Applied Research Note: Alpha-monolaurin stimulates the antibody response elicited upon infectious bronchitis vaccination of broilers

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Primary Audience: Nutritionists, Researchers, Feed Formulators, Microbiologists, Veterinarians

SUMMARY

Alpha-monolaurin has been demonstrated to have antipathogenic properties and is therefore used as feed additive for broilers to prevent infectious diseases and improve production performance. As its antiviral effect is thought to be exerted by disintegrating the viral envelope, α-monolaurin might counteract with the current vaccination programs used in poultry production that are based on administering live attenuated viral strains. In this study, the effect of a commercially available formulation of α-monolaurin (FRAC12 Dry) on infectious bronchitis (**IB**) vaccination was evaluated in Ross 308 broilers. In chickens orally vaccinated with live infectious bronchitis virus (**IBV**), supplementation of FRA C12 Dry did not seem to affect the uptake of vaccine IBV, though indication for enhanced viral clearance of this virus was seen after 30 d in the supplemented birds. Furthermore, anti-IBV antibody titer values were significantly higher in orally IBV-vaccinated animals receiving FRA C12 Dry compared with the control group receiving blank feed. Taken together, the results indicate that α-monolaurin does not influence oral IB vaccination efficacy but, in contrast, has the potency to stimulate the immune response that is elicited upon vaccination. This further supports the use of α-monolaurin as a feed additive for broilers. Follow-up studies are, however, necessary to evaluate if better protection can be established upon challenge with IBV and whether a similar effect can be observed for other pathogens.

Key words: alpha-monolaurin, broiler, feed additive, infectious bronchitis, vaccination

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DESCRIPTION OF PROBLEM

Lauric acid (C12) is a medium-chain fatty acid that is commonly found in natural products, including coconut oil. But also its α-mono glyceride form, α-monolaurin, is found, for instance, in human breast milk and displays antibacterial and antiviral activity. Alpha-monolaurin can be produced by esterification of lauric acid to the sn-1 position of a glycerol molecule and also exerts an antipathogenic effect, likely by solubilizing the lipids and phospholipids in the bacterial cell membrane or viral envelope, which results in

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destabilization or disintegration of the pathogen (Isaacs et al., 1986). Moreover, α-monolaurin is biologically more active in neutralizing viruses and bacteria compared with nonesterified lauric acid (reviewed by Lieberman et al. (2006)). In addition, α-monolaurin is not affected by the pH range encountered in the gastrointestinal tract and hence is stable throughout the complete gastrointestinal tract and resistant to enzymatic breakdown by lipases in the mouth, stomach, and small intestine (reviewed by Mu and Hoy (2004)). Commercially available formulations of α-monolaurin are therefore marketed as valuable feed additives that improve the health status and the production performance of poultry (Fortuoso et al., 2019). However, due to its antiviral properties, α-monolaurin could counteract the effect of live attenuated vaccines, especially those administered orally. This is of concern, as antiviral vaccination of poultry is often performed with live vaccines allowing mass application (orally, e.g., via drinking water or spray), as, for example, is the case for some commercial infectious bronchitis (**IB**) vaccines. The main objective of this study was to evaluate the effect of supplementing a commercial available formulation containing α-monolaurin (FRA C12 Dry) on vaccine uptake and elicited antibody response in Ross 308 broilers orally vaccinated with live attenuated infectious bronchitis virus (**IBV**).

MATERIALS AND METHODS

Broilers and Housing

One-day-old male maternally derived antibody (**MDA**) positive broiler chickens (Ross 308) were obtained from a commercial hatchery in Belgium and did not receive any antimicrobial or anti-inflammatory drugs at the hatchery or at the study site. Broilers originated from breeders that have been vaccinated with live vaccines of different serotypes (Mass, 4/91, QX, D274) and have been boosted with an inactivated multivalent vaccine containing Mass and D274 antigens. Identification was established with wing tags. Broilers were housed in isolators (each 2 m^2 and 1 m high) equipped with HEPA-filtered air circulation with positive air pressure to prevent access of external viruses. Their design and construction are in accordance with the EU Directive 2010/63/EU. Potable drinking water originating from the public water system for human consumption was provided *ad libitum* and a one-phase feeding regimen was applied. The study and its methodology were approved by the ethical committee of Poulpharm BVBA (application P17139-ISO).

Experimental Design

The study included 2 treatment groups that were separated by assigning one isolator to each treatment group to avoid cross-contamination. Fifty broilers, 25 broilers per isolator, were randomly allocated to one of the 2 isolators upon arrival. The basal composition of the commercial feed was the same for both treatment groups. The control group received blank feed during the entire study, whereas the feed of the treated group was supplemented with the test product FRA C12 Dry (provided by FRAmelco BV, Raamsdonksveer, The Netherlands) at 3 kg/ton from the first day onward. FRA C12 Dry is a mixture of mono-, di-, and triglycerides of lauric acid on a silica carrier (E551a, silicic acid, precipitated and dried). Besides the main ingredient, α-monolaurin, FRA C12 Dry also contains a small amount of free glycerol and free lauric acid. The lauric acid used for the esterification process originates from palm kernel oil.

On day 1 and day 15, all broilers of both treatment groups were individually vaccinated with one dose ($\geq 10^3$ EID_{50}) of live attenuated IB vaccine (Nobilis IB Ma5, MSD Animal Health, Merck & Co., USA). The vaccine suspension was prepared according to the manufacturer's guidelines and administered by oral gavage. On days 15 and 30, the number of birds per isolator was respectively reduced to 20 and 10 to prevent overcrowding. The general health status of all birds was monitored daily. All broilers (still) included in the study were individually weighed upon arrival (day 1) and at the end of the study (day 40). In addition, the individual body weight of birds that were taken out of the isolators for density purposes (day 15 and 30) was registered, as well as the feed consumption (days 1, 15, 30, 40).

Tracheal swabs were obtained on days 5, 9, 15, and 30 and stored at 4°C. IBV RNA was detected in the tracheal samples by RT-qPCR.

Therefore, RNA was extracted from the tracheal samples with the innuPREP Virus DNA/RNA kit (AJ Innuscreen GmbH, Berlin, Germany), and subsequently the presence of IBV RNA was analyzed by RT-qPCR using the Kylt IBV detection kit (AniCon Labor GmbH, Höltinghausen, Germany). On days 15, 30, and 40, blood was collected from the jugular vein and serum was obtained after centrifugation (3,200 × *g* for 15 min) and stored at –20°C. The anti-IBV antibody titers in serum samples were determined by ELISA with the Infectious Bronchitis Antibody Test Kit (Biochek, Reeuwijk, The Netherlands) (the cutoff value of the ELISA kit as specified by the manufacturer was 833). All assays were performed according to the manufacturer's instructions.

The main study parameters of this trial were the presence of IBV RNA in the trachea, anti-IBV antibody titers in the serum, individual body weight, daily weight gain per isolator, and feed conversion ratio per isolator (daily feed intake divided by daily weight gain).

Statistical Analysis

Statistical analysis was performed with R version 3.6.0 © 2019 (The R Foundation for Statistical Computing, Vienna, Austria). Two-sided Wilcoxon rank-sum tests were carried out to compare the 2 treatment groups with respect to body weight and anti-IBV antibody titer. The statistical significance of differences in proportion IBV RNA-positive birds between treatment groups was evaluated by two-sided Fisher's exact tests. Results were considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Sustainable and efficient animal production is important in poultry husbandry. Birds in commercial flocks are, however, often subjected to multiple stress factors, such as high stocking densities and suboptimal climate conditions, which makes them highly susceptible for infectious diseases. This results in decreased reproduction and growth performance and high mortality rates. Preventive and therapeutic measures are currently employed to combat infections of bacterial and viral origin. There is, however, an urgent need of alternative ways to prevent and treat infections with microorganisms as (1) the use of antibiotics is restricted due to the increasing number of resistant pathogens, and (2) vaccination does not necessarily guarantee full protection, as exemplified for IBV. This coronavirus of domestic fowl replicates in the respiratory tract as well as in other tissues such as the kidneys, gonads, and alimentary tract and induces IB, a disease characterized by clinical signs such as depression, coughing, and nasal and ocular discharge. Although live and inactivated IB vaccines are commercially marketed, their effectiveness is often mediocre as they fail to provide sufficient cross-protection against other IBV serotypes (reviewed by (Cavanagh 2007)). IBV infections therefore remain an important source of economic loss within the poultry industry.

Alpha-monolaurin, a monoester formed from lauric acid, may become an important feed additive due to its antipathogenic activity and its stability in the gastrointestinal tract (reviewed by (Mu and Hoy 2004) and (Lieberman et al., 2006)). In this study, the effect of a commercial available formulation of α-monolaurin (FRA C12 Dry) on vaccination with Nobilis IB Ma5 was evaluated in Ross 308 broilers to address the question whether this compound could interfere with orally administered live attenuated vaccines. The choice of pathogen/vaccine can be justified by the fact that IBV has relevant disease-causing potential in poultry, as described earlier, and vaccination with Nobilis IB Ma5 is often applied in Europe.

Two treatment groups, housed in separate isolators, were included in this study to assess the effect of supplementing FRA C12 Dry; the feed of the treated group was supplemented with the test product, whereas the control group received blank feed. Animals of both treatment groups were orally vaccinated on days 1 and 15. No serious health problems were observed during the trial and no significant differences were observed in body weight throughout the complete trial (Figure 1A). In addition, the average daily weight gain was comparable between the control and treated group (65.61 and 65.07 g, respectively) and the feed conversion ratio equaled 1.38 for both treatment groups. The lack of improvement of these parameters may be partially linked to the strictly controlled

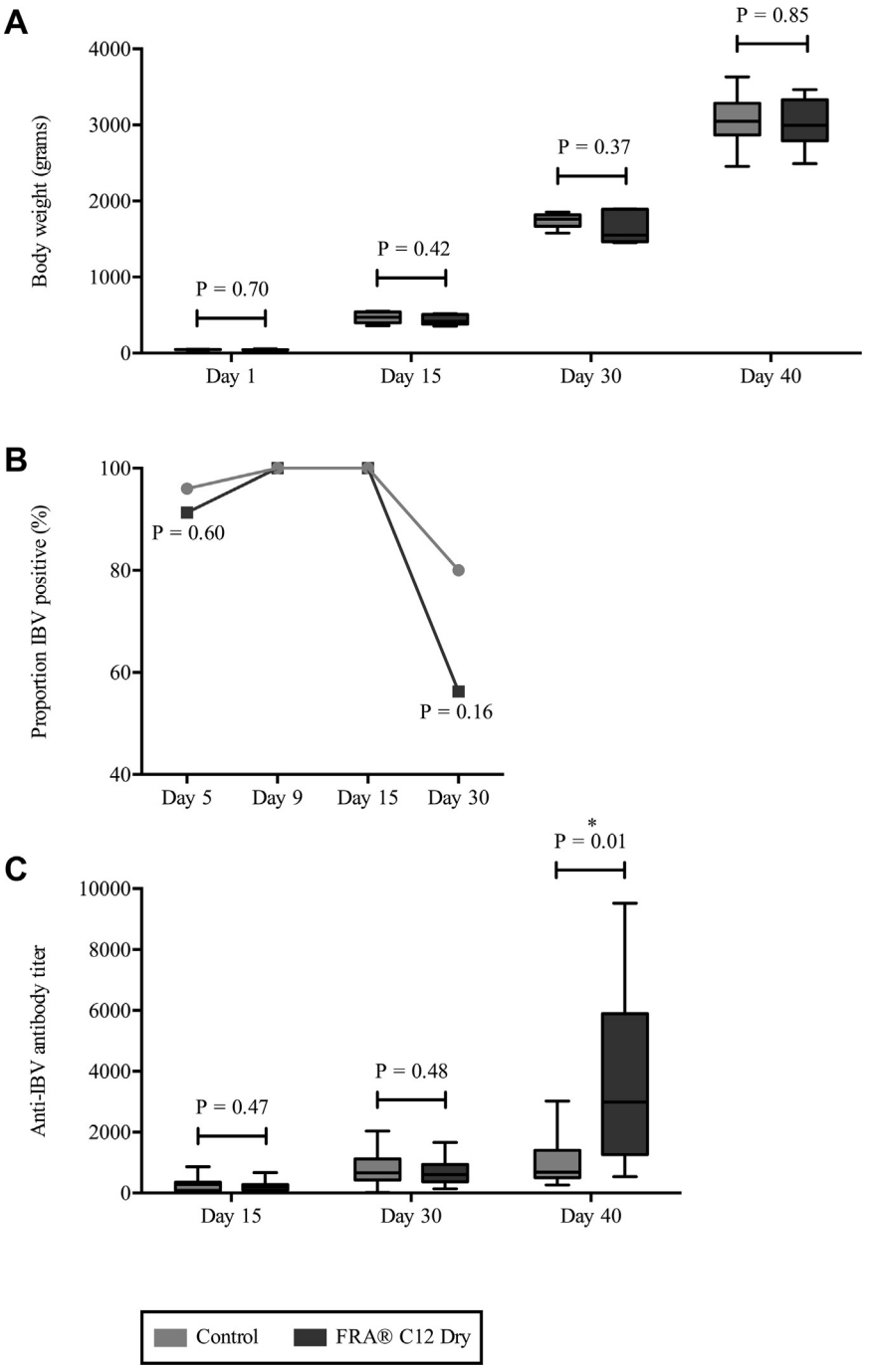

Figure 1. Effect of FRA C12 Dry on body weight, proportion IBV positives, and anti-IBV antibody titer. (A) The body weight of each animal (still) included in the study was registered on day 1 and day 40, whereas birds that were taken out of the isolators for density purposes were individually weighed on day 15 and day 30. (B) IBV RNA in tracheal swabs obtained on days 5, 9, 15, and 30 was detected by RT-qPCR and the proportion of IBV-positive animals per treatment group was calculated. (C) The IBV antibody titer in serum samples acquired on days 15, 30, and 40 was evaluated by ELISA. All boxplots show the median, interquartile range, and min/max values. Significant differences ($P \leq 0.05$) are indicated by an asterisk.

conditions during the trial, such as housing in HEPA-filtered isolators that restrict access of external pathogens.

Faster Clearance of IBV in Birds Receiving FRA C12 Dry

Feeding a diet supplemented with a commercial formulation of α-monolaurin (FRA C12 Dry) does not affect IBV vaccine uptake at current vaccination doses. Although some birds were still negative for IBV on day 5, respectively 4 and 9% for the control and treatment groups at days 9 and 15 all birds from both groups tested positive (Figure 1B). On day 30, the percentage of IBV-positive birds decreased, indicating that some birds established elimination of the virus. Interestingly, the percentage of IBV-positive birds in the treated group was markedly lower compared with the control group (56 and 80%, respectively), though not statistically significant. This could suggest that faster clearance of vaccine-derived IBV occurs in birds receiving α-monolaurin. As there was no effect on vaccine clearance at earlier time points, a direct antiviral effect of α-monolaurin (FRA C12 Dry) was not observed in this trial. Alternatively, FRA C12 Dry may have supported the immune response elicited by vaccination, which could explain the differences in the percentage of IBV-positive birds on day 30.

FRA C12 Dry Stimulates Anti-IBV Antibody Production

Vaccination with live attenuated IBV was demonstrated to result in the development of antibodies (Cook et al., 1999). To test the hypothesis that FRA C12 Dry stimulates the immune response, anti-IBV antibody titers were determined after vaccination. The titers gradually increased from day 15 to day 40 for both the control and treatment group. At days 15, 30, and 40 respectively 5, 40, and 30% of the control animals and 0, 31 and 80% of the FRA C12 Dry group had seroconverted (titer above the cutoff value of the ELISA). Interestingly, significantly higher anti-IBV titer values were detected at day 40 in vaccinated birds receiving FRA C12 Dry compared with vaccinated control birds (Figure 1C). Together with the observation that more birds were able to establish faster clearance of vaccine IBV, this may suggest that α-monolaurin indeed strengthens the immune response, though it should be noted that an increase in antibody titers does not necessarily guarantee better protection against future infections.

Although vaccine uptake was not impacted in this study, an improved antibody response against IBV was observed.

Earlier studies suggest a downregulating activity of α-monolaurin on the immune system during infections, which makes the increased antibody response, as specific immune response, a finding that requires further investigation (Li et al., 2009; Zeinab et al., 2014; Haase et al., 2015; Zhang et al., 2016). In conclusion, α-monolaurin does not seem to interfere with, but rather stimulates, the immune response elicited by IB vaccination, which would further support its use as a feed additive. Follow-up studies are, however, required to (1) examine if supplementing α-monolaurin also results in better protection against several pathogenic IBV strains upon challenge, (2) further characterize the interaction between α-monolaurin and the immune system, and (3) investigate whether α-monolaurin induces a similar effect upon vaccination against and challenge with other pathogens.

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This research was funded by FRA melco BV. The sponsor approved the study design, but was not involved in the collection, analysis, and interpretation of the data, nor in writing the report. The sponsor revised the written manuscript and agreed to submit the article for publication.

DISCLOSURES

Authors E.P.C.W.D. and O.D. are employees of FRAmelco BV, which produces the test product FRA C12 Dry used in this study.

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