2	-	-	2	-	-	2	-

Table 2: Ration analysis over three periods of trial. A (APC group), B (citric acid group) and C (control group)

Ration analysis	0-14 day			15-28 day			+29 day		
	A	B	C	A	B	C	A	B	C
Energy (Kcal/kg)	2902	2902	2950	3082.4	3022.7	3102.7	3130.3	3070.5	3144.6
Protein (%)	21.19	21.19	21.5	20.48	20.35	20.21	19.13	18.99	19
Lysine	1.267	1.268	1.30	1.289	1.285	1.164	1.121	1.117	1.111
Methionine	0.600	0.600	0.611	0.641	0.638	0.606	0.596	0.592	0.592
Met. + Cys.	0.948	0.948	0.960	0.980	0.973	0.938	0.920	0.913	0.898
Ca	0.537	0.537	1	0.528	0.527	1.01	0.528	0.527	0.911
P available	0.320	0.320	0.50	0.257	0.255	0.500	0.256	0.254	0.45
Na	0.201	0.201	0.16	0.174	0.174	0.162	0.151	0.15	0.15
Fiber	4.16	4.16	4.20	4.05	4.01	3.98	3.86	3.82	3.81

natural product (APC) from Agrar Production and Consulting (APC) Company in Austria on broiler's performance, immunity and gut performance.

Materials and Methods

The experiment was designed as a completely randomized test with 6 treatments and 4 replicates. Treatments included normal diet (group C), normal diet + 2% citric acid (group B) and normal diet + 0.2% APC (group A), with a basal diet (Table 1) and it's content (Table 2). Regarding the special formula of the APC which uses 30-40% lower calcium (Ca) and phosphorus (P) than NRC, all these groups were treated with 2 levels (H, high and L, low) of these minerals and final treatments were; A_H, A_L, B_H, BL, C_H and C_L, and each treatment had 4 replicates. 960 sexed one day old Ross 308 chicken (480 pair male and female) were divided equally into 24 cages (0.5×0.5m). Chicken were kept under the Ross recommended procedure and received 6 different diet formulas based on corn-soybean meal which contained 2900-3100Kcal metabolizable energy and 19-21% protein as recommended by NRC over the different periods of production. But, Ca and P ratio were two levels for each group, NRC recommended (H) and 35% less than NRC (L). Chicken had free access to food and water, no antibiotics were used at all, and they were vaccinated against Newcastle disease (ND, days 10, 20 and 30, eye drop), infectious bronchitis (IB, days 1, 8 and 18, spray) and Gumboro (BD, days 14 and 24, drinking). Food consumption and weight gain were recorded weekly for 7 weeks, and blood samples were collected with 10 days interval via brachial vein. On days 21 and 42 two chicken of each replicate from both sexes were sacrificed with central ketamin injection. The ileum content plus duodenal and jejunal contents and tissues were collected for microbial, pH, and histological studies, respectively. The obtained data were analyzed by GLM using SAS software and Mean±SEM were compared by Duncan's range test.

Results

Results of the analyzed data for body weight, food consumption and feed conversion ratio are shown in Table 3. As this Table indicates the body weight gain is higher in APC group and citric acid group is between control and APC groups. The same situation is also comparable for food consumption and feed conversion ratio, which APC group is much better. The results of

Table 3: Body weight (BW, gr), food consumption (FC, gr) and feed conversion ratio (FCR) of three groups of trial over different periods of production. A (APC group), B (citric acid group) and C (control group)

Age (day)	Bw (gr)			Bw gain (gr)	
Group	21 day	42 day	47 day	22-42 day	43-47 day
A	560.4±9.0[a]	1918.7±22.7[a]	2244.0±37.3[a]	1358.4±15.0[a]	325.2±21.3
B	543.1±10.1[ab]	1871.2±57.2[a]	2191.8±45.6[a]	1328.2±47.6[a]	320.6±29.0
C	514.8±5.9[b]	1680.2±25.8[b]	2029.6±8.2[b]	116.5±29.9[b]	349.5±23.1
	FC (gr)			FC (gr)	
A	445.7±5.3	3447.1±80.0	4219.9±58.2	2630.6±79.1	772.9±35.6
B	469.5±7.6	3506.2±101.1	4295.6±84.4	2665.6±86.8	789.4±19.0
C	445.8±3.0	3641.8±53.8	4445.9±72.9	2773.6±55.4	804.1±19.4
	FCR			FCR	
A	1.383±0.012[a]	1.750±0.023[a]	1.837±0.033[a]	1.903±0.035[a]	2.414±0.198
B	1.430±0.012[ab]	1.810±0.002[ab]	1.917±0.015[a]	1.976±0.030[ab]	2.635±0.214
C	1.480±0.015[b]	2.037±0.038[b]	2.077±0.027[b]	2.309±0.068[b]	2.319±0.143

Numbers are Mean ± SEM of collected data. Values with common superscript letters indicate no significant difference ($p<0.05$).

intestinal pH, and microflora content, and serum electrophoresis are shown in Table 4. This Table shows lower intestinal pH, higher intestinal lactobacillus content and higher gamma globulin for APC group. Compared these results statistically shows significant differences ($p<0.05$) among treatments for some parameters. Histological studies of intestine also confirmed the better situation of apical cells of the intestine for APC group (results not shown).

Discussion

It is well established that the gastrointestinal normal microflora plays an important role in the health and well-being of poultry. Various pathogenic microbes, such as Escherichia coli, have been implicated to reduce the growth of poultry. Possible mechanisms for this reduction of growth are: toxin production, utilization of nutrients essential to the host, and suppression of microbes that synthesize vitamins or other host growth factors. Avians possess the same basic structures for nutrient extraction as other vertebrate, a tubular intestine, but specific variation within the avian gastrointestinal tract (GIT) includes a crop for storage of feed, proventriculus (simple stomach), gizzard, and paired ceca (Duke, 1986). The pH values of specific sections of the chicken GIT are: crop 4.5, proventriculus 4.4, gizzard 2.6, duodenum 5.7 to 6.0, jejunum 5.8, ileum 6.3, colon 6.3, ceca 5.7, and bile 5.9 (Farner, 1942). These pH values in specific areas of the avian GIT selectively allow establishment of a specific microbial population in birds. Regarding these facts about the poultry digestive system in this study they are considered with each other. The best results belong to group A (APC) for all factors, group B is intermediate and group A is in the third situation. It seems the measured factors are correlated, because as the pH lowers, BW and FCR are improved and ratio of Lactobacillus to Escherichia coli also increases. The percentage of gamma globulin is changed significantly, and is relatively higher for group A. These results confirm the findings about the relation between gut circumstances and performance of broilers. The pH values are close to the Farner (1942) report, and seems acidic in broiler's digestive system, which allow establishment of specific pH and microorganisms, particularly Lactobacillus spp (Sarra *et al*., 1985). The mutual situation between microflora and pH prevents of *E. coli* growth and this conditions make the absorptive area more beneficial (Dofing and Gottschal, 1997), as the histological studies confirmed these changes (results not shown). On the other hand acidic conditions make the nutrients more available (Boling *et al*., 2001) which monitors better performance. As mentioned earlier no antibiotic was used in this study, and group A had the least mortality rate compared with other groups, probably related to better immunity and lower pathogens in their gut. APC as a natural additive stabilizes the pH in the digestive system and lower concentration of Ca with the mineral source synergizes this situation. Because Ca has a high acid binding capacity which is able to neutralize the H^+ ions and at lower Ca level it works better. But using the low Ca level in other groups made some problems such as paralysis and tremor, that sounds NRC level of Ca is standard for normal diets, but might be manipulated by organic acids or natural prebiotics such as APC.

Increasing pressures from consumer concerns make it necessary to reduce the use of antibiotics in feed due to the negative human health issues by antibiotic resistance. In 1994, Bates et al. first reported that vancomycin-resistant enterococci (VRE) could be traced to farm animals in Great Britain and they suggested that farm animals could be a reservoir for the VRE infection.

Table 4: Total colony count, Colibacillus and Lactobacillus (Log No/gr), and their ratio of the ileum content, and serum gammaglobulin in three different groups of the trial. A (APC group), B (citric acid group) and C (control group)

Group	A	B	C
Total microflora of the ileum (Log No/gr)	7.6±2.1[a]	9.3±2.5[ab]	11.6±2.8[b]
Colibacillus (Log No/gr)	3.4±1.1[a]	5.6±1.8[ab]	7.4±2.1[b]
Lactobacillus (Log No/gr)	4.2±1.3	5.5±2.1	7.2±3.1
LactobacillusColibacillus ratio	1.3±0.3[a]	0.7±0.5[ab]	0.5±0.4[b]
Intestinal pH	6.22±0.22[a]	6.51±0.45[ab]	6.71±0.31[b]
Serum gammaglobulin (mg%)	38±5[a]	26±4[b]	19±5[b]

Numbers are Mean ± SEM of collected data. Values with common superscript letters indicate no significant difference ($p<0.05$).

They isolated 62 of VRE from nonhuman sources, of which 22 were from farm animals and 5 from uncooked chicken. Roy *et al.* (2002) also reported that 91 of the 92 Salmonella samples isolated from poultry products, live poultry, and poultry environment were resistant to erythromycin, lincomycin, and penicillin. Many measurements have been developed to reduce the use of antibiotics as growth promoters. Enhanced biosecurity of poultry farm (Tablante *et al*., 2002), genetic selection of poultry resistant to disease (Gross *et al*., 2002), and vaccination to pathogenic microbes (Williams, 2002) have successfully protected poultry production from disease loss. Competitive exclusion (Nurmi and Rantala, 1973) is also a popular strategy for preventing poultry from intestinal infectious disease due to the effective inhibition of pathogenic bacteria (La *et al*. 2003) among hundreds of microbial population (Vaughan *et al*., 2000) in the gut. Probiotic (Fuller, 1989) with defined bacteria and prebiotic with ability to aid growth of beneficial bacteria have been reported to enhance poultry growth (Jernigan *et al*., 1985; Fernandez *et al*., 2002). Organic acids, enzymes, and mycotoxin binding agents also have positive effects on poultry growth (Chaveerach *et al*. 2004; Raju and Devegowda, 2000). In this relation it seems interesting to focus on using natural products such as flavonoids or organic acids in animal diet to reduce the microflara content, particularly pathogens and in this case improve the performance and immunity of the host instead of using antibiotics.

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