Revista Brasileira de Zootecnia
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ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.40, n.12, p.2776-2782, 2011

Performance and intestinal health of broilers inoculated with nalidixic acid-resistant *Salmonella* Typhimurium and treated with organic acids

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ABSTRACT - An experiment with 630 one-day-old chicks experimentally inoculated with *Salmonella* Typhimurium was performed to evaluate the effects of an organic acid blend (benzoic, fumaric and 2-hydroxi-4-methylltio-butanoic - HMTBa) on intestinal health and performance. The experimental challenge with *Salmonella* Typhimurium occurred in two different routes of administration: via crop on the first day after hatching, and via feed, offered from seven to 14 days of age. These groups were treated with organic acids in a 3 × 2 (agent *versus* acid) factorial arrangement. Chicks treated with organic acids had higher weight gain, mean live weight and better feed conversion at 14 days of age, and higher weight gain at 28 days of age. Chicks treated with organic acids inoculated via crop had higher duodenum villi height than animals of the control group. Jejunum villi were higher in chicks of the negative control group compared with birds supplemented with organic acids. Small intestine pH in chicks of the inoculated group was lower compared with control group throughout the trial period. HMTBa acid in the dosage of 0.4% improves intestinal health and performance when nalidixic acid resistant *Salmonella* Typhimurium is experimentally inoculated.

Key Words: birds, contamination, control feed, conversion, salmonellosis

Introduction

For the last years, paratyphic infections have been classified as of high risk for both Brazilian poultry industry and public health and *Salmonella enterica* subsp. *enterica* sorovar Typhimurium has been considered one of the most important of these diseases. Then, *Salmonella* Typhimurium control in poultry facilities is essential to avoid trading restrictions and to minimize the financial hazards of the Brazilian Industry, which developed some criteria to control these pathogens until the period before slaughtering and processing.

The presence of a contaminated stock, environment and feeds is the major ways to introduce *Salmonella* into the production system are (Tessari et al., 2003). By this means, contamination of diet, silos or feeders enables the transmission of *Salmonella* to the bird stock (Berends et al., 1996). Dibner & Buttin (2002) stated that organic acids can reduce *Salmonella* contamination and, consequently, minimize the subclinical infections in birds, and contribute to improve nutrient absorption, increasing the expression of the digestive and immune system potentialities.

It is hard to compare results among experiments that tested organic acids in blends without considering their particular way of action. Benefits depend on the organic acid which is presented in blends and their doses, so, the correct use of these compounds requires more information about their way of action on the different gastrointestinal pathogens.

Because of this necessity to control the pathogens, this experiment was proposed and conducted to observe the activity of a blend of organic acids (benzoic, fumaric and 2-hidroxi-4-metiltiobutanoic) on the performance and intestinal health of broilers inoculated with *Salmonella enterica* subsp. *enterica* sorovar Typhimurium.

Material and Methods

Chicks were allotted in the Avian Diseases Experimental Section with proper isolation facilities for the study of these pathogens. These facilities were built to assure that birds would not be contaminated, blocking insects, rodents, wild birds and people. The contact established between the chicks and people was restricted to individuals particularly dressed and with specific biosecurity care established for this experiment. The necropsy of the chicks considered negative control confirmed the absence of *Salmonella*.

Received October 15, 2009 and accepted July 7, 2011.
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A total of 630 day-old chicks was distributed into six treatments and seven replicates of 15 birds each were used for a period of 28 days. A completely randomized design was used in a 3 × 2 factorial arrangement, with three inoculated agents (0.85% saline solution as a placebo, and *Salmonella enterica* subsp. *enterica* sorovar Typhimurium inoculated in the crop and via feed) and the use of the organic acids proposed (0 or 4 kg/T of the organic acid blend) (Table 1).

The inoculum was prepared as described by Fernández et al. (2001) and nalidixic acid resistant *Salmonella* Typhimurium was obtained from the broiler samples used by Rezende et al. (2008) in her experiment. Feed was formulated based on corn-soybean meal according to recommendations by Rostagno et al. (2005) and were offered *ad libitum*. The organic acid blend was composed of benzoic acid (22.44%), fumaric acid (41.34%) and 2-hidroxi 4-methyl-tiobutanoic acid (28.40%), offered to the birds through the ration during all the experimental period. The contaminated feed was made at the dose of 0.4% of total ration. In the group challenged via ration, *Salmonella* inoculum (0.5 mL of the inoculum/500 g feed) was prepared by means of manual homogenization in the plastic bags.

Chicks and feed were weighed on the 1st, 7th, 14th and 21st days of age mean body weight, weight gain, feed intake and feed conversion were calculated. On the 7th, 14th and 21st and 28th days of age, chicks fasted for four to six hours before the necropsy for elimination of the chime. One bird per experimental unit was weighed and necropsied. The small intestine relative weight and height of each bird was established.

Samples of duodenum and jejunum were collected for histomorphometrical evaluation and processed, following the standard methodology by Luna (1968), at 7, 14 and 28 days of age. At the same ages, the small intestine and jejunum content was collected from one bird per replicate and the pH values of the samples were determined according to the methodology used by Silva et al. (2000).

Results were submitted to ANOVA and means compared by Tukey test at 5% probability using SAS (2004).

Results and Discussion

Organic acid supplementation resulted in better results of feed conversion ($P<0.05$) compared with control treatments (Table 2). These results are in accordance with Partanen & Mroz (1999), who claimed that organic acids, associated with nutritional assessments, handling and biosecurity, can maintain intestinal health, which can affect feed conversion. Birds inoculated with *Salmonella* Typhimurium had worst feed conversion compared with the control group (Table 2), for both ways of inoculation tested.

Table 1 - Experimental design of the treatment

S. Typhimurium challenge and the method of inoculation	Organic acids	Number of chick	*S.* Typhimurium dose
No – saline solution at 0.85%	No	105	No challenge
No – saline solution at 0.85%	Yes	105	No challenge
Yes, into the crop on the 1st day of age	No	105	5.0×10^2 CFU
Yes, into the crop on the 1st day of age	Yes	105	5.0×10^2 CFU
Yes, through feed from 7th to 14th days of age	No	105	5.0×10^2 CFU/kg
Yes, through feed from 7th to 14th days of age	Yes	105	5.0×10^2 CFU/kg

Table 2 - Mean weight, weight gain, feed intake and feed conversion for broilers from 1 to 14 days of age inoculated with *Salmonella* Typhimurium, and treated with an organic acid blend

	Starter weight (g)	Mean weight at 14 days of age (g)	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)
			Organic acids (A)		
Without	42.79	431.02	388.23	490.38	1.303b(1)
With	42.33	455.68	413.53	484.70	1.179a
			Inoculated agent (S)		
Placebo	42.68	455.14	402.45	402.49b	1.012a
ST-crop[2]	42.62	426.80	384.18	521.16a	1.414b
ST-ration[3]	42.37	458.11	415.74	538.98a	1.297b
			Variation factors (%)		
Organic acids (OA)	<0.001	0.032	0.030	0.351	0.028
Inoculated agents (IA)	<0.001	0.081	0.079	<0.001	<0.001
OA × IA	<0.001	0.036	0.035	0.850	0.064
CV (%)	0.39	8.09	8.96	3.99	14.18

[1] Distinct letters on the column indicate difference (Tukey, 5%); [2] ST-crop:*Salmonella* Typhimurium inoculated via crop; [3] ST-ration: *Salmonella* Typhimurium inoculated via ration. CV = coefficient of variation.

The evaluation of the interaction between inoculated agents and organic acids (Table 3) showed the best mean weight and weight gain from 1 to 14 days of age for chicks inoculated with the bacteria via crop and treated with the organic acid blend. Le Ny (2005) reported that organic acids can, primarily, inhibit the pathogen establishment because of the intracellular pH reduction, which affects DNA replication and protein synthesis.

The group inoculated via crop with *Salmonella* Typhimurium ($P<0.05$) showed lower values for both mean weight at 14 days of age and weight gain from 1 to 14 days of age compared with the chicks inoculated via ration (Table 3). This result can be explained by the chick age at inoculation, because the birds were challenged via crop on the first day of age, and via ration at seven days of age. The age is an important factor for the microbiota composition, and in younger animals the bacteria can easily colonize the intestines. The microbiota is formed by the contact of the chick with the microorganisms presented in the hatching chamber, in the transportation boxes or in the facilities and equipments. Desmidt et al. (1997) reported that, by aging, the specific and non-specific immunological defense mechanisms are capable of impairing *Salmonella* infection, which can be attributed to the development of the cellular and humoral immune system on the first days of life. Beal et al. (2004) orally challenged chicks at one, three and six weeks of age with 10^8 CFU of *Salmonella* Typhimurium and verified that older birds, with a well-developed immune system, showed a faster response to eliminate the inoculated agents. Barrow (2000) confirmed that the invasion mechanism and capacity of *Salmonella* depends on the age of bird and the way the agent contaminates the organs.

By evaluating chick performance from 1 to 28 days of age (Table 4), it was verified that chicks that received *Salmonella*, via both inoculation ways, presented best feed conversion ($P<0.05$) and, on the other hand, worse feed conversion ($P<0.05$) when compared with the control birds. Similar results were observed by Xie et al. (2000), who verified significant performance reduction when birds were inoculated with a *Salmonella* Typhimurium lipopolysaccharide (LPS).

Birds treated with the organic acid blend showed increased efficiency in weight gain from 1 to 28 days of age compared with the control treatment (Table 4). Organic acids can reduce colonization by *Salmonella* (Van Immerseel et al., 2004) and increase performance (Snow et al., 2004; Rafacz-Livingston et al., 2005 a,b). In the evaluation of the activity of organic acids on broiler performance, Skinner et al. (1991) found a positive effect of the fumaric acid (0.125%) until 49 days of age with linear reduction of mortality until 0.5% of the diet. Viola & Vieira (2007) tested different mixtures of organic acids in diets and verified that the body weight gain was not affected by treatments.

In this study, chicks inoculated via crop at 7, 14 and 28 days of age which did not receive organic acids showed lower villus height in the duodenum compared with the group that received organic acids (Table 5). At 28 days of age, jejunum villi presented lower heights ($P<0.05$) compared with treatments which did not receive organic acids. These results suggest that the lower the contamination by non-desirable microorganisms in the intestine, the higher the villus height. Intestinal integrity and health can be related to the results obtained, because broilers which received organic acids had significant results for feed conversion at 14 days of age and weight gain at 28 days of age when compared with the group that did not receive the organic acid blend.

According to Daskiran et al. (2004) and Leeson et al. (2005), acidifiers help the intestinal integrity maintenance

Table 3 - Development of interaction between inoculated agents and organic acids used for mean weight at 14 days of age and weight gain from 1 to 14 days of age for broilers inoculated with *Salmonella* Typhimurium via crop and via ration, treated with an organic acid blend

	Mean weight at 14 days of age (g)		
	Inoculated agents		
Organic acids	Placebo	ST-crop[2]	ST-ration
Absence	441.02Aab	393.44Bb	458.60Aa
Presence	449.26Aa	460.16Aa	457.62Aa
	Weight gain from 1 to 14 days of age (g)		
	Inoculated agents		
Organic acids	Placebo	ST-crop[2]	ST-ration
Absence	397.98Aab	350.49Bb	416.21Aa
Presence	402.92Aa	417.87Aa	415.27Aa

[1] Distinct letters on the row indicate difference (Tukey, 5%); [2] ST-crop:*Salmonella* Typhimurium inoculated via crop; [3] ST-ration: *Salmonella* Typhimurium inoculated via ration.

Table 4 - Mean weight, weight gain, feed intake and feed conversion of broilers from 1 to 28 days of age inoculated with *Salmonella* Typhimurium, and treated with an organic acid blend

	Starter weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)
		Organic acids (A)		
Without	1125.10b(1)	1082.31b	1513.90	1.534
With	1201.13a	1158.80a	1574.46	1.505
		Inoculated agent (S)		
Placebo	1155.16	1112.48	1406.75b	1.398a
ST-crop[2]	1170.25	1127.63	1599.55a	1.565b
ST-ration[3]	1163.92	1121.55	1626.25a	1.596b
		Variation factors (%)		
Organic acids (OA)	0.001	0.001	0.069	0.176
Inoculated agents (IA)	0.847	0.846	<0.001	<0.001
OA × IA	0.194	0.190	0.173	0.456
CV (%)	5.97	6.20	6.79	4.60

[1] Distinct letters on the column indicate difference (Tukey, 5%); [2] ST-crop: *Salmonella* Typhimurium inoculated via crop; [3] ST-ration: *Salmonella* Typhimurium inoculated via ration. CV = coefficient of variation.

Table 5 - Villus height and crypt depth of duodenum and jejunum of chicks inoculated with *Salmonella* Typhimurium via crop

	7 days		14 days		28 days	
	Duodenum					
	Height (µm)	Depth (µm)	Height (µm)	Depth (µm)	Height (µm)	Depth (µm)
Negative control[a]	997.73a	237.43	1131.79a	213.03	1283.54a	157.28c
Negative control + acid[b]	975.32a	187.49	931.10a	181.13	1063.07b	263.76a
ST- crop	851.81b	168.04	566.15b	174.44	876.80c	175.06bc
ST- crop + acid[d]	1021.88a	198.16	1197.04a	249.03	1109.60b	218.00b
CV (%)	5.22	22.47	13.20	29.43	6.30	10.99
P	<0.001	0.742	<0.001	0.620	<0.001	<0.001
	Jejunum					
	7 days		14 days		28 days	
	Height (µm)	Depth (µm)	Height (µm)	Depth (µm)	Height (µm)	Depth (µm)
Negative control[a]	602.89b	237.43a	575.60	170.42	613.31b	139.72
Negative control + acid[b]	718.77a	111.00b	585.70	170.67	823.93a	131.84
ST-crop	493.66c	86.72b	881.80	190.81	584.09b	119.00
ST-crop + acid[d]	665.07ab	90.30b	620.00	216.24	617.59b	64.95
CV (%)	7.42	12.63	24.25	27.40	14.26	49.78
P	<0.001	<0.001	0.470	0.394	<0.001	0.753

Distinct letters on the same column and age differ by Tukey test (5%).
[a] Negative control; [b] Negative control of the acid; [c] Inoculated via crop; [d] Inoculated via crop and treated with acid.
CV = coefficient of variation.

due to the reduced microbiological challenges of the mucosa, increasing the capacity of feed use by the animals and reducing the maintenance wastes of intestinal tissues. According to Salazar et al. (2008), the way of action of the organic acids in the intestinal epithelium can reduce the pathogenic bacteria population because of their capacity to decrease intestinal medium pH, which hampers the bacteria adhesion to the mucosa, reducing epithelium damage. In the same way, Maiorka et al. (2004) observed that the presence of trophic agents, such as nutrients in the intestinal lumen stimulate the mucosa development, originating a mitotic process, in addition to increasing the number of cells and the villus height.

At 21 days of age, the small intestine weight was lower (P<0.05) with the organic acid addition (Table 6). Higher weight (P<0.05) was observed at 7, 14 and 28 days of age, for treatments inoculated with *Salmonella*, in all the inoculation ways compared with the placebo group.

The higher small intestine weight in the challenged groups does not seem to be related to a better intestinal health state or nutrient absorption capacity. This fact was confirmed by the data presented in Table 5 because at 7, 14, and 28 days of age, chicks inoculated via crop showed mean values (P<0.05) for duodenum villus height compared with the other analyzed groups. Another explanation is that the finding might be due to the *Salmonella* activity in the intestine, because microbiological challenges can result in intestinal inflammation and, sometimes, necrosis of the tissues can occur (Apajalahti, 2005).

Table 6 - Small intestine weight and height of broilers at 14, 21 and 28 days of age inoculated with *Salmonella* Typhimurium via crop and via ration and treated with an organic acid blend

	7 days		14 days		21 days		28 days	
	Weight (%BW)	Height (%BW)	Weight (%BW)	Height (%BW)	Weight (%BW)	Height (%BW)	Weight (%BW)	Height (%BW)
				Organic acids (OA)				
Absence	7.37	51.12	4.98	21.91	4.41a	16.64	3.42	11.87
Presence	7.07	50.91	4.71	22.20	3.77b	16.55	3.41	11.00
				Inoculated agents (IA)				
Placebo	6.29b	54.22a	4.33b	24.32a	3.06b	16.04	2.40b	11.54
ST-crop	8.16a	47.81b	4.88b	20.90b	4.73a	16.51	3.86a	11.68
ST-ration	-	-	5.33a	20.95b	4.48a	17.23	3.98a	11.10
				Variation Factor (%)				
Organic acids (OA)	0.255	0.931	0.130	0.683	0.028	0.950	0.992	0.360
Inoculated agents (IA)	<0.001	0.013	<0.001	<0.001	<0.001	0.777	<0.001	0.470
OA × IA	0.235	0.682	0.003	0.257	0.083	0.133	0.051	0.136
CV (%)	9.61	12.44	11.82	10.58	22.51	26.89	12.69	11.23

Distinct letters on the same column indicate differences by Tukey test (5%). CV = Coefficient of variation. % WG = bird weight gain.

The group inoculated with placebo had higher small intestine length (P<0.05) compared with the groups inoculated with the pathogen via both tested ways at 7 and 14 days of age (Table 6). This fact can be due to *Salmonella* infection, which caused tissue and cell disorders which reduced the absorption area and worsened feed conversion at 7 and 14 days of age.

Through the analysis of the interaction between inoculated agents and organic acids of the small intestine weight at 14 days of age (Table 7), it was possible to verify that the small intestine weight of chicks that were inoculated via ration and received the organic acid blend was lower (P<0.05) compared with the birds that did not receive the organic acids in the diet. This is a possible result of the organic acids activity against the pathogenic agent.

The inclusion of the organic acid blend did not affect small intestine pH or ceca content (P<0.05) during the studied periods (Table 8). It was observed that the small intestine pH was statistically higher in the placebo group when compared with the inoculated chicks. The ceca pH was statistically different at 7 (Table 8) and 21 days of age (Table 9) and the placebo group showed the highest values.

Table 7 - Development of statistical interaction for small intestine weight at 14 days of age for broilers inoculated with *Salmonella* Typhimurium via crop and via ration and treated with organic acids blend

	Inoculated agents		
	Placebo	ST-crop	ST-ration
Organic acids in ration			
Absence	4.43Ab	4.63Ab	5.89Aa
Presence	4.23Aa	5.13Aa	4.76Ba

Different lowercase letters on the column (row) indicate statistical difference by Tukey test (5%).

Table 8 - Values of small intestine and ceca content pH of broilers at 7, 21 and 28 days of age inoculated with *Salmonella* Typhimurium via crop and via ration and treated with a blend of organic acids

	7 days of age		21 days of age		28 days of age	
	Small intestine	Ceca	Small intestine	Ceca	Small intestine	Ceca
			Organic acids (OA)			
Absence	6.67	6.59	6.44	6.68	6.24	6.65
Presence	6.37	6.61	6.51	6.53	6.05	6.82
			Inoculated agents (IA)			
Placebo	6.82a	7.15a	6.70a	6.68	6.88a	6.89
ST-crop	6.22b	6.04b	6.24b	6.45	5.86b	6.63
ST-ration	-	-	6.33b	6.69	5.70b	6.68
			Variation factors (%)			
Organic acids (OA)	0.102	0.940	0.515	0.161	0.166	0.118
Inoculated agents (IA)	0.002	<0.001	0.004	0.114	<0.001	0.099
OA × IA	0.127	0.591	0.304	0.009	0.533	0.496
CV (%)	7.25	8.94	5.76	4.98	7.22	4.89

Distinct letters in the column and row indicate statistical differences by Tukey test (5%).

Table 9 - Development of statistical interaction between inoculated pathogenic agent and the use of organic acid blend in ration for ceca content pH at 21 days of age in chicks inoculated with *Salmonella* Typhimurium

	Inoculated agents		
Organic acids	Placebo	ST-crop	ST-ration
Without	6.53	6.70	6.81
WIth	6.83a	6.21b	6.56ab

Different lowercase letters on the column (row) indicate statistical difference by Tukey test (5%).

It is likely that *S.* Typhimurium determined higher intestinal fermentation and, as a consequence, reduced the pH. Lower pH indicates fermentation and production of volatile fatty acids (Huang et al., 2006). In a similar way, Chambers et al. (1997) verified that the samples with the highest pH values, in CFU of *Salmonella* were also the ones with lowest cecal pH.

Conclusions

Organic acids blend (benzoic, fumaric and 2-hidrox-4-methyltiobutanoic), offered at 0.4% in ration increases performance and intestinal health for broilers challenged with *Salmonella* Typhimurium between 1 and 28 days of age.

Acknowledgements

The authors thank CNPq and Novus do Brasil for financial and material support and Karla Avanço for helping them translate this article.

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