

Aquatize® Combats Necrotic Enteritis

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“More and more poultry consumers worldwide are requesting, nay demanding, that their broilers and turkeys be grown without the use of dietary antibiotics. The successful commercial production of broilers and turkeys, in the absence of antibiotics, is dependent on the control of necrotic enteritis” - (1) Bill Dudley-Cash, Nov. 6, 2006, Feedstuffs 78(46).

Non-antibiotic products that effectively combat bacterial pathogens can be difficult to find. But recent regulations that have tightened the laws governing antibiotics or banned them outright have forced animal producers to solve old problems like necrotic enteritis through alternative means. Aquatize®, a non-antibiotic stabilized sodium chlorite/chlorate solution approved by the EPA for use in animal drinking water, has been effective in combating a wide range of pathogens, including the eleven microbial pathogens listed below. In the EPA test Aquatize® killed 99.999% of these microbes at dilutions of 1:2000 or 1:5000 and at exposure times ranging from 5 to 10 minutes -- an impressive accomplishment.

<i>Campylobacter jejuni</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>
<i>Escherichia coli</i> 0157:H7	<i>Listeria monocytogenes</i>	<i>Pasturella multocida</i>
<i>Pseudomonas aeruginosa</i>	<i>Salmonella enteritidis</i>	<i>Salmonella typhimurium</i>
<i>Shigella dysenteriae</i>	<i>Streptococcus suis</i>	

We report here about three scientific studies that evaluated Aquatize® administered via drinking water in preventing necrotic enteritis in broilers and for reducing bacterial pathogen infection in chickens. Aquatize® outperformed or was on par with several **antibiotics** and was able to prevent major losses in weight, feed conversion and mortality in challenged birds. In these studies, birds were infected with both *Eimeria sp* and *Clostridial sp* by the two laboratories that conducted **these** investigations.

Below is a description of the first study using Aquatize® that was conducted by Dr. James L. McNaughton and Mr. Thomas L. Haschen, both of the PARC Institute in Easton, Maryland (5). A model of intestinal necrosis was chosen for study that was easy to reproduce in a laboratory setting and that had economic relevance for the production of meat animals. The NE model included challenging young broiler chicks with 200,000 *Eimeria acervulina* per bird on the 5th day followed by several hundred million CFU of *Clostridium perfringens* (CP) on the 7th day. To start the test, healthy, newly hatched broiler chicks (Ross x Cobb males from a commercial source) were randomly placed into wire-floor cages at 100 birds per group in 10 pens of 10 birds/pen.

Birds and feed were weighed on day 21 (end of trial). The test groups included:

Uninfected Control	(UC)
Infected Control	(IC)
Infected Control + 66 ppm salinomycin and 55 ppm bacitracin	(IC+M)
Infected Control plus Aquatize® (1:2,000) in the drinking water	(IC+A)
Infected Control + Aquatize® + Sodium Bicarbonate	(IC + A + B)
Infected Control + Sodium Bicarbonate at 0.30%	(IC + B)
Infected Control + Disinfectant Iodine at 1:256 dilution	(IC + I)
Infected Control + Chlorine Dioxide at 1:1024 dilution	(IC + O)

As is clear from the data in the Table 1, infection (IC) severely depressed growth and feed conversion and resulted in a very high lesion score that was significantly different from the uninfected-control chicks (UC). Treatment of infected birds with salinomycin and bacitracin (IC + M) helped the birds overcome about 50% of the depression from the NE. In marked contrast, broilers receiving Aquatize® in the drinking water at a 1:2,000 dilution (IC + A) recovered 75% of the weight and had a feed conversion that was not different statistically from uninfected controls. Moreover, in the Aquatize® group mortality was fully overcome compared to IC and 85% of the fecal bacterial counts were eliminated.

Table 1: Weight, Feed Conversion, Mortality, Intestinal Lesions and Fecal CFU

<u>Trt Group</u>	<u>21-D Weight</u> (grams)	<u>Feed Conv.</u> (gr/gr)	<u>Lesion Score</u>	<u>Mortality</u> (%)	<u>Fecal CFU</u> (% reduced)
UC	520 ^a	1.358 ^a	0.14 ^a	3.0	11%
IC	383 ^d	1.476 ^d	2.64 ^d	8.0	----
IC+M	430 ^c	1.414 ^c	1.61 ^c	4.0	24%
IC+A	485 ^b	1.372 ^b	0.74 ^b	3.0	85%
IC+A+B	505 ^{ab}	1.364 ^{ab}	0.53 ^b	3.0	92%
IC+B	416 ^c	1.452 ^d	1.82 ^c	4.0	35%
IC+I	424 ^c	1.410 ^c	2.22 ^d	4.0	46%
IC+O	512 ^{ab}	1.368 ^{ab}	0.69 ^b	5.0	88%

Letters that are different are statistically significantly different at $p < 0.05$.

Intestinal lesions were scored as: 0 = no redness present; 1 = red spots and/or strikes present; 2 = ¼ to ½ of the intestine is red; and 3 = intestine is completely red and covered.

Mixing a small amount of sodium bicarbonate with the Aquatize® stock solution allowed the birds to fully recover in weight, feed conversion, the number of lesions, mortality and to exhibit a reduction in fecal bacteria of 92%. The combination of Aquatize® and bicarbonate gave better improvements in performance, livability and bacterial reduction than two other competing products, a Disinfectant Iodine® (IC + I) and Chlorine Dioxide (IC + O). Bicarbonate alone helped the birds overcome NE, but we do not have an explanation for that effect (IC+B).

These results suggest that including Aquatize® and sodium bicarbonate in the drinking water helped to overcome the impact of NE in broilers. Obviously, such an observation needs to be tested more thoroughly in the field to be sure it is not an artifact of a single laboratory observation.

Later, Dr. Greg Mathias and Dr. Chuck Hofacre, DVM of Southern Poultry Research, conducted a second investigation of NE. Day-old broiler chicks (Cobb x Cobb males) were obtained from a commercial hatchery and assigned to five (5) treatment groups of eight (8) Petersime battery cages/each, using a randomized, complete block design. Birds received routine vaccination at the hatchery. Weight gain (measured on days 5, 14, and 28, end of test), feed conversion, and a reduction in mortality and intestinal lesions were measured. The birds were fed starter and grower commercial rations *ad libitum*.

At five (5) days Bacitracin MD and Aquatize® treatments were started:

Treatment Design

	<u>Group</u>	<u>Treatment</u>	<u>Disease Challenge</u>
Positive Control	1	None	No
Negative Control	2	None	Yes
Bacitracin MD	3	50 gr/ton	Yes
Aquatize®	4	1:2000 dilution	Yes
Aquatize®	5	1:5000 dilution	Yes

On day 14, birds in groups 2-5 were given 5,000 oocysts of *E. acervulina* and 200 oocysts of *E. maxima* per bird, and then on days 18-20 birds were given a total of 400,000,000 CFU of *Clostridia sp* per bird by gavage in daily amounts of 1.0 ml water. On day 22, 4 birds from each cage (a total of 160 birds) were sacrificed and examined for the presence of lesions in the digestive tract, an indicator of necrotic enteritis. Throughout the experiment all birds that died were necropsied and the cause of death determined.

Mortality in the positive control (group 1) was 2%, but mortality was increased by the NE in all other groups. Birds that received BMD (group 3) had a mortality of 16%. In marked contrast, mortality in the presence of Aquatize® was 8% (group 4) and 10% (group 5).

The intestinal lesion score of birds in uninfected, **positive** control (group 1) was zero, but NE led to a lesion score of 9 in the negative control (group 2). BMD treatment did not lower the intestinal lesion score at all (group 3), but remarkably both treatments with Aquatize® reduced the lesions score to 3.5 and 3.9, in (groups 4 and 5), respectively.

Table 2: Weight, Lesion Scores and Feed Conversion in NE Challenged Birds

<u>Treatment</u>	<u>Group</u>	<u>Treatment</u>	<u>Lesion Score</u>	<u>Wt. Change</u> <u>14-28 days</u>	<u>F/G</u> <u>28 days</u>
Positive Control	1	None	0.8	0.760 ^a	1.294 ^a
Negative Control	2	None	9.0	0.696 ^c	1.390 ^c
BMD	3	50 gr/ton	9.0	0.745 ^b	1.324 ^b
Aquatize®	4	1:2000 dilution	3.5	0.719 ^{bc}	1.348 ^b
Aquatize®	5	1:5000 dilution	3.9	0.718 ^b	1.325 ^b

Different letters next to the number indicates that it is statistically significantly different from a number with a different letter, at $p < 0.05$.

Birds that were not challenged (group 1) with infectious NE gained the most weight and had the best feed conversion and these were statistically significantly different from all the other groups. Although numerical differences in weight and feed conversion were observed between the BMD (group 4) and the two Aquatize® groups (groups 4 & 5) when the data were examined the differences were not statistically different. The BMD and two Aquatize® groups of birds outperformed the challenged, negative control birds (Group 2), and these differences were statistically significant.

In a third investigation (6) investigators administered either salinomycin (SAL-66 ppm) of COCCIVAC®-B (CVB) to male broiler chicks (Ross x Cobb) housed in wire-floor cages (8 replications and 5 birds/rep) and then challenged all groups of chicks with *Eimeria acervulina* oocysts (200,000/bird on day 5 post-hatch) and *Clostridium perfringens* (hundreds of millions/bird on day 7 post-hatch) to determine the impact of either Aquatize® (AQ) or Bacitracin (BA-55 ppm) in the prevention of NE. Birds and feed were weighed at 21-days and intestinal lesion scores were determined on each bird.

The treatments included the following:

- T1 = No antibiotics, coccidiostat, no Aquatize®, T2 = COCCIVAC® - B,
- T3 = Salinomycin (66 ppm), T4 = Aquatize® (1:2000 dilution),
- T5 = Bacitracin –MD (55 ppm)
- T6 = COCCIVAC® – B + Aquatize® (1:2000 dilution)
- T7 = COCCIVAC® – B + Bacitracin-MD (55 ppm)
- T8 = Salinomycin (66 ppm) + Aquatize® (1:2000 dilution)
- T9 = Salinomycin (66 ppm) + Bacitracin-MD (55 ppm)

Broiler chicks serving as a negative control (T1) had significantly lower body weights, worsened feed conversion, and the worst lesion scores of all treatments. For the test groups without anticoccidials (T4 & T5) Aquatize® had significantly better live performance and intestinal lesion scores than BA alone. T2 (CVB) and T3 (SAL) warded off the impact of NE equally. The combination of Aquatize® with CVB (T6) or Aquatize® with SAL (T8) yielded the best lesion scores and also exhibited good mortality scores and performance measures of weight and feed conversion.

Table 3: Weight, Mortality, Feed Conversion and Lesion Scores in NE Model

<u>Group</u>	<u>Body Weight, gr</u>		<u>F/G</u>	<u>Mortality (%)</u>	<u>Lesion Score</u>
	<u>Initial</u>	<u>Final</u>			
T1	35.49 ^a	626.7 ^e	1.455 ^e	22.50 ^b	1.608 ^e
T2	35.14 ^a	673.0 ^{cd}	1.369 ^c	12.50 ^{ab}	1.090 ^{cd}
T3	35.04 ^a	693.3 ^{bc}	1.372 ^c	10.00 ^{ab}	1.031 ^{bcd}
T4	35.07 ^a	651.5 ^{de}	1.395 ^d	17.50 ^{ab}	0.942 ^{bc}
T5	35.10 ^a	649.2 ^{de}	1.410 ^d	17.50 ^{ab}	1.256 ^d
T6	35.27 ^a	721.9 ^{ab}	1.334 ^{ab}	7.50 ^a	0.494 ^a
T7	35.36 ^a	726.5 ^a	1.328 ^{ab}	7.50 ^a	0.794 ^b
T8	35.09 ^a	720.1 ^{ab}	1.346 ^b	5.00 ^a	0.456 ^a
T9	35.38 ^a	730.8 ^a	1.319 ^a	7.50 ^a	0.838 ^{bc}

Different letters next to the number indicates that it is statistically significantly different from a number with a different letter, at $p < 0.05$.

These three experiments indicate that the addition of Aquatize® in the drinking water of young, disease-challenged broiler chicks reduced mortality, reduced intestinal lesions, improved weight gain and improved feed conversion. These observations were made in direct comparison with untreated, disease-challenged birds as well as disease-challenged birds that were treated with the usual antibiotics, vaccines and other chemicals used by industry as weapons against NE in chicks and turkey poults. While we do not suggest that Aquatize® could replace rotational treatments for NE or vaccines for causative agents, it is clear from these studies that Aquatize® is a viable weapon for producers to use in the fight against bacterial and protozoa infection and can be included in the animal producers' arsenal in the absence of antibiotics and without affecting the bottom line. The use of Aquatize® could make such programs more effective.

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