

## ANTIMICROBIAL ACTIVITY OF MENTOFIN AND ITS EFFECT ON ANTIBODY RESPONSE OF BROILERS TO NEWCASTLE DISEASE VIRUS VACCINE

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### ABSTRACT

Mentofin is a herbal product containing 10 % eucalyptus oil, 10% menthol, 33% liquid builders and 47% saponins. Its antimicrobial activity *in vitro* against Newcastle Disease (ND) virus and urease producing bacteria, and its effect on antibody response of broilers to Newcastle Disease (ND) virus vaccine were evaluated. Mentofin at 0.5 % concentration inactivated the lentogenic strain of ND virus within 15 minutes at interaction temperature of 37 °C and at 0.01% inactivated *Proteus vulgaris* in nutrient broth while 0.0001% concentration inactivated the same bacteria in urease broth. For evaluating its effect on antibody response of broilers to ND virus vaccine, one hundred day old broiler chicks were divided in to four groups (A, B, C and D) with 25 birds in each. Each bird of group A and B was vaccinated against ND and each bird of group A and C was treated with Mentofin while birds of group D served as negative control. Anti-ND-HI antibody titer of all birds was monitored on 14, 21, 28 and 35 days of age. Mentofin treated broilers showed higher consistent antibody titer as compared to untreated broilers. These birds when given challenge infection on 35 days of age showed same protection as that of untreated vaccinated birds. However, un-vaccinated and Mentofin treated broilers showed higher protection than those of non-treated unvaccinated. Mentofin did not show any effect on weight gain and feed conversion ratio of the treated broilers. Moreover, droppings from Mentofin treated birds showed no urease producing bacteria while 100 percent droppings of the Mentofin untreated birds showed urease producing bacteria.

**Key words:** Mentofin, anti-NDV HI antibody titer, FCR, Urease producing bacteria, Ammonia.

### INTRODUCTION

Poultry sector is one of the important segments of agriculture industry of Pakistan. Broiler meat contributes 23.8 percent of the total meat production in the country (Economic Survey of Pakistan 2009-2010). The broilers suffer from co-infections resulting in significant economic losses due to high morbidity / mortality and higher feed conversion ratio (FCR) (Nili and Asasi, 2002; Ley, 2003). Immunity failure in chickens occurs quite often because of biological or chemicals induced immune suppression, variation of avian pathogens and irregular use of vaccines (Xie and Song, 2005). Immuno-suppressed flocks may have high susceptibility to secondary infections and poor feed conversion ratio and response to commonly used vaccines (Sharma *et al.*, 2000). In poultry production, application of immunostimulants is an essential requirement to improve the immunity of broilers. Some herbal products whose mode of action is unknown, are effective immunopotentiating agents. Mentofin is one of such products containing 10 % eucalyptus oil, 10% menthol, 33% liquid builders and 47% saponins. It is used in poultry to reduce *Escherichia coli* (*E. coli*) related lesions, mortality with acute infectious bursal disease

(IBD) and reactions of Newcastle disease (ND) vaccination. It has positive effect on weight gain and improves the FCR of broilers (Carli *et al.*, 2008). It reduces the morbidity and specific lesions after infectious bronchitis virus (IBV) challenge infection (Barbour *et al.*, 2008). Eucalyptus and peppermint oils potentiate both innate-cell mediated and humoral immune responses in chickens. Administrations of these volatile oils have a potent immunomodulatory effect on immune response of birds to vaccines (Awaad *et al.*, 2010). Present study was therefore, designed to investigate the antimicrobial activity of Mentofin and its effect on antibody response of broilers to ND virus vaccine

### MATERIALS AND METHODS

**Source of chicks:** One hundred day old broiler chicks were purchased from Big Bird Hatchery, Raiwind Road, Lahore and were transported to Animal House of Department of Microbiology, Faculty of Veterinary Science (FVS), University of Veterinary and Animal Sciences (UVAS), Lahore.

**Experimental design:** The broiler chicks were divided into 4 groups (A, B, C and D) with 25 birds in each. All groups were kept in separate cages in the same room. All

the birds were given fresh water and feed containing toxin binder (Mycosorb) *ad lib*. All the birds of each group were given measured amount of the feed.

**Vaccination:** On day one, NDV and IBV vaccine (Bivalent) was administered to each bird of group A and B using spray method. On day 10 post first treatment (PFT), oil based inactivated avian influenza virus (H<sub>9</sub>N<sub>2</sub>) vaccine was administered to each bird of all groups (0.3ml/bird: subcut: mid-dorsal side of neck). On day 11 PFT, live attenuated D-78 vaccine against IBD was administered to birds of all groups through drinking water. On day 21 PFT, live attenuated NDV and IBV Vaccine were administered to each bird of group A and B through drinking water.

**Mentofin treatment:** Mentofin (EWABO, Germany) was administered to birds of group A and C in drinking water (0.25ml/litre) at age of 5-7, 15-17, 25-31 and 39-41 days (Barbour *et al.*, 2008).

**Blood collection:** Blood samples (3 ml) from each bird of all groups were collected from wing vein on day 7, 14, 21, 28 and 35 of age using 5 ml disposable sterile syringes. Each of the syringes was properly marked and kept undisturbed at 4 °C for overnight and subsequently at 37 °C for 2 hours. The serum thus separated from each syringe was separated and transferred to properly labeled serum vials. The serum samples thus collected were stored at -40 °C till required for further processing.

**Challenge infection:** Each bird of each experimental group was given challenge infection on 35 days of age using velogenic strain of the ND virus obtained from University Diagnostic Laboratory, UVAS, Lahore (0.2 ml: intranasal: 100 units of EID<sub>50</sub>). Clinical signs / symptoms, morbidity and mortality were recorded for 10 days post challenge infection. The dead birds were opened and postmortem changes were recorded (Alexander, 2003).

**Feed conversion ratio:** Measured amount of feed was given to each group of the birds. Weight gain of birds of each group was recorded at weekly interval i.e., on 7, 14, 21, 28 and 35 days of age. At end of the experiment, FCR at weekly basis was calculated of each group by using following formula;

$$FCR = \text{Feed consumed} / \text{Weight gain}$$

**Detection of urease producing bacteria:** Samples of dropping of birds of each group (5 samples/ group) were collected at 35 days of age and were mixed in normal saline. Then these suspensions were inoculated into tubes containing nutrient and urea base broth (LAB<sup>®</sup>, UK). The tubes were incubated at 37°C for 48 hours.

**Antibody response:** Each serum sample was processed for monitoring anti-NDV-HI antibodies using hemagglutination inhibition (HI) test (Allan *et al.*, 1978).

Vaccine vial of live lentogenic strain of ND virus was purchased and processed for determination of HA titer. Its 4HA was determined for its application in HI test.

**Antimicrobial activity:** *In vitro* antiviral activity of Mentofin against NDV was tested at different concentrations, time and temperature (Barbour *et al.*, 2010). *In vitro* antibacterial activity of Mentofin against urease producing bacteria (*Proteus vulgaris*) was determined at different dilutions and time according to the dilution technique (Tilton and Howard, 1987).

**Statistical analysis:** The data regarding HI titer was processed for calculation of geometric mean titer (Swayne *et al.*, 1998) and data about weight gain was analyzed using ANOVA (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

Mentofin treated broilers showed higher consistent antibody (anti-NDV HI antibody titer) response as compared to untreated broilers (Table 1). Essential oils of Eucalyptus and Peppermint in the Mentofin preparation might have enhanced the anti-NDV-HI antibodies in broilers (Awaad *et al.*, 2010). The herbal product treated birds show increased antibody response to either NDV or IBDV vaccine (Barbour *et al.*, 2008). Similarly, layers treated with Polyimmune show immuno-augmenting effect to NDV vaccine (Shabbir *et al.*, 2008). However, broilers treated with Habek Mint (*Mentha longifolia*) do not show any modulation in antibody response to NDV vaccine (Al-Ankari *et al.*, 2004).

Broilers treated with either Mentofin and vaccinated with NDV or vaccinated only showed 87 % protection. on 35 days age to challenge infection. However, non-vaccinated broilers treated with Mentofin showed higher protection (37%) as compared to that of in non treated unvaccinated birds (25%). Presumably Mentofin might have increased non specific resistance of the birds. However, vaccinated broilers treated with Mentofin show 35% protection, untreated but vaccinated birds 25% and untreated and unvaccinated birds 0% protection to challenge with velogenic strain of ND Virus (Awaad *et al.*, 2010). Biomin<sup>®</sup> IMBO (herbal product) enhanced humoral immune response to live NDV vaccine, but did not decrease post virulent NDV challenge mortality (Mohammadamin and Qubih, 2010).

In *in vitro* studies revealed that , 0.75, 0.50 and 0.25% concentration of Mentofin inactivated the lentogenic strain of ND Virus within 5, 10 and 15 minutes, respectively at interaction temperature of 37 °C. Concentration the Mentofin is inversely proportional to time required to inactivate the virus (Barbour *et al.* 2010). Methanol extract of leaves, hexane extract of leaves as well as the hexane and chloroform extract of seeds of Neem (*Azadirachta indica*) are effective for inhibiting

NDV and IBDV replication in VERO cells and in chicken embryos (Helmy *et al.*, 2007).

Mentofin did not induce any effect on weight gain and in FCR (Table 2). However, in some experimental studies Mentofin treatment showed positive effect on weight gain and FCR of broilers (Barbour *et al.*, 2008). Feeding of Habek Mint (*Mentha longifolia*) causes improvement in the mean body weight, daily average weight gain, feed intake and FCR (Al-Ankari *et al.*, 2004).

All samples of droppings from Mentofin treated birds did not show urease producing bacteria while 100% droppings of the untreated birds showed the growth of same bacteria. Herbal products might be working as antibacterial agent against urease producing bacteria and ultimately mitigating the environmental level of ammonia. However, essential oil blend (CRINA) did not reduce the intestinal numbers of *C. perfringens* (spore forming bacteria) compared to a non-supplemented control group (Abildgaard *et al.*, 2010). It is observed that Mentofin at more than one percent concentration in nutrient broth inactivated the *Proteus vulgaris* while its 0.0001 % inactivated the bacteria in urea broth (Table 3&4). In urea broth alkaline pH might have augmented the antibacterial activity of the Mentofin. Eucalyptus oil has antibacterial activity against non spore forming

bacterial species such as *Haemophilus influenzae*, *H. parainfluenzae*, and *S. maltophilia* and *S. pneumoniae* (Cermelli *et al.*, 2008)

In conclusion Mentofin mitigate ammonia production in poultry sheds and as immuno-stimulant in poultry. This effect may enhance the non specific resistance of the birds against mild respiratory problems

**Table 1: Effect of Mentofin on the antibody response of broilers to Newcastle disease virus vaccine**

Treatment (Group of Broilers) n=25	Geometric mean titer of anti-NDV-HI * antibodies at different age (days) of broilers			
	14	21	28	35
Vaccinated + Mentofin treated (A)	6.1(69)	6.4(84)	7.2 (147)	7.5 (181)
Vaccinated (B)	5.4 (42)	6.1 (64)	6.6 (97)	6.9 (119)
Mentofin treated and non-vaccinated (C)	-	-	-	-
Non Vaccinated + Non Treated (D)	-	-	-	-

Figures in Table shows GMT on the basis of well number of the serum dilution while figures in parenthesis indicate the GMT on basis of dilution data of the serum

**Table 2: Effect of Mentofin on Weight gain and FCR of broilers on weekly basis**

Treatment (Group) n=25	Weight (grams) and FCR of vaccinated broilers at different age (days)				
	7	14	21	28	35
Vaccinated + Mentofin Treated (A)	108.4(1.1)	285.2(1.5)	574.9(1.6)	945.1 (1.7)	1256.1 (2.0)
Vaccinated (B)	117.8(1.0)	309.2(1.4)	595(1.5)	993.0 (1.6)	1267.8 (2.0)
Mentofin Treated (C)	127.2(0.9)	349.2(1.2)	680(1.4)	1096.7(1.5)	1374 (1.8)
Non Vaccinated + Non Treated (D)	121.8(1.0)	349.7(1.2)	676.1(1.4)	1073.0 (1.5)	1350.7 (1.9)

F Figures in parenthesis show FCR values of the respective group of birds and figure outside the parenthesis show the values of weight gain. There is no significant difference in weight gain and FCR values in the same column of respective group of birds.

**Table 3: In Vitro Antibacterial Activity of Mentofin against Proteus vulgaris in Nutrient Broth**

Dilutions of Mentofin	Incubation Time for Interaction of Mentofin with the Urease Producing Bacteria (hours)			
	8	16	24	48
10 <sup>-1</sup>	+	-	-	-
10 <sup>-2</sup>	+	-	-	-
10 <sup>-3</sup>	+	+	+	+
10 <sup>-4</sup>	+	+	+	+

*Proteus vulgaris* was isolated from broiler droppings and was inoculated in each dilution of Mentofin in nutrient broth. Each tube was incubated at 37 °C for 48 hours. Loop full material from each dilution was transferred to urea agar on 8, 16, 24 and 48 hours of incubation. Growth of the bacteria and development of pink color on the urea agar is indication that Mentofin did not inactivate the bacteria.

**Table 4: In Vitro Antibacterial Activity of Mentofin against Proteus vulgaris in Urea Broth**

Dilutions of Mentofin	Incubation Time (hours) for Interaction of Mentofin with the Urease Producing Bacteria			
	8	16	24	48
10 <sup>-1</sup>	+	-	-	-(8.0)
10 <sup>-2</sup>	+	-	-	-(8.0)
10 <sup>-3</sup>	+	-	-	-(9.0)
10 <sup>-4</sup>	+	-	-	-(9.0)
10 <sup>-5</sup>	+	+	-	-(10.0)

*rProteus vulgaris* was isolated from broiler droppings and was inoculated in each dilution of Mentofin in urea broth. Each tube was incubated at 37 °C for 48 hours. Loop full material from each dilution was transferred to urea agar on 8, 16, 24 and 48 hours of incubation. Growth of the bacteria and development of pink color on the urea agar is indication that Mentofin did not inactivate the bacteria. Figures in parenthesis indicate the pH of the medium. Positive sign + indicates that bacteria was remained viable and grew well on urea agar.

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