

Application of Mentofin® in Broilers with Clinical Infectious Bursal Disease to Reduce *Escherichia coli* Related Problems after Vaccination against Newcastle Disease

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Abstract: In this study Mentofin® was used in commercial broiler chickens naturally exposed to infectious bursal disease virus (IBDV) and administered live Newcastle disease virus (NDV) vaccine to evaluate the ability of Mentofin® to reduce *Escherichia coli*-related respiratory lesions, immunomodulate Newcastle disease (ND) vaccine response, change pharyngeal aerobic bacterial counts, and have certain impacts on specific production parameters. Mentofin® was added to the drinking water (100 ml/500 l water) of broiler chickens aged between 3 and 5 days. ND and IBD vaccinations were administered via drinking water to 15-day-old chickens. NDV vaccination was given again when the chickens were 20 days old. No difference was found between the control and Mentofin® groups regarding oropharyngeal aerobic bacterial counts. Acute IBD was diagnosed in both groups because the antibody levels were unprotective and varied widely. The mean NDV-hemagglutination inhibition antibody titers obtained with NDV vaccination in both groups were above the protective level, and the titers were considered uniform. The data showed that Mentofin® was able to reduce the lesions caused by *E. coli* after NDV vaccination. Mentofin® also reduced the rate of mortality in broilers with IBD interaction to the live NDV. In conclusion, Mentofin® reduced both the occurrence of *E. coli*-related lesions and the mortality rate usually observed after administering live NDV to broilers with clinical IBD.

Key Words: *E. coli*, chicken, IBDV, NDV vaccine, Mentofin

İnfeksiyöz Bursal Hastalıklı Broiler Piliçlerde Newcastle Hastalığına Karşı Aşılama Sonrası *Escherichia coli*'ye Bağlı Problemleri İndirgemek Amacıyla Mentofin® Kullanımı

Özet: Bu çalışmada, saha kaynaklı İnfeksiyöz Bursal Hastalık Virusunu ve canlı NDV aşısına maruz kalan ticari broiler piliçlerde Mentofin® kullanılarak, Mentofinin *Escherichia coli*'ye bağlı solunumla ilgili lezyonları indirgeyebilmesi, ND aşılması bağışıklık yanıtı, faringeal aerobik bakteri miktarı değişimi ve spesifik üretim parametreleri üzerindeki belirli etkileri değerlendirildi. Mentofin® 100 ml / 500 l suya doze edilerek 3-5 yaşındaki günler arası içme suyuna uygulandı. Onbeş günlük yaşındaki civcivlere içme suyu yoluyla ND ve IBD aşılımları yapıldı. ND aşısı 20. günde tekrarlandı. Mentofin® ve kontrol grupları arasında orofaringeal aerobik bakteri miktarı bakımından farklılık bulunamadı. Antikor yanıtındaki geniş ölçekli değişkenlik ve korumasızlıktan dolayı her iki grupta da akut IBD teşhis edildi. NDV aşılama sonrası sağlanan ortalama NDV-Hemagglütinasyon İnhibisyon-antikor titreleri 2 grupta da koruyucu düzeyin üzerindeydi ve titreler uniform bulundu. Veriler Mentofin®'in NDV aşılması sonrası *E. coli*'ye bağlı lezyonları indirgeyebileceğini gösterdi. Mentofin® ayrıca NDV canlı aşısı ile aşılama sonrası IBD'li broiler piliçlerde mortaliteyi azaltıcı etkiye sahiptir. Sonuç olarak Mentofin® klinik IBD ile infekte broiler piliçlerde canlı NDV aşılama sonrası gözlenen *E. coli*'ye bağlı lezyonları ve mortaliteyi indirgeyebilmektedir.

Anahtar Sözcükler: *E. coli*, piliç, IBDV, NDV aşısı, Mentofin

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Introduction

Escherichia coli-related problems in broiler chickens are among the most important causes of economic loss to Turkey's poultry producers. Pathogenic *E. coli* strains are found in the intestinal tract of hatched chicks (1), suggesting rapid spread after hatching. Pathogenic and non-pathogenic *E. coli* can be transferred from feces to the litter and environment. Dust in poultry houses may contain between 10^5 and 10^6 cfu *E. coli* g⁻¹. This bacterium can survive for long time periods in dry field conditions (1).

The most common type of coli septicemia that affects chickens originates in the respiratory tract. *E. coli* gains access to the circulatory system following damage to the respiratory mucosa by infectious or noninfectious agents (1,2). Infectious bronchitis virus (IBV), Newcastle disease virus (NDV), mycoplasmas, and ammonia are the most common predisposing agents for *E. coli* infiltration (3).

Susceptibility to *E. coli* infection could increase due to NDV or IBV vaccination in young chicks, resulting in coli septicemia. Five days after administration of a vaccine strain of NDV, clearance of aerosol-administered *E. coli* was reduced (4). Vaccination or infection with these vaccine viruses close to slaughter can also cause coli septicemia or airsacculitis, resulting in the necessity to cull.

In Turkey very virulent infectious bursal disease virus (IBDV) is widely seen in commercial broilers, because of insufficient biosecurity measures and improper vaccination due to undetected variability in maternal IBDV vaccine antibody titers. Mentofin® is a natural product comprised mainly of essential oils, which has been proven safe for broilers and layers (5-7, unpublished data). In addition, previous experimental and field trials demonstrated the efficacy of this product in preventing respiratory problems, improving performance, and stimulating the immune system when it was used in drinking water (7,8, unpublished data).

The purpose of the present study was to determine if treating broilers with Mentofin® could prevent *E. coli*-related respiratory lesions in the first days of broiler life and after vaccination against ND. In addition, we evaluated the impact of Mentofin® on production, antibody response to ND vaccine, and pharyngeal aerobic bacterial count under field conditions.

Materials and Methods

Chickens

In a broiler production facility, 2 commercial broiler houses, each with a 10,000 bird capacity and the same production conditions, were used. Into each house, 10,000 commercial broiler chicks with a healthy appearance were placed into a 450-m² space.

Mentofin® Application

Mentofin® (EWABO Chemikalien GmbH & Co. KG, Germany) was administered at the dose of 100 ml/500 l of drinking water for 3 consecutive days. The first Mentofin® application via drinking water was started when the chickens were 3 days old and stopped when they were 5 days old. The second application was for 3 consecutive days starting when the chickens were 18 days old.

Vaccination Schedule

Vaccinations were scheduled by the responsible veterinarian in the broiler company. ND (Intervet Clone 30) and IBD (Polymed-Tabic) vaccinations in drinking water were administered to 15-day-old chickens. A second dose of ND vaccine (Intervet Clone 30) was given to 20-day-old chickens.

Bacteriological Examination

In all, 10 oropharyngeal swabs from each of the chickens in both groups were taken at 5, 22, and 28 days of age to compare aerobic bacterial counts. Each swab was put into a tube containing 1 ml of 0.9% NaCl solution (physiological saline solution; PSS) and vortexed in order to suspend the organisms in the diluent. This bacterial suspension was diluted to 10^{-10} . Then, 20 µl of each dilution was spread onto Muller Hinton agar (Oxoid, CM337) with 10% sheep blood. After 24 h of aerobic incubation at 37 °C, colonies were counted on the agar surface and the number of aerobic bacteria in the main suspension was determined.

Serological Tests

In total, 50 blood samples, 25 from the control group and 25 from the Mentofin® group, were collected at each of the following days of age: 1, 14, 28, 35, and 44. Blood was also collected at the age of 1 day to determine the presence of *Mycoplasma gallisepticum* (MG) antibodies by serum plate agglutination test and ELISA, and to determine maternally-acquired antibody titers to

IBDV with a commercial ELISA (Biocheck[®]) and to NDV with the hemagglutination inhibition (HI) test. At days 28 and 44, only ND-HI antibody titers were evaluated in the serum samples, while IBDV-ELISA titers together with ND-HI antibody titers were evaluated at 14 and 35 days of age.

Lesion Scoring

Each dead chicken was necropsied and lesions were scored according to the criteria previously determined and mentioned below. The lesion score index was calculated by dividing the total of lesion scores by the number of dead chickens.

Lesion Scoring Criteria

A score of 0 was given if there were no lesions in any of the organs, i.e. if completely transparent air sacs and pink-colored lungs were seen. If there was congestion in the lungs and the trachea, and slight turbidity in the air sacs, it was scored as 1. In the case of airsacculitis and pneumonia, purulent tracheitis, and systemic lesions in other organs, such as the liver, spleen, etc., a score of 2 was given.

Production Parameters

Body weight gain, feed conversion rate (FCR), feed consumption, water consumption, and daily mortality were recorded. Environmental and house temperatures were recorded daily. Any other unplanned farm practices were also recorded during the study.

Statistical Analysis

The Kruskal Wallis test was used to compare the total aerobic bacterial counts from oropharyngeal swab samples of the control and Mentofin[®] groups; however, the Mann-Whitney U test was used to compare ND-HI antibody titers between the Mentofin[®] and the control groups. Statistical analyses were performed with SPSS software.

Results

Total Aerobic Bacteria in Oropharyngeal Swabs

Total aerobic bacterial counts detected in the oropharyngeal swabs of chickens in the control and Mentofin[®] groups are given in the Table. No statistically significant difference was observed between the control and Mentofin[®] groups.

Table. Aerobic bacterial counts [colony forming units (cfu) swab⁻¹] in the oropharynx of chickens at different ages in the control and Mentofin[®] groups.

Age (days)	Number of Bacteria	
	Control Group	Mentofin [®] Group
5 (ama) ^a	1.7 x 10 ¹⁰	3.7 x 10 ¹⁰
22 (bma) ^b	3.3 x 10 ¹⁰	1.6 x 10 ¹⁰
28 (ama) ^a	2.5 x 10 ¹⁰	2.8 x 10 ¹⁰

^a(ama): after Mentofin[®] application.

^b(bma): before Mentofin[®] application.

Serological Test Findings

None of the sera had antibodies against MG according to the serum plate agglutination test and ELISA.

Considering maternally-derived IBDV-antibodies in 1-day-old chicks, the mean titers in the Mentofin[®] and control groups were 14090 and 12347 with CVs (coefficient of variation) of 29% and 38%, respectively, indicating that both flocks had uniform and sufficient amounts of protective antibody titers against IBDV. These titers were rechecked on 14th day of age and were still at the protective level of 14,317 in control group and 13,365 in the Mentofin[®] group, with acceptable uniformity of CV (control group: 14%; Mentofin[®] group: 20%). At 35 days of age mean ELISA-antibody titers with CVs in the Mentofin[®] and control groups were 1461/64% and 1631/53%, respectively (Figure 1).

During the study ND-HI antibody titers were examined 5 times at different ages. The mean ND-HI antibody titer change over time is shown in Figure 2.

On the first day of the study, chicks in the Mentofin[®] and control groups had protective maternally-derived antibody titers against NDV; however, a significant difference was found between the antibody titers of the Mentofin[®] group and those of the control group ($P < 0.01$). Blood sera were examined for ND antibodies at 14 days of age, which was 3 days before the ND-IBD vaccination, and the mean HI antibody titers in both groups almost reached the minimum protective level. There was no significant difference in antibody titers between the 2 groups. On the ninth day following the second ND vaccination and the first Mentofin[®] application antibody titers in the Mentofin[®] and control groups

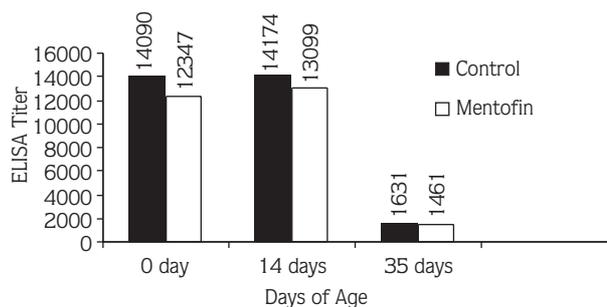


Figure 1. ELISA antibody titers to IBDV in the control and Mentofin® groups at different ages.

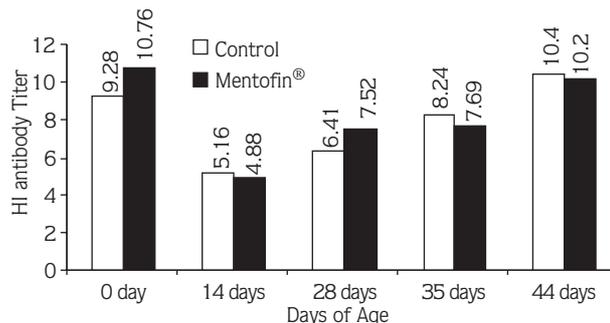


Figure 2. Comparison of the mean ND-HI antibody titers (log₂) in the control and Mentofin® groups at different ages.

increased to the protective level (2 log 7.52 and 2 log 6.41, respectively), associated with uniformity. It was observed that antibody levels in the Mentofin® group were higher than those of the control group ($P < 0.01$). ND antibody titers were monitored at 35 and 44 days of age and a gradual increase was observed in the titers with maintenance of uniformity on these days. At 35 days of age the antibody titers of the chickens in the control group were higher than those in the Mentofin® group ($P < 0.05$), but this difference in antibody levels was not observed at 44 days of age (Figure 2).

Lesion Score

It was found that lesion scores in the Mentofin® group dramatically decreased just after the second ND vaccination at 20 days of age, in comparison to the control group. Lesion score changes during the study are given in Figure 3.

Mortality

During the study mortality rates in the control and Mentofin® groups were 6.60% and 4.50%, respectively. Between 18 and 29 days of age 40 chickens in the Mentofin® group died, while there were 95 deaths in the control group. Details of mortality are given in Figure 4.

Results of Production Parameters

At the end of the study mean body weight in the control group was 1953 g, while it was 1962 g in the Mentofin® group.

FCR in the Mentofin® and control groups was 1.8711 and 1.9471, respectively.

Discussion

One of the interesting findings of our study was the diagnosis of acute IBD in both the Mentofin® group and the control group. This means that there was a previous IBDV contamination in the houses and the broilers in the flocks were infected by the virus when the maternal antibody titer to IBDV decreased below the protective level. Our IBDV-ELISA antibody titer examinations support this hypothesis, as in the first 2 antibody examinations the maternal antibody titers levels were protective and uniform in both groups, and 20 days after IBD vaccination the titers in both flocks were unprotective and multiform, indicating that if IBDV was present in the environment, the flocks would easily have been infected and disease-related lesions would have been observed. The low and multiform antibody titers suggested that vaccination against IBDV in both groups was unsuccessful, although the reasons are unknown; thus, IBDV-related lesions were observed (hemorrhages and severe edema in the bursa of Fabricius). The presence of the virus in the bursa of Fabricius was confirmed by agar gel precipitation test (data not shown). These results confirmed that the study groups were naturally infected with IBDV just after the level of maternally-derived antibodies decreased. Thus, in this study flocks infected with acute IBD showed no signs of immunosuppression.

In order to determine if the addition of Mentofin® to the drinking water of broiler chickens caused any change to the number of aerobic bacteria in the oropharynx, we examined the sampled swabs from this body region on the 5th day after Mentofin® treatment, and before and after Mentofin® treatment on days 22 and 28 of the study. We did not observe any statistically significant difference between the control and the Mentofin®

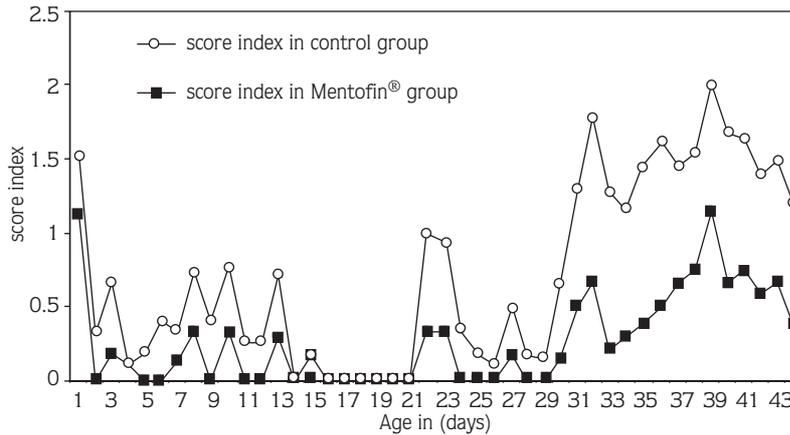


Figure 3. A comparison of lesion score index variations in Control and Mentofin® groups.

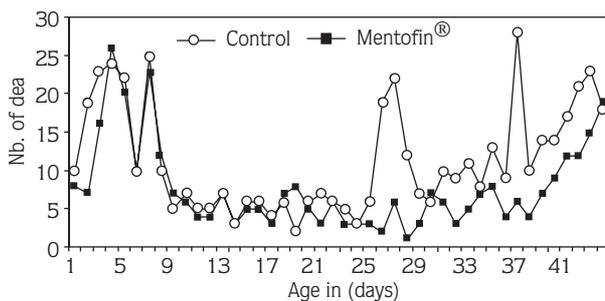


Figure 4. Daily mortality in the Mentofin® and control groups at different ages.

groups, showing that Mentofin® had no in vivo antibacterial affect on the aerobic bacteria colonizing the oropharynx region of the broiler chickens: however, the antimicrobial effect in different body regions, especially in the respiratory system, still remains questionable.

ND HI-antibody titers were routinely examined to evaluate the effect of Mentofin® on humoral immune response of the chickens. Some statistical differences between the groups were observed before and after ND vaccinations at 15 and 20 days of age; however, mean HI-antibody titers in both groups were above the protective level and the titers were considered uniform, which means that the chickens in both groups were properly immunized by live ND vaccine.

The most prominent findings of this study were the decrease in lesion scores and mortality rates in the Mentofin® group, particularly after the second Mentofin® application and ND vaccination. There was a 4-fold

decrease in *E. coli*-related lesions in the Mentofin® group when compared to the control group between the ages of 21 and 24 days (Figure 1). For the duration of the study the control group consistently had higher lesion scores than the Mentofin® group; however, between days 29 and 34 the difference in lesion scores between the groups was markedly greater. These data clearly indicate that Mentofin® was able to prevent the lesions caused by *E. coli* after ND vaccination.

An increase in *E. coli*-related daily mortality was observed in the controls starting at 25 days of age, while there was no mortality increase in the Mentofin® group (Figure 4). The increased level of mortality in the control group persisted until the end of the study. These findings also support the decrease in the lesion scores in the Mentofin® group.

FCR and body weight gain are considered to be sensitive indicators of non-specific body response against any substances used in live animals. In this study, broilers in the Mentofin® group showed an improvement in feed conversion (1.8711) compared to the control birds (1.9471). Also, mean body weight in the Mentofin® group was higher than in the control group. These data clearly indicate that Mentofin® had a positive effect on feed conversion and body weight gain.

The results of the study suggest that Mentofin® could be used to reduce *E. coli*-related lesions and mortality in broilers with acute IBD and ND vaccination reactions, and could have a positive effect on ND-HI antibody response produced just after ND vaccination and on improving the FCR of broilers.

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