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Broiler Chick Responses to Anorectic Agents: 1. Dietary Acetic and Propionic Acids and the Digestive System

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PINCHASOV, Y. AND S. ELMALIAH. *Broiler chick responses to anorectic agents: 1. Dietary acetic and propionic acids and the digestive system.* PHARMACOL BIOCHEM BEHAV 48(2) 371-376, 1994. – The effects of various levels of acetic or propionic acid supplementation on feed intake and the gastrointestinal tract were studied in female broiler chicks. Voluntary feed intake and body weight gain decreased with the inclusion of the acids. Results indicate that acetic and propionic acids exert a similar anorectic effect on chicks fed diets differing in energy content. The inhibitory effect of these acids on voluntary feed intake could not be attributed to alterations in feed utilization, to differences in pancreatic α-amylase activity, or to differences in the acid-base balance. The increase in cecal capacity and volatile fatty acid (VFA) content is probably due to increased utilization of nonstarch polysaccharides at that site, the latter compensating for feed inhibition. This reflects a slight adaptive response of the cecum to anorectic agents.

Acetic acid Anorectic agents Broiler chicks Cecum Feed restriction Gastrointestinal tract Pancreatic α-amylase Propionic acid

THE use of feed restriction in poultry production is well known, and in some branches even essential. Quantitative feed restriction has commonly been used to control pullet growth, by feeding a predetermined amount of a balanced diet (10, 15,26). An alternative to physical feed restriction is qualitative restriction, via a self-restricting diet (9,14,16,24). This is achieved by supplementing drugs or chemicals to a balanced diet, or by feeding a diet deficient in one or several essential nutrients, such that the birds will voluntarily restrict themselves when fed ad lib.

Several short-term studies examining the use of dietary acetic and propionic acids in chicks (4,6,17,25) have confirmed their inhibitory effects on feed intake. However, the mechanism by which feed inhibition is effected is uncertain. Regulation of voluntary intake, although known to be controlled by the CNS, can be mediated through different mechanisms and compartments (i.e., the digestive, peripheral blood, or central systems). To determine the role of the gastrointestinal tract (GIT) in feed regulation effected by dietary supplementation of acetic and propionic acids, the capacity of GIT segments and the rate of pancreatic α-amylase secretion were examined in fast-growing chicks. These anorectic compounds were supplemented to diets containing 2500 or 2750 kcal metabolizable energy (ME) per kg.

The avian digestive tract has long been recognized to contain volatile fatty acids (VFAs), the greatest concentration being in the cecum (1). The production of VFAs in the latter depends mainly on dietary fiber intake (7,11,12,20). It is not known, however, whether dietary supplementation of acetic and propionic acids affects cecal VFAs. The production of VFAs in the cecum were therefore examined in the present study.

MATERIALS AND METHODS

Experimental Animals

Broiler breeder females of the Arbor Acres strain, 120 days old, were brooded in battery cages located in a temperature- and ventilation-controlled room. They were provided with a commercial starter diet and had free access to food and water for the first week of age. At seven days of age, 96 selected birds were weighed, wing-banded, and randomly distributed in individual cages. They were fed ad lib diets supplemented

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TABLE 1
EXPERIMENTAL DIET COMPOSITIONS

	Calculated Composition (g/kg)									
ME (kcal/kg)	2500	2500	2500	2500	2500	2750	2750	2750	2750	2750
Protein	162	162	162	162	162	179	179	179	179	179
Met + Cys	5.36	5.37	5.38	5.35	5.20	5.91	5.89	5.85	5.91	5.77
Lysine	7.75	7.89	8.19	7.77	7.83	9.26	9.40	9.71	9.28	9.35
Calcium	10.0	10.0	10.0	10.0	11.0	10.0	10.0	10.0	10.0	11.5
Available phosphorus	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
Acetic acid		10	30				10	30		
Propionic acid				10	30				10	30

Vitamins were provided per kg diet: vitamin A, 8500 IU; cholecalciferol, 1700 ICU; α-tocopherol acetate, 10 mg; vitamin K (menadione sodium bisulfite), 2 mg; riboflavin, 5 mg; Ca-pantothenate, 10 mg; niacin, 20 mg; vitamin B_{12}, 10 μg; folic acid, 500 μg; pyridoxine, 1.5 mg; choline chloride, 200 mg; ethoxyquin, 125 mg. Minerals were provided per kilogram diet: Mn, 80 mg; Zn, 50 mg; Fe, 25 mg; Cu, 2 mg; I, 1.2 mg; Co, 200 μg; Se, 100 μg.

with acetic and propionic acids (as sodium and hemicalcium salts, respectively; Sigma Chemical Co., St. Louis). These compounds were supplemented at levels of 0%, 1%, and 3% of diets containing 2500 or 2750 kcal ME per kg (Table 1) and examined in a block design (two compounds by two energy levels by three doses by eight birds each) to 21 days of age. Body weights and feed intakes were monitored individually each week. Necropsy was carried out at 21 days of age on four birds per subgroup. The birds were killed by cervical dislocation, and the pancreas was removed, weighed, and frozen (−20°C). The GIT segments were cut and weighed with and without contents, and the pH of the ingesta was measured for each segment. Cecal ingesta was kept frozen (−80°C) for VFA determination.

Pancreatic α-Amylase Assay

The activity of pancreatic α-amylase (EC 3.2.1.1) was determined using a modification of Bernfeld's (2) procedure. The pancreas was homogenized in 50 volumes of ice-cold deionized water with an Ultra-Turrax homogenizer (setting 5, 30 s). The homogenate was centrifuged at 12 000 × *g* for 15 min (0–4°C) and a 100-μl aliquot of the supernatant was used for enzyme assay. Starch solution (1 g/100 ml; Merck, Darmastadt, Germany) gelatinized in 20 mM phosphate-buffered saline (PBS), pH 6.9, containing 10 mM NaCl, was used as a substrate. This solution (1 ml) was added to duplicate supernatant samples and incubated for 3 min at 37°C. For each assay, a soluble starch solution (0–8 mg/ml; Merck) was hydrolyzed with α-amylase from *Aspergillus oryzae* (16 U/ml; Sigma) as a standard. One unit of amylase activity was defined as that hydrolyzing 1 mg starch per 3 min at 37°C. The increase in reducing power following hydrolysis, as measured by dinitrosalicylic acid (DNS) reagent, was used to determine amylolytic activity. After the addition of DNS (2 ml), reaction tubes were heated for 5 min in boiling water and cooled to room temperature, and absorbance was measured at 550 nm. Colorimetric determination was carried out using a computerized microplate reader (Bio-Tek Instruments, EL309, Winooski, VT).

Volatile Fatty Acids in Cecal Ingesta

VFAs in the cecal contents were determined as described for ruminants (22), using gas–liquid chromatography (Hewlett

TABLE 2
BODY WEIGHT (BW), BW GAIN, FEED AND ENERGY INTAKES, AND GAIN : FEED RATIO OF FEMALE BROILER CHICKS FED AD LIB DIETS WITH ACETIC OR PROPIONIC ACIDS SUPPLEMENTED AT LEVELS VARYING FROM 0% TO 3% OF DIETS CONTAINING 2500 AND 2750 kcal ME/kg

	Energy		Acid		Dose				Main Effects Significance of *F* Values		
Variable	2500	2750	Acetic Acid	Propionic Acid	0	1	3	Root MSE*	Energy	Acid	Dose
Feed intake (g/bird)	745	786	780	751	820	782	693	72	0.005	0.048	0.0001
ME intake (kcal/bird/2 weeks)	1861	2162	1951	1877	2050	1957	1733	180	0.006	0.048	0.0001
Body weight (g)											
7-day-old	152	148	153	148	152	147	152	16	NS	NS	NS
21-day-old	552	591	585	559	606	563	546	66	0.004	0.057	0.001
BW gain (g)	400	443	432	411	454	417	394	62	0.001	NS	0.001
Gain : feed ratio	0.54	0.56	0.55	0.55	0.55	0.53	0.57	0.08	NS	NS	NS

*MSE = mean square error. n = 28–42 birds per group. No significant interactions were noted between main effects. NS = not significant ($p > .05$).

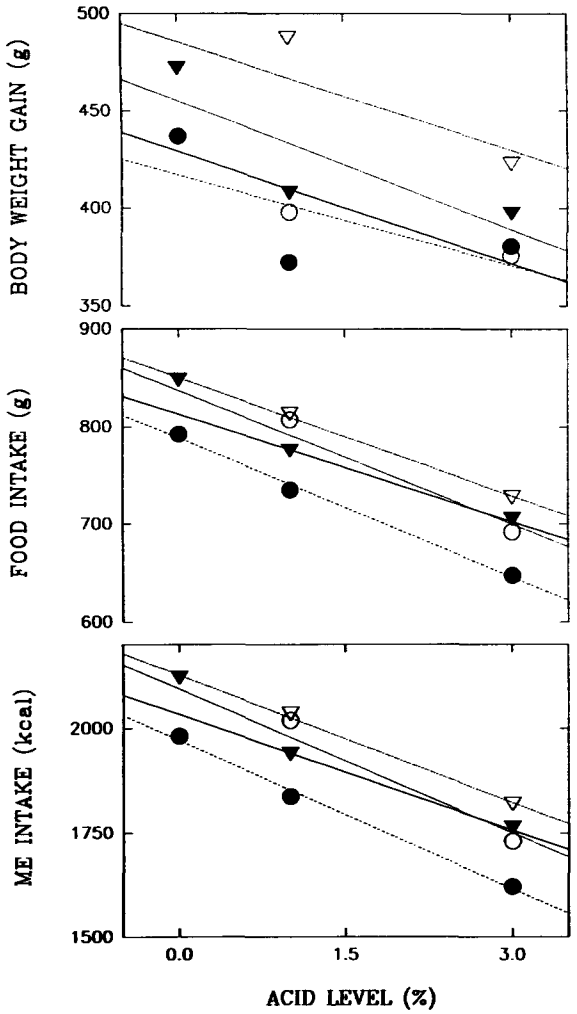

FIG. 1. Voluntary ME and feed intakes and body weight gain in 21-day-old chicks fed ad lib diets with acetic (○,▽) or propionic (●,▼) acids supplemented for two weeks at levels varying from 0% to 3% of diets containing 2500 (circles) and 2750 (triangles) kcal ME/kg. Each symbol represents the average of eight birds.

Packard, 5890A, Avonadale, PA). After defrosting to 0–4°C, 0.5-ml aliquots of the samples were homogenized in two volumes of deionized water and centrifuged for 5 min in 12 000 × *g* (4°C). The supernatants were then decanted and deproteinized with 10 volumes of *m*-phosphoric acid (25%, w/v). Immediately thereafter, 1-μl aliquots, as well as a standard solution, were subjected to gas chromatography and the VFAs were quantified by an integrator (Hewlett Packard, 3396A).

Statistical Analysis

Analysis of variance was carried out using the general linear models (GLM) procedure of base SAS® software (19). A nonrandomized factorial design was used in which dietary energy and anorectic compounds and their doses (and all interactions) were assigned as main effects. All measurements were carried out on individual birds.

RESULTS

The anorectic effects of acetic and propionic acids on feed intake and body weight gain were examined in chicks 7–21 days of age. Since no significant interactions were noted between main factors (except for cecal VFA content), results are shown as main effects. Voluntary feed intake decreased significantly in a dose-dependent manner with the inclusion of the acids, to a greater extent with propionate supplementation ($p < .048$; Table 2 and Fig. 1). A low-energy diet (2500 kcal/kg) further decreased feed intake ($p < .005$) when supplemented with either acid, as compared to the 2750-kcal diet. A similar trend was observed for ME intake (Table 2 and Fig. 1). Body weight and body weight gain decreased considerably with acid supplementation and with decreasing dietary energy level; however, no significant differences were noted between the acids. Gain : feed ratios were not affected by any tested factor.

Although the gizzard was unaffected, the relative weight of the proventriculus increased significantly with increased dosage of either acid (Table 3). The relative weights of the cecum and its capacity (cecum + content) increased significantly in a dose-dependent manner with inclusion of either acid. In comparison to acetate supplementation, much higher cecal weights ($p < .02$) and capacities ($p < .011$) were observed in chicks provided with dietary propionate (Table 3). A slight increase in cecal weight ($p < .016$) and capacity (NS) was observed in birds fed the 2750-kcal diet, as compared to the low-energy diet.

The pH in the proventriculus was not influenced by any tested factors (Table 3); however, it tended to increase with the inclusion of either acid. Duodenal pH was not affected by acid supplementation (Table 3), but a slightly more acidic pH was observed in birds fed the low-energy diet ($p < .006$), versus the 2750-kcal one. A similar energy effect was observed with the cecal pH (Table 3); however, it also increased gradually ($p < .0001$) with the inclusion of either acid. The relative weight of the pancreas was not affected by acid supplementation or energy levels. Total, specific (U/g), and relative (U/g body weight) activities of pancreatic α-amylase were not influenced by dietary anorectants, but significantly higher activities ($p < .001$, .005, and .01, respectively) were observed with the 2750-kcal diet versus the 2500-kcal one (Table 3).

Total VFA concentration in the cecal ingesta was significantly higher in birds fed the 2750 kcal diet compared to birds fed the 2500 kcal one, and it increased to a greater extent with acid supplementation in the 2750-kcal diet (Table 4). Examination of the cecal ingesta indicated that the VFAs were composed, in molar proportion, mainly of acetate (64 to 68%), whereas propionate levels were less than 15%. As can be seen from Fig. 2, the cecal acetate level was higher when acids supplemented to the 2750-kcal diet (significant Dose × Acid interaction; Table 4). Cecal propionate, however, increased (to a peak value with acetate supplementation) with the inclusion of either acid at both energy levels (Fig. 2). A greater increase in cecal propionate ($p < .0001$) was observed with dietary supplementation of acetate versus propionate. Cecal butyrate was higher ($p < .033$) in birds fed the 2750-kcal diet versus birds fed the 2500-kcal diet (Table 4), displaying a pattern opposite to that observed for cecal propionate (Fig. 2).

DISCUSSION

The expected inhibitory effect of dietary supplementation with acetic and propionic acids on voluntary feed intake was evident in the current study. Feed and ME intakes decreased significantly in a dose-dependent manner with the inclusion of either acid, to a greater extent with propionate supplementation (Table 2 and Fig. 1). These observed responses are in accordance with results obtained from short- (4,6,17) and

TABLE 3

GASTROINTESTINAL SEGMENT WEIGHTS, INTESTINAL INGESTA pHs, PANCREAS WEIGHT AND PANCREATIC α-AMYLASE ACTIVITY OF 21-DAY-OLD FEMALE BROILER CHICKS FED AD LIB DIETS WITH ACETIC OR PROPIONIC ACIDS SUPPLEMENTED AT LEVELS VARYING FROM 0% TO 3% OF DIETS CONTAINING 2500 AND 2750 kcal ME/kg

	Energy		Acid		Dose				Main Effects Significance of F Values		
Weights of Organs	2500	2750	Acetic Acid	Propionic Acid	0	1	3	Root MSE*	Energy	Acid	Dose
					g/100 g BW						
Proventriculus	0.62	0.59	0.60	0.61	0.57	0.59	0.65	0.08	NS	NS	0.034
Gizzard	2.44	2.36	2.38	2.41	2.41	2.38	2.39	0.38	NS	NS	NS
Cecum	0.59	0.67	0.59	0.67	0.60	0.59	0.71	0.10	0.016	0.020	0.007
Cecum with content	1.51	1.57	1.43	1.65	1.35	1.52	1.75	0.28	NS	0.011	0.002
pH of intestinal ingesta											
Proventriculus	4.12	4.13	4.10	4.15	3.98	4.15	4.24	1.08	NS	NS	NS
Duodenum	6.05	6.27	6.11	6.21	6.17	6.16	6.16	0.27	0.006	NS	NS
Cecum	5.91	6.14	6.03	6.03	5.74	6.07	6.28	0.32	0.019	NS	0.0002
Pancreas weight	0.31	0.31	0.31	0.32	0.33	0.31	0.31	0.05	NS	NS	NS
Pancreatic α-amylase activity											
Units	3231	3884	3552	3563	3687	3636	3348	644	0.001	NS	NS
U/g	1780	1938	1817	1901	1843	1864	1869	183	0.005	NS	NS
U/g BW	5.34	6.14	5.66	5.82	5.68	5.87	5.67	1.02	0.010	NS	NS

*MSE = mean square error. n = 8–20 birds per group. No significant interactions were noted between main effects. NS = not significant ($p > .05$).

long-term (16) studies. Anorectic agents exhibited a synergistic effect with low-energy diets (2500 kcal ME/kg) on reducing feed intake in the present study, enabling further decreases in feed and ME intakes via a self-restricting diet.

The mechanism by which short-chain fatty acids inhibit feed intake is still unknown. The insignificant effect of dietary anorectants on the gain : feed ratio (Table 2) indicates that feed intake is inhibited by a peripheral blood or central mechanism, not through an alteration in feed utilization. This hypothesis is also supported by the anatomic and biochemical measurements in the GIT. Neither the relative weight of the pancreas nor its amylolytic activity were influenced by acid supplementation (Table 3). On the contrary, compensatory increases in the relative weight of the cecum and its capacity were found with supplementation (Table 3). This is due to increased severity of feed inhibition (18), resulting in increased utilization of the nonstarch polysaccharides (NSPs) at this site (5,12,20,23).

There is evidence that the inhibitory effect on feed intake is not due to the high acidity of the supplemented anorectants affecting acid–base balance. First, VFAs were incorporated into the diets as sodium or calcium salts (Table 1). Secondly, the pHs of the proventriculus and duodenum ingesta were not affected by the supplementation (Table 3). This assumption is also supported by results from a previous study (17) in which propionic acid neutralized with KOH still significantly depressed feed intake in a linear manner. In addition, a decrease in cecal acidity (a gradual increase in cecal pH) was observed with the incorporation of either acid (Table 3), probably as a result of the diluting effect of increased cecal content.

TABLE 4

VOLATILE FATTY ACID (VFA) CONCENTRATIONS (μmol/g content) IN CECAL INGESTA OF 21-DAY-OLD FEMALE BROILER CHICKS FED AD LIB DIETS WITH ACETIC OR PROPIONIC ACIDS SUPPLEMENTED AT LEVELS VARYING FROM 0% TO 3% OF DIETS CONTAINING 2500 AND 2750 kcal ME/kg

	Energy		Acid		Dose			Main Effects Significance of F Values						
VFAs	2500	2750	Acetic Acid	Propionic Acid	0	1	3	E	A	D	E × D	E × A	D × A	E × D × A
Acetic	22.8	26.2	24.0	25.7	23.0	24.9	24.8	0.040	NS	NS	NS	NS	0.017	0.057
Propionic	4.90	4.20	5.19	4.76	2.86	4.22	5.73	NS	0.001	0.0001	NS	NS	0.0001	0.0002
Butyric	6.16	8.10	6.06	7.48	8.56	6.36	7.18	0.033	NS	NS	NS	NS	NS	NS
Iso-valeric	0.80	0.79	0.86	0.85	0.58	0.80	0.91	0.020	0.016	0.002	0.057	0.012	0.002	0.003
Total	34.6	39.3	36.1	38.8	35.0	36.3	38.6	0.007	NS	NS	NS	NS	0.011	0.020

*NS = not significant ($P > .05$), E = energy, A = acid, D = dose.

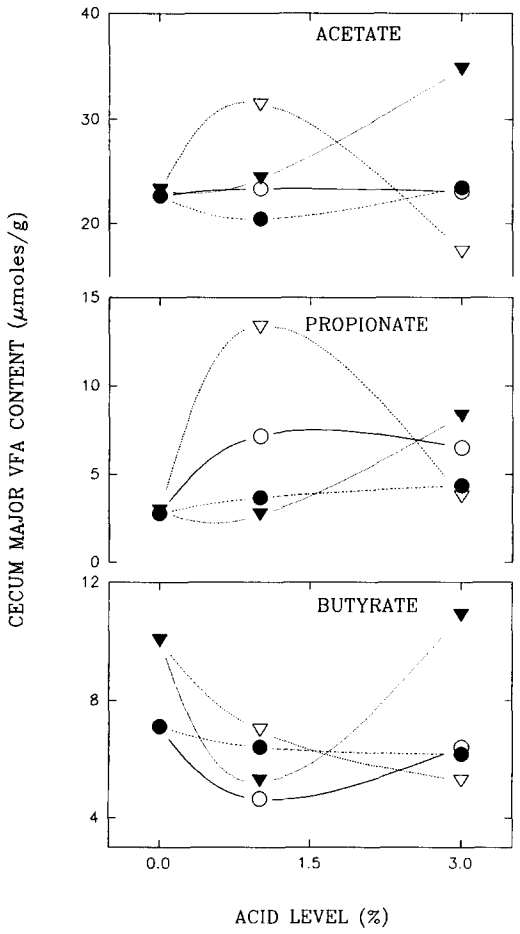

FIG. 2. Volatile fatty acid (VFA) concentration in the cecal ingesta of 21-day-old chicks fed ad lib diets with acetic (○,▽) or propionic (●,▼) acids supplemented at levels varying from 0% to 3% of diets containing 2500 (circles) and 2750 (triangles) kcal ME/kg. Each symbol represents the average of four birds.

According to Bolton and Dewar (3), acetic and propionic acids provided to fowl as calcium salts (2.5%) completely disappear from the ingesta of the gut sections before reaching Meckel's diverticulum. If this is the case in the birds used in the present study, the reason for the slight increase in total cecal VFAs cannot be attributed to the dietary supplementation. A positive relationship between dietary fiber intake and cecal acidity or VFA production has been reported for pigs (8) and rats (7,11,12). This is in agreement with results of the present study, in which a lower cecal pH was observed in birds fed the low-energy diet (i.e., high dietary crude fiber). The inhibitory effect of dietary VFAs on feed intake is assumed to extend the time during which ingesta can stay in the GIT. This, in turn, enables the cecal flora to extensively digest the NSPs and to increase net VFA production and energy salvage of the feed-restricted chick. Assuming cecal VFAs are produced by NSP fermentation, net VFA production and energy salvage have been estimated to be 9–9.8 kJ/g fermented NSP in nonruminant omnivores (13). Cecal VFA production from microbial fermentation in fowl has been estimated to contribute from 5% to 15% of the daily energy requirements for maintenance (20). The marked increase in cecal capacity could be due to increased utilization of the NSPs at that site, the latter compensating for the feed inhibition effected by the anorectants.

Annison et al. (1) examined avian digesta along the digestive tract and showed the greatest concentration of VFAs to be present in the cecum, composed mainly of acetic, propionic, and butyric acids. The results of the present study indicate that VFAs in the cecal ingesta are comprised, in molar proportion, of approximately 70% acetate, whereas the level of propionate is less than 15% (Table 4). It must be noted that cecal propionate concentration increased with the inclusion of either acid, to a greater extent with dietary acetate (Table 3 and Fig. 2). Moreover, increased supplementation of the acids altered neither acetate nor butyrate levels in the cecum, but increased the molar concentrations of propionate and isovalerate. The observed difference between acetate and propionate concentrations may be attributed to rate of transport, due to different mechanisms of absorption (21).

Results of the present study indicate that acetic and propionic acids exert a similar anorectic effect on chicks fed diets differing in energy content. The inhibitory effect of these acids on voluntary feed intake could not be attributed to alterations in feed utilization, to pancreatic secretory rate of α-amylase, or to differences in acid-base balance. The marked increase in cecal capacity was probably due to increased utilization of the NSPs at that site, the latter compensating for feed inhibition.

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