Efficacy of a Nutrient Synergy Against Colibacillosis in Poultry

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ABSTRACT

The objective of this study was to evaluate the efficacy of a Nutrient Synergy (NS; Blend of nine nutrients) in maintaining the performance and alleviating the pathologic effects in broilers challenged with a high and a low dose-*E. coli*, following a primary challenge with H9N2-avian influenza virus. Six groups of broilers were included (19 birds/group). Each bird in groups 1-4 received at an age of 20 d. a primary intratracheal challenge of 2 HA units of H9N2 virus. At the age of 23 days, birds of groups 1 and 3 received a high dose-*E. coli* challenge in the right thoracic air sac (1.5x10 9 cfu/0.5ml/bird), while birds of groups 2 and 4 received a low dose-*E. coli* challenge in the same route (1.5 x 106 cfu/ml). The initiation of a NS-daily administration, intraesophageally, was according to the manufacturer instructions (Epican Forte®) (976mg/Kg of body weight). The treatment was restricted to birds in groups 3, 4, and 5, effective the age of 17 days and until 28th day of age. Birds of group 6 were unchallenged and untreated.

However, the average weight and feed conversion at 28 days of age was significantly improved ($p < 0.05$) in the NS-treated group compared to NS-deprived group, with similar low dose-*E. coli* challenge. The frequency of ocular exudates-sign and diarrhea at 2 days post the *E. coli* challenge dropped significantly ($p < 0.05$) in the NS-treated groups in comparison to deprived birds, with a similar dose of *E. coli* challenge. The frequency of diarrhea was kept low at 5d. post-challenge, with the high dose of *E. coli* in birds treated with NS ($P<0.05$). The frequencies of the right and left thoracic airsacculitis, and the frequency of pancreatitis were reduced significantly in NS-treated birds with low-dose *E. coli* in comparison to similarly challenged birds, deprived of NS ($p < 0.05$). However in the high-dose *E. coli* challenge groups, the NS treatment lowered only the frequency of abdominal airsacculitis ($P<0.05$).

INTRODUCTION

Due to immense reported failures from around the world in treatment against drug-resistant *E. coli* infections in poultry, a critical need in research emerged targeting the development of materials to be administered in poultry to help the host (bird) to maintain homeostasis and standard performance under the burden of significant infections by primary and secondary *E. coli* infections.[1,2] The Nutrient Synergy developed by Dr. Rath Institute in Santa Clara, USA (Epican Forte®) had nine molecules, including amino acids, vitamins, minerals, and a molecule purified from green tea .[3,4] The evaluation of the efficacy of such a developed material against colibacillosis required standardization of a successful *E. coli* challenge following primary infections, such as those caused by low pathogenic avian influenza viruses,[5] or other primary viruses .[6,7]

The purpose of this work was to evaluate the efficacy of a developed material known as Epican forte® in broilers against a standardized high and low dose-*E. coli* challenge following a primary infection by H9N2 virus.

MATERIALS AND METHODS

Birds

One hundred and fourteen day-old broilers were divided into six groups (19 birds/group), with no significant difference in mean weight among the groups ($p>0.05$). All birds were reared on the floor in separate

Table 1. Mortality percentage in each group at the end of the experiment (28 days of age)

Group*	*E. coli* challenge	NS treatment	Mortality %
1	High	-	52.63^{c}
2	Low	-	10.52^{b}
3	High	+	63.16^{c}
4	Low	+	10.52^{b}
5	-	+	0.0^{a}
6	-	-	0.0^{a}

* Groups were challenged intrathoracically with 0.5 ml of an *E. coli* suspension in the right airsac at 3 days following an intratracheal challenge with 0.5 ml of a 2HA units of H9N2 Avian Influenza Virus given at 20 days of age. The *E. coli* suspension given to groups 1 and 3 had a transmittance of 3%, corresponding to a dose of 2.9x109 CFU/ml. Groups 2, and 4 were given 10-3dilution of the *E. coli* suspension given to groups 1 and 3. Group 6 was the control, left unchallenged. Group 5 remained unchallenged and received a daily oral administration, of 976mg/Kg body weight of Nutrient Synergy (NS), along with groups 2 and 4, starting at 17 days of age.

a,b,c Percentages in a column with different alphabetical superscripts are significantly different ($P<0.05$).

Table 2. Average weight and feed conversion ratio of each group at 28 d. of age

Group*	*E. coli* challenge	NS treatment	Average weight (g)**	Feed Conversion Ratio
1	High	-	765.0^{a}	3.46^{a,b}
2	Low	-	925.0^{b}	2.44^{a}
3	High	+	752.1^{a}	3.84^{b}
4	Low	+	1082.3^{c}	2.36^{a}
5	-	+	1049.5^{b,c}	2.38^{a}
6	-	-	1035.5^{b,c}	2.40^{a}

* Groups were challenged intrathoracically with 0.5 ml of an *E. coli* suspension in the right air sac at 3 days following an intratracheal challenge with 0.5 ml of a 2HA units of H9N2 Avian Influenza Virus given at 20 days of age. The *E. coli* suspension given to groups 1 and 3 had a transmittance of 3%, corresponding to a dose of 2.9x109 CFU/ml. Groups 2, and 4 were given the 10-3dilution of the *E. coli* suspension given to groups 1 and 3. Group 6 was the control, left unchallenged. Group 5 remained unchallenged and received a daily oral administration, of 976mg/Kg body weight of Nutrient Synergy (NS), along with groups 2 and 4, starting at 17 days of age.

** Average weight of the remaining living birds in each group.

a-c Averages in a column with different alphabetical superscripts are significantly different ($P<0.05$).

groups.

H9N2 Challenge

The primary virus (H9N2-Avian Influenza Virus) was administered intratracheally to each 20 d-old bird in groups 1-4. The challenge per bird was equivalent to 2HA units/0.5 ml of H9N2 virus.

High and Low Dose-*E. coli* Challenge

Two types of *E. coli* challenges were used namely, a high dose *E. coli* challenge (1.5 x 109 cfu/0.5 ml/bird) and a low dose *E. coli* challenge (1.5 x 106 cfu/0.5ml/bird) as described previously.[5] Both challenges were administered in the right thoracic airsacs, and allocated as follows: the high dose-*E. coli* challenge was administered to each bird in groups 1 and 3, and the low dose-*E. coli* challenge was administered to each bird in groups 2 and 4. Birds in groups 5 and 6 remained unchallenged with H9N2 and *E. coli*.

Nutrient Synergy Treatment

The Nutrient Synergy (NS) treatment was initiated on daily basis, between the ages of 17-28 days and at 976 mg/kg body weight. The NS was administered intra-esophageally in 1 ml volume/ bird. The NS-treated groups were 3, 4 and 5.

Clinical Signs, Mortality, Weights and Feed Conversion

The frequency of clinical signs in each of the 6 groups was recorded at an age of 25 days (2 d. post *E. coli* challenge), and 28 days (5 d. post *E. coli* challenge) including: ocular exudates, conjunctivitis, rales, diarrhea, huddling, nasal discharge, and thick oral saliva. The cumulative mortality percent up to the age of 28 d was recorded; the weight of each bird was recorded at 16 and 28 d of age. The weight gain and the feed consumption during this period were recorded. Accordingly, the feed conversion to weight reflected the period between 16-28 days of age. It is worth noting that the NS administration to groups 3, 4, and 5 started at the age of 17 d.

Gross Lesions

The frequency of each of nine gross lesions in birds of the 6 groups is recorded at the sacrifice day (28 d of age). The gross lesions included: tracheitis, right thoracic airsacculitis, left thoracic airsacculitis, abdominal airsacculitis, splenomegaly, pericarditis, perihepatitis, enteritis and pancreatitis.

Statistics

The One Way ANOVA followed by Tukey's

test were used for weight and feed conversion comparison among the six groups. The Chi-square Test was used for the comparison of frequencies of each sign, lesion, and percent mortality among the groups (α=0.05). Both tests were performed using a statistical computing software (SPSS 15.0, SPSS Inc., Chicago, USA).

RESULTS

Results of the experiment are shown in Tables 1-5. Similar percent mortality ($p > 0.05$) occurred in the NS-treated birds versus those that were deprived of treatment in the high dose challenged birds (63.11% vs. 52.6%, respectively) and in the low dose challenged ones (10.52% vs. 10.52%; Table 1). No mortality was seen in group 5, a reflection of the safety of the NS. In addition, the controls in group 6, deprived of NS-treatment and challenge, had a 100% survival.

The average weight at 28 days of age was significantly improved ($p < 0.05$) in the NS-treated group 4 (1,082.3g) compared to the NS-deprived group 2 (925.0 g), with similar low *E. coli* dose challenge (Table 2). This improvement in weight of group 4 treated with NS was associated with improvement in feed conversion to live weight (ratio of 2.36) in comparison to the NS-deprived group 2 (Feed conversion ratio of 2.44) (Table 2)

Birds in all groups had an absence of morbidity signs, at the initiation of the NS treatment (17 d. of age); however, at 2 days post the E. coli challenge (25 d of age), the frequency of the sign of ocular exudates was significantly lower ($p < 0.05$) in the NS-treated groups 3 and 4 in comparison to the frequency in NS-deprived birds (groups 1 and 2) and in both, the high and low *E. coli*-dose challenged birds (Table 3). In addition, at 25 and 28 d. of age, the diarrhea frequency was reduced significantly ($P<0.05$) in group 3 (NS-treated and challenged with high E. coli) compared to group 1 (NS-deprived and challenged with a dose similar to birds of group 3; Tables 3 and 4)

Three lesions had a significant reduction in frequency due to the NS treatment ($p<0.05$), namely, the two frequencies of the right and left thoracic airsacculitis, and the frequency of pancreatitis in birds with low dose *E. coli* challenge (group 4) compared to NS-deprived group 2 (Table 5). A low frequency of the abdominal airsacculitis lesion was observed in group 3, treated with NS in comparison to the frequency of the same lesion in group 1, deprived from NS and challenged with a similar high *E. coli* dose ($p <0.05$).

Table 3. Morbidity signs in surviving birds at 25 days of age (2 days post *E. coli* challenge)

Group*	*E. coli* challenge	NS treatment	Frequency of birds with specific sign / Tested surviving number						
			Ocular exudates	Conjun-ctivitis	Rales	Diarrhea	Huddling	Nasal discharge	Thick oral saliva
1	High	-	8/10^{c}	2/10^{a}	9/10^{b,c}	9/10^{c}	9/10^{b}	0/10^{a}	0/10^{a}
2	Low	-	9/18^{b,c}	2/18^{a}	15/18^{b,c}	13/18^{b,c}	0/18^{a}	0/18^{a}	0/18^{a}
3	High	+	2/10^{a,b}	2/10^{a}	10/10^{c}	4/10^{a}	10/10^{b}	0/10^{a}	0/10^{a}
4	Low	+	0/17^{a}	0/17^{a}	9/17^{b}	8/17^{a,b}	0/17^{a}	0/17^{a}	0/17^{a}
5	-	+	0/19^{a}	0/19^{a}	0/19^{a}	8/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}
6	-	-	0/19^{a}	0/19^{a}	0/19^{a}	6/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}

* Groups were challenged intrathoracically with 0.5 ml of an *E. coli* suspension in the right air sac at 3 days following an intratracheal challenge with 0.5 ml of a 2HA units of H9N2 Avian Influenza Virus given at 20 days of age. The *E. coli* suspension given to groups 1 and 3 had a transmittance of 3%, corresponding to a dose of 2.9x109 CFU/ml. Groups 2, and 4 were given 10-3dilution of the *E. coli* suspension given to groups 1 and 3. Group 6 was the control, left unchallenged. Group 5 remained unchallenged and received a daily oral administration, of 976 mg/Kg body weight of Nutrient Synergy (NS), along with groups 2 and 4, starting at 17 days of age.

a-c Frequencies in a column with different alphabetical superscripts are significantly different ($P<0.05$).

Table 5. Frequency of birds (cumulative of dead and surviving) showing lesions through the 5 days post *E. coli* challenge)

Group*	*E. coli* challenge	NS treatment	Frequency of birds with specific lesion / Number tested								
			Tracheitis	Airsacculitis Thoracic right	Airsacculitis Thoracic left	Airsacculitis Abdominal	Spleno-megaly	Peri-carditis	Peri-hepatitis	Enteritis	Pancreatitis
1	High	-	12/19^{c}	14/19^{b}	14/19^{b}	13/19^{c}	1/19^{a}	8/19^{b}	6/19^{b}	14/19^{a}	13/19^{a,b}
2	Low	-	7/19^{b,c}	12/19^{b}	8/19^{b}	4/19^{a,b}	1/19^{a}	2/19^{a}	1/19^{a}	17/19^{a}	18/19^{b}
3	High	+	13/19^{c}	15/19^{b}	9/19^{b}	5/19^{b}	0/19^{a}	7/19^{b}	7/19^{b}	11/19^{a}	9/19^{a}
4	Low	+	3/19^{a,b}	1/19^{a}	0/19^{a}	0/19^{a}	1/19^{a}	1/19^{a}	0/19^{a}	13/19^{a}	9/19^{a}
5	-	+	1/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	14/19^{a}	16/19^{b}
6	-	-	1/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	0/19^{a}	15/19^{a}	16/19^{b}

* Groups were challenged intrathoracically with 0.5 ml of an *E. coli* suspension in the right air sac at 3 days following an intratracheal challenge with 0.5 ml of a 2HA units of H9N2 Avian Influenza Virus given at 20 days of age. The *E. coli* suspension given to groups 1 and 3 had a transmittance of 3%, corresponding to a dose of 2.9x109 CFU/ml. Groups 2, and 4 were given a 10-3dilution of the *E. coli* suspension given to groups 1 and 3. Group 6 was the control, left unchallenged. Group 5 remained unchallenged and received a daily oral administration of 976 mg/Kg body weight of Nutrient Synergy (NS), along with groups 2 and 4, starting at 17 days of age.

a-c Frequencies in a column with different alphabetical superscripts are significantly different (P<0.05*)*

Table 4. Morbidity signs in surviving birds at 28 days of age (5 days post *E. coli* challenge*)*

Group*	*E. coli* challenge	NS treatment	Frequency of birds with specific sign/tested surviving number						
			Ocular exudates	Conjunctivitis	Rales	Diarrhea	Huddling	Nasal discharge	Thick oral saliva
1			0/9^{a}	0/9^{a}	6/9^{b}	7/9^{b}	5/9^{b}	0/9^{a}	0/9^{a}
2	High	-	0/17^{a}	0/17^{a}	12/17^{b}	8/17^{a,b}	1/17^{a}	0/17^{a}	0/17^{a}
3	Low	-	0/7^{a}	0/7^{a}	5/7^{b}	2/7^{a}	3/7^{b}	0/7^{a}	0/7^{a}
4	High	+	0/17^{a}	0/17^{a}	9/17^{b}	6/17^{a,b}	0/17^{a}	0/17^{a}	0/17^{a}
5	Low	+	0/19^{a}	0/19^{a}	0/19^{a}	6/19^{a,b}	0/19^{a}	0/19^{a}	0/19^{a}
6	-	+	1/19^{a}	1/19^{a}	0/19^{a}	9/19^{a,b}	0/19^{a}	0/19^{a}	0/19^{a}

* Groups were challenged intrathoracically with 0.5 ml of an *E. coli* suspension in the right air sac 3 days following an intratracheal challenge with 0.5 ml of a 2HA units of H9N2 Avian Influenza Virus given at 20 days of age. The *E. coli* suspension given to groups 1 and 3 had a transmittance of 3%, corresponding to a dose of 2.9x109 CFU/ml. Groups 2, and 4 were given a 10-3dilution of the *E. coli* suspension given to groups 1 and 3. Group 6 was the control, left unchallenged. Group 5 remained unchallenged and received a daily oral administration of 976mg/Kg body weight of Nutrient Synergy (NS), along with groups 2 and 4, starting at 17 days of age.

a,b Frequencies in a column with different alphabetical superscripts are significantly different (P<0.05).

DISCUSSION

It is apparent that the high dose challenge with *E. coli* following the H9N2 resulted in significant mortalities compared to the low mortality induced by the low dose challenge of *E. coli*. This fact confirms that the level of exposure to *E. coli* in poultry in the presence of a primary infection determines the severity of collibacillosis.8 The interception with NS in birds of groups 3 and 4 did not result in reduction of mortality compared to similarly challenged groups 1 and 2 that were deprived of NS administration (Table 1). This data most likely indicated that the benefits of NS were confined to the survivors from the challenge, as shown in improvement of performance, and in reduction of certain signs and lesions (Tables 2-5). Actually, previous research on benefits of NS on the surviving host challenged with the viruses only was documented.[3,4] Certain components of NS, including the green tea extract, vitamin C, selenium, magnesium, and copper, were proven to improve the regeneration of tissues damaged by infection, the healing of tumors, and even the reduction of viral multiplication.[9,10,11]

The low *E. coli* challenge in group 4 treated with NS helped the survivors to gain a significantly higher weight with better feed conversion, compared to the similarly challenged group 2 that was deprived of NS treatment ($P<0.05$; Table 2). This is an indication to poultry managers that the farms should have a better management of the litter, drinking water, and ventilation to keep the exposure to *E. coli* to a minimum, which enables the NS to have its significant positive effect on performance in weight, and in economic savings due to improvement of feed conversion to body weight. This improvement could be due to the impact of the NS components on maintaining the integrity of the digestive system absorptive cells,[12,13,14] and in providing important elements and vitamins needed for anabolic pathways in the broilers.[15]

It is confirmative from the performance data shown in Tables 1 and 2 that it is preferred to keep broilers free of primary and secondary infections to obtain the lowest mortality, and appropriate weight gain, as shown in groups 5 and 6, a data that is in agreement with other previously documented literature.[16] In addition, it is clear that in the presence of a high exposure to a primary avian influenza of H9N2 subtype, the exposure to *E. coli* has to be kept to its lowest level for the NS to have its positive effect on live weight, as shown in group 4, obtaining a mean weight of 1,082.3 g at 28 d. of age, a mean that is even higher than the controls, and resulting in the best feed conversion of 2.36 (Table 2).

The improvement of performance by NS in the birds given a low dose challenge by *E. coli* could be due to the alleviation of certain signs and lesions, such as the significant low frequency in birds of group 4 of the sign of ocular exudates ($P<0.05$; Table 3), and for the three lesions--the right thoracic airsaculitis, the left thoracic airsaculitis, and the pancreatitis (Table 5). The alleviation of the mentioned sign and the three lesions is important in poultry health, since they affect the overall performance of weight gain and feed conversion.[8] By the way, the presence of airsac inflammation will incriminate the carcss in the slaughter house, resulting in its exclusion from the food chain, and in serious economic losses to the poultry industry.[17] It is worth noting that the frequencies of signs of ocular exudates, diarrhea, and the abdominal airsaculitis lesion were significantly lowered ($P<0.05$) by the NS, even in the high dose challenge by *E. coli*, compared to a similarly challenged group deprived of the NS (Tables 3, 4, and 5). Such alleviations by NS of important signs and lesions, even in the birds challenged with high dose of *E. coli*, is of significance in this new approach of treating against collibacilosis.

In conclusion, the new approach of using a NS in broilers to reduce the injurious effects of H9N2/*E. coli* challenges is of paramount importance, since it is targeting to improve the resistance in the host to this ailment, and not the elimination of the infection by chemotherapy. The significant positive effect of NS in improvement of performance and health were mostly apparent in birds infected with H9N2 and a low dose of *E. coli* challenge. The administration of NS to broilers exposed to in the field LPAI-H9N2 avian influenza, while keeping the exposure to *E. coli* to a minimum, is expected to improve their production. This last suggested claim will be investigated in the near future

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