

Evaluation of Essential Oils in the Treatment of Broilers Co-infected with Multiple Respiratory Etiologic Agents

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ABSTRACT

This study evaluated the use of Mentofin,[®] a natural Eucalyptus/Peppermint essential oil-based preparation, in treatment of broilers with multiple viral and bacterial infections in their respiratory system, a condition that is prevalent in poultry industry of most parts of the world. Eighty day-old broiler chicks were divided into four treatments (two replicate pens/treatment with 10 birds/pen). Birds of the four treatments were all vaccinated

with live Infectious Bursal Disease Virus (IBDV) (intra-esophageally) and with live Newcastle disease virus (intra-ocularly), at 10 and 18 days of age. Each bird in treatments 1 and 2 was challenged intra-tracheally at 7 days of age, with 1.6x 10⁴cfu / 50µl / bird of viable *Mycoplasma gallisepticum* organism.

The birds in treatments 1 and 2 were each challenged intratracheally at 28 days of age, with 0.5 ml of a mixture containing H9N2-avian influenza virus (2 HA units / bird) and intermediate Infectious Bronchitis virus (IBV) (10x log 3.6 EID₅₀ / bird), and another intrathoracic challenge with a 0.1

ml mixture of H9N2 (2 HA units / bird) and IBV (10 x log 3.6 EID50 / bird).

Mentofin treatment was given to birds of treatments 2 (challenged) and 3 (un-challenged) in 250 ml Mentofin / liter of drinking water at 14-16 days of age (starting treatment at 7 d post the MG challenged and 4 d post the first vaccination, and ending treatment at 2d before the second vaccination). The Mentofin treatment was repeated in birds of groups 2 and 3 in the same concentration of drinking water, at 30-32 d of age (starting treatment at 2 days post IB/H9N2 challenge). The 4th treatment group was the control, deprived of all challenges and Mentofin treatment.

The Mentofin treatment reduced the mortality in challenged and unchallenged birds, improved the feed conversion in unchallenged birds, reduced specific signs and lesions, homogenized the creatinine level in challenged birds, as indicated by the low Coefficient of Variation, reduced the immune reaction to the H9N2 challenge and to IBDV intermediate strain, and boosted the vaccine immunity to the NDV vaccine.

INTRODUCTION

The broilers in the developing countries suffer usually of coinfections, resulting in significant economic losses due to high morbidity or mortality, and in costly higher feed conversion.^{1,2} The absence of the implementation of National Poultry Improvement Plans (NPIP) in many developing countries³ prevents the eradication of important economic infections such as that caused by *Mycoplasma gallisepticum* (MG). The presence of this disease with another predominant respiratory viruses, namely Infectious Bronchitis Virus (IBV) and Avian Influenza Virus (H9N2), on a wide scale in the Middle Eastern countries, and many other developing countries,^{4,9} predisposes the broiler flocks to magnification of the pathological effects induced by MG. This results in a serious respiratory disease complex caused by the interaction among MG, IBV, live vaccine viruses, and H9N2-avian influenza.^{1,2,10}

The treatment by antimicrobial agents

in the developed countries is faced by many restrictions due to contamination of carcasses by these medications and the potential of drug resistance in bacteria.¹¹⁻¹³ The search for holistic approaches using natural herbal medicines has expanded in the last 5 years^{10,14-16} due to their safety.^{17,18} In addition, the synergism in the many active ingredients that are present in their essential oils prevents the emergence of bacterial resistance to their antimicrobial activity.^{17,19,20}

The purpose of this study is to evaluate the natural essential oils present in Mentofin® in treatment of live vaccinated-broilers, co-infected with MG, IBV, and H9N2. The evaluation will include the broiler performance (mortality, feed conversion ratio, body weight), morbidity signs and lesions frequency, selected serum biochemical profile, immune responses, and immune reactions to challenge and vaccines.

MATERIALS AND METHODS

Birds and Vaccines

Eighty day-old broiler chicks were divided into four treatments (two replicate pens/treatment with 10 birds/pen). Birds of the four treatments were all vaccinated with live Infectious Bursal Disease Virus (IBDV) (intra-esophageally) (Cevac®IBD L, Ceva, Hungary), and with live Newcastle disease virus (intra-ocularly) (Cevac®New L, Ceva, Hungary), at 10 and 18 days of age.

Challenges

Each bird in treatments 1 and 2 was challenged intratracheally at 7 days of age with live *Mycoplasma gallisepticum* (MG) equivalent to 1.6x 10⁴cfu / 50µl/ bird.

The birds in treatments 1 and 2 were each challenged intratracheally again at 28 days of age with 0.5 ml of a mixture containing the H9N2 avian influenza virus (2 HA units / bird) and intermediate Infectious Bronchitis virus (IBV) (10x log 3.6 EID50 / bird), and with another challenge delivered intrathoracically, between the last two ribs, in 0.1 ml/ bird containing a 0.1 ml mixture of H9N2 (2 HA units / bird) and IBV (10 x log 3.6 EID50 / bird).

Treatment with Mentofin

Mentofin treatment was given to birds of treatments 2 (challenged) and 3 (unchallenged) in 250 ml Mentofin / liter of drinking water at 14-16 days of age (starting treatment at 7 d post the MG challenge and 4 d post the first vaccination, and ending treatment at 2d before the second vaccination). This Mentofin treatment was repeated in birds of treatments 2 and 3 in the same concentration of drinking water, at 30-32 d. of age (starting treatment at 2 days post IB/H9N2 challenge). The 4th treatment group was the control, deprived of all challenges and Mentofin treatment.

Sampling and Analysis

Blood was drawn from individual birds at 18, 28, and 42 days of age for studying the immunity to NDV (HI test),²¹ IBDV (ELISA) (IDEXX Laboratories, One IDEXX Drive, Maine 04092, USA), and H9N2 (HI test)²¹ and for specific serum biochemical profile studies, using the VetTest Chemistry Analyzer (IDEXX Laboratories). The live body weight and feed conversion were de-

termined at 18, 28, and 43 days of age. Six morbidity signs were recorded for each bird at 10 different ages (7, 14, 21, 28, 29, 30, 31, 32, 35, and 43 days of age). The cumulative mortality, seven gross lesions in each bird, and Bursal Weight Index (BWI) were examined at the market age of 43 days. The Bursal Weight Index (BWI) was obtained by dividing the weight of Bursa over the live body weight of the bird and multiplying by 100.

RESULTS AND DISCUSSION

The two Mentofin treated groups 2 and 3 (treated-challenged, and treated-unchallenged) had the lowest mortality of 5.0 and 0.0%, respectively, compared to 15.0% in the untreated-challenged group and 10.0% in the untreated-unchallenged controls. The BWI and mean body weight at market age were similar in all treatments (Table 1).

There was a consistent improvement of feed conversion ratio (FCR) in Mentofin treated-unchallenged birds (group 3) at 18, 28, and 43 days, resulting in the lowest cumulative FCR of 1.70 compared to

Table 1. Mortality %, Bursal Weight Index (BWI), and Mean Body Weight at market age of 43 d. old birds

Group*	Mortality%	BWI	Mean Body Weight (kg)
1	15.0	0.050	2.4
2	5.0	0.046	2.1
3	0.0	0.044	2.5
4	10.0	0.054	2.5

*Group 1 = untreated-challenged, Group 2 = treated-challenged, Group 3 = treated-unchallenged, Group 4 = untreated-unchallenged; challenges = at 7 days of age (MG), and at 28 days of age (H9N2 and IBV); treatment with Mentofin between 14-16, and 30-32 days of age.

Table 2. Feed Conversion Ratios at 18, 29, and 43 days of age, among differently treated birds

Group*	FCR at different ages		
	18 days	28 days	43 days
1	1.29	1.75	1.94
2	1.35	1.79	1.99
3	1.16	1.60	1.70
4	1.26	1.61	1.81

*Group 1 = untreated-challenged, Group 2 = treated-challenged, Group 3 = treated-unchallenged, Group 4 = untreated-unchallenged; challenges = at 7 days of age (MG), and at 28 days of age (H9N2 and IBV); treatment with Mentofin between 14-16, and 30-32 days of age.

Table 3. Percentage of birds showing specific morbidity signs at 32 days of age (4 days post H9N2/IBV challenge)

Group*	Percentage of birds with specific signs					
	rales	SIOS**	conjunctivitis	Ocular exudates	Nasal discharge	diarrhea
1	100.00 ^a	0.00	5.00	55.00 ^a	0.00	38.75
2	85.00 ^a	0.00	0.00	15.00 ^b	0.00	35.00
3	5.00 ^b	0.00	0.00	0.00 ^b	0.00	15.00
4	5.00 ^b	0.00	0.00	0.00 ^b	0.00	12.50

*Group 1 = untreated-challenged, Group 2 = treated-challenged, Group 3 = treated-unchallenged, Group 4 = untreated-unchallenged; challenges = at 7 days of age (MG), and at 28 days of age (H9N2 and IBV); treatment with Mentofin between 14-16, and 30-32 days of age.

**SIOS = Swelling of infraorbital sinuses

a-b Percentages in a column with different alphabetic superscripts are significantly different ($P < 0.05$).

Table 4. Percentage of birds showing specific morbidity signs at 35 days of age (7 days post H9N2/IBV challenge)

Group*	Percentage of birds with specific signs					
	rales	SIOS**	conjunctivitis	Ocular exudates	Nasal discharge	diarrhea
1	100.00 ^a	0.00	5.00	55.00 ^a	0.00	38.75
2	85.00 ^a	0.00	0.00	15.00 ^b	0.00	35.00
3	5.00 ^b	0.00	0.00	0.00 ^b	0.00	15.00
4	5.00 ^b	0.00	0.00	0.00 ^b	0.00	12.50

*Group 1 = untreated-challenged, Group 2 = treated-challenged, Group 3 = treated-unchallenged, Group 4 = untreated-unchallenged; challenges = at 7 days of age (MG), and at 28 days of age (H9N2 and IBV); treatment with Mentofin between 14-16, and 30-32 days of age.

**SIOS = Swelling of infraorbital sinuses

a-b Percentages in a column with different alphabetic superscripts are significantly different ($P < 0.05$).

untreated-unchallenged controls (1.81) at 43 days of age (Table 2).

This apparent improvement in production (mortality and FCR) induced by Mentofin treatment was associated with wide reduction in rales and ocular exudates frequency at 4 and 7 days post H9N2/IB challenge, while the reduction in ocular exudates frequency in the Mentofin treated-challenged birds extended up to market age (15 days post H9N2/IBV challenge) (Tables 3-5). It is documented in literature that the main active ingredients of Eucalyptus and peppermint help in clearing the air passages from mucous,^{10,22,23} which was confirmed in the lower rales-frequency in this work. Such clearance of air passages helps in more efficiency-passage of air, and higher

exchange of oxygen. This is most likely the reason behind the better production performance of the treated broilers.

Moreover, the production improvement was also associated with reduction of frequency of abdominal airsacculitis and pericarditis lesions in Mentofin treated-challenged vs untreated-challenged birds (Table 6). This observation could be due to the role of essential oils in reducing the infectivity of the complex of agents, thus lowering the development of secondary infections by *E. coli* that is responsible for development of lesions in the airsacs and pericardium.^{24,25} Both Mentofin treated group 2 (Mentofin treated-challenged) and group 3 (Mentofin treated-unchallenged) had the lowest frequency of hydropericardium condition compared to

group 1 (untreated-challenged) and group 4 (untreated-unchallenged) (Table 6). The role of essential oil active ingredients in lowering the frequency of hydropericardium condition in broilers will be the subject of future investigation.

In comparing the two challenged groups 1 and 2 at the market age of 43 days old, the Mentofin reduced the Coefficient of Variation (CV)% in serum creatinine level of group 2-birds to 19% compared to 29% in untreated birds of group 1 (Table 7). This reduction in CV reflects the homogeneity in the kidney health of the multiply challenged birds that were treated with Mentofin (group 2) compared to similarly challenged birds that were deprived of the treatment (group 1). This lowering of the CV, reflecting homogeneity in kidney function of treatment 2 compared to treatment 1, could be the reason behind having lower hydropericardium frequency due to better water balance obtained by healthier kidneys.²⁶⁻²⁸ It is worth noting that the mean level of creatinine in all groups were within the normal range for chicken.²⁹

The serum aspartate aminotransferase showed higher allowable levels at 42 days of age in the Mentofin treated-unchallenged birds of group 3 (486.0 U/L), compared to untreated-unchallenged control group 4 (367.2 U/L), and in group 2 (Mentofin treated-challenged) (313.2 U/L) compared to group 1 (untreated-challenged) (286.7 U/L)

(Table 8), a pattern reflecting higher liver activity in Mentofin treated birds compared to deprived ones. This is most likely due to the 1,8-cineole major active component of Mentofin that has been documented in its protection of liver failure against endotoxemic shocks.³⁰ This protection of the liver by Mentofin treatment could have helped in reducing the mortalities in challenged broilers, as shown in Table 1.

Regarding immune reactions to challenge, the Mentofin reduced the immune reactions at 43 days of age to H9N2 in birds of group 2 (treated-unchallenged) (titer = 820.7) compared to group 1-birds (untreated-challenged) (titer = 1133.7) (Table 9), and the immune reaction to IBDV intermediate strain (Table 10), while improving the response to NDV vaccination in Mentofin treated-challenged birds (titers in group 2 vs group 1 birds were 1008.2 vs 829.7, respectively) and improved it also in Mentofin treated-unchallenged birds (titer of 513.8) compared to group 4 (untreated-unchallenged) (titer of 186.7) (Table 11).

This reduction in immune reactions to injurious H9N2 and intermediate strain of IBDV by Mentofin treatment is favorable, to avoid immune injuries that affect directly the performance of these birds.²⁴⁻³¹

CONCLUSION

The Mentofin treatment reduced the mortality in challenged and unchallenged birds,

Table 5. Percentage of birds showing specific morbidity signs at 43 days of age (15 days post H9N2/IBV challenge)

Group*	Percentage of birds with specific signs					
	rales	SIOS**	conjunctivitis	Ocular exudates	Nasal discharge	diarrhea
1	63.35 ^a	0.00	65.95 ^a	35.40 ^a	0.00	29.86
2	64.60 ^a	0.00	41.65 ^a	0.00 ^b	0.00	21.11
3	5.00 ^b	0.00	5.00 ^b	0.00 ^b	0.00	30.00
4	0.00 ^b	0.00	0.00 ^b	0.00 ^b	0.00	31.25

*Group 1 = untreated-challenged, Group 2 = treated-challenged, Group 3 = treated-unchallenged, Group 4 = untreated-unchallenged; challenges = at 7 days of age (MG), and at 28 days of age (H9N2 and IBV); treatment with Mentofin between 14-16, and 30-32 days of age.

**SIOS = Swelling of infraorbital sinuses

a-b Percentages in a column with different alphabetic superscripts are significantly different (P<0.05).

improved the feed conversion in unchallenged birds, reduced specific signs and lesions, homogenized the creatinine level, as indicated by the low Coefficient of Variation in challenged birds, and improved the liver function (serum aspartate aminotransferase) in both challenged and unchallenged birds, while reducing the immune reaction to the H9N2 challenge and to IBDV intermediate strain, and boosting the vaccine immunity to NDV.

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