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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Research Article
Effect of Digestrom® and Poultry Star® on the Body Performance and Immunity Status of Broiler Chickens

Sahar Abdulhasan D.

Department of Public Health, College of Veterinary Medicine, University of Wasit, Kut, Iraq

Abstract

Objective: This study was carried out to evaluate the positive effects of two different commercial products (Digestrom® and Poultry Star®) on body performance and the immune response against Newcastle disease after challenge with a virulent local strain in broiler chickens routinely vaccinated against Newcastle disease. **Materials and Methods:** A total of 250 one-day-old broiler chicks were randomly distributed to five groups (G1-G5) with 50 chicks per group and treated as follows, G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×10^{11} CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×10^{11} CFU kg^{-1}) as feed additives, G4: Positive control (vaccinated but not treated), G5: Negative control (vaccinated and treated). All groups except G5 (negative control) were vaccinated with NDV LaSota and 14-days-old IBDV D78 intermediate at 5, 10 and 20 days of age via drinking water. At the age of 25 days, all groups were challenged with the virulent strain of Newcastle disease at 100 ELD_{50} 10^6. **Results:** The current study showed the highest variable antibody titer (Abs) in the third group that was fed Digestrom® and Poultry Star® with significant increases ($p<0.05$) in serum total protein, albumin and globulin levels, average daily feed intake and average daily gain ($p<0.05$) as well as a significant decrease ($p<0.05$) in morbidity and mortality, followed by the other groups (G1 and G2) that were fed with one product, compared to the positive control group (G4). Meanwhile, the results were significantly lower in the negative control group (G5) than in all other groups. The results following challenge with virulent NDV were very high in G5 and very low in G3. **Conclusion:** Dietary inclusion of 150 ppm Digestrom® improved body performance, which led to increased serum total protein levels. Moreover, both individual and combined application of Digestrom® and Poultry Star® increased the immune response against NDV, with synergistic effects observed with combined treatment.

Key words: Digestrom®, Poultry Star®, Newcastle disease, ELISA, growth performance, poultry diet

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Corresponding Author: Sahar Abdulhasan D., Department of Public Health, College of Veterinary Medicine, University of Wasit, Kut, Iraq

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have been used as feed additives in poultry diets for more than 50 years to enhance growth performance and prevent bacterial diseases. However, the world has turned to antibiotic alternatives as feed additives due to residual antibiotics in animal products and their tendency to increase the resistance of harmful bacteria to antibiotics, essential oils have been used as alternatives to antibiotics as they have shown antimicrobial effects[1]. The role of antimicrobial agents is to change the membrane permeability of pathogenic bacteria to ions such as H+ and K+[2]. Moreover, the essential oils and extracts of herbs and spices improve appetite and digestion in addition to exerting antimicrobial effects[3,4]. Because of bacterial resistance to antibiotics, especially entropathogenic bacteria in newly hatched broiler chicks, new preventive measures have been used to reduce bacterial infection, such as using probiotics as feed additives, which plays a significant role in reducing feed intake and improving body and productive performance[5]. Due to continued restriction on the use of long-term drugs, many countries have used alternative feed additives to prevent digestive problems. Previously, public health has relied on antibacterial growth promoters. Because of new trends in the use of feed additives, the use of antibiotic growth promoters has been reduced. Herbal substances such as Digestrom®, which are known for their positive effects on the intestines, have become the focus of public discussion. Herbal extracts, probiotics and enzymes help improve poultry performance[6,7]. Internal secretions, bile production and body enzymes depend on natural digestive enhancers, which optimize nutrient digestion while producing the lowest amounts of harmful metabolic materials. Guo *et al.*[8] proved that some isolates of probiotics have inhibitory effects on certain *E. coli* strains, such as K88 and K99. Zohair[5] demonstrated that the use of probiotics in poultry diets has a significant role in reducing colonies of *E. coli* and reducing pathological and histological lesions caused by *E. coli* infection. Talebi *et al.*[9] demonstrated the role of probiotics in improving immunity after vaccination against Gamboro and Newcastle disease (ND), where the increase was significant compared to the untreated groups, in addition to improving body performance by increasing body weight and feed conversion. Essential oils and probiotics enhance cellular and humoral responses in broiler chickens, which play important roles in resistance to many bacterial and viral infections[10]. Hashemipour *et al.*[11] reported that dietary supplements with thymol and carvacrol improve the immune response in broiler chickens. However, there is much *in vitro* evidence that demonstrates the efficacy of plant extracts as anti-viral, bacterial and fungal agents, moreover, a few *in vivo* studies have confirmed the positive effects of these alternatives as growth promoters[12]. Therefore, the present study aimed to investigate the positive effects of Digestrom® and Poultry Star® as feed additives on the growth performance and immune status of broiler chickens.

MATERIALS AND METHODS

Commercial products: The commercial blend of phytogenics (CBP: 150 mg kg^{-1}) includes oregano [OEO, 300 ppm] and EO [500 ppm] (3). CBP (Digestrom® Biomin GmbH, Herzogenburg, Austria) contains a blend of oregano, anise and citrus peel essential oils based on SPME-GC analysis and is administered at a dose of 150 mg kg^{-1} of the diet (4). Carvacrol is the active and main compound of the essential oil blend, making up approximately 102 g of chemical components per kg of CBP. OEO is reported to be present at 300 and 500 ppm and is available as a powder called Oregano-Stim (Meriden Animal Health Ltd., Luton, UK), containing 5% essential oils from Origanum vulgare sub sp. In addition, 95% of the essential oil blend consists of Hirtum plants, which is an inert natural feed and carrier[13].

Poultry Star® is an Australian product made from a mixture of beneficial bacterial isolates including lactic acid bacteria as well as a mixture of *Enterococcus* spp., *Bifidobacterium* spp., *Pediococcus* spp. *Lactobacillus* spp. for 2×10^{11} CFU kg^{-1} of probiotics.

Experimental design: Two hundred and sixty broiler chicks in good condition (Rose 308, a Belgian of Origin) were bought from Falcon Hatchery (Baghdad). A total of 10 chicks were slaughtered to estimate the maternal immunity against Newcastle disease virus (NDV). The remaining chicks were then randomly divided into 5 groups (G1 to G5) with 50 chicks in each group: G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×10^{11} CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×10^{11} CFU kg^{-1}) as feed additives, G4: Positive control (vaccinated but not treated), G5: Negative control (vaccinated and treated). All groups except G5 (negative control) were vaccinated with NDV LaSota and 14-days-old IBDV D78 intermediate at 5, 10 and 20 days of age via drinking water. At the age of 25 days, all groups were challenged with the virulent strain of Newcastle disease at 100 ELD_{50} 10^6.

Sample collection: Blood samples were collected from the chicks at different intervals. Five to ten chicks were randomly taken from each group after 12 h of fasting. Then, approximately 2-5 mL of blood was collected by a disposable syringe via the jugular vein. Blood samples were placed in tubes containing gel coagulate. Serum was separated after centrifugation at 1000 ×g for 10 min and then stored at -20°C until analysis.

Enzyme linked immunosorbent assay (ELISA) (Synbiotics-USA): ELISA was performed in accordance with the manufacturer's protocol. The ProFLok® NDV indirect ELISA kit (symbiotics-USA) was used as a quick test for antibodies against Newcastle disease in infected and vaccinated chicks[13].

Measuring total protein levels in blood serum: Total serum protein levels were calculated according to Bayureat (Biuret method) using a commercial kit (RANDOX® mbH, Germany)[14].

Measuring the level of albumin in blood serum: The level of serum albumin was calculated using a bromocresol green method with a commercial kit (TC® mbH, Germany) produced according to the researcher's specifications[14].

Measuring the level of globulin in blood serum: The concentration of globulin was calculated indirectly by the following equation after determining the concentrations of serum total protein and albumin:

$$\text{Globulin concentration (gm dL}^{-1}\text{)} = \left(\text{Total protein concentration-Albumin concentration}\right)$$

Challenge test: Twenty chicks were challenged with virulent local Newcastle isolate at a titer of 100 ELD_{50} 10^6 according to methods proposed by Reed and Muench[15]. Clinical signs (respiratory and neurological) and mortality were recorded daily.

Statistical analysis: A statistical analysis system (SAS) was used to analyze the effect of the factors used in the study on parameters[16]. Extraction of the least significant difference at multiple levels was performed to compare the means in the current study.

RESULTS

Newcastle immunity: To evaluate maternal immunity against Newcastle disease, 10 serum samples were collected from 260 one-day-old birds before dividing the chicks into groups. The results of the ELISA analysis showed a good maternal immune response (4248.9±170.9). The present study was conducted to evaluate the efficacy of Digestrom® and Poultry Star® as feed supplements in improving the immune response against Newcastle disease with appropriate vaccination programs during different periods. Table 1 shows a significant increase ($p<0.05$) in the level of antibodies against Newcastle disease at ages of 7, 14 and 21 days. The third group that was fed Digestrom® and Poultry Star® exhibited the significant increase ($p<0.05$) in antibody titer, followed by the remaining groups (G1, G2 and G4, sequentially), compared to the negative control group (G5), which showed the significant decrease ($p<0.05$) in immune response. At 35 days after challenge with virulent local NDV isolate (100 ELD_{50} 10^6), a significant increase ($p<0.05$) in antibody titer was observed in all groups. The highest increase was observed in the G3 followed by G1, G2 and G4, while G5 showed a significant decrease ($p<0.05$) in antibody titer.

Serum total protein, albumin and globulin concentrations: On day 35 after challenge with a local virulent isolate of NDV, significant increase in serum total protein levels ($p<0.05$) were observed among the groups as shown on Table 2. The third group that was feed both Digestrom® and Poultry Star® showed significantly increased ($p<0.05$) total protein, albumin and globulin levels with a low A/G ratio, followed by other groups (G1, G2 and G4, sequentially), while G5 showed a significant decrease ($p<0.05$) in the concentration of serum proteins.

Effect of dietary supplements on broiler chicken growth performance: On days 0-21, a significant increase ($p<0.05$) in average daily feed intake and average daily gain was observed in broiler chickens fed both Digestrom® and Poultry Star®. The third group fed both Digestrom® and Poultry Star® exhibited the significant increase ($p<0.05$), followed by the other groups (G1, G2 and G4, sequentially), while the G5 did not exhibit any significant increase ($p>0.05$). In addition, F:G was not significantly changed ($p>0.05$) among the treated groups during the experimental period of 0-21 days due to the addition of Digestrom® and Poultry Star® as feed additive. G5 showed a significant decrease ($p<0.05$) in average daily feed intake and daily gain after 25 days, this result was observed at 21-35 days of age. Meanwhile, the other groups, especially G3, showed a significant increase ($p<0.05$) in average daily feed intake and daily gain as well as a significant decrease in F:G during the breeding period of 0-35 days (Table 3).

Table 1: Effects of Digestrom® and Poultry Star® on the titer of antibodies (Mean±SE) against Newcastle disease in broiler chickens at different periods as determined by ELISA

	Periods			
Groups	7 days	14 days	21 days	35 days
1	1048.70±44.56^{a}	1869.70±45.7^{b}	2375.9±106.8^{b}	5102.70±166.1^{b}
2	1044.80±48.9^{a}	1761.30±66.7^{b}	2132.7±82.2^{c}	4509.40±183.6^{c}
3	1171.20±57.4^{a}	2143.90±61.8^{a}	2875.4±93.1^{a}	5698.30±98.4^{a}
4	1063.50±52.1^{a}	1545.50±79.8^{c}	1872.8±33.9^{d}	4089.10±138.1^{d}
5	1086.10±52.3^{a}	692.10±49.9^{d}	221.3±18.6^{e}	1839.40±106^{e}
LSD	145.72	176.55	214.3	405.26

Number of samples: 10 from each group. *Challenge with virulent local ND isolate on day 25, G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}) as feed additives, G4: Positive control, G5: Negative control. The different letters adjacent to the means of the same column refer to significant differences among treatment means (p<0.05). LSD: Least significant difference

Table 2: Effects of Digestrom® and Poultry Star® on the serum proteins (mg dL^{-1}) in broiler chickens after challenge

	Index			
Groups	Total protein	Globulin	Albumin	A/G ratio
1	2.389±0.09^{b}	2.079±0.05^{ab}	4.468±0.08^{b}	0.88
2	2.118±0.03^{c}	1.891±0.05^{bc}	4.010±0.06^{c}	0.89
3	2.682±0.05^{a}	2.214±0.04^{a}	4.897±0.06^{a}	0.82
4	1.957±0.02^{c}	1.665±0.08^{c}	3.622±0.07^{d}	0.85
5	1.205±0.06^{d}	1.108±0.04^{d}	2.313±0.09^{e}	0.92
LSD	0.256	0.256	0.319	

Number of samples: 5 from each group. A/G, Albumin/globulin ratio, *Challenge with virulent local ND isolate on day 25, G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}) as feed additives, G4: Positive control, G5: Negative control. The different letters adjacent to the means of the same column refer to significant differences among treatment means (p<0.05). LSD: Least significant difference

Table 3: Effects of Digestrom® and Poultry Star® on broiler chicken growth performance

	Groups					
Index	G1	G2	G3	G4	G5	LSD
0-21 days (mean±standard error)						
ADFI (g/day)	61.48±0.35^{b}	59.18±0.40^{c}	65.74±0.71^{a}	57.17±0.52^{d}	56.74±0.63^{d}	2.270
ADG (g/day)	39.38±0.14^{b}	37.80±0.14^{c}	42.16±0.25^{a}	35.95±0.16^{d}	35.42±0.15^{d}	0.747
F:G	1.56±0.01	1.56±0.01	1.55±0.02	1.59±0.02	1.60±0.02	NS
22-35 days (mean±standard error)						
ADFI (g/day)	139.90±0.85^{b}	137.10±0.77^{b}	145.08±0.52^{a}	137.16±0.68^{c}	138.18±0.48^{c}	2.830
ADG (g/day)	80.40±0.65^{b}	78.26±0.47^{bc}	85.02±0.48^{a}	71.70±0.63^{c}	61.08±0.42^{d}	2.261
F:G	1.73±0.02^{c}	1.74±0.01^{c}	1.70±0.01^{c}	1.91±0.01^{b}	2.26±0.01^{a}	0.067
0-35 days (mean±standard error)						
ADFI (g/day)	100.20±1.83^{Ab}	98.80±1.36^{b}	106.10±1.59^{a}	96.60±1.49^{c}	85.4±1.31^{d}	6.396
ADG (g/day)	56.30±0.50^{b}	54.68±0.4b^{c}	59.44±0.55^{a}	53.16±0.40^{c}	38.8±0.36^{d}	1.961
F:G	1.77±0.01^{b}	1.80±0.01^{b}	1.78±0.03^{b}	1.81±0.02^{b}	2.2±0.01^{a}	0.100

Number of samples: 5 from each group. *Challenge with virulent local ND isolate at day 25, G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}) as feed additives, G4: Positive control, G5: Negative control. The different letters adjacent to the means of the same column refer to significant differences among treatment means (p<0.05). LSD: Least significant difference

Clinical signs and mortality of broiler chickens: Table 4 shows morbidity and mortality rates during the 10-days period after challenge with a virulent local isolate of NDV at 25 days, including respiratory and neurological signs and the daily deaths among a total of 20 chicks per group. The vaccinated groups with three booster doses of ND (*La Sota*) vaccine in drinking water showed low rates of morbidity and mortality, especially G3, which was fed both Digestrom® and Poultry Star® as feed additive, followed by the remaining groups (G1, G2 and G4, sequentially), compared to the G5 negative control group, which exhibited the highest rates of morbidity and mortality.

DISCUSSION

The results of the present study showed the synergistic effect of Digestrom® and Poultry Star® as a feed additive, which was more efficient than their individual effects

Table 4: Development of clinical signs and mortalities at 10 days after challenge with local NDV isolate at 35 days of age

Index	Morbidity (%)	Mortality (%)
Groups		
1	60 (12)[c]	10 (2)[c]
2	60 (12)[c]	20 (4)[c]
3	50 (10)[d]	10 (2)[c]
4	70 (14)[b]	30 (6)[b]
5	100 (20)[a]	100 (20)[a]

Number of chicks in each group: 20. Number in parentheses indicate the number of chicks showing clinical signs or death, G1: Received Digestrom® as a feed additive at a dose of 150 mg kg^{-1}, G2: Received Poultry Star® as a feed additive at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}), G3: Received Digestrom® at a dose of 150 mg kg^{-1} and Poultry Star® at a dose of 0.5 g kg^{-1} (2×1011 CFU kg^{-1}) as feed additives, G4: Positive control, G5: Negative control. The different letters adjacent to the means of the same column refer to significant differences among treatment means ($p<0.05$). LSD: Least significant difference

on the immune response against Newcastle disease before and after challenge with a virulent local isolate of NDV ($100\ ELD_{50}10^6$). These results are consistent with the results of Hashemipour *et al.*[11], whom confirmed that the use of thymol and carvacrol at a dose of 200 ppm with probiotics in broiler chickens enhances the immune response (cellular and humoral) by increasing the titers of IgG and other antibodies. Alp *et al.*[17] showed a significant increase ($p<0.05$) in the level of IgG antibodies in the serum of vaccinated chicks fed oregano (300 ppm kg^{-1}). Hong *et al.*[18] showed an increase in IgG in chicks fed CBP at 125 ppm, which is consistent with the results observed in G1 of the current study, which was fed CBP at 125 ppm. This result indicates the inhibitory effect of carvacrol on the production of prostaglandin E2, thereby proving that carvacrol is an anti-inflammatory agent[19]. Acamovic and Brooker[10] demonstrated the immunostimulant effect of the polyphenol fraction of thymol and OEO and their relationship with the mononuclear phagocyte system and cellular and humoral immunity. The results of G2 agree with Ahmad[20] and Ng *et al.*[21], whom explained the role of bacterial *lactobacillus acidophilus* in enhancing immunity by stimulating phagocytosis activity and activating B lymphocytes to increase IgG production. Probiotic increase the number of protective white blood cells in the body, which stimulates pattern recognition receptors that detect pathogen-associated molecular patterns (PAMPs) and Toll-like (TLRs) receptors of dendritic cells in the lamina propria, in turn, stimulates dendritic cells, which promotes the phagocytic process and stimulates other immune cells (T and B cells)[22]. The increase in the concentration of serum proteins (albumin and globulin) in the treatment group was consistent with the findings of Zhang *et al.*[23], whom reported that the increase in total serum proteins indicates an improvement in the immune response. Havenaar and Spanhaak[24] demonstrated the role of natural probiotic bacteria in improving humoral immunity by inhibiting many pathogenic bacteria that act as immunosuppressants by destroying the intestinal lining, liver and other vital organs that produce necessary proteins, such as albumin and globulin. The improvement in average daily feed intake and daily weight and the reduced FCR in the treatment groups (Digestrom® and Poultry Star®) on day 0-35 agree with the findings of Lee *et al.*[25,26], whom reported the role of feed additives (phytogenic and probiotic) in improving digestion and appetite and in promoting the production of endogenous enzymes, which enhance the digestion of nutrients and the gut passage rate in broiler chicks. As Amad *et al.*[27] showed the role of phytogenic and probiotic feed additives in improving the digestibility of nutrients in the ileum. Several researchers have also indicated the importance of phytogenic compounds on the physical performance of broiler chickens[28,29]. In the current study, the use of Digestrom® (150 ppm) in broiler chickens helped increase the daily weight on days 22-35. The results were in agreement with those of Khattak *et al.*[12], whom demonstrated that the use of OEO, especially thymol and carvacrol, at a dose of 200 ppm is mainly responsible for improving body weight and increasing the conversion efficiency of broiler chickens. Hashemipour *et al.*[11] showed that broiler feed diets containing OEO improve the average daily gain and daily feed intake during the period from 25-42 days. The results also agreed with those of Alp *et al.*[17], whom demonstrated that feed supplementation with OEO regulates feed consumption and increases feed conversion in broiler chickens at 21-35 days compared to those in the control group. The results of the present study are consistent with the results of Amad *et al.*[27], whom found significant differences in BWG and ADFI (growth period and total phase) between broiler chickens fed OEO and those fed probiotics. The ADFI and ADG results of G2 agreed with those of Crawford[30], whom demonstrated that the mechanism of probiotics for improving body weight is not well known but involves improving feed consumption, increasing the efficiency of the protein consumed[31], fermenting other carbohydrates[32], increasing the effectiveness of digestive enzymes[33] and producing some essential amino acids and vitamins in the gastrointestinal tract. There is a positive correlation between the increase in probiotic dose and the productive performance of birds[34]. Fioramonti *et al.*[35] reported the role of probiotic bacteria in affecting the main functions of the gastrointestinal tract, such as digestion and absorption. Therefore, the growth performance of chicks in the current study by using Digestrom® and Poultry Star® may be attributed to the effect of essential oils on digestion. This explanation is supported by Langhout[14] and Williams and Losa[36], whom reported a high immune response in broiler chickens fed phytogenic and beneficial bacteria.

CONCLUSION

In conclusion, there is a difference in the response of broiler chickens to individual or combined phytogenic and probiotic feed additives. Dietary inclusion of 150 ppm Digestrom® improved body performance, which led to greater serum total proteins. Moreover, the titer of antibodies against NDV was increased by feeding 150 mg kg^{-1} Digestrom® and Poultry Star® individually or in combination.

SIGNIFICANCE STATEMENT

This study discovered possible individual and synergistic effects of Digestrom® and Poultry Star® that may be beneficial for enhancing immunity against ND in broiler chickens. This study will help researchers understand the synergistic role of natural beneficial bacteria and phytogenic oils in improving body performance and immune responses against dangerous diseases such as ND. Thus, a new theory on these micro nutrient combinations and possibly other combinations, has been introduced.

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