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Preliminary Evaluation of Dietary Natustat™ Versus Histostat® (Nitarsone) for Control of *Histomonas meleagridis* in Broiler Chickens on Infected Litter

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Abstract: Histomoniasis (infectious enterohepatitis) is a disease of broiler chickens, turkeys and game fowl which are reared on litter or exposed to range. It is caused by the protozoan organism *Histomonas meleagridis*. Histomonads are spread in chicken feces, *Heterakis gallinarum* (cecal worm) eggs or earthworms. Symptoms may include: poor feed conversion ratio and body weight, diarrhea with sulfur-colored droppings, cecal and liver lesions, darkening of the facial regions and sometimes death. Morbidity, mortality and culling may reach 20% in chickens. In the U.S.A., nitarsone (4-nitrophenyl-arsonic acid; Histostat®, Alpharma, Inc., Fort Lee, NJ) is used as an aid in prevention of histomoniasis. In this trial, Natustat™ (Alltech, Inc., Nicholasville, KY), a proprietary plant derived product, was used at 1.925 kg/Tonne and compared with nitarsone in the diets of unsexed Hubbard HiY broiler chicks to 42 d of age on histomonad infected litter from broiler breeders. Infected and uninfected control groups were also included. There were 4 pens each containing 150 chicks (unreplicated live performance) and 25 birds per pen were randomly sampled at 28, 35 and 42 d of age for cecal (0-4) and liver lesion (0-3) scoring. The histomonad infected litter group had significantly elevated cecal and liver lesion scores compared to the uninfected control group, indicating the challenge model was effective. At each sampling point, Natustat™ was statistically equivalent to nitarsone for lowering cecal or liver lesion scores and these treatments gave results significantly better than infected control.

Key words: Broiler, histomoniasis, caecal worm, Natustat™, nitarsone

Introduction

Histomoniasis (infectious enterohepatitis) a disease of broiler chickens, turkeys and game fowl which are reared on litter or exposed to range, is caused by the fragile protozoan organism *Histomonas meleagridis* (Skinner, 2000). First symptoms are a decline in feed consumption (loss of appetite), inactivity, drooping wings (drowsiness) and unkempt feathers, followed by a drop in body weight and appearance of diarrhea with sulfur-colored droppings (Renwald, 1970). Other symptoms include poor feed utilization, increased thirst, darkening of the facial regions and sometimes death (Hall *et al.*, 1975). With mild infections, birds show no overt symptoms except that they expel cecal cores. It has become apparent that infected farms may suffer clinical outbreaks year after year (McDougald, 1997).

The disease primarily affects the ceca and is characterized by cecal inflammation, roughness and thickening of the cecal wall and large cheese-like (caseous) cecal cores. Chickens are more resistant to histomoniasis than turkeys and are less likely to experience liver mottling deep into the tissue and head discoloration (Renwald, 1970; McDougald, 1997). Lesions of uncomplicated histomoniasis are confined to the ceca and liver, thus the reason for the synonymous term, enterohepatitis. The ceca are ballooned and walls may be thickened, necrotic and ulcerated. Caseous cores within the ceca may be blood tinged. In addition, peritonitis may be present if ulcers have perforated the ceca walls. Livers are swollen and display circular depressed areas of necrosis. Smaller lesions may coalesce to form larger ones. The lesions are yellowish to yellow-green and extend deeply into the underlying liver tissue (Mississippi State University). The virulence of *Histomonas* infections varies greatly in chickens. Some strains of the parasite produce mild to severe lesions in the ceca whereas other strains produce moderate to severe lesions in both the ceca and liver. Extra mortality occurs in some outbreaks and not in others (McDougald, 1997).

Histomoniasis may occur in association with roundworm and cecal worm (nematode) parasites, enteritis, coccidiosis or combinations of these, often making the individual diseases difficult to distinguish. Occasionally, acute attacks may cause cecal bleeding similar to that in cecal coccidiosis (Renwald, 1970) and histomoniasis may be misdiagnosed as cecal coccidiosis (McDougald and Casey, 1982).

The histomonad is passed in the fecal material of infected birds. In many instances, the organism is shed within the eggs of the cecal worm, *H. gallinarum* of chickens, turkeys and game birds. Free-living

histomonads do not survive for long in nature but those in cecal worm eggs may survive for several years. Consequently, most histomonad transmission is considered to be due to ingesting infected cecal worm eggs. Transmission may also occur by the earthworm. Chickens are frequently infected without showing signs of the disease. These chickens may shed enormous numbers of protozoan organisms, many of which are protected by cecal worm eggs.

Nitarsone (4-nitrophenylarsonic acid; Histostat®, Alpharma, Inc., Fort Lee, NJ) is for use in the feed of chickens and turkeys at 0.1875kg/Tonne as an aid in prevention of histomoniasis. Nitarsone may be used in combination with bacitracin methylene disalicylate or zinc bacitracin (Skinner, 2000). Cases of drug resistant *H. meleagridis* have not been reported in the literature and there is no evidence that birds develop a protective immunity against *H. meleagridis*, even following recovery from an active illness, so immunization against the organism is not an option, according to McDougald (1997). Histomonads are extremely difficult to eliminate from farms with a history of the disease. As such, preventive medication is the best way to control the disease.

Natustat™ is a proprietary product included in this study to determine if it is effective against the protozoan *H. meleagridis*. This study was designed to compare *H. meleagridis* exposed broilers fed Natustat™ (1.925kg/Tonne) to identically challenged broilers fed Histostat (0.1875kg/Tonne) and non-supplemented feed. A non-challenged non-supplemented group was also included.

Materials and Methods

Birds, treatments and diets: A total of 600 day-old unsexed Hubbard HiY broiler chicks were used in this study with 150 chicks placed in each of four pens. Each pen was 3.05m x 3.66 m and 0.86 m high. Four 22.7 kg capacity tube feeders and four Plasson Mark IV waterers were placed in each pen. The pens contained approximately 5 cm depth of new wood shavings topped with 2.5 cm of *H. meleagridis* infected litter from a broiler breeder flock, except in the uninfected control group which had 7.5 cm of new wood shavings. Heat was provided by propane heaters and negative pressure air exchange was used for ventilation.

The 4 treatment groups included: one non-challenged, non-supplemented control (CON); and three histomonad infected treatments, non-supplemented control (CON+h); nitarsone supplemented at 0.1875kg/Tonne (NIT+h) and Natustat™ supplemented at 1.925 kg/Tonne (NAT+h).

The nutritionally balanced corn-soy starter and grower basal diets (Table 1) were prepared in mash form by a commercial mill. The broiler starter diet was fed from 0 to 21 d and the grower diet from 21 to 42 d of age. No coccidiostat or antibiotic growth promoter was used.

This study was conducted in compliance with the United States Department of Agriculture (USDA) Animal Welfare Act.

***Histomonas meleagridis* exposure system:** Prior to the study, used litter was collected from a local broiler breeder flock and was confirmed to contain cecal worms infected with histomonads. This litter was used as the infected top dressed litter for inoculating the broiler chicks in designated groups from the time of placement.

Disease verification: After d 7, all broiler chickens were observed daily for clinical signs of infection from *H. meleagridis*, such as lethargy, abnormal fecal discharge and death. On d 28, 35, and 42, a random sample of 25 chickens per pen were caught, euthanized and necropsied. Their ceca and livers were scored as follows:

Cecal scores:

0 Normal - no lesions observed;
1 Slight thickening of cecal wall(s);
2 Moderately thickened cecal wall(s) or slight/moderate cecal core;
3 Moderate thickening of cecal wall(s) plus cecal cores; and
4 Ceca(s) grossly enlarged due to presence of large cecal core.

Liver scores:

0 Normal - no lesions observed;
1 Slight mottling;
2 Moderate mottling;
3 Concentrated areas of necrosis.

In addition, all deaths were presented for necropsy. The ceca and liver of each broiler chicken that died after challenge were scored using the same systems as described for sampled birds.

Statistical analysis: As there was only one pen per treatment, live performance data were obtained but no statistical analysis was possible. The live performance response variables, BW, FCR, mortality-adjusted FCR were recorded at d 28, 35 and 42. Cecal lesion scores and liver lesion scores from 25 birds per treatment at 28-, 35- and 42-d of age were statistically evaluated by one-way Analysis of Variance (ANOVA). Arc sine transformed data were used for cecal lesion scores and liver lesion scores. The level of probability for significance has been indicated for each mean in results (Table 2 and 3). When ANOVA was significant at $P<0.05$, means were separated by Tukey's test (Statistix 8, 2003).

Table 1: Experimental basal starter (0-21 d) and grower (21-42 d) diets for broiler chickens

	Starter Diet	Grower Diet
Yellow Corn 7.5%	54.07	59.68
Soybean meal (47.5)	37.16	32.06
Animal-vegetable fat	4.06	4.00
Dicalcium phosphate	1.64	1.44
Limestone	1.96	1.81
Salt	0.30	0.25
Choline chloride (60%)	0.08	0.05
Vitamin premix[A]	0.04	0.05
Trace mineral premix[A]	0.07	0.04
Tri-basic copper chloride (58% Cu)	0.03	0.03
L-Lycine Hcl	-	0.03
DL-Methionine	0.21	0.21
Selenium premix	0.05	0.05
Calculated analysis		
Crude protein %	22.00	20.00
Crude fat %	6.48	6.56
Crude fibre %	2.80	2.73
Poultry ME kcal/kg (kcal/lb)	3086 (1400)	3142 (1425)
Calcium %	1.10	1.00
Available P %	0.47	0.42
Lysine %	1.28	1.15
Methionine %	0.57	0.54
Met + Cys %	0.94	0.88
Added Se ppm	0.30	0.30

[A]vitamin and trace mineral premixes were provided by a commercial turkey company and met or exceeded National Research Council (1994) requirements. No coccidiostat or antibiotic growth promoter was used.

Results and Discussion

Live Performance: The results indicated that the challenge model using infected broiler breeder litter was effective at lowering BW and increasing FCR and mortality in the CON+h group compared to the CON group.

On all weighing days, the average BW for the NAT+h group was higher than those in the CON+h group (Table 2). This indicates that supplementation with Natustat™ alleviates the decrease in body weight associated with *H. meleagridis* infection. Furthermore, FCR was improved in the NAT+h group compared to the CON+h group. Previous reports have indicated that a loss of appetite and a reduction in body weight are among the first symptoms observed in histomoniasis (Renwald, 1970). Additionally, poor feed utilization has been reported as a symptom of histomoniasis (Hall *et al.*, 1975).

Total mortality was 3.33% for NAT+h compared to 8.00, 16.67, and 26.67% in the CON, CON+h, and NIT+h groups. On necropsy it was observed that some birds had enteritis type lesions. Enteritis-like mortality was lowest in the NAT+h group (0.67%), followed by 4.00%, 5.33%, in the NIT+h and CON groups respectively, with the infected, non-supplemented CON+h group having 16.67%.

Cecal lesion scores: As shown in Table 3, cecal lesion scores in the present study were significantly higher ($P < 0.001$) for the CON+h broiler chickens at 28, 35, and 42 d of age compared to the CON chickens, indicating that the histomonad infected litter caused an outbreak of histomoniasis. At 28 and 35 d, the NIT+h and NAT+h chickens had cecal lesion scores that were statistically equivalent and lower than CON+h birds. At 42 d, the NAT+h birds had significantly lower caecal lesion scores (0.44) than the NIT+h (0.96) birds. These results indicate that Natustat™ can play an important protective role regarding the suppression of caecal lesions in chickens challenged with a *H. meleagridis* infection.

Liver lesion scores: Liver mottling deep into tissue is associated with moderate to severe histomoniasis (Salsbury Laboratories, Inc., 1982). Although liver lesion scores in broiler chickens in the present trial were relatively low (0 to 1.16 range), significant treatment differences were detected (Table 3). At 28, 35, and 42 d, broiler chickens in the CON+h group had significantly higher ($P < 0.001$) liver lesion scores (0.76, 1.16, and 0.84, respectively) than the CON group (0 at each sampling). The NIT+h treatment (0, 0, and 0.04) and the NAT+h treatment (0.04, 0.24, and 0) results were statistically equivalent. This result indicates that for broiler chicken liver protection during histomoniasis,

Table 2: Body weight, feed conversion ratio, mortality-adjusted feed conversion ratio, total mortality, and "enteritis-like" mortality of unsexed Hubbard HiY broiler chickens at 28, 35, and 42 d of age as influenced by *Histomonas meleagridis* infection and dietary Natustat™ or Histostat® (nitarsone) - 1 pen/treatment; no statistical analysis

	Body Weight (kg)	Feed/Body wt.	Adj. Feed/Body wt.	Total Mortality (%)
Age: 28 dy				
CON	0.871	1.622	1.622	0
CON +h	0.768	1.884	1.824	6.00
NIT +h	0.786	2.150	1.750	24.67
NAT + h	0.870	1.662	1.649	1.33
Age: 35 day				
CON	1.362	1.854	1.817	3.33
CON +h	1.183	2.101	1.904	16.00
NIT +h	1.334	2.066	1.733	26.67
NAT + h	1.221	1.906	1.868	3.33
Age: 42 day				
CON	1.709	2.547	2.347	8.00
CON +h	1.612	2.697	2.434	16.67
NIT +h	1.860	2.717	2.279	26.67
NAT + h	1.683	2.125	2.086	3.33

[1]Treatment abbreviations are: CON = non-challenged, non-supplemented control; and 3 histomonad infected treatments, CON+h = non-supplemented control; NIT+h (nitarsone; 4-nitrophenylarsonic acid; Histostat®, Alpharma, Inc., Fort Lee, NJ) at 0.1875kg/Tonne; and NAT+h = Natustat™ (Alltech, Inc., Nicholasville, KY) at 1.925 kg/Tonne.

[2]There were 150 broiler chicks per pen initially; 25 were removed at 28 d (~125 remained), 35 d (~100 remained), and at 42 d (~75 remained) for necropsy and scoring

Table 3: Cecal lesion scores and liver lesion scores of unsexed Hubbard HiY broiler chickens (25 sampled/pen each time) at 28, 35, and 42 d of age as influenced by *Histomonas meleagridis* infection and dietary Natustat™ or Histostat® (nitarsone)

	Caecal Lesion scores (0-4)			Liver Lesion Scoes (0-3)		
	Day 28	Day 35	Day 42	Day 28	Day 35	Day 42
CON	0.08^{c}	0.08^{c}	0.20^{c}	0^{b}	0^{b}	0^{b}
CON+h	1.84^{a}	1.56^{a}	1.56^{a}	0.76^{a}	1.16^{a}	0.84^{a}
NIT+h	1.04^{b}	0.64^{c}	0.96^{b}	0^{b}	0	0.04^{b}
NAT+h	0.76^{b}	0.64^{c}	0.44^{c}	0.04^{b}	0.24^{c}	0^{b}
SEM	0.09	0.08	0.09	0.04	0.07	0.05
P value[2]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Treatment abbreviations are: CON = non-challenged, non-supplemented control; and 3 histomonad infected treatments, CON+h = non-supplemented control; NIT+h (nitarsone; 4-nitrophenylarsonic acid; Histostat®, Alpharma, Inc., Fort Lee, NJ) at 0.1875kg/Tonne and NAT+h = Natustat™ (Alltech, Inc., Nicholasville, KY) at 1.925 kg/Tonne.

[2]Arc sine transformed data used in the statistical analysis.

the Natustat™ and nitarsone (Histostat®) performed similarly.

Conclusions: This study was designed to compare *H. meleagridis* challenged broilers fed Natustat™ (1.925kg/Tonne) to identically challenged broilers fed nitarsone (0.1875kg/Tonne) and non-supplemented broilers (CON+h) and non-challenged, non-supplemented (CON) broilers in a 6-wk floor-pen study. It was noted that d-42 average live weights were heaviest for the NAT+h group followed by the NIT+h group. At d-42 FCR was best in the NAT+h group. Mortality was lowest in the CON and NAT+h groups at each day observed. Cecal lesions were lowest at d 28, 35 and 42 in the CON group while highest on those same days in the CON+h group indicating that histomonad organisms were infecting the ceca of challenged broilers. On d-42 the NIT+h group had a significantly higher ($P < 0.05$) cecal lesion score value than the NAT+h group (Table 3). Among the treated groups, there were no differences ($P>0.05$) in d-28 and d-42 liver lesion scores. On d 35 the NIT+h group had no liver lesions observed (score = 0) making it different ($P<0.05$) from those of the NAT+h group. Data from this study suggest that Natustat™ supplementation allows *H. meleagridis* challenged birds to perform similar to non-challenged non-supplemented birds and better than challenged non-supplemented birds. Cecal lesion scores were generally lower for Natustat™ supplemented birds compared to nitarsone

supplemented birds, while liver lesions were generally lower for birds in the NIT+h group.

References

Hall, C.F., R.R. Bell, T.B. Blount, W.O. Cawley, S.E. Glass, J.E. Grimes, L.C. Grumbles and S.A. Naqi, 1975. A Manual of Poultry Diseases (B-1031). Texas A & M University, College Station, p: 46.

McDougald, L.R., 1997. Blackhead disease (histomoniasis) in chickens. Poultry Digest 56: 8, 10-11.

McDougald, L.R. and J.M. Casey, 1982. Blackhead disease in chickens. Poultry Tips (Feb.), Cooperative Extension Service, University of Georgia, Athens.

National Research Council (NRC), 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, D. C.

Renwald, R.T., 1970. Gland-O-Lac Poultry Health Book. Omaha, Nebraska, pp: 8, 27.

Salsbury Laboratories, Inc., 1982. Salsbury Manual of Poultry Diseases, 7th Ed. Charles City, Iowa, pp: 7-9.

Skinner, J.T., 2000. Effects of nitarsone as an aid in prevention of blackhead in turkeys challenged with *Histomonas meleagridis* carried by nematodes. Pages 97-99. in: Proc. Arkansas Poult. Symp., Springdale.

Statistix 8, 2003. User's Manual. Analytical Software, Tallahassee, Florida.