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ORIGINAL ARTICLES

Evaluation of an Essential Oil in Treatment of Immunosuppressed-coinfected Broilers

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ABSTRACT

The purpose of this study is to evaluate the essential oil of *Eucalyptus spp.* and peppermint in treatment of immunosuppressed broilers, challenged with *Mycoplasma gallisepticum* (MG) at 5 days of age, live B1 and LaSota NewCastle disease vaccine strains at 10 and 16 d. of age respectively, Infectious Bursal Disease Virus (IBDV) at 21 days of age, and with Infectious Bronchitis Virus (IBV) at 26 days of age. Fifty day-old broilers chicks were divided into 5 groups (10 chicks/group). Groups 1 and 2 were replicates of each other, challenged with MG (5 days of age), IBDV (21 days of age), and IBV (26 days of age), and given B1 and LaSota live vaccine against NDV at 10 and 16 days of age. The essential oil treatment in groups 1 and 2 was in drinking water at 0.25 ml/liter of water and for the periods of 12 – 14, 18 – 20, 23 – 25, and 27 – 29 days of age, almost 2 days after selected challenges with a live disease-causing virus or a vaccine virus. Group 3 was challenged and vaccinated with similar strains as groups 1 and 2, but deprived of the essential oil treatment. Group 4 was deprived of all challenges and vaccinated with B1 and LaSota at the same time of vaccination of groups 1 - 3, but administered the essential oil with a similar time-treatment pattern followed in groups 1 and 2. Group 5 was deprived of challenges and essential oil treatment, but vaccinated similarly with B1 and LaSota. Results showed that the essential oil treatment was able to reduce the morbidity and rales, just 2 days post the IBV challenge. This treatment had a consistent reduction in the frequency of rales at 2, 3 and 4 days post the IBV challenge. In addition, the treatment in challenged groups 1 and 2 was also able to reduce the frequency of five out of eight observed lesions, namely the abdominal airsacculitis, enteritis, splenomegaly, bursal congestion and liver enlargement. Concerning the mean Body Weight Gain, there was no statistical differences between the 5 experimental groups ($p<0.05$). A clear improvement in Feed Conversion occurred in challenged groups 1 (3.12) and 2 (3.02), treated with the essential oil, in comparison to the similarly challenged group 3 (3.69) that was deprived of the treatment ($p<0.05$). Another improvement by the essential oil was in the consistent higher magnitude of acquired humoral responses to both NDV and IBDV in the multiply challenged groups 1 and 2, in comparison to the similarly challenged group 3 that was deprived of the treatment.

Key words: Broilers; Coinfected; Essential oil; Immunosuppressed

Introduction

The broilers in the developing countries, suffer usually of coinfections resulting in significant economic losses due to high morbidity or mortality and due to higher feed conversion (Nili and Asasi, 2002; Ley, 2003). The absence of the implementation of National Poultry Improvement Plans (NPIP) in many developing countries (Emsley, 2006) prevents the eradication of important economic infections such as that caused by *Mycoplasma gallisepticum* (MG). The presence of this disease and another predominant respiratory virus (Infectious Bronchitis Virus - IBV) on a wide scale in the Middle Eastern countries and many other developing countries (Barbour *et al*., 1997; Nili and Asasi,

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2002) predisposes the broiler flocks to magnification of the pathological effects induced by immunosuppressive agents, such as the Infectious Bursal Disease Virus (IBDV), resulting in a serious respiratory disease complex caused by the interaction among MG, IBDV, IBV, and even live vaccine viruses (Ley 2003).

The IBDV-immunosuppressed broilers, infected with MG are proven to suffer significantly from immune reactions even from live vaccine strains, such as the B1 and the LaSota strains (Kleven, 2003; Snyder *et al*., 1986). Most of the field cases in broilers of the developing countries suffer of co-infections with immunosuppressive IBDV, associated with respiratory conditions involving MG and IBV, in addition to significant reactions induced by live NewCastle Disease Virus (NDV) vaccines (Kleven, 2003).

The purpose of this study is to evaluate the natural essential oils present in Mentofin® in its treatment of IBDV-immunosuppressed broilers, vaccinated with B1 and LaSota live strains, and administered a sequence of controlled challenges of MG and IBV.

Material and Methods

Birds and Treatments

Fifty day-old broiler chicks were vaccinated intra-occularly with B1 (10 days of age) and LaSota (16 days of age) live NDV vaccine strains. The 50 birds were divided into 5 groups (10 chicks/group). Birds in groups 1 and 2 were replicates of each other, challenged intratracheally at 5 days of age, each with 2 HA units/0.1 ml of MG/bird, corresponding to 2.7 x 10^5 CFU/bird. In addition, each bird in the groups 1 and 2 was administered intra-esophageally an intermediate strain of IBDV at 21 days of age, and at 40.0 $\log_{10}$ $TCID_{50}$/1ml saline/bird, while the IBV (Massachusetts strain) was administered at 26 days of age, both intra-tracheally and in the right thoracic air sac routes, with 30.0 $\log_{10}EID_{50}$/0.1 ml/route/bird. Both groups 1 and 2 were treated with Mentofin® in the water at 0.25 ml/liter of drinking water at 4 intervals namely, 12 - 14, 18-20, 23 - 25, and 27 - 29 days of age, almost 2 days after each challenge with a live virus. Group 3 was challenged and vaccinated in a similar pattern as groups 1 and 2, but deprived of the Mentofin® treatment. Group 4 was vaccinated similarly, deprived of the challenges, but treated with Mentofin® and at the same time intervals as groups 1 and 2. Group 5 was vaccinated similarly, but deprived of the challenges and the Mentofin® treatment. A summary of the design and treatments of this study is presented in Table 1.

All birds received the same grower ration with the following percentage of the major components namely, Protein (20%), Fat (5.45%), Fiber (2.7%) and a ME value of 3125 Kcal/Kg. The rest of the components in the diet have their concentrations adjusted according to the NRC (1994) values.

Signs and Lesions

Two signs were observed and recorded in each bird of the 5 groups and at the following days of age, 28 (2 days post IB challenge), 29 (3 days post IB challenge), and 30 (4 days post IB challenge, i.e., the day of sacrifice for recording lesions). The 2 signs were: morbidity and rales. Eight gross lesions were recorded at 30 days of age namely, tracheitis, right thoracic airsaculitis, left thoracic airsaculitis, abdominal airsaculitis, enteritis, splenomegaly, bursal congestion, and enlarged liver.

Weight Gain, Bursal Index and Feed Conversion

The weight gain during the period between the ages of 5 until 30 days of age was calculated for the five groups by subtracting the weight of birds in each group at the age of 30 days from the weight at 5 days. The Bursal Index was calculated for each bird by dividing the bursal weight over the live body weight X 100, and the mean was obtained from the sum of Bursal indices of all birds within a group divided by their numbers. The Feed Conversion was calculated by the division of the weight of the consumed feed by the live weight of the birds in each group.

Quantification of humoral responses to NDV and IBDV

The indirect ELISA (IDEXX®, Maine, USA) was used to quantify the humoral responses in sera of each bird to NDV and IBDV at 29 days of age. The optical densities read by the ELISA reader (BIO-TEK, Vermont, USA) were interfaced to the computer program supplied by IDEXX®; xCheck Assay Management System v. 3.3, for computation of the titers from the optical densities of color resulting from the enzymatic assays.

Statistics

The SPSS v.15 (Statistical Package for the Social Sciences, Chicago, USA) computing statistical program was used to analyze the data obtained for this randomized complete design. The comparison of the means was done by one-way ANOVA followed by Tukey's test for mean separation, while the frequencies or percentages of birds showing a specific assessed parameter were compared among the different treatments by the CHI-square method. Significant differences in the means and frequencies are reported at a $p = 0.05$.

Results and discussions

The birds did not have any signs of morbidity at 5 days of age, an observation that reflected the proper health status of the experimental birds that will be subjected later to different treatments. At 26 days of age, no morbidity was observed in any bird of the 5 groups in spite of the MG and IBDV challenges, and of previous vaccination with B1 and LaSota live strains.

At 2 days post challenge with IBV (28 days of age)(Fig. 1), the morbidity was the highest in birds of group 3, challenged with IBV and deprived of the Mentofin® treatment. Group 3 had also the highest frequency of rales (7/10)(70%). It is confirmed in literature that IBV challenge will show early signs of morbidity and rales especially when the challenge is intra-tracheal and/or intra-thoracic (Cavanagh and Naqi, 2003).

This pattern of the highest frequency in the rale sign was maintained in group 3 at 3 days post the IBV challenge (Fig. 2). This is also in agreement with previous works showing that the sign of rales due to IBV-intrathoracic challenge in presence of immunosuppression and MG infection will persist for 10 days (Adler *et al*., 1962; Pejkovski *et al*., 1979).

The consistency in the highest frequency of rales in IBV-challenged group 3, deprived of Mentofin®, was also maintained at 4 days post the IBV challenge (Fig. 3). The reduction in the frequency of rales following the IBV challenge, due to the treatment by essential oils of Mentofin ® in groups 1 and 2 in comparison to the high frequency of rales in the similarly challenged group 3 that is deprived of Mentofin®, is most likely due to the synergistic effects of the compounds present in the essential oils of Mentofin®, that were proven before to alleviate the pathologic effects of MG (Barbour *et al*., 2006), and the immune injuries due to live vaccine strains (Barbour *et al.*, 2005). Such alleviations of pathological injuries by Mentofin® could have helped in birds of groups 1 and 2 to have less frequency of signs following another co-infection obtained by a controlled challenge of IBV. A previous reported histopathologic work showed a significant protection of the respiratory system tissues by Mentofin® against a co-infection by MG and other viral infections, in which the essential oils helped in maintaining the cilia and goblet cells of the epithelial layer of the chicken trachea (Barbour *et al*., 2006).

The frequency of gross lesions in the 5 groups is shown in Table 2. The Mentofin® treatment in challenged groups 1 and 2 was able to reduce the frequency of five out of 8 observed lesions in comparison to similarly challenged group 3, deprived of Mentofin®. The five reduced lesions, in groups 1 and 2, by Mentofin® were: Abdominal airsacculitis, enteritis, splenomegaly, bursal congestion, and enlargement of the liver. Other workers have showed the impact of essential oils of *Eucalyptus spp.* and Peppermint in protection of the respiratory (Cermelli *et al*., 2007; Mckay and Blumberg, 2006), digestive (Bakkali *et al*., 2008; Mckay and Blumberg, 2006), and lymphoid systems (Serafino *et al*., 2008; Samarth, 2007).

Table 1: Nature of treatments showing the challenging three disease-causing agents and the brackted age in days at which each was delivered to the specific groups and the assigned treatment by essential oil of Mentofin®

Groups	Challenges* (Ages)			Mentofin® treatment**
	MG (5 d.)	IBDV (21 d.)	IBV (26 d.)	
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	No
4	No	No	No	Yes
5	No	No	No	No

*challenged groups 1, 2, and 3 received intratracheally a volume of 0.1 ml of 2 HA units of MG challenge, containing 2.7 x 10^5 CFU, an esophageal challenge by 1 ml of 40.0 x $\log_{10}$ $TCID_{50}$ of an IBDV-intermediate strain, and two equal challenges each of 30 $\log_{10}$ EID_{50} IB (Massachusetts strain)/0.1 ml in trachea and in the right airsac, respectively. The five groups were vaccinated with live B1 and Lasota vaccine strains against NewCastle Disease Virus, in the eye, at 10 and 16 days of age respectively.

** The treatment with Mentofin® was in the drinking water at 0.25 ml/liter and at 4 intervals namely, 12-14, 18-20, 23-25, and 27-29 d. of age.

Table 2: Frequency of birds showing specific gross lesions at 30 days of age (4 days post the IBV challenge)

Group	Frequency of birds with specific lesion / Number tested							
		Airsacculitis						
	Tracheitis	Right thoracic	Left thoracic	Abdominal	Enteritis	Splenomegaly	Bursal Congestion	Enlarged Liver
1	$7/9^{ab}$	$5/9^{b}$	5/9	0/9	$1/9^{a}$	0/9	$1/9^{ab}$	$1/9^{a}$
2	$10/10^{b}$	$7/10^{b}$	5/10	1/10	$3/10^{ab}$	1/10	$1/10^{ab}$	$5/10^{b}$
3	$6/10^{a}$	$7/10^{b}$	4/10	2/10	$6/10^{b}$	2/10	$4/10^{b}$	$6/10^{b}$
4	$4/9^{a}$	$1/9^{a}$	2/9	2/9	$2/9^{ab}$	0/9	$1/9^{ab}$	$3/9^{ab}$
5	$6/10^{a}$	$2/10^{a}$	2/10	1/10	$2/10^{ab}$	0/10	$0/10^{a}$	$1/8^{aa,b}$

Frequencies in a column with different alphabetical superscripts are significantly different ($p<0.05$)

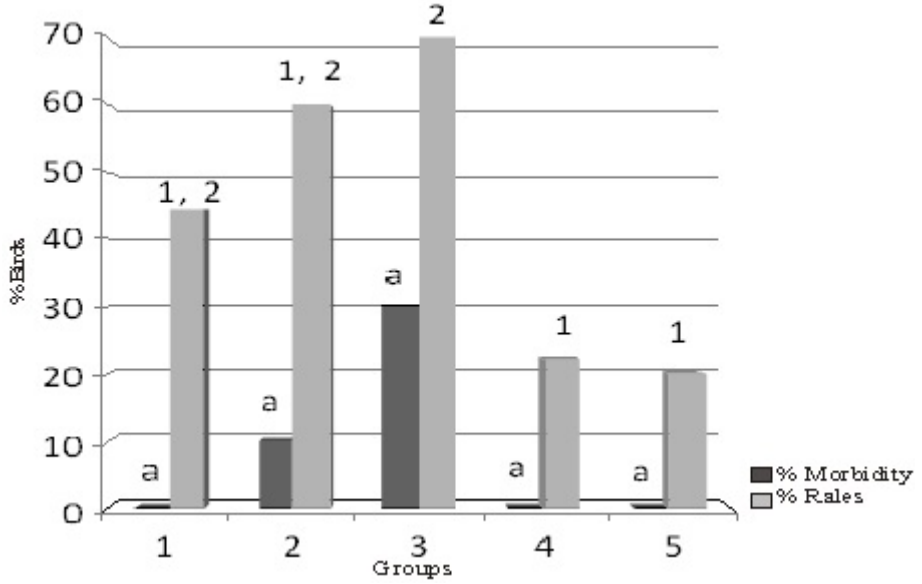

Fig. 1: Percentage of birds in each of the 5 groups showing morbidity and rales at 2 days post the IBV challenge. Groups 1, 2, and 3 were challenged. Groups 1, 2, and 4 were administered Mentofin®. Group 5 is the control-unchallenged and non-treated with Mentofin®. Percentages with different alphabets and Arabic numericals shown at the top of histogram, for morbidity and rales respectively, are significantly different at $p<0.05$.

The production performance of the 5 groups is shown in Table 3. The mean Body Weight Gain between the age of 5 to 30 days didn't differ significantly ($P>0.05$) among the 5 groups. The Bursal Weight Index was within the normal range in groups 4 and 5, deprived of IBDV challenge (Chiville, 1967). This is a sign of the success of the IBDV challenge in groups 1, 2, and 3, inducing a certain degree of atrophy in their Bursa in comparison to the unchallenged groups 4, and 5. This atrophy in the Bursa of Fabricius most likely leads to immunosuppression (Lukert and Saif, 2003), which could affect negatively the feed conversion (Sharma *et al*., 2000). Actually the highest Feed Conversion ratio was obtained in the challenged group 3, deprived of Mentofin® (Feed Conversion = 3.69), in comparison to similarly challenged groups 1 and 2 (administered Mentofin®) showing respective mean Feed Conversions of 3.12 and 3.02. It is worth noting that the best low feed conversion ratio was obtained by group 5, deprived of challenges and Mentofin® treatment. It is previously documented that the immunosppression induced by IBDV will induce subclinical infections in broilers resulting in higher feed conversion (Sharma *et al*., 2000). The treatment by Mentofin®, just 2 days post the IBDV challenge, could have reduced the infectivity, replication, and/or immune injuries by this virus in groups 1 and 2, thus improving their feed conversion ratio.

Table 3: Means of body weight gain, Bursal Weight index, and feed conversion

Group	Mean		
	Weight Gain*	Bursal Weight Index**	Feed Conversion***
1	1042.2^{a}	0.149^{a}	3.12^{ab}
2	1055.6^{a}	0.164^{ab}	3.02^{ab}
3	1046.5^{a}	0.199^{ab}	3.69^{b}
4	1022.8^{a}	0.252^{b}	3.18^{ab}
5	1125.6^{a}	0.233^{ab}	2.54^{a}

^{a,b}Means in a column with different alphabetical superscripts are significantly different ($p<0.05$)
* Weight gain between the age of 5 to 30 d.
**Bursal Weight Index is the weight of Bursa of Fabricius divided by the live weight of the bird x 100
*** Feed conversion is the weight of consumed feed divided by the live weight of the bird.

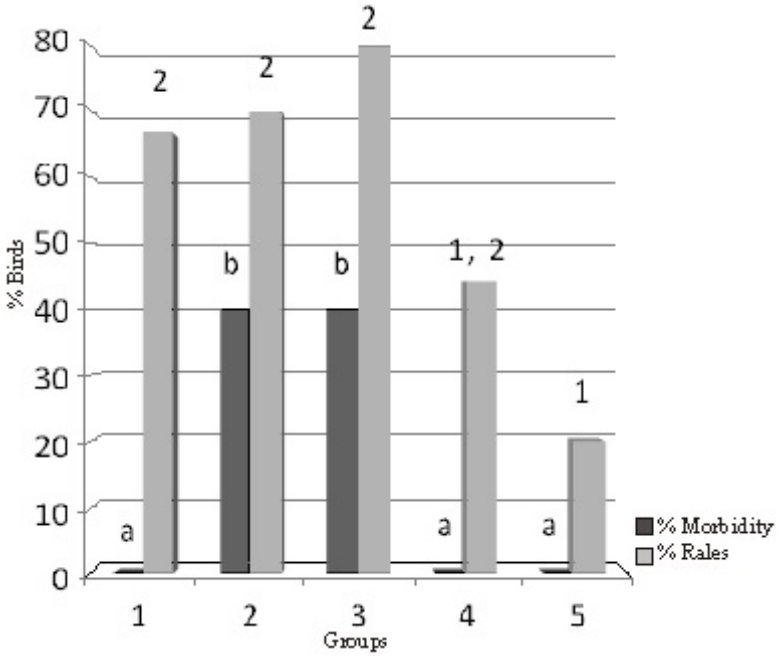

Fig. 2: Percentage of birds in each of the 5 groups showing morbidity and rales at 3 days post the IBV challenge. Groups 1, 2, and 3 were challenged. Groups 1, 2, and 4 were administered Mentofin®. Group 5 is the control-unchallenged and non-treated with Mentofin®. Percentages of a sign with different alphabets and Arabic numericals shown at the top of histogram, for morbidity and rales respectively, are significantly different at $p<0.05$.

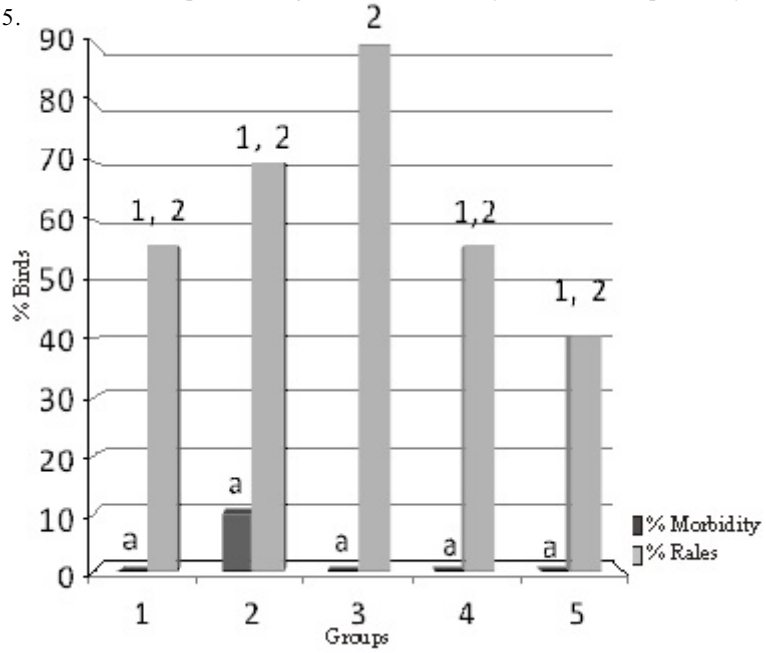

Fig. 3: Percentage of birds in each of the 5 groups showing morbidity and rales at 4 days post the IBV challenge. Groups 1, 2, and 3 were challenged. Groups 1, 2, and 4 were administered Mentofin®. Group 5 is the control-unchallenged and non-treated with Mentofin®. Percentages of a sign with different alphabets and Arabic numericals shown at the top of histogram, for morbidity and rales respectively, are significantly different at $p<0.05$.

The humoral immunity quantified by the indirect ELISA showed higher consistent titers to NDV and IBDV in multiply-challenged groups 1 and 2, treated with Mentofin®, in comparison to that obtained in similarly challenged group 3, deprived of the Mentofin® (Figs. 4 and 5). Previous literature showed the immunomodulation effect of essential oils of Eucalyptus and Peppermint in improving the NDV titers in poultry (Çarli *et al*., 2008).

In spite of the competition to the immune system in birds infected with MG, IBDV, IBV, and the live vaccine strains of NDV, still the Mentofin® helped in improving the humoral responses to NDV and IBDV in groups 1 and 2 in comparison to similarly vaccinated and challenged group 3 deprived of the Mentofin® treatment (Figs. 4, 5). It is worth noting that the absence of competition among different infecting agents in group 5, challenged only with vaccine strains of NDV, resulted in the best NDV-titer of 1294.70 (Fig. 4).

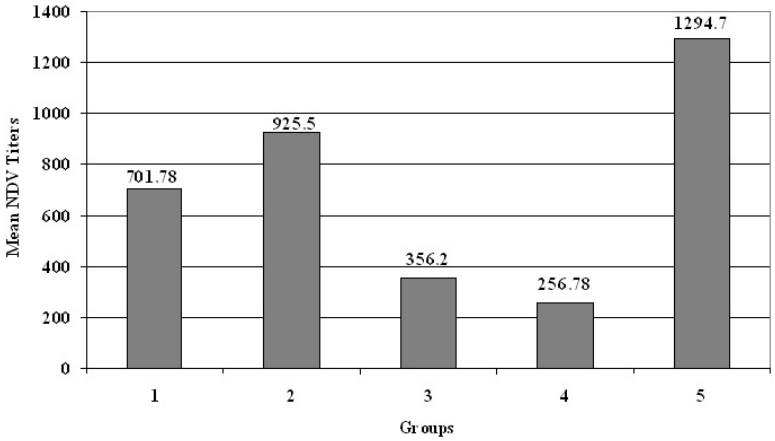

Fig. 4: Mean NDV antibody titers in different experimental groups at 29 days of age. All means did not differ significantly ($p>0.05$).

The lowest negligible IBDV titers were obtained in groups 4 and 5 deprived of IBDV challenge, a sign of high biosecurity in separation of the differently treated groups of birds in this experiment. It is worth noting that no report is yet documented in literature on the impact of essential oils on improvements of immune responses to immunosuppressive viruses, such as the IBDV.

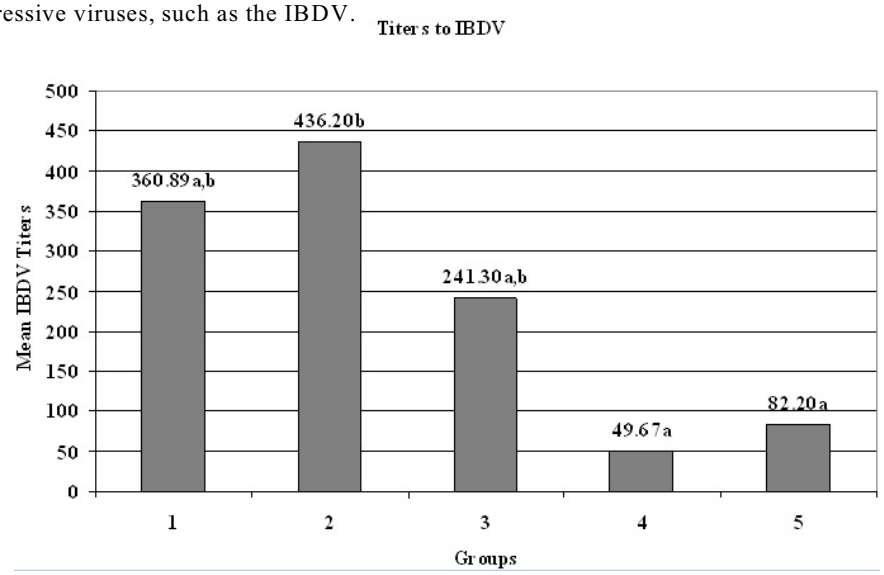

Fig. 5: Mean IBDV antibody titers in different experimental groups at 29 days of age. Mean IBDV titers followed by different alphabetic superscripts shown over the histogram are statistically different ($p<0.05$).

In conclusion, the Mentofin® was able to reduce the morbidity right after the IBV challenge (2 days post IBV challenge), and to have a consistent reduction in frequency of rales at 2, 3, and 4 days post the IBV challenge and to reduce specific lesions at 4 days post the IBV challenge. A clear improvement in Feed Conversion occurred in challenged groups 1 and 2, treated with Mentofin®, in comparison to similarly challenged group 3 that is deprived of Mentofin®. This result is significant in saving on the cost of feed, by highering the efficiency of transformation of feed to body weight. Another improvement by Mentofin® was in the consistent higher magnitude of acquired humoral responses to both NDV and IBDV in the multiply challenged groups 1 and 2, in comparison to similarly challenged group 3, deprived of Mentofin®.

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