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Fumaric Acid in Broiler Nutrition: A Dose Titration Study and Safety Aspects

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Abstract: Six isonitrogenous (22.8% CP) and isocaloric diets (13 MJ ME/kg) containing 0, 1.25, 2.50, 3.75, 5.0 and 7.5% fumaric acid (FA), respectively, were fed for 26 days to 12 (replicates) x 8 (chicks per replicate) Lohmann-Hybrid newly hatched male chicks (48 g body weight) per treatment. The diets consisted mainly of wheat and soybean meal. Mortality ranged between 0 and 4 %. Final body weight (and feed efficiency) amounted to 1,506 (756), 1,597 (767), 1,532 (754), 1,485 (759), 1,342 (738) and 1,378 g (747 g gain/kg feed) for the groups with 0, 1.25, 2.50, 3.75, 5.0 and 7.5% FA, respectively. The 1.25% FA group showed significantly ($p<0.05$) better weight gain than all other groups and better feed efficiency than the groups with 5.0 and 7.5% FA. Higher gain was associated with higher feed intake. Body weight of the 5.0 and 7.5% FA groups was significantly lower than that of all other groups. It is concluded that 1.25% FA may promote growth of broilers, but the effect disappears with further increasing doses. The relative weight of heart, liver and spleen was not affected by the treatment. Pathological findings summarized to 5/10 in the 7.5% FA group, to 1/10 in the control and to 2-3/10 in the other groups. The 7.5% FA group showed also the highest incidence of kidney alterations and slightly thinner duodenal wall. From the findings the margin of safety is concluded to be about 3% (3.75/1.25) considering 1.25% FA in the broiler diet as the optimally effective concentration.

Key words: Fumaric acid, broiler nutrition, wheat meal, soybean meal

Introduction

Due to recent withdrawal of antibiotic feed additives in the EU (Regulation 1831/2003), alternatives are highly demanded. Fumaric acid ($C_4H_4O_4$; molecular weight: 116) is approved at EU-level as a preservative to protect cereals and other feedstuffs against microbial decomposition. Fumaric acid is antimicrobially active (Alp *et al.,* 1999; Radecki *et al.,* 1987), reduces undesirable bacteria in the gastro-intestinal tract (e.g. *E. coli*) and finally improves growth rate up to an inclusion rate of 4 % in pigs and poultry (Bolduan, 1987; Eidelsburger and Kirchgessner, 1994; Falkowski and Aherne, 1983; Kirchgessner and Roth, 1988; Kirchgessner *et al.,* 1991; Patten and Waldroup, 1988). But Schmack (2001) described adverse effects in calves (also renal dysfunction) after acidifying milk substitutes with fumaric acid. Some pathological effects were also observed when a fumaric acid ester was used in human therapy of psoriasis, including nephrotoxic effects (Hohenegger *et al.,* 1989). An upper safe level of fumaric acid for animals could not be established so far (SCAN, 2003).

It should therefore be re-evaluated, if and at which levels of fumaric acid promotes growth in broiler and which dose could be considered as the upper safe level.

Materials and Methods

The experiment was conducted in the battery house of the Institute of Animal Nutrition, Nutrition Diseases and Dietetics at the Faculty of Veterinary Medicine of Leipzig University, Germany. Steel wire cages were used having a surface area of 0.80 m² (100 x 80 x 60 cm) per cage. Also, steel trays were arranged under each cage to collect broiler droppings and wastage feed. Temperature and ventilation were controlled automatically. Light was provided continuously. Feed and water was offered for *ad libitum* intake.

Experimental design and diets: A total of 576 Lohmann-Hybrid day old male broiler chicks were randomly distributed to 72 cages in 3 tiers with 8 birds per cage. Six treatments were allocated to corn and soybean based pelleted diets (Table 1) containing 0, 1.25, 2.50, 3.75, 5.00 and 7.50% fumaric acid (FA), respectively, given for 26 days. The diets were formulated to be isonitrogenous and isocaloric.

Experimental procedure: Birds were vaccinated against Infectious Bursal Disease using spray method in the hatchery as well as against New Castle Disease at 8th day of age through drinking water. Dead and abnormal birds were promptly removed, weighed and gross

Table 1: Formulation (% ingredients) of the experimental diets

	Fumaric acid (%)					
Ingredients	0	1.25	2.5	3.75	5.0	7.5
Wheat	36.200	36.200	36.200	36.200	36.200	36.200
Soybean seed	44.000	44.000	44.000	44.000	44.000	44.000
Soybean oil	8.000	8.000	8.000	8.000	8.000	8.000
Wheat starch	5.80	4.85	3.90	2.95	1.95	0.050
Cellulose	1.75	1.45	1.15	0.85	0.60	-
Fumaric acid	-	1.250	2.500	3.750	5.000	7.500
Monocalciumphosphate	1.450	1.450	1.450	1.450	1.450	1.450
Calciumcarbonate	1.850	1.850	1.850	1.850	1.850	1.850
Sodium chloride	0.465	0.465	0.465	0.465	0.465	0.465
Vitamin-premix[a]	0.250	0.250	0.250	0.250	0.250	0.250
Trace element premix[b]	0.125	0.125	0.125	0.125	0.125	0.125
DL-Methionin (99%)	0.160	0.160	0.160	0.160	0.160	0.160
Total	100	100	100	100	100	100

[a]2.5g vitamin premix: Vitamin A, 13.500 I.U; Vitamin D_3, 1.500 IU; a-DL-Tocopherolacetate, 50 mg; Menadion, 2 mg; Thiamin, 3mg; Riboflavin, 5mg; Pyridoxin, 4mg; Cobalamine, 15µg; Biotin, 250µg; Folic acid, 200µg; Nikotenic acid, 60µg; Ca-pantothenate, 30mg; Cholin, 750mg; Ascorbic acid, 150mg. [b]1.25g trace element premix: Fe, 125.00mg; Zn, 100.00mg; Cu, 10,00mg; Mn, 50,00mg; Se, 0.25mg; I, 2.50mg; Co, 0.20mg

Table 2: Chemical composition (g/100g air dry sample) of the experimental diets

	Fumaric acid (%)					
Components	0	1.25	2.5	3.75	5.0	7.5
Dry matter	92.75	92.94	92.75	93.17	93.31	93.64
Crude protein	23.02	23.34	23.18	22.92	21.74	22.51
Crude fibre	5.18	5.26	4.58	4.46	4.47	4.21
Ether extract	11.02	10.99	11.04	11.36	12.56	14.14
Ash	6.87	7.08	6.89	7.05	7.19	6.92
Nitrogen free extract	46.66	46.27	47.06	47.38	47.35	45.86
Organic matter	85.88	85.86	85.86	86.12	86.12	86.72
Starch	30.54	28.27	30.16	29.22	29.40	29.97
Sugar	5.02	3.88	3.73	4.10	3.37	4.28
ME (MJ kg-1 feed)*	13.10	12.61	12.90	12.86	13.03	13.90

*ME in MJ/kg = g Crude protein x 0.01551 + g Crude Fat x 0.03431 + g Starch x 0.01669 + g Sugar x 0.01301, legal German formula for feed control (Weinreich *et al.*, 2002).

necropsy was conducted. Weight of other birds of the same cage and rest feed in the feeder were recorded. Feed supplied, refusal and wastage were recorded throughout the trial. Birds were weighed per week basis and latest at the end of the trial (26 days). At the end of trial 60 birds were selected (1 bird/cage and 10 per treatment) based on cage average for gross pathology. Weight of sacrificed bird and their heart, liver and spleen were recorded.

Chemical and data analysis: Proximate feed analysis were performed according to VDLUFA (2000). Data were monitored by computer Excel program and analysed using the SPSS 11.5 program by analysis of variance (ANOVA) and Tukey's B test for treatment differences (Steel and Torrie, 1980).

Results

Growth, feed consumption and feed efficiency: Supplementing 1.25% FA resulted in highest weight gain (Table 3) from the start of the trial and continued until end of the trial (26 days: 1549 g, $p<0.05$). Weight gain of 2.5 and 3.75 % FA groups was lower (1484g and 1438g, respectively) and at the level of the control (1459g, $p>0.05$). Adverse effects on weight gain ($p<0.05$) were observed for 5.0 and 7.5% FA (1295g and 1331g, respectively). During end of the trial (day 26) the live weight of the birds attained significantly ($p<0.05$) higher in 1.25% FA group (1597g) where 0, 2.5, 3.75, 5.0 and 7.5% FA groups showed 1506, 1532, 1485, 1342 and 1378g consequently (Fig. 1).

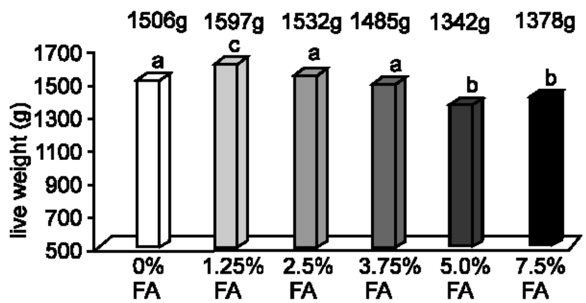

Fig. 1: Final live weight (g) of fumaric acid (FA) fed broiler chicks during 26 days trial.

The 1.25% FA started with highest feed intake (172 g per bird in the 1st week). But, groups offering 2.5, 3.75 and 7.5% FA was not different from that of the control (159, 154 and 150 g, respectively, vs. 158g) in the same week and lowest feed intake (146g) was recorder for 5.0% FA group ($p<0.05$). Cumulative feed intake for 1.25 % FA group was 2022 g, not significantly ($p<0.05$) higher than for the control (1930g) and the group with 2.5% FA (1969g). However, the 5.0 and 7.5 % FA groups consumed significantly ($p<0.05$) less (1754 and 1781g, respectively) than all other groups.

Feed conversion efficiency (g LWG/Kg FI) was calculated for 1.25% FA group (767g gain/kg feed), but significantly

Table 3: Live weight gain (LWG, g), feed intake (FI, g) and feed conversion efficiency (FCE, g LWG 1000 g-1 FI)) of fumaric acid fed broiler chicks during 26 days trial

	Days of trial	Fumaric acid (%) 0	1.25	2.5	3.75	5.0	7.5
Weight gain	1-7	$147^{ab}\pm12$	$165^{d}\pm9$	$153^{b}\pm8$	$145^{ab}\pm9$	$133^{c}\pm10$	$140^{ac}\pm8$
	1-14	$503^{a}\pm30$	$539^{c}\pm18$	$510^{a}\pm20$	$489^{a}\pm21$	$434^{b}\pm30$	$456^{b}\pm21$
	1-21	$1021^{a}\pm66$	$1088^{c}\pm41$	$1033^{a}\pm38$	$998^{a}\pm50$	$871^{b}\pm52$	$919^{b}\pm47$
	1-26	$1459^{a}\pm82$	$1549^{c}\pm49$	$1484^{a}\pm48$	$1438^{a}\pm48$	$1295^{b}\pm65$	$1331^{b}\pm49$
Feed intake	(1-26)	$1930^{ac}\pm107$	$2022^{c}\pm71$	$1969^{ac}\pm69$	$1898^{a}\pm98$	$1754^{b}\pm92$	$1781^{b}\pm71$
FCE	(1-26)	$756^{ac}\pm10$	$767^{c}\pm9$	$754^{ac}\pm16$	$759^{ac}\pm21$	$738^{b}\pm17$	$747^{ab}\pm16$
Mortality (%)		2.1	3.1	0	3.1	4.2	1.0
(died/treatment)		(2)	(3)	(0)	(3)	(4)	(1)

^{abc}Means having uncommon superscripts in the same row differ significantly (p<0.05).

Table 4: Gross pathology in different treatments from 60 sacrificed birds

Organ	Observation	Fumaric acid (%) 0	1.25	2.5	3.75	5.0	7.5
Liver	Enlarged liver	-	-	1*	1	-	1#
	Hepatomegalie	-	1	-	-	-	-
	Bright liver	-	-	1,1#	1	1#	-
Heart	Enlarged	-	1	-	-	-	-
Kidney	Radish clay in colour	-	-	-	-	1	1
	Grey in colour	-	-	-	-	-	1
	Radish brown marble	-	-	-	-	-	1#
	Capsulated blood	-	-	1#	-	1#	-
Pancreas	Bloody	-	1	-	-	-	-
Breast Muscle	Spotted	1	-	1*	-	-	-
All muscle	Spotted	-	-	-	-	-	1#
Duodenum	Dilatation	-	-	-	-	-	1,1
Abnormal birds		1	3	3	2	2	5
Normal birds		9	7	7	8	8	5
Total birds		10	10	10	10	10	10

#*Same superscript in the same treatment indicates same bird

different (p<0.05) from the control (756) and the 2.5 (754) and 3.75 (759) % FA groups (Table 3). Poorest value was obtained for the 5.0% FA group (738), which significantly (p<0.05) different from all other groups except 7.5% FA.

Mortality, pathological condition and organ weight: The mortality of birds was within the range 0 to 4% irrespective of the treatment (Table 3). During the course of the trial, 13 of 576 birds died from the experiment. Two of the mortalities occurred in the control group (15.4%) and for the remaining mortalities, specific causes of death were not associated with any treatment.

Pathological records showed were attributed to nonspecific cardiovascular problems. At the end of the trial, no increase in pathological findings was observed due to continuous inclusion of FA up to a level of 5.0 % (Table 3). The 7.5% FA group showed some more abnormalities (5/10 birds compared to 1-3/10 for the other treatments). Additional to the findings the organs liver, kidney and in muscle tissue, 2/10 birds showed a dilatation of the duodenum (thin wall) only in 7.5% FA group (Table 4). It is also mentionable that in 7.5% FA caused no other abnormalities in any organs, which tends to conclude that addition of 7.5% FA might cause duodenal dilation.

Absolute (data not shown) and relative weight of heart and spleen (Table 5) was not statistically different (p>0.05) but absolute liver weight reflected the differences. The 5% FA group showed the lowest absolute and relative liver weight, but these finding can hardly attributed to the treatment, because the 7.5% FA group was again in the normal range.

Discussion

Addition of 1.25% FA in feed caused higher (p<0.05) weight gain but already 2.5 and 3.75% FA tended to approximate growth to control level. Eidelsburger and Kirchgessner (1994) demonstrated that up to 1.0% FA has a positive effect in broilers, which is in agreement with our results. There is probably only a small optimum dose range for the supplementation of FA to broiler diets. However, the diets used in the experiment contained cereals of good quality and a dose response to FA may appear different from that shown, if feedstuffs of minor quality had been used.

Patten and Waldroup (1988) found that 1.0% FA in broiler resulted in higher body weight gain but did not influence feed utilization. Our studies showed also the absence of a significant effect on feed efficiency. However, Vogt *et al.* (1981), reported that FA significantly improved feed utilization curve-linearly, with the peak response occurring at an inclusion rate between 1.0 and 1.5%. This result can be regarded as a confirmation of the above conclusion that the lowest level of FA tested in our experiment was probably near to the maximum effective level.

In poultry, hydrochloric acid and specific enzyme secretion are functional from hatching. HCl and pepsinogen are secreted in the proventriculus, in quantities much larger than in mammals (Moran, 1982) and on a continuous basis. Lower amounts of an organic acid supplementation as needed for piglets may therefore suffice to give the same magnitude of improvements in growth rate on poultry. As a matter of fact, the optimal dosage for piglets is about 2% and higher (Kirchgessner and Roth, 1976).

Table 5: Slaughtering weight (g) and weight (% live weight, lw) of heart, liver and spleen of 10 birds per treatment

	Fumaric acid (%)					
Parameters	0	1.25	2.5	3.75	5.0	7.5
Live weight (lw)	$1540^{a}\pm100$	$1675^{b}\pm48$	$1583^{a}\pm75$	$1544^{a}\pm61$	$1394^{c}\pm72$	$1432^{c}\pm104$
Relative to (1)	100	109	103	100	91	93
Heart	$12.2^{a}\pm1.32$	$12.6^{a}\pm1.96$	$13.1^{a}\pm1.79$	$12.6^{a}\pm1.65$	$12.5^{a}\pm1.90$	$12.1^{a}\pm1.52$
Relative to lw	0.79	0.75	0.83	0.82	0.90	0.84
Liver	$45.4^{ab}\pm6.67$	$48.6^{a}\pm7.76$	$47.1^{a}\pm7.53$	$43.7^{ab}\pm3.59$	$38.7^{b}\pm5.91$	$42.0^{ab}\pm6.00$
Relative to lw	2.95	2.90	2.98	2.83	2.78	2.93
Spleen	$2.7^{a}\pm0.67$	$2.7^{a}\pm0.67$	$2.8^{a}\pm0.42$	$2.9^{a}\pm0.32$	$2.7^{a}\pm0.68$	$2.6^{a}\pm0.52$
Relative to lw	0.18	0.16	0.18	0.19	0.19	0.18

^{abc}Means having uncommon superscripts in the same row differ significantly ($p<0.05$).

Kirchgessner *et al.* (1991) reported that up to 2.0% FA did not increase feed consumption in broiler. On contrary, Bolduan (1987) found in piglets that at a low level of 1.2% FA causes rapid emptying of stomach resulting in higher feed intake. Also unpublished studies (Gropp, 1974) suggest that in piglets the growth promotion efficacy of FA is strongly correlated with an increased feed intake. A higher feed intake might therefore be expected also in poultry at optimal dosages of FA, as observed in the broiler. Consequently, feed efficiency is only slightly improved due to addition of FA. Skinner *et al.* (1991) demonstrated that the addition of FA (up to 0.25 %) significantly improved body weight of females and average weight gain of both sexes but found no effect on feed utilization of broilers. Feed consumption was significantly increased when diets contained 0.125 or 0.50% FA. In another trial with male broilers the body weight was significantly ($p<0.05$) improved by the addition of 0.125 and 0.25% FA. There were no significant differences in feed consumption; feed utilization was improved by the addition of all levels of FA.

Dietary acidification lowers the gastric pH (Blank *et al.,* 1999) creates better environment for pepsin action and increases gastric proteolysis and amino acid digestibility (Vogt *et al.,* 1981; Blank *et al.,* 1999; Taylor, 1962). The acid anion has been shown to complex with Ca, P, Mg and Zn, which results in an improved digestibility of these minerals. Furthermore, organic acids serve as substrates in the intermediary metabolism (Kirchgessner and Roth, 1988; Giesting *et al.,* 1991). Moreover, Blank *et al.* (1999) could demonstrate that FA, as a readily available energy source, has a local tropic affect on the mucosa of the small intestine in weaning pigs. Other scientists focus on the antimicrobial action of FA reducing the microbial count in the GIT (Vogt *et al.,* 1981).

The inclusion of FA up to the 5 % level did not cause any health problems or serious pathological findings. This is at least partly confirmed by Patten and Waldroup (1988) who showed that organic acids (including FA) up to 2.0% in the diet resulted in no health problems. No kidney alterations have been observed in the control and in the 1.25% FA group. The groups with 2.5 and 5.0% showed 1/10 kidney with capsulated blood, no discoloration in the 2.5 and 3.75% FA group, but 3 kidney alterations in the 7.5% FA group. High FA (5.0 and 7.5% in the diet) resulted in a severe significant depression of live weight gain, feed intake and feed efficiency. A very conservative assessment would at least not exclude a potential influence of 7.5% FA on kidney function for these high dosages as known for humans (Kolbach and Nieboer, 1992).

The highest level tolerated without toxic symptoms was 3.75%, but beneficial effects on broiler performance were absent at this dosage. The level of margin for FA in broiler diets would than be approximately 3 (3.75/1.25), assuming that 1.25 % FA is the highest recommended dose.

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