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Extracts of laminarin and laminarin/fucoidan from the marine macroalgal species *Laminaria digitata* improved growth rate and intestinal structure in young chicks, but does not influence *Campylobacter jejuni* colonisation

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ABSTRACT

Promoting growth performance, while limiting the proliferation of bacteria such as *Campylobacter jejuni,* in the post-hatch period is a key goal of the broiler industry. Therefore, the objective of this study was to evaluate the effects of supplementing the diet with Laminarin and Fucoidan extracts on growth performance, small intestinal morphology and function, immune response and *Campylobacter jejuni* colonisation following an experimental challenge in young chicks following dietary supplementation. The experiment consisted of three diets: 1) basal diet; 2) basal diet + 250 ppm Laminarin (LAM); 3) basal diet + 250 ppm LAM + 80 ppm FUC (LAM/FUC). Day old Ross chicks (n = 135), were housed in groups of three, with 15 replicates per treatment group. On day three, all chicks were orally gavaged with 0.1 ml 10^6 colony forming units of *C. jejuni.* On day 13, caecal digesta samples were collected for quantification of *C. jejuni* and *Lactobacillus.* Ileal tissue was also collected post-slaughter in order to examine small intestinal morphology and the gene expression of targets related to gut health. The mean total intake for the chicks fed the LAM, LAM/FUC and basal diets during the experiment (day 0–13) were 411 g, 411 g and 373 g respectively, with chicks fed the LAM and LAM/FUC supplemented diets having increased feed intake compared to the basal diet fed chicks ($P < 0.05$). Chicks offered diets containing LAM and the LAM/FUC combination had higher ($P < 0.05$) total weight gain (262 g and 254 g respectively) compared to the basal diet 243 g. Dietary inclusion of LAM/FUC combination increased feed conversion ratio (FCR) (1.68 g/g vs 1.77 g/g) compared to the basal diet ($P < 0.05$). Chicks offered the LAM and LAM/FUC diet had increased ileal villus width compared to chicks offered the basal diet, while chicks fed the LAM diet had increased villus height compared to chicks fed the basal diet ($P < 0.05$). Chicks offered the both the LAM and LAM/FUC supplemented diets had increased gene expression of the tight junction protein *CLDN1,* compared to chicks fed the basal diet ($P < 0.05$). The gene expression of the measured intestinal nutrient transporters was unaffected by dietary supplementation ($P > 0.10$). There was no effect of LAM or LAM/FUC extracts on the proliferation of *C. jejuni* or on *Lactobacilli* numbers in the caeca. In conclusion, supplementation with LAM or LAM/FUC in the post-hatch period improved growth rate, positively modified small intestinal architecture and impacted the intestinal immune response, but did not impact the extent of *C. jejuni* proliferation.

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Supplementation of the diet with LAM provided beneficial effects over supplementation with LAM/FUC in relation to FCR, villus height and aspects of the immune response.

1. Introduction

The post-hatch period is a critical time in chick production. There are rapid changes in the environment of the chick during this period that create significant challenges to the development and maturation of their digestive, immune and thermo-regulatory systems (Maiorka et al., 2006). Additional external challenges include the ban on antibiotic growth-promoters in EU member states in 2006. This ban has wide ranging implications including negative effects on chick growth as well as the uncontrolled proliferation of pathogenic microorganisms. *Campylobacter* is of particular concern to the consumer; these gram-negative bacteria are commensal in poultry but pathogenic in humans. *Campylobacter* species are the most common cause of acute bacterial gastroenteritis in humans with *Campylobacter jejuni* (*C. jejuni*) responsible for over 90% of cases (Ruiz-Palacios, 2007; European Food Safety et al., 2011; Smith et al., 2016). Such challenges have led to the search for alternative approaches to improve chick performance while suppressing the proliferation of pathogenic bacteria such as *C. jejuni* in the chick.

Seaweed extracts containing the polysaccharides fucoidan and laminarin, have been exploited as novel sources of bioactive compounds and alternatives to antibiotics due to their range of biological effects (Sweeney and O'Doherty, 2016). Fucoidan and laminarin are the main water soluble polysaccharides of brown algae (Michel et al., 1996). The inclusion of seaweed extracts in the diets of post-weaned pigs improves growth performance in the absence of in-feed antibiotics (Leonard et al., 2010; O'Doherty et al., 2010; McAlpine et al., 2012). While older studies in the literature have utilised relatively crude seaweed extracts consisting on mixtures of compounds, advances in extraction technologies (Garcia-Vaquero et al., In press) have allowed the characterisation of the specific bioactives in seaweed and a number of studies have now been performed which highlight the different modes of action of fucoidan and laminarin.

Fucoidan is a sulphated polysaccharide, containing L-fucose as one of the major monosaccharides (Berteau and Mulloy, 2003). It is a non-digestible polysaccaride that appears to have a predominately prebiotic effect in the large intestine. Fucoidan has a capacity to positively alter the microbial profile of the intestinal tract of the post-weaned pig through increase in beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (O'Doherty et al., 2010; Smith et al., 2011; Mukhopadhya et al., 2012; Walsh et al., 2013). Laminarin represents a group of low-molecular weight polysaccharides of β-(1–3)-linked glucans with β-(1–6)-linked side chains of varying distribution and length (Read et al., 1996). Dietary laminarin improves growth rate and feed efficiency in weaning piglets (Walsh et al., 2013; Heim et al., 2014a). Laminarin interacts with epithelial cells of the small intestine to improve nutrient digestibility, villus architecture and increase the expression of the nutrient transporters: *GLUT1*, *GLUT2*, and *SGLT1* in weaned piglets (Sweeney et al., 2012; Walsh et al., 2013; Heim et al., 2014b). Dietary laminarin is also taken up by the Peyer's patches and is presented to underlying dendritic cells to influence cytokine production. Infact dietary supplementation of pigs with laminarin from *Laminaria digitata*, downregulated the expression of a panel of inflammatory cytokines in the colon and liver (Ryan et al., 2010; Sweeney et al., 2012). Interestingly, supplementation of laminarin derived from *L. digitata* increased the expression of the pro-inflammatory cytokines *IL6* and *IL8* in *ex-vivo* lipopolysaccaride-challenged colonic tissue (Smith et al., 2011; Bahar et al., 2012). These data suggest that laminarin decreases the expression of cytokines in the homeostatic GIT but increases the capacity of pro-inflammatory cytokines to respond to a pathogenic challenge.

The application of fucoidan and laminarin to the post-hatch diet in broilers is of immense interest. The potential for laminarin to enhance intestinal nutrient absorption and immune function would have positive effects on growth rate. The addition of fucoidan to the laminarin diet had the potential to additionally stimulate a healthy gut microbiota and to suppress *Campylobacter* colonisation during the post-hatch period. Hence, the objective of this study was to determine if nutritional supplementation with a purified laminarin extract or a laminarin/fucoidan extract from the new-born chick stage, can enhance feed efficiency and growth rates in the developing chicks and suppress *C. jejuni* numbers in the caecum of experimentally *C. jejuni* challenged chicks.

2. Materials and methods

2.1. Experimental diets

The experiment was a complete randomised design consisting of three treatment groups: 1) basal diet (Chick Starter, Gain Poultry Feeds Glanbia, Ireland); 2) basal diet supplemented with 250 ppm purified LAM; 3) basal diet supplemented with a seaweed extract containing 250 ppm LAM and 80 ppm FUC combination (BioAtlantis Ltd., Tralee, Co. Kerry, Ireland). The purified LAM and the extract of LAM/FUC from the brown algae *Laminaria Digitata* were generated as previously described by Walsh et al. (2013). The LAM/FUC diet also contained alginate (180 ppm) and mannitol (120 ppm). Feed samples were retained for chemical analysis and the results are presented in Table 1.

2.2. Animals and management

Day old male Ross chicks (n = 135) were sourced from Carton Bros. (Monaghan, Ireland) and were managed in accordance with