Algal beta-(1,3)-glucan and its effect on infectious bursal disease vaccination in poultry

Beta-(1,3)-glucanen uit algen en hun effect op vaccinatie tegen infectieuze bursitis bij pluimvee

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ABSTRACT

Vaccination is used as a control and prevention tool for infectious bursal disease (IBDV) in poultry. A new strategy for improving vaccination efficiency is the use of in-feed immune modulating ingredients. The aim of this study was to test if the use of beta-(1,3)-glucans in feed can enhance IBDV vaccination. The trial was conducted in ROSS 308 broilers. In the study, a negative control group (not vaccinated, not supplemented), a positive control group (vaccinated, not supplemented) and a treated group (vaccinated, supplemented with beta-(1,3)-glucan) were included. All broilers, except the negative control, were orally vaccinated at 18 days of age with a live IBDV vaccine. Blood samples were taken at day 18 and 35 to measure antibody titers against IBDV. Serological analysis showed the presence of maternal derived antibodies at time of vaccination in some birds. The beta-(1,3)-glucan supplemented birds showed decreased CV% (coefficient of variation) and significantly increased average antibody titers compared to non-supplemented vaccinated birds. Additionally, the beta-(1,3)-glucan group showed increased number of birds with antibody titers above the titer threshold for protective immunity. The results obtained in the current trial clearly indicate that beta-(1,3)-glucan can increase seroconversion and serological response to IBDV vaccination.

SAMENVATTING

Vaccinatie is de preventiemaatregel bij uitstek in geval van infectieuze bursitis (IBDV) bij pluimvee. Het gebruik van immuun-modulerende ingrediënten via het voer is een nieuwe strategie om het succes van de vaccinatie te verbeteren. Het doel van deze studie was na te gaan of de toevoeging van beta-(1,3)-glucanen in het voer de vaccinatie tegen IBDV kan verbeteren. De studie werd uitgevoerd bij vleeskuikens (ROSS 308). Er werden drie groepen dieren opgenomen: een negatieve controlegroep (met niet-gevaccineerde, onbehandelde dieren), een positieve controlegroep (met gevaccineerde maar onbehandelde vleeskuikens) en een gesupplementeerde groep (met gevaccineerde dieren behandeld met beta-(1,3)-glucanen). Alle vleeskuikens in de studie, behalve de negatieve controledieren, werden oraal gevaccineerd op dag 18 met een levend, geïnactiveerd IBDV-vaccin. Bloedstalen voor serologie werden genomen op dag 18 en dag 35. Uit de resultaten van het serologisch onderzoek bleek dat sommige dieren nog beschermd waren door maternale antistoffen op het tijdstip van vaccinatie. De dieren gesupplementeerd met beta-(1,3)-glucanen hadden een lagere CV% (variatiecoëfficiënt) en significant hogere specifieke IBDV-antistoftiters dan niet-gesupplementeerde dieren. Ook waren er in de groep die behandeld werd met beta-glucaan meer dieren met antistoftiters boven de titer voor beschermende immuniteit. De resultaten van deze proef duiden aan dat beta-(1,3)-glucanen de seroconversie en serologische respons op IBDV-vaccinatie kunnen verhogen.

INTRODUCTION

Vaccination plays a vital role in poultry health management. Since the ban on the use of antibiotic growth promotors in animal production, there has been a growing interest in immunization and it is an important tool to reduce the use of therapeutic antibiotics. The primary reason for vaccinating poultry is to reduce the losses due to morbidity and mortality caused by all kind of pathogens (Marangon and Busani, 2007). There are many diseases that are prevented by vaccinating animals against them. One of the most common viral infections in chickens, i.e. infectious bursal disease (IBDV), also known as Gumboro disease, is caused by the IBDV virus. This virus destroys B-lymphocytes in the bursa of Fabricius and affects cell-mediated immunity, leading to mortality and immunosuppression resulting in poor performance with significant economic impact (Sharma et al., 2000; Müller et al., 2012; Ingrao et al., 2013). Vaccination is most important in IBDV prevention and control (Marangon and Busani, 2007; Müller et al., 2012). The vaccine helps to prevent IBDV by inducing a cell-mediated immunity and by boosting the animals' humoral immune system to produce antibodies that in turn fight the invading virus, protecting them against IBDV (Jakka et al., 2014). There are a lot of factors determining vaccination efficiency. Sharif and Ahmad (2018) defined a vaccination failure as: "when the animals do not develop adequate antibody titer levels and/or are susceptible to a field disease outbreak, following vaccine administration". Often, the vaccine is thought to be the cause, but a lot can go wrong between the moment of vaccine development and preparation, and the production of neutralizing antibodies by the animal (Marangon and Busani, 2007; Sharif and Ahmad, 2018). Monitoring antibody titers is important to guarantee efficient vaccination and to allow adjustment of vaccination programs, e.g. in case of protective IBDV maternal antibodies acquired through the egg yolk, the IBDV live vaccine is neutralized, consequently resulting in vaccine failure (Marangon and Busani, 2007; Müller et al., 2012).

A tool for improving vaccination efficiency, not systematically used yet, is in-feed supplementation, which modulates the immune system. It has been shown that response to vaccination can be improved by using immune modulating ingredients administered through the feed, such as beta-glucans (An et al., 2008; Kovacocyova et al., 2014; Vojtek et al., 2017; Horst et al., 2018). Antigen-presenting cells, such as macrophages and dendritic cells, can recognize beta-(1,3)-glucan carbohydrate structures by specific receptors on their surface, such as the dectin-1 receptor, (Medzhitov, 2007; Goodridge et al., 2009; Soltanian et al., 2009). In response to binding beta-(1,3)-glucan, those immune cells become more active in engulfing, killing and digesting invading pathogens and initiate a signaling cascade stimulating the attraction, formation and activation of other immune cells (Soltanian et al., 2009).

One organism effectively producing beta-(1,3)-glucans, is the alga, *Euglena gracilis*, as it stores the molecule as a carbohydrate product in its cytoplasm (Krajcovic et al., 2015). This new algal-derived beta-(1,3)-glucan is available as an in-feed solution for all animal species. The aim of this study was to examine if the use of this new algal-derived beta-(1,3)-glucan in feed can enhance IBDV vaccination efficiency in poultry.

Table 1. Composition of the starter and grower basal diet.

Ingredients (%)	Starter (0-14 days)	Grower (15-35 days)
Corn	40.00	10.00
Soybean meal	22.45	12.60
Wheat	16.99	50.40
Soy	15.00	18.59
Vitamin/mineral premix	1.33	1.33
Limestone	1.09	1.39
L-lysine hydrochloride	1.09	1.02
Animal fat	1.00	3.00
Monocalcium phosphate	0.56	0.33
DL-Methionine	0.28	0.24
Soya oil	0.10	0.00
L-Threonine	0.07	0.00
Sodium chloride	0.03	0.02

MATERIALS AND METHODS

Birds and management

The trial with the broilers was carried out during six weeks in a semi-commercial facility in Belgium. In total, 96 individually tagged, one-day-old male Ross 308 broilers were divided into three treatment groups. The 32 birds (replicates) per treatment group were housed in eight separate pens (four birds/pen). The pens were littered with wood shavings and the birds were fed using a two-phase feeding scheme: starter (day 1 to 14), grower-finisher (day 14 to 38). Drinking water and feed (mash form) were provided ad libitum. The composition of the basal diet is shown in Table 1.

Treatments were assigned to the pens using a randomized complete block design. The treatments differed between the three groups: a negative control group (no vaccination, basal diet), a positive control group (vaccination, basal diet) and a treatment group (vaccination, basal diet supplemented with 50 g/T beta-(1,3)-glucans from *Euglena gracilis (*Aleta™, Kemin Europa NV), throughout the whole feeding period. The negative control group was housed in a designated isolated area and a strict protocol (specific sequence, protective clothing) was followed when entering the broiler house. On day 18, the broilers in the vaccinated groups were individually vaccinated

with an oral live freeze-dried IBDV vaccine (Nobilis® Gumboro D78, MSD Animal health) (Fantay et al., 2015).

Blood sampling

On day 18 and 35, blood samples were taken from the wing vein (vena cutanea ulnaris) of all animals. Serum was separated by centrifugation at 3000 g for ten minutes and the serum antibody titers (IgY) against IBDV were measured using a commercial IBDV ELISA kit (Biochek, United Kingdom) following the instructions of the manufacturer. Measuring antibody titers on day 18 is important to detect if maternal antibodies, which can interfere with the vaccine, are still present, consequently making the bird less susceptible to the vaccination.

Analysis of variance (ANOVA) was applied for statistical analysis (Statgraphics Centurion XVI software, Statpoint Technologies, Warrenton, VA, USA). Means were compared using Fisher's least significant difference procedure. All statements of significance were based on a P-value less than or equal to 0.05.

RESULTS AND DISCUSSION

Because of the endemic situation, it is advised to vaccinate birds against IBDV in Belgium (DGZ, 2018). Vaccination is performed by a live vaccine via drinking water or by a recombinant vaccine via subcutaneous injection. Laying and breeder hens are vaccinated at day 1, day 20 or day 28, depending on the presence of passive immunity acquired through the yolk. Broilers are vaccinated between day 10 and 18, depending on their immune status (WVPA, 2015). To guarantee protection against IBDV, it is important to determine the optimal vaccination age. The optimal time of vaccination is routinely determined by serological examination of serum (ELISA) in one-to-three-days old broilers using the Deventer formula (Smialek et al., 2016). If serology shows the presence of maternal antibodies, vaccination should be postponed to a later age, as these antibodies neutralize the vaccine (Marangon and Busani, 2007; Müller et al., 2012). Still, routine serology is often neglected, and vaccination is performed at standard ages (DGZ, 2016). It is clear that the advised standard vaccination program regarding IBDV is not easily applicable in the field.

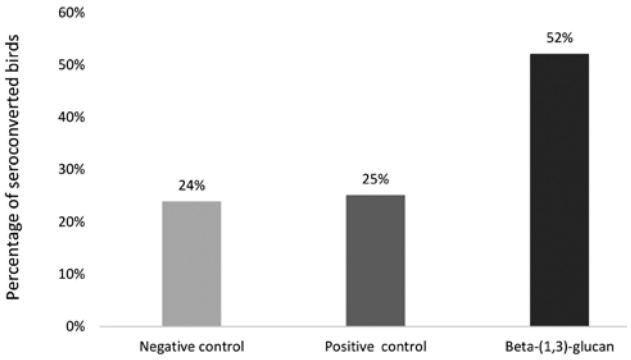

Figure 1. Percentage of seroconverted birds (positive birds) at day 35 (average of 32 replicate birds per group).

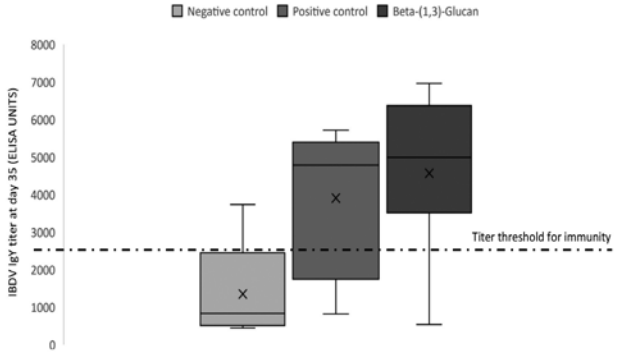

Figure 2. Box plot of IBDV titers (infectious bursal disease virus; ELISA units) per treatment at day 35 (the boxes show the first quartile, median and third quartile; the whiskers show the minimum and maximum; the x shows the mean value).

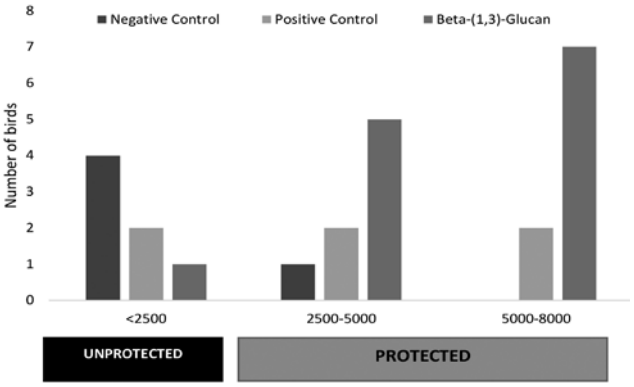

Figure 3. Number of birds with IBDV antibody titers in certain titer classes at day 35.

Table 2. Average specific IBDV MDA (infectious bursal disease virus maternal antibodies; ELISA units) at day 18 and IgY titer (ELISA units) at day 35 (the values are shown as means of 32 replicate birds ± standard deviation).

	MDA (ELISA units)	IgY (ELISA units)	CV %
Negative control	531.5 ± 192.5	1348.7 ± 1361.7a	101.0
Positive control	513.8 ± 137.6	3905.8± 1984.5a	50.8
Beta-(1,3)-glucan	548.4 ± 174.2	4563.3 ± 2165.9b	47.5
	P-value = 0.402	P-value= 0.007	

In the present study, the presence of maternal antibodies (in about 30% of the birds) is clearly shown in case of IBDV as documented in the literature (Naqi et al., 1983; Alam et al., 2002; Müller et al., 2012). Serological analysis at day 18 showed the presence of maternal IBDV specific antibodies (MDA) in 22 out of the 96 birds with an average antibody titer of 530 ELISA units (Table 2). No statistical difference was observed in average MDA titers between groups. A clear presence of passive immunity could interfere with IBDV vaccination programs; therefore, serology should be performed on a routine base to determine the optimal time of vaccination. As the optimal antibody titer for vaccination should be equal or below 100-250 ELISA units (Biochek, 2017) when using an intermediate live vaccine such as Nobilis Gumboro D78, birds showing passive protection at day 18 were taken out of the present trial.

At 35 days, differences in seroconversion to the vaccines were observed amongst all treatment groups (Figure 1). In total, 52% of the birds reacted positively to the vaccine in the beta-(1,3)-glucan supplemented group, while only 25% and 24% of the birds seroconverted in the positive and negative control group, respectively. Results of the average IBDV titers are shown in Table 2. Nobilis Gumboro D78 vaccines should induce immunity 6-7 days after vaccination and protective immunity should remain 31 days as claimed by the vaccine producer. This was clearly shown by the serological analysis on day 35 (17 days after vaccination). The non-vaccinated birds showed a non-protective average IgY titer compared to both vaccinated groups with protective average titers. Furthermore, the average IBDV titer at day 35 was significantly increased in the beta-(1,3)-glucan supplemented group (4563.3 ELISA units) compared to the positive (3905.8 ELISA units) and negative (1348.7 ELISA units) control group (Table 2).

To evaluate the success of vaccination, two parameters need to be considered: the mean antibody titers (as a measure of the immune response to the vaccine) and the coefficient of variation (CV%) (showing the uniformity of the vaccination response in a group or flock). For live vaccine applications, the CV% should be below 60% for effective and homogenous vaccination (Biochek, 2017), which was the case in both vaccination groups (50.80% and 47.46%) of the present study (Table 2). A numerical decrease of the CV% was observed in the beta-(1,3)-glucan supplemented group (47.46%), compared to the positive control group (50.80%). The CV% was above 60% in the non-vaccinated group, confirming the hypothesis that the positive antibody titers in this group were generated by circulation of the vaccine virus in the house and not by active immunization.

The average antibody titers at day 35 in broilers vaccinated with Nobilis Gumboro D78 should be above 2500 ELISA units to guarantee protection (Biochek, 2017). Considering the antibody titers in the vaccination groups were above the protective threshold and had a CV% below 60%, the vaccination performed in the current trial can be considered successful. Additionally, the broilers in the beta-(1,3)-glucan supplemented group showed significantly higher antibody titers (4563.3 ELISA units) and lower CV (47.5 %) than the non-supplemented vaccinated control group, providing a significantly better vaccination efficiency.

When the antibody titers observed in the different treatment groups were plotted and compared to the threshold for immunity of 2500 ELISA units in case of Nobilis Gumboro D78 vaccination in broilers, the following conclusions could be made (Figure 2): In the negative control group, 20% of the birds reacting positively to the vaccine showed protective immunity, hypothetically due to circulation of the vaccine strain in the broiler house as noted before, although protective measures to prevent circulation of the virus in the poultry house were taken, such as a designated isolated area for the negative control birds, strict treatment sequence and protective clothing. Further virological analysis to confirm this hypothesis was not performed. In the vaccinated groups, 66.67% and 84.62% of birds seroconverting to the vaccine (which were 25% and 52%) showed titers above the protective immunity threshold in positive control and beta-(1,3)-glucan supplemented group, respectively. Beta-(1,3)-glucan supplementation increased the number of birds showing protective immunity compared to the non-supplemented birds, as can be observed in Figure 3. This effect has been demonstrated previously by Horst et al. (2018), who investigated the effect of beta-(1,3)-glucan supplementation on NDV vaccination. In that study, beta-(1,3)-glucan supplementation in birds resulted in an increased number of birds with NDV antibody titers above the immunity threshold compared to the non-supplemented group.

CONCLUSION

The results of the trial in the present paper show that beta-(1,3)-glucan can increase IBDV seroconversion and serological response. Beta-(1,3)-glucan supplemented birds showed increased average antibody titers and decreased CV% compared to non-supplemented vaccinated birds. Additionally, the beta-(1,3)-glucan supplemented group showed an increased number of birds with antibody titers above the titer threshold for protective immunity. These data prove that beta-(1,3)-glucan supplementation can increase the success rate of an efficient vaccination.

REFERENCES

Alam J., Rahman M.M., Sil B.K., Khan S.R., Giasuddin M., Sarker M.S.K. (2002). Effect of maternal derived antibody on vaccination against infectious bursal disease

(Gumboro) with live vaccine in broiler. *International Journal of Poultry Science 1*, 98-101.

An B.K., Cho B.L., You S.J., Paik H.D., Chang H.I., Kim S.W., Yun C.W., Kang C.W. (2008). Growth performance and antibody response of broiler chicks fed yeast derived beta-glucan and single-strain probiotics. *Asian-Australasian Journal of Animal Sciences 21*, 1027-1032.

Biochek (2017). Interpretation and application of results manual. Biochek, United Kingdom. Accessed July 2019. https://www.biochek.com/wp-content/uploads/2018/01/BIOCHEK-INTERPRETATION-Manual.pdf.

DGZ (2016). *Activiteitenverslag 2016 Diergeneeskundige Begeleiding Pluimvee*. DGZ, Belgium. Accessed July 2019. https://www.dgz.be/sites/default/files/Rapport_Diergeneeskundige_Begeleiding_DGZ_2016_pluimvee.pdf.

Fantay H., Balcha E., Tesfay, A., Afera B. (2015). Determining optimum time for administration of live intermediate vaccine of infectious bursal disease to chickens at Mekelle farm. *Journal of Veterinary Science & Technology 6*, 1-4.

Goodridge H.S., Wolf A.J., Underhill D.M. (2009). Beta-glucan recognition by the innate immune system. *Immunological Reviews 230*, 38-50.

Horst G., Levine R., Chick R., Hofacre C. (2018). Effects of beta-1,3-glucan (Aleta™) on vaccination response in broiler chickens. *Poultry Science 98*, 1643-1647.

Ingrao F., Rauw F., Lambrecht B., van den Berg T. (2013). Infectious Bursal Disease: A complex host-pathogen interaction. *Developmental and Comparative Immunology 41*, 429-438.

Jakka P., Reddy Y.K., Kirubaharan J.J., Chandran N.D. (2014). Evaluation of immune responses by live infectious bursal disease vaccines to avoid vaccination failures. *European Journal of Microbiology and Immunology 4*, 123-127.

Kovacocyova K., Reichel P., Seidel H., Brenesselova M., Balasz K. (2014). Influence of beta-glucan and vaccination against *Lawsonia intracellularis* on selected immune indices in weaned piglets. *Acta Veterinaria-Beograd 64*, 105-114.

Krajcovic J., Vesteg M., Schwartzbach S.D. (2015). Euglenoid flagellates: a multifaceted biotechnology platform. *Journal of Biotechnology 202*, 135-145.

Marangon S., Busani L. (2007). The use of vaccination in poultry production. *Revue scientifique et technique (International Office of Epizootics) 26*, 265-274.

Medzhitov R. (2007). Recognition of microorganisms and activation of the immune response. *Nature 449*, 819-826.

Müller H., Mundt E., Eterradossi N., Islam M.R. (2012). Current status of vaccines against infectious bursal disease. *Avian Pathology 41*, 133-139.

Naqi S.A., Marquez B., Sahin N. (1983). Maternal antibody and its effect on Infectious Bursal Disease immunization. *Avian Diseases 27*, 623-631.

Sharif A., Ahmad T. (2018). Preventing vaccine failure in poultry flocks In: Wang N. and Wang T. (editors). *Immunization – Vaccine Adjuvant Delivery System and Strategies, IntechOpen.* United Kingdom, 79-94.

Śmiałek M., Śmiałek A., Koncicki A. (2016). Practical aspects of estimation of optimal time for vaccination of chicken against IBDV with use of "Deventer formula". *Polish Journal of Veterinary Sciences 19*, 425-427.

Soltanian S., Stuyven E., Cox E., Sorgeloos P., Bossier P. (2009). Beta-glucans as immunostimulant in vertebrates and invertebrates. *Critical Reviews in Microbiology 35*, 109-138.

Vojtek B., Mojzisova S., Smrco P., Drazovska M. (2017). Effects of orally administered beta-1,3/1,6-glucan on vaccination responses and immunological parameters in dogs. *Food and Agricultural Immunology 28*, 993-1002.

WVPA (2015). Entschema advise. WVPA, Belgium. Accessed July 2019. https://www.dgz.be/sites/default/files/WVPA_pluimvee_entschema_advies_2015.pdf.

Sharma J.M., Kim I.-J., Rautenschlein S., Yeh H.-Y. (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental and Comparative Immunology 24*, 223-235.