Appraisal of acidulants and acid-binding capacity in corn-soybean meal diets on productive variables, nutrient digestibility and gastrointestinal ecology of broilers

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ABSTRACT

An attempt has been made to ascertain the effects of acid-binding capacity (ABC) and acidifiers in corn-soybean meal diet on gut microbiota, nutrient digestibility and productive variables of broilers. In a 3×2 factorial experiment, 3 levels of ABC (650, 800 and 950 mEq/kg) and 2 mixes of acidifiers (1: fumaric acid and sodium diacetate, 2: tartaric acid and calcium propionate) were assessed. *Coliform* population and digestibility of dry matter and organic matter did not change by experimental factors or their interactions. Combination of tartaric acid and sodium diacetate resulted in severe mortality in starter phase. The ABC_3 (ABC at pH 3) at 800 mEq/kg of diet significantly increased the feed intake and body weight gain of broilers, but at the level of 650 mEq adversely affected on feed intake and body weight gain of broilers during starter and overall periods.

Keywords: acidifier, digestibility, growth, intestine, chickens

RÉSUMÉ

Evaluation des acidifiants et de la capacité de fixation des acides dans les régimes maïs-tourteau de soja sur les paramètres de production, la digestibilité des nutriments et l'écologie gastro-intestinale des poulets de chair

Cette étude vise à déterminer les effets des acidifiants et la capacité de fixation des acides (ABC) dans deux régimes type sur le microbiote intestinal, la digestibilité des nutriments et le svariables de production des poulets de chair. Dans une étude de type 3 × 2, 3 niveaux d'ABC (650, 800 et 950 mEq / kg) et 2 mélanges d'acidifiants (1: acide fumarique et diacétate de sodium, 2: acide tartrique et propionate de calcium) ont été évalués. La population de coliformes et la digestibilité de la matière sèche et de la matière organique n'ont pas été influencées par les composés testés. La combinaison d'acide tartrique et de diacétate de sodium a entraîné une forte mortalité sévère en phase initiale. L'ABC3 (ABC à pH 3) à 800 mEq/kg aliment a significativement augmenté l'ingestion alimentaire et le gain de poids corporel des poulets de chair, mais affecte négativement ces paramètres au niveau de 650 mEq/kg.

Mots clés : acidifiant, digestibilité, croissance, microbiote, poulets

Introduction

Different organic acids such as acetic, malic, fumaric and citric acid are used in broiler nutrition for various aims. These additives have been evaluated in several trials in poultry feeding as antibacterial [2-3, 15] or for enhancement of nutrient digestibility [4] and the other aims [41].

Some workers indicated that these additives can result in better efficiency under suboptimal conditions such as low phosphorous rations [34] or wheat based diets [22]. For instance, Sacakli et al. [48] reported that addition of 2.5 g organic acid (lactic acid + formic acid)/kg diet with low-level available phosphorus improved tibia ash. In addition, while diets comprised of 4 or 6% citric acid, body weight gain and tibia ash were maximized at lower available phosphorus levels than when diets contained no citric acid [11].

Acid-binding capacity (ABC) is a new launched concept among the numerous characteristics of feed ingredients [36]. The ABC of a feed is the amount of acid required to reduce the pH of feed to a special level [33, 36, 47]. Due to different chemical composition of various feed ingredients, their ABC can be remarkably different. Therefore, various diets because of their ingredients do not have similar ABC.

Some studies have shown reductions in intestinal pH and improvements on intestinal development [42], ileal amino acid digestibility, calcium retention and performance with reduced dietary ABC [10, 23]. Most studies have evaluated the ABC of the diet by using different sources or levels of calcium [42, 47] or by adding a buffer to the diet [10] while little research has tried to assess the influence of ABC by using a combination of commonly used ingredients on poultry [47]. It was hypothesized that dietary ingredients can influence the function of digestive system like enzymes and gastric acid secretions and subsequently alter broiler performance.

The objective of the current trial was to study the effect of different levels of ABC_3 in optimal (corn based diet) condition on nutrients digestibility, microbiota and some other traits of digestive tract, litter situation and growth performance in broilers.

Materials and Methods

TREATMENTS, BIRDS AND HOUSING

A 2×3 factorial arrangement was conducted with 2 mixes of acidifiers (1: fumaric acid and sodium diacetate, 2: tartaric acid and calcium propionate) and 3 levels of acid-binding capacity (ABC) (650, 800 and 950 mEq/kg). The ABC_3 of all ingredients was assessed before feed formulation as previously described by Gilani et al. [27]. The basal diets were formulated (Tables 1, 2, and 3) to meet the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide [7]. Each of the 6 assay mash diets was fed to 4 replicates of 10 chicks each for 41 days. A total of 240 day-old straight run Ross 308 chicks were purchased form a local hatchery. All the chicks were weighed individually (37 g) and randomly divided into 24 floor pens. Each pen (1 m^2) was equipped with a manual feeder and two nipple drinkers and the floor was covered with clean wood shavings.

Feed as mash form and drinking water were provided *ad libitum* throughout the trial. House temperature was 32°C in the first week and then gradually decreased to 24°C by the end of the third week. A lighting schedule of 24 h illumination with approximately 20 lx was used for the entire period. Chicks were vaccinated for Infectious Bronchitis on day 4 and Newcastle Disease on 4, 11, and 20 day of age. The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Mashhad, Iran.

PERFORMANCE INDICES

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured during the experiment. Feed intake was determined from the difference between supplied and residual feed in each pen and was adjusted for mortality. The FCR was calculated as the ratio between feed intake and weight gain. Also, European production efficiency factor [7] and growth rate [13] were calculated.

Acidifiers	**Tartaric acid-Sodium diacetate**			**Fumaric acid-Calcium propionate**		
ABC_3[1] (mEq/kg)	**650**	**800**	**950**	**650**	**800**	**950**
Ingredients (%)						
Corn	52.98	50.58	46.16	52.78	51.30	48.23
Soybean meal	33.63	35.43	36.31	36.67	35.15	35.72
Fish meal	5	4	4	5	4.11	4.14
Vegetable oil	3.96	4.7	6.17	4.03	4.47	5.49
Limestone	0.92	0.93	0.92	0.92	0.84	0.31
Dicalcium phosphate	1.5	1.63	1.64	1.5	1.61	1.61
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.35	0.16	0.15	0.35	0.36	0.36
DL-Methionine	0.33	0.34	0.35	0.33	0.34	0.34
L-Lysine HCl	0.20	0.20	0.20	0.20	0.20	0.19
L-Threonine	0.01	0.02	0.02	0.01	0.02	0.02
Tartaric acid	0.62	0	0	0	0	0
Sodium diacetate	0	1.5	3.6	0	0	0
Fumaric acid	0	0	0	0.72	0	0
Calcium propionate	0	0	0	0	1.1	3.09
Calculated nutritional value (%)						
Metabolizable energy (kcal/kg)	3025	3025	3025	3025	3025	3025
Crude protein	22.7	22.7	22.7	22.7	22.7	22.7
Calcium	1.05	1.05	1.05	1.05	1.05	1.05
Available phosphorus	0.50	0.50	0.50	0.50	0.50	0.50
Sodium	0.19	0.26	0.39	0.19	0.19	0.19
Lysine	1.44	1.43	1.43	1.44	1.44	1.43
Methionine	0.75	0.75	0.75	0.75	0.75	0.75
Methionine + Cystine	1.07	1.07	1.07	1.07	1.07	1.07
Threonine	0.94	0.94	0.94	0.94	0.94	0.94

[1]ABC_3 of ingredients (mEq/kg): corn=191, soybean meal=1111, fish meal=645, limestone=12000, dicalcium phosphate=2645, vitamin premix=1836, mineral premix=1467, common salt=181, vegetable oil=364, DL-methionine=119, L-lysine=76, L-threonine=126, tartaric acid=-5021, fumaric acid=-4407, calcium propionate=10516, sodium diacetate=6851

[2]vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Table I: Composition of starter (0-10 d) diets including two mixes of acidifiers, and three levels of ABC_3 (650, 800, and 950 mEq/kg)

Acidifiers	Tartaric acid-Sodium diacetate			Fumaric acid-Calcium propionate		
ABC$_3$[1] (mEq/kg)	650	800	950	650	800	950
Ingredients (%)						
Corn	51.96	49.47	46.02	52.38	50.94	47.30
Soybean meal	35.99	36.72	35.33	35.70	35.95	37.90
Fish meal	1.77	1.61	3.32	1.97	2	1
Vegetable oil	6.18	7	8	5.91	6.37	7.53
Limestone	0.75	0.74	0.71	0.74	0.78	0.01
Dicalcium phosphate	1.64	1.66	1.45	1.61	1.61	1.74
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.32	0.19	0.17	0.32	0.32	0.34
DL-Methionine	0.28	0.28	0.27	0.27	0.28	0.28
L-Lysine HCl	0.10	0.09	0.05	0.09	0.09	0.08
L-Threonine	0.02	0	0	0	0	0
Tartaric acid	0.50	0	0	0	0	0
Sodium diacetate	0	1.74	4.18	0	0	0
Fumaric acid	0	0	0	0.50	0	0
Calcium propionate	0	0	0	0	1.16	3.31
Calculated nutritional value (%)						
Metabolizable energy (kcal/kg)	3150	3150	3150	3150	3150	3150
Crude protein	21.5	21.5	21.5	21.5	21.5	21.5
Calcium	0.9	0.9	0.9	0.9	1.10	1.15
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.16	0.38	0.48	0.16	0.16	0.16
Lysine	1.24	1.24	1.24	1.24	1.24	1.24
Methionine	0.61	0.61	0.62	0.61	0.61	0.62
Methionine + Cystine	0.95	0.95	0.95	0.95	0.95	0.95
Threonine	0.83	0.86	0.86	0.83	0.86	0.86

[1]ABC$_3$ of ingredients (mEq/kg): corn=191, soybean meal=1111, fish meal=645, limestone=12000, dicalcium phosphate=2645, vitamin premix=1836, mineral premix=1467, common salt=181, vegetable oil=364, DL-methionine=119, L-lysine=76, L-threonine=126, tartaric acid=-5021, fumaric acid=-4407, calcium propionate=10516, sodium diacetate=6851

[2]vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Table II: Composition of grower (11-24 d) diets including two mixes of acidifiers, and three levels of ABC$_3$ (650, 800, and 950 mEq/kg)

Acidifiers	Tartaric acid-Sodium diacetate			Fumaric acid-Calcium propionate		
ABC$_3$[1] (mEq/kg)	650	800	950	650	800	950
Ingredients (%)						
Corn	57.80	55.53	52	57.50	54.73	53.46
Soybean meal	30.28	29.80	24.60	30.34	30.89	31.15
Fish meal	1.00	1.76	6.00	1.09	1.00	1.00
Vegetable oil	6.23	7.00	8.00	6.33	7.25	7.67
Limestone	0.83	0.85	1.00	1.09	0.22	0
Dicalcium phosphate	1.69	1.55	1.37	2.09	2.76	1.64
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.19	0.18	0.15	0.34	0.34	0.34
DL-Methionine	0.22	0.22	0.21	0.22	0.23	0.23
L-Lysine HCl	0.08	0.07	0.26	0.08	0.07	0.07
Tartaric acid	0.50	0	0	0	0	0
Sodium diacetate	0.67	2.53	5.00	0	0	0
Fumaric acid	0	0	0	0.50	0	0
Calcium propionate	0	0	0	0	2	3.95
Calculated nutritional value (%)						
Metabolizable energy (kcal/kg)	3200	3200	3200	3200	3200	3200
Crude protein	19	19	19	19	19	19
Calcium	0.90	0.90	1.05	1.08	1.32	1.40
Available phosphorus	0.42	0.42	0.47	0.49	0.60	0.42
Sodium	0.21	0.50	0.80	0.16	0.16	0.16
Arginine	1.20	1.20	1.17	1.20	1.21	1.20
Lysine	1.06	1.06	1.23	1.06	1.06	1.06
Methionine	0.52	0.52	0.54	0.52	0.52	0.52
Methionine + Cystine	0.83	0.83	0.83	0.83	0.83	0.83
Threonine	0.71	0.71	0.71	0.71	0.71	0.71

[1]ABC$_3$ of ingredients (mEq/kg): corn=191, soybean meal=1111, fish meal=645, limestone=12000, dicalcium phosphate=2645, vitamin premix=1836, mineral premix=1467, common salt=181, vegetable oil=364, DL-methionine=119, L-lysine=76, L-threonine=126, tartaric acid=-5021, fumaric acid=-4407, calcium propionate=10516, sodium diacetate=6851

[2]vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Table III: Composition of finisher diets including two mixes of acidifiers, and three levels of ABC$_3$(650, 800, and 950 mEq/kg)

TOTAL TRACT DIGESTIBILITY OF NUTRIENTS AND ANALYSES OF DIGESTA AND LITTER

Gastrointestinal transit time of diets was measured at d 16 by covering the pen floors with clean paper 4 h after feed withdrawal. Then the time between the offering of the diets containing 0.3% chromic oxide and the appearance of three spotted green excreta in each pens was considered as gastrointestinal passage rate of feed [51]. Feeding of these diets was continued until 21 day. A sample of the diet and excreta from each pen was stored at -20 °C for further analyses of dry and organic matter. Also, crude protein content in the diets and excreta samples was determined by Kjeldahl standard procedure of AOAC [5]. Chromium in the diets and excreta was determined as described by Fenton and Fenton [24]. Nutrient digestibility was calculated using the formula of Leeson and Summers [37]. Moreover, fresh voided excreta were scored for wetness on a scale of 1 to 5. Scale 1 represents normally formed excreta and scale 5 represents very watery excreta at 21 day of age [44].

Three male birds with the average weight of each treatment were slaughtered at 21 and 41 d of age. Then, small intestine was excised and digesta in different parts of digestive tract was obtained. The pH of digesta in crop and ileum was measured using a pH meter (Model 691, Metrohm, Switzerland) as described by Esmaeilipour et al. [22]. Moreover, a little jejunal contents were carefully kept in sterile dishes at -20 °C until analyses in the laboratory. The samples were homogenized and then one gram of each sample was collected and transferred into 10 ml sterile saline solution for dilution. After that, each sample was propagated on selective agar plates as follows. The EMB agar (Eosin methylene blue) was utilized for coliforms and MRS agar (Man, Rogosa, and Sharpe agar medium) was used for lactobacillus bacteria [6]. The number of bacterial colonies was counted after incubation at 37 °C for 48 hours. Then, the typical colonies were expressed as $\log_{10}$ colony-forming units per milliliter (CFU/mL). Then, the rest of jejunal digesta was centrifuged at 3000×g for 15 min to obtain the supernatant (0.5 mL) for viscosity. The viscosity (as centipoises [cP]) of the supernatant was measured with a digital viscometer (Brookfield Engineering Laboratories Inc., USA). Furthermore, pH and humidity of litter at 41 day were quantified as described by Safaeikatouli et al. [49].

STATISTICAL ANALYSES

All data were analyzed using the General Linear Model procedure of the Statistical Analysis System [50]. Tukey's Studentized Range (HSD) test was used to compare the means. All statements of significance were based on probability of $P<0.05$.

Results

PERFORMANCE INDICES

The effects of acidifier [Tartaric acid-sodium diacetate (Tar-Sod), or fumaric acid – calcium propionate (Fum-Ca)], ABC_3 and their interactions on feed intake, body weight gain, growth rate, mortality and European production efficiency factor of broilers are shown in Tables 4, 5 and 6, respectively. Type of basal diet did not influence feed intake.

Main effects		Feed intake (g)				Body weight gain (g)			
		Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall
Acid	Tar-Sod[2]	216.4	866.5	2216.2^{b}	2399.6^{b}	168.5	664	1373^{b}	2290.0^{b}
	Fum-Ca	230.0	885.2	2513.0^{a}	3628.2^{a}	166.3	701	1501^{a}	2369.8^{a}
	SEM	4.75	28.2	62.54	73.38	3.03	12.57	23.60	31.02
ABC_3	650	192.7^{b}	886.4	2159.6^{c}	3239.5^{c}	156.4^{b}	657.5	1417.0^{b}	2234.1^{b}
	800	240.4^{a}	894.8	2596.7^{a}	3732.0^{a}	173.1^{a}	701.6	1511.2^{a}	2386.0^{a}
	950	236.4^{a}	846.4	2337.4^{b}	3420.3^{b}	172.7^{a}	690.9	1384.5^{c}	2248.1^{a}
	SEM	5.82	35.06	78.99	90.11	3.68	15.50	29.50	38.70
Interactions									
Tar-Sod	650	131.2^{c}	688.2^{b}	1752.6	2573.1	92.3^{c}	567.2	1257.3	1903.3
Tar-Sod	800	266.4^{a}	905.2^{ab}	2487.8	3659.5	193.2^{ab}	727.1	1367.9	2288.3
Tar-Sod	950	250.7^{ab}	947.6^{a}	2455.2	3653.5	183.4^{bc}	714.9	1355.3	2253.7
Fum-Ca	650	264.1^{a}	955.9^{ab}	2609.6	3829.7	199.7^{a}	740.5	1444.5	2384.9
Fum-Ca	800	247.1^{ab}	888.5^{ab}	2709.6	3845.2	181.9^{bc}	731.2	1518.9	2432.1
Fum-Ca	950	213.2^{b}	750.8^{ab}	2294.5	3258.6	140.8^{b}	764.7	1384.3	2199.8
SEM		11.65	70.12	160.18	180.41	7.83	31.02	60.42	0.066
Sources of variation		**P-Values**							
Acid		0.051	0.640	0.002	0.002	0.599	0.045	0.005	0.0008
ABC_3		<0.0001	0.558	0.001	0.001	0.003	0.134	0.009	0.010
Acid × ABC_3		0.030	0.033	0.108	0.080	<0.0001	0.143	0.579	0.233

[1]ABC_3 is acid-binding capacity of a feedstuff at titer point of 3 (pH=3).

[2]Tar-Sod: Tartaric acid-sodium diacetate, Fum-Ca: fumaric acid-calcium propionate.

[a-d]Means within each column for every effect with no common superscript are significantly different ($P<0.05$).

Table IV: Effects of type of mixes of acidifiers (Acid), acid-binding capacity (ABC_3)[1], and their interactions on feed intake and body weight gain of each bird during starter (0-10 d), grower (11-24 d), finisher (25-42 d), and overall period

Main effects		Feed conversion ratio				Growth rate			
		Starter	Grower	Finisher	Overall	Starter	Grower	Finisher	Overall
Acid	Tar-Sod[2]	1.29 ^{b}	1.30	1.61	1.50	136.4	122.3 ^{b}	88.0	112.1 ^{b}
	Fum-Ca	1.41 ^{a}	1.27	1.68	1.53	137.2	127.1 ^{a}	90.9	114.0 ^{a}
	SEM	0.024	0.048	0.039	0.030	1.00	1.50	1.20	0.39
ABC_3	650	1.25 ^{c}	1.35	1.51 ^{c}	1.46	132.1 ^{b}	123.8	90.96	113.2
	800	1.43 ^{a}	1.30	1.73 ^{a}	1.56	138.2 ^{a}	125.7	90.91	113.8
	950	1.37 ^{b}	1.22	1.69 ^{b}	1.52	140.0 ^{a}	124.6	86.91	112.1
	SEM	0.030	0.060	0.049	0.036	1.20	1.89	1.50	0.75
Interactions									
Tar-Sod	650	1.48 ^{b}	1.21	1.39	1.43	107.0 ^{d}	124.3	92.4	113.2
Tar-Sod	800	1.37 ^{c}	1.24	1.82	1.60	145.0 ^{a}	122.5	83.3	111.1
Tar-Sod	950	1.36 ^{c}	1.33	1.81	1.62	143.0 ^{b}	123.6	84.0	111.3
Fum-Ca	650	1.32 ^{c}	1.30	1.80	1.60	146.2 ^{a}	122.0	85.1	112.0
Fum-Ca	800	1.36 ^{c}	1.21	1.78	1.58	142.7 ^{b}	125.1	88.7	113.7
Fum-Ca	950	1.51 ^{a}	1.11	1.66	1.47	131.6 ^{c}	131.0	89.6	113.4
SEM		0.058	0.119	0.110	0.068	2.40	3.50	3.22	2.50
Sources of variation		P-Values							
Acid		0.0008	0.657	0.224	0.510	0.546	0.027	0.096	0.001
ABC_3		0.0005	0.324	0.008	0.160	0.0002	0.748	0.095	0.056
Acid × ABC_3		0.0009	0.146	0.057	0.175	<0.0001	0.072	0.096	0.152

[1]ABC_3 is acid-binding capacity of a feedstuff at titer point of 3 (pH=3).
[2]Tar-Sod: Tartaric acid-sodium diacetate, Fum-Ca: fumaric acid-calcium propionate
[a-d] Means within each column for every effect with no common superscript are significantly different (P<0.05).

Table V: Effects of mixes of acidifiers (Acid), acid-binding capacity (ABC_3)[1], and their interactions on feed conversion ratio and growth rate of each bird during starter (0-10 d), grower (11-24 d), finisher (25-42 d), and overall period

Main effects		Mortality				European production efficiency factor
		Starter	Grower	Finisher	Overall	
Acid	Tar-Sod[2]	17.80 ^{a}	1.22	1.11	5.11 ^{a}	341.3 ^{b}
	Fum-Ca	1.67 ^{b}	0.53	1.13	1.06 ^{b}	373.3 ^{a}
	SEM	1.668	0.514	0.650	0.538	10.60
ABC_3	650	25.26 ^{a}	1.93	0.44	6.83 ^{a}	347.3
	800	1.79 ^{b}	0.00	0.80	0.77 ^{b}	368.3
	950	2.15 ^{b}	0.71	2.11	1.65 ^{b}	356.3
	SEM	2.043	0.645	0.816	0.676	13.56
Interactions						
Tar-Sod	650	44.71 ^{a}	2.73	0	10.89 ^{a}	312.3
Tar-Sod	800	1.43 ^{b}	0	1.43	0.95 ^{b}	344.5
Tar-Sod	950	2.87 ^{b}	0	1.59	1.71 ^{b}	331.4
Fum-Ca	650	1.43 ^{b}	1.79	1.79	1.70 ^{b}	353.3
Fum-Ca	800	1.43 ^{b}	0	0	0.34 ^{b}	371.4
Fum-Ca	950	0 ^{b}	0	0	0 ^{b}	364.8
SEM		4.087	1.385	1.746	1.446	27.137
Sources of variation		P-Values				
Acid		<0.0001	0.337	0.982	<0.0001	0.032
ABC_3		<0.0001	0.102	0.290	<0.0001	0.502
Acid × ABC_3		0.031	0.767	0.534	0.046	0.525

[1]ABC3 is acid-binding capacity of a feedstuff at titer point of 3 (pH=3).
[2]Tar-Sod: Tartaric acid-sodium diacetate, Fum-Ca: fumaric acid-calcium propionate
[a,b] Means within each column for every effect with no common superscript are significantly different (P<0.05).

Table VI: Effects of mixes of acidifiers (Acid), acid-binding capacity (ABC_3)[1], and their interactions on mortality percentage of birds during starter (0-10 d), grower (11-24 d), finisher (25-42 d), and overall period and European production efficiency factor of broilers

Combination of Fum-Ca as compared with Tar-Sod resulted in more feed intake and weight gain and better growth rate and European production efficiency factor (EEPF) in finisher phase and in overall. Combination of Tar-Sod particularly in starter phase resulted in sever mortality and negative effects on the EEPF.

The ABC_3 at 800 mEq/kg of diet significantly increased the feed intake and body weight gain of broilers but at the level of 650 mEq had adverse effect on feed intake and body weight gain of broilers during starter and overall period. The FCR and growth rate were not affected by ABC_3 in overall study, although 650 mEq ABC_3 had deleterious effect on these criteria in the starter phase. Interrelationships of type of acidifier and ABC_3 during starter and grower phases was significantly pronounced for feed intake. Diet containing Tar-Sod and 650 mEq/kg in starter phase remarkably decreased feed intake of broilers but increased the feed intake as compared to the other treatments. However, feed intake in finisher phase and overall study did not significantly influenced by these treatments.

Diets comprising Tar-Sod and 650 mEq/kg caused to severe mortality in starter phase, whereas during the rest of production did not have any significant effect on mortality that is probably related to inducible tolerance or adaptation of chickens to the organic acids and low level of ABC_3. Nevertheless, remarkable difference for mortality remained among treatments during whole study.

NUTRIENTS DIGESTIBILITY, GUT MICROFLORA AND ANALYSES OF DIGESTA AND LITTER CONTENT

The effects of acidifier (Tar-Sod or Fum-Ca), ABC_3, and their interactions on digestibility of dry matter, organic matter, and crude protein and jejunal population of *Lactobacillus* and *Coliforms* at 21 d are presented in Table 7. A possible explanation may be based on competitive exclusion which proliferation of undetermined bacteria restricted the *Lactobacillus* population. However, *coliforms* population and digestibility of dry matter and organic matter did not change by experimental factors or their interactions.

The effects of type of acidifier (Tar-Sod or Fum-Ca), ABC_3 and their interactions on jejunal digesta viscosity (cP) and excreta wetness at 21 d and jejunal digesta viscosity, litter pH and relative humidity at 41 d are given in Table 8.

Combination of Fum-Ca compared with Tar-Sod remarkably decreased percentage of litter moisture. Also, 650 mEq/kg ABC_3 brought about less litter moisture at 41 d. Diets comprising Tar-Sod and high ABC_3 (950 mEq/kg) caused to more litter moisture.

Discussion

These results are contradictory with Rynsburger [47] which demonstrated low level of ABC through reduction of calcium sources in the diet did not influence intestinal pH and body weight gain of broilers, but increased the feed intake and FCR. However, Daskiran et al. [20] added a commercial organic acid (Luctacid®) to the diet of broilers exposed to heat stress. They noted that the best survivability was for the birds consuming acidifier from grower phase, not starter.

It appears that there are great discrepancies among various results about organic acids. Feed consumption and utilization were improved by the addition of fumaric acid in broilers ration with no effect on mortality [52]. Improvement of feed intake and FCR due to addition of 0.5% citric acid in broilers diet was reported by Chowdhury et al. [18]. Boling et al. [12] mentioned that citric acid did not improve performance of laying hens fed a corn-soybean meal diet containing sufficient calcium. Also, calcium propionate and calcium formate did not influence weight gain, but deteriorated FCR [29]. The addition of citric acid reduced the body weight gain but did not change the feed intake and FCR [14]. The inclusion of 20 g/kg of citric acid decreased feed intake but it did not affect body weight gain of broilers at 24 d of age [21]. Also, in another study, citric acid decreased feed intake and weight gain of broilers [4]. Pirgozliev et al. [43] indicated that fumaric acid reduced feed intake of broilers. Islam et al. [30] have conducted a research with fumaric acid from 0 to 7.5 percent of diet in broilers. They concluded that safety margin is about 3%; and 1.25% of this organic acid in the diet was the optimally effective concentration. Runho et al. [46] observed that fumaric acid improved significantly FCR in broilers fed diets from 0.25 to 1%, because a reduction of consumption was noticed, but growth was unaffected. However, growth promoting effects of organic acids in poultry do not seem to be as remarkable as in pigs [35].

Along with the current results, Mohammadpour et al. [39] indicated that acidifiers in suboptimal diets containing wheat without exogenous enzyme had some beneficial effects on bone mineralization. Also, comparison of femur physical traits and histological study of tibial growth plate at 21 and 41 days of age may indicate that younger chickens are more susceptible to the alteration of diets.

Organic acids have provided an approach for microbial mitigation in poultry. For instance, citric acid supplementation significantly decreased ileal *Coliforms* contents [8]. Pirgozliev et al. [43] indicated that fumaric acid declined *Coliforms* in ileum. Also, Moharrery and Mahzonieh [40] mentioned that malic acid has potential for reduction of *E. coli*. Abdel-Mageed [1] mentioned that 0.2% dietary butyric acid glycerides improved *Lactobacillus spp.* and pH in ileum and ceca of Japanese quails. Also, reduction of gram negative bacteria [57] and increase *Lactobacillus* counts in the gastrointestinal tract of broilers have been reported [19, 26]. Van Immerseel et al. [55] have indicated significantly reduced levels of Salmonella in the cecum of birds fed organic acids. Calcium formate did not reduce *Coliforms* [32]. However, in an experiments with broiler chickens, increasing amounts of fumaric acid (0.5, 1.0 and 2.0%) did not offer protection

Main effects		Apparent digestibility of nutrients (%) Dry matter	Organic matter	Crude protein	Microbial population (log10/mL) Lactobacillus	Colliforms
	Tar-Sod[2]	92.29	85.98	85.37	4.47	4.34
Acid	Fum-Ca	93.23	87.89	86.53	3.59	4.32
	SEM	0.550	0.843	0.499	0.340	0.107
	650	92.13	86.07	86.86	4.21	4.57
ABC_3	800	92.79	87.38	85.79	3.60	4.18
	950	93.34	87.36	85.19	4.33	4.24
	SEM	0.639	1.049	0.633	0.412	0.139
Interactions						
Tar-Sod	650	91.34	82.80	85.64	5.04	4.83
Tar-Sod	800	92.52	85.19	86.21	4.93	4.19
Tar-Sod	950	92.20	87.20	85.50	5.09	3.79
Fum-Ca	650	94.85	90.27	88.28	5.09	4.14
Fum-Ca	800	93.72	89.14	87.95	4.52	3.80
Fum-Ca	950	94.60	88.35	88.01	4.94	4.54
SEM		1.618	2.191	1.308	0.980	0.408
Sources of variation		P-Values				
Acid		0.229	0.109	0.804	0.064	0.901
ABC_3		0.470	0.538	0.145	0.402	0.075
Acid × ABC_3		0.843	0.241	0.630	0.162	0.600

[1]ABC_3 is acid-binding capacity of a feedstuff at titer point of 3 (pH=3).
[2]Tar-Sod: Tartaric acid-sodium diacetate, Fum-Ca: fumaric acid-calcium propionate

Table VII: Effects of mixes of acidifiers (Acid), acid-binding capacity (ABC_3)[1], and their interactions on apparent digestibility of nutrients and microbial population in jejunum of broilers at 21 d

Main effects		41 day Jejunal digesta viscosity (Cp)	Litter pH	Relative humidity of litter (%)	21 day Excreta wetness	Jejunal digesta viscosity (Cp)
	Tar-Sod[2]	3.01	7.43	45.47 [a]	3.16	2.78
Acid	Fum-Ca	2.77	7.41	39.17 [b]	2.58	2.79
	SEM	0.133	0.073	1.379	0.24	0.106
	650	3.15	7.43	38.51 [b]	3.06	2.94
ABC_3	800	2.85	7.48	47.77 [a]	3.56	2.61
	950	2.68	7.35	45.67 [a]	3.00	2.79
	SEM	0.171	0.104	1.726	0.298	0.138
Interactions						
Tar-Sod	650	3.21	7.47	36.23 [b]	2.98	2.77
Tar-Sod	800	2.83	7.38	53.18 [a]	2.50	2.12
Tar-Sod	950	3.87	7.40	52.21 [a]	3.00	2.92
Fum-Ca	650	2.94	7.38	38.26 [b]	2.75	2.68
Fum-Ca	800	2.70	7.58	34.04 [b]	2.50	2.68
Fum-Ca	950	2.54	7.51	41.54 [ab]	2.00	2.43
SEM		0.449	0.208	3.452	0.573	0.277
Sources of variation		P-Values				
Acid		0.179	0.797	0.003	0.090	0.971
ABC_3		0.122	0.524	0.021	0.421	0.246
Acid × ABC_3		0.512	0.680	0.005	0.869	0.806

[1]ABC_3 is acid-binding capacity of a feedstuff at titer point of 3 (pH=3).
[2]Tar-Sod: Tartaric acid-sodium diacetate, Fum-Ca: fumaric acid-calcium propionate
[a,-b] Means within each column for every effect with no common superscript are significantly different (P<0.05).

Table VIII: Effects of mixes of acidifiers (Acid), acid-binding capacity (ABC_3)[1], and their interactions on jejunal digesta viscosity (Cp), pH and relative humidity of litter at 41 d and excreta wetness and jejunal digesta viscosity (Cp) at 21 d from cecal *Salmonella* colonization or carcass contamination following oral challenge with *Salmonella typhimurium* [56]. Also, Izat et al. [31] demonstrated that levels of total organisms and *Coliforms* were not reduced by dietary calcium formate.

Low gastrointestinal pH has adverse impacts for colonization of acid-intolerant bacteria such as *Coliforms*. Organic acids have an antimicrobial effect because they diffuse through the bacterial cell membrane and then dissociate into anions and protons, and eventually disturb the intracellular electron-balance or acts by suppressing the cellular enzyme and transport systems [38, 45, 53]. Acids with a high pK_a (which are 50 per cent dissociated), longer-chain-length acids and unsaturated acids are the most effective.

Chaveerach et al. [16] indicated that the efficiency of organic acids in controlling microbes such as Campylobacter is influenced by concentration, form of the acid, and the degree of any dissociation. The results of the use of the mixtures of organic acids (formic, acetic, propionic, and hydrochloric acids) demonstrated that although the amounts of accumulated undissociated acids of each acid in combinations are less than in individual use of acids at the same low pH levels, particular at pH 4.5, the mixtures gave a higher reduction rate. They strongly demonstrated that the combination of organic acids gives a remarkably high bactericidal effect on *Campylobacter jejuni/coli* viability in low pH aqueous solutions. The reason for this synergistic effect is still unknown.

Some organic acids such as citric acid improved the digestibility of dry matter and crude protein in broilers [4]. Garcia et al. [25] demonstrated that 10,000 ppm of formic acid in finisher diet of broilers improved ileal digestibility of dry matter and crude protein. Also, acidifier improved nitrogen retention in quails [17]. Supplementation of fumaric acid to starter diets of pigs during the initial 3 to 4 wk after weaning increased the ileal digestibilities of crude protein, and amino acids [10]. According to Van Der Sluis [54], the positive effect of organic acids on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings.

Dietary formic acid under conditions of good hygiene had slightly positive effect on the ileal digestibility of nutrients [28]. Neither, the addition of citric acid did not alter apparent ileal digestibility of crude protein [14]. Pirgozliev et al. [43] indicated that fumaric acid did not change nutrients digestibility. It can be concluded that the decrease in secretions from the gastrointestinal tract in the presence of fumaric and sorbic acids may be a mechanism involved in the mode of action of dietary organic acids.

The results of the study by Biggs and Parsons [9] indicated that feeding organic acids did not have any consistent effects on amino acids (AA) digestibility, or cecal microbial numbers. For instance, digestibility of most AA was reduced by gluconic acid in some treatments, whereas citric acid (3%) increased AA digestibility at 4 d but not at 21 d. For cecal microbial populations at 21 d, *bifidobacteria* were reduced when 2% gluconic acid and 3% citric acid were fed to chicks in a corn-soybean meal diet, whereas 4% gluconic acid had no effect when fed in a dextrose-casein diet to chicks.

The discrepancies of different results might be due to differences in the type and concentration of organic acid, chemical form, pKa value, type of microorganism, animal species, site and location in gastrointestinal tract and buffering capacity of the feed [28]. Further studies are required to fully explore dose-response effects on broiler performance and the broilers physiology.

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