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Effects of a laminarin-rich algal extract on caecal microbiota composition, leukocyte counts, parasite specific immune responses and growth rate during *Eimeria tenella* infection of broiler chickens

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ABSTRACT

Coccidiosis, infection with protozoan parasites of genus *Eimeria*, is a major problem in poultry husbandry worldwide. The disease is currently managed by coccidiostats and live vaccines, but these approaches are not sustainable. Hence, it is important to identify new means to control the infection and/or ameliorate its detrimental effects on gut health. Laminarin, a β-glucan found in marine brown algae, has prebiotic and bioactive properties that could be beneficial in coccidiosis control. The present study aimed to examine the potential of laminarin as an immunostimulatory and microbiota-regulatory compound in broiler chickens infected with *E. tenella*. Chickens were continuously fed a diet supplemented with a laminarin-rich algal extract (AE) from first feed and subsequently infected with *E. tenella* at 19 days old. The outcome of infection including caecal microbiota and some immune parameters were monitored during the experiment. Results showed that AE supplementation affected some lymphocyte subpopulations, with increased numbers of TCRγ/δ+CD8-, B-cells and CD4-CD8αβ+ cells and lower numbers of CD4+CD8αα+ cells in blood and increased proportions of CD4-CD8αβ+ spleen cells compared to those in control chickens. The AE diet did not affect parasite excretion, lesion scores or *E. tenella* specific T-cell responses. However, reductions of *E. tenella* induced contraction of *Bifidobacteriaceae* and expansion of *Clostridiaceae* in caecal microbiota were observed for AE fed chickens compared to chickens fed the control diet. Thus, AE feed supplementation induced some immunostimulatory activity in chickens and affected some of the alterations in caecal microbiota evoked by *E. tenella* infection.

1. Introduction

In domestic fowl coccidiosis is an enteric disease caused by obligate intracellular protozoan parasites of the genus *Eimeria*, phylum Apicomplexa (Chapman et al., 2013; Mesa-Pineda et al., 2021). *Eimeria* spp. have a monoxenous life cycle and ten different *Eimeria* species that can infect chickens have now been identified (Blake et al., 2021). The disease is a major threat to health and welfare in all types of modern poultry husbandry as well as causing substantial production losses for the industry yearly. For example, at 2016 prices the expenses of prophylaxis, treatment and production loss due to coccidiosis have been estimated to generate costs equivalent to £ 0.16 per broiler chicken produced (Blake et al., 2020). The direct impact of *Eimeria* infection on chickens varies from subclinical with effects on growth rate and feed conversion to severe clinical signs of disease and death depending on factors such as the infecting *Eimeria* species, infection dose and immune status of the chicken (Chapman et al., 2013; Mesa-Pineda et al., 2021). Furthermore, an important negative consequence of *Eimeria* infection is the disruption of intestinal microbiota homeostasis it causes with an additional increased risk of colonization by pathogenic bacteria such as *Clostridium perfringens* (Moore, 2016) and *Salmonella enterica* serovars Typhimurium or Enteritidis (Arakawa et al., 1981; Qin et al., 1995). For example, many studies have shown alterations of relative numbers and diversity in the caecal microbiota upon infection of chickens with

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E. tenella that exclusively infects and replicates in caecal cells (Campos et al., 2022; Huang et al., 2018; Kimura et al., 1976; Macdonald et al., 2017). With respect to alterations in bacterial relative abundance after *E. tenella* infection the outcome varies between reports and is likely influenced by factors such as sampling time after infection, diet, and other husbandry aspects. However, commonly decreases of Lactobacillaceae and Bifidobacteriaceae and increases of Clostridiaceae and Enterobacteriaceae have been reported. Disturbances in gut microbiota, so called dysbiosis, may in addition to facilitate pathogen colonisation also cause other detrimental consequences to the chicken such as intestinal inflammation and reduced digestion and uptake of nutrients (Ducatelle et al., 2023).

The means currently available to control *Eimeria* infections in chickens are prophylactic medication with chemotherapeutic drugs, so called coccidiostats, and live vaccines. Both these approaches carry drawbacks, for example Eimeria parasites develop resistance to coccidiostats, and there is a limited supply of the live vaccines as well as a high cost and ethical concerns about their manufacturing (Soutter et al., 2020; Witcombe and Smith, 2014). Hence, it is highly prioritised to develop new methods to control or reduce the negative effects of *Eimeria* infections in poultry production. Strategies to explore in this work include means to aid the chicken immune response to limit parasite replication by immunomodulatory compounds or to maintain and restore the gut microbiota to limit the disturbances caused by parasite infection. There are also reports that the caecal microbiota contributes to the inflammatory response and caecal pathology in *E. tenella* infection (Tomal et al., 2023a; Tomal et al., 2023b). Thus, modulation of microbiota may have additional beneficial effects in *Eimeria* control.

A substance with both prebiotic and bioactive properties is laminarin, a soluble β-glucan storage-polysaccharide found in marine brown algae (Karuppusamy et al., 2022). In mammals, it has been shown that laminarin is recognised by innate immune receptors such as Dectin-1, which in turn elicits immunomodulatory effects on both innate and adaptive immunity (Bonfim-Mendonça et al., 2017; Goodridge et al., 2009). In chickens, the receptor/s recognising laminarin have not yet been identified but immune modulatory effects have nonetheless been observed upon in-feed addition of laminarin to chickens, for example as increased intestinal mRNA expression of interleukin-17A, tumor necrosis factor-α, and Toll-like receptors 2 and 4 (Sweeney et al., 2017; Venardou et al., 2021) as well as altered composition of circulating T-cell populations (Ivarsson et al., submitted). The prebiotic effects are well studied in mammals and include stimulation of mainly *Bacteroides* sp. (Gotteland et al., 2020). In chickens, an increase of *Bifidobacterium* spp. in the caecal microbiota was observed in response to laminarin supplementation (Venardou et al., 2021). In addition, more general beneficial effects such as increased expression of genes involved in nutrient absorption and intestinal integrity (Venardou et al., 2021), as well as positive effects on growth rate and small intestine architecture and increased feed intake (Sweeney et al., 2017) have been observed in broiler chickens provided a laminarin-rich extract.

Taken together, laminarin as feed supplement induce several effects that in theory seem beneficial for chickens during *Eimeria* infections. Laminarin may boost the chicken immune responses to combat the parasite and/or ameliorate harmful secondary effects of Eimeria infection such as dysbiosis or tissue damage. The aim of the present study was to further investigate the potential of laminarin as an immunostimulatory and microbiota-regulatory compound in broiler chickens when infected with *E. tenella*. Therefore, the effects of a laminarin-rich algal extract (AE) on parasite replication, caecal microbiota composition, leukocyte counts, parasite specific immune responses and growth rate after *E. tenella* infection of broiler chickens were studied.

2. Materials and methods

2.1. Chickens, housing, and experimental design

The experiment comprised 64 mixed sex Ross 308 broiler hybrid chickens hatched at a commercial hatchery and reared under SPF-conditions at the animal facilities at the Swedish Veterinary Agency, Uppsala, Sweden from day-old (<72 h from hatch). Chickens were group housed with feed and water *ad libitum* in cages with chopped straw bedding in rooms under negative pressure ventilation. After *E. tenella* infection, infected and uninfected chickens were housed in separate rooms.

At arrival at the animal facilities chickens were randomly allocated to feed groups, control or AE, n = 32 for each group (Supplementary material Table 1). At eight days of age, experimental day – 11, chickens were individually weighed and identified and subsequently allocated to four sampling groups (n = 8/group) within each feed group to achieve an equal mean weight. At 19 days of age, experimental day 0, chickens from two sampling groups from each feed group were inoculated orally with 1000 live *E. tenella* oocysts per bird. Chickens were monitored for clinical signs of disease daily. Blood samples were collected from the jugular vein and chickens were individually weighed at several occasions throughout the experiment (Supplementary material Table 1). On experimental days 7 and 10, respectively, chickens one sampling group of each infected or uninfected feed group were sacrificed by cervical dislocation.

2.2. Feed

The experimental diets were a control diet and a diet with inclusion of 0.075 % AE that provided 300 ppm laminarin. Both diets were pelleted and formulated (Supplementary material table 2) according to nutritional requirements of ROSS 308 (Aviagen, 2019). The algal extract was from *Saccharina latissima* cultivated at sea on longlines in the Koster archipelago outside Tjärnö marine laboratory on the Swedish West Coast (Thomas et al., 2022) and was obtained as described in Ivarsson et al., (2023). The laminarin content of the algal extract was determined enzymatically by measuring of the β 1,3/1,6-glucan content (K-YBGL 12/16, Megazyme) and was 43.5 % on DM-basis.

2.3. Experimental E. tenella infection and parasitological analyses

Sporulated *E. tenella* oocysts used for inoculation were prepared as earlier described (Wattrang et al., 2016) from a pure *E. tenella* Houghton strain (Chapman and Shirley, 2003) isolate. Viability of inoculation oocysts was estimated based on approx. 12 % of oocysts losing infectivity per month of storage in 2 % dichromate at 4 °C and the oocysts used had been stored for 5 months.

From experimental day 5 to day 9 days all faeces produced by the chickens were collected once a day and numbers of oocysts per gram faeces (OPG) were determined as described earlier (Wattrang et al., 2016; Wattrang et al., 2019). From chickens sacrificed on experimental day 7 both caeca were collected, weighed with content and cut open longitudinally with sterile scissors and a sample of content (pooled from both caeca) was collected and stored at – 80 °C for microbiota DNA extraction. The remaining caecal contents were carefully collected into M/15 phosphate buffer with 0.02 % EDTA, pH 7.5 (National Veterinary Institute) and lesion scoring according to Johnson and Reid (1970) was performed with the slight modification that half scores were given when lesions were judged in-between two defined scores. The caecal mucosa, down to the serosa layer from both caeca was collected using a scalpel and added to the caecal contents and homogenised with a food blender for 10–20 sec. Trypsin (#11564416, Gibco, Thermo Fisher Scientific) to a final concentration of 1.5 % (w/v) in M/15 phosphate buffer was added to the homogenate and samples were incubated at 37 °C for 30 min. Samples were subsequently filtered through double gauze and

oocysts were pelleted from the filtrate by centrifugation. Pelleted oocysts were re-suspended in water and counted in a light microscope.

2.4. DNA extraction from microbiota samples, sequencing and bioinformatic analysis

Caecal content samples collected at experimental day 7 and 10, respectively, were thawed and microbiotal DNA was isolated using the QIAamp PowerFecal Pro DNA Kit (#51804, Qiagen). The procedure followed the manufacturer's instructions with the exception that samples were homogenised on a FastPrep-24 5 G (MP Biomedicals), 30 sec at 5 m/s twice. Sequencing of the 16S rRNA gene V3-V4 region was performed on a NovaSeq instrument (Illumina) with PE250 libraries by Biomarker Technologies (BMKGENE) GmbH (Münster, Germany).

The reads were processed using the Nephele platform (v2.25.4) The quality trimming used settings for paired end reads with a sliding window of size 4, quality cut-off of 12 and a minimum length of 30. The DADA2 (v1.18) amplicon pipeline was run with default settings for chimera removal, merging paired reads and taxonomic assignment with database SILVA (v138.1). Nephele's downstream diversity pipeline was run with a sampling depth of 34623, corresponding to the minimum number of counts found with DADA2.

For the statistical analysis, R (v4.2.2) was used and the DADA2 outputs were loaded into phyloseq (v1.42.0) to investigate abundance, through bar plots of relative abundance, alpha diversity using Chao1 and Shannon diversity measures, and to carry out a principal coordinate analysis with weighted UniFrac distance matrix. The data was further loaded into DEseq2 to investigate differences in the abundance between conditions by creating pair wise contrasts, a significance threshold of 0.01 was used for the adjusted p-value. Additionally, correlation testing was carried out between the normalized counts from DEseq2 and each sample condition using Pearson and Spearman methods.

Code used found on: https://github.com/AndraAlma/micro

2.5. Blood leukocyte counts

Absolute counts of heterophilic granulocytes, monocytes, thrombocytes, lymphocytes and some subpopulations of lymphocytes (B-cells, CD4+CD8-, CD4+CD8αα+, CD4-CD8αβ+, CD4-CD8αα+, TCRγ/δ+CD8-, TCRαα+CD8αβ+, and TCRαα+CD8αα+ cells) were determined in EDTA stabilised blood samples collected on experimental days −11, −5, 2 and 4 using a previously described no-lyse, no-wash flow cytometry-based method (Wattrang et al., 2020). Two panels of monoclonal antibodies were used in this analysis (Table 1) and the gating strategies are described in Supplementary material Figs. 3 and 4. Samples were recorded for 1 min reduced flow rate (corresponding to approximately 11,000 events in the CD45+ gate in panel 1 and approximately 3000 events in the lymphocyte gate in panel 2, respectively; Supplementary material Figs. 3 and 4) in a BD FACSVerse™ Flow cytometer (BD Biosciences) equipped with 488 nm blue, 633 nm red and 405 nm violet lasers. Results were analysed using the FACSDiva software version 9.0 (BD Biosciences).

2.6. In vitro induction of E. tenella-specific IFN-γ production and T-cell activation

On experimental day 8 blood was collected from the jugular vein into sterile heparinised blood collection tubes (#368494, BD Vacutainer ®, BD Life Sciences). At sacrifice of chickens on experimental day 10 spleens were collected into test tubes with sterile phosphate buffered saline (PBS; without Ca^{2+} and Mg^{2+} at pH 7) and stored on ice. From these samples PBMC and spleen cells, respectively, were purified by Ficoll-Paque PLUS (GE Healthcare Life Sciences) gradient centrifugation as previously described (Wattrang et al., 2023). Both spleen cells and PBMC were suspended in growth medium; RPMI 1640 (HyClone, Cytiva) supplemented with 200 IU penicillin/ml, 100 μg streptomycin/ml, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol and 5 % foetal calf serum (FCS; Gibco® #10082147, ThermoFisher Scientific). The final mononuclear cell concentration was adjusted to 10^7 live cells /ml.

For IFN-γ induction, 100 μl of cell suspension and 100 μl of growth medium alone, or growth medium supplemented with sonicated *E. tenella* antigen (Wattrang et al., 2019) at a final concentration of 5 μg/ml were added per well to quadruplicate (PBMC) or triplicate (spleen cells) wells in ELISpot plates (ELISpot Plus: Chicken IFN-γ (ALP) kit, #3125–4APW, Mabtech) prepared according to the manufacturer's instructions. Plates were incubated for 18 h at 40 C, 5 % CO_2 in a humid atmosphere where after spots were visualised according to kit instructions. Plates were scanned in an iSpot Spectrum Reader ELR078IFL (Autoimmune Diagnostika GmbH, Strassberg, Germany) and spots were

Table 1
Monoclonal antibodies used for immunolabelling and combinations (panels) used to identify leukocyte populations in whole blood (panels 1 and 2) and phenotype activated chicken T-cells in culture (panel 3).

				Panel		
Abbreviation	Clone	Specificity	Fluorochrome	1	2	3
CD45-PerCp/Cy5.5	UM16–6[a]	Chicken CD45, all isoforms (Huhle et al., 2017)	Peridinin chlorophyll-cyanine 5.5[c]	X	-	X
CD41/61-FITC	11C3[a]	Chicken integrin CD41/61 (GPIIb-IIIa)	Fluorescein[d]	X	X	-
MRC1L-B-AF647	KUL01[b]	Chicken mannose receptor MRC1L-B (Staines et al., 2014)	Alexa Fluor 647[d]	X	-	-
Bu1-PACBLU	AV20[b]	Chicken Bu1 (ChB6)	Pacific Blue™[d]	X	-	-
MHCII-RPE	2G11[b]	Major histocompatibility complex class II	R-phycoerythrin[d]	X		
TCRγ/δ- PerCp/Cy5.5	TCR−1[b]	Chicken γ/δ T-cell receptor	Peridinin chlorophyll-cyanine 5.5[c]	-	X	-
CD8β-RPE	EP42[b]	β-chain of chicken CD8	R-phycoerythrin[d]	-	X	-
CD8α-Cy5	3–298[b]	α-chain of chicken CD8	Cyanine 5[d]	-	X	X
CD4-PACBLU	CT−4[b]	Chicken CD4	Pacific Blue™[d]	-	X	X
CD25-PE/Cy7	AV142[a]	Chicken CD25, interleukin−2 receptor α-chain	R-phycoerythrin-cyanine 7[c]	-	-	X
TCRγ/δ-Fitc	TCR−1[b]	Chicken γ/δ T-cell receptor	Fluorescein[d]	-	-	X
TCRα/Vβ$_1$-RPE*	TCR−2[b]	Chicken α/Vβ$_1$ T-cell receptor	R-phycoerythrin[d]	-	-	X
TCRα/Vβ$_2$-RPE*	TCR−3[b]	Chicken α/Vβ$_2$ T-cell receptor	R-phycoerythrin[d]	-	-	X
CD8β-APC/Cy7	EP42[b]	β-chain of chicken CD8	Allophycocyanin-cyanine 7[c]	-	-	X

X: used in panel, -:not used in panel
* TCRα/Vβ$_1$-RPE and TCRα/Vβ$_2$-RPE were analysed together as TCRα/β+ cells.
a Purchased from Bio-Rad Antibodies.
b Purchased from SouthernBiotech.
c Fluorochrome conjugated using Lightning-Link® conjugation kits (abcam) according to the manufacturer's protocol.
d Fluorochrome conjugated by manufacturer.

enumerated using the software AID EliSpot Software version 7.0. The mean number of IFN-γ producing cells (spots) were determined for each multiplicate of wells and the net number of *E. tenella* antigen induced IFN-γ producing cells (the mean number of IFN-γ producing cells in *E. tenella* antigen stimulated cultures - the mean number of IFN-γ producing cells in medium cultures) for each individual chicken was calculated.

Induction of blast transformation and CD25 expression was performed Induction of blast transformation and CD25 expression was performed (Wattrang et al., 2023) and analysed by flow cytometry as previously described (Wattrang et al., 2015; Wattrang et al., 2023) with the instrument and software described in Section 2.5. For the current assay sonicated *E. tenella* antigen (Wattrang et al., 2019) at a final concentration of 5 μg/ml, or murine monoclonal antibody to chicken CD3 (anti-CD3; clone CT-3, #8200, SouthernBiotech) at 0.1 μg/ml were used as inducers and the cultured mononuclear cells were labelled with monoclonal antibody panel 3 (Table 1) and LIVE/DEAD® fixable Aqua dead stain (#L34957, ThermoFisher Scientific). The gating strategy was previously described (Wattrang et al., 2023) and is shown in Supplementary material Fig. 5. Results for *E. tenella* induced blast transformation and CD25 expression were calculated as net proportions (the proportion of blasts/CD25 + cells in *E. tenella* antigen stimulated cultures - the proportion of blasts/CD25 + cells in medium cultures). Because the net proportions were non-normally distributed, and therefore 95 % confidence intervals (CI) of these were asymmetrical, the arithmetic means and CI were estimated based on arcsin (square-root (x/100)) transformation implementing the method of Land (1974).

2.7. Statistical analysis

All data were tested for normality and homoscedasticity by use of diagnostic plots of residuals in the statistical software SAS (version 9.4). The leukocyte population data was log transformed before analysis. Leukocyte population, lesion score, net numbers of *E. tenella* induced IFN-γ producing cells and growth performance data after infection were analysed with Mixed procedure with feed treatment and infection as fixed factors. The interaction feed treatment × infection was also included in the model, and the weight at infection day zero was included in the model for the performance data and the respective value for the day −5 of leukocyte populations was included in the model for the leukocyte population data. Cage was included as a random factor in the model. The p-values were Tukey Kramer adjusted for pair wise comparisons. The data before infection was analysed with GLM procedure with feed treatment as fixed factor. The results from the statistical analysis are presented in (Supplementary material table 6).

2.8. Nucleotide sequence data

Nucleotide sequence data reported in this paper are available in the ENA database under project accession number **PRJEB65461**.

3. Results

3.1. Growth and leukocyte counts prior to the E. tenella infection

Chickens were weighed and blood samples for absolute counts of different leukocyte populations were collected on experimental days −11 (8 days of age) and −5 (14 days of age) before the *E. tenella* infection. During this time period no differences betweens the two feed groups were observed for body weights or daily weight gain (DWG; Supplementary material figure 7). For leukocyte counts in general, heterophils, total population of lymphocytes and lymphocyte subpopulations showed increasing numbers while monocytes and thrombocytes showed similar numbers during this period (Figs. 1 and 2, Supplementary material figure 8). Statistically significant differences between the feed groups were observed mainly for lymphocyte numbers at these time points. Total numbers of lymphocytes were higher for chickens in the AE feed groups compared to those for chickens in control feed groups at day −5 and this difference could be pinpointed to higher numbers of B-cells ($p = 0.002$; Fig. 1F), CD4-CD8αβ+ T-cells ($p = 0.02$; Fig. 2B) and TCRγ/δ+CD8- T-cells ($p = 0.05$; Fig. 2C). For CD4 +CD8αα+ T-cells (Supplementary material figure 8) chickens in the AE feed groups showed lower number compared to the control feed groups on both days −11 ($p = 0.02$) and −5 ($p = 0.05$). In addition, at day −11 chickens in the AE feed groups had higher thrombocyte counts ($p = 0.003$, Fig. 1B) compared to chickens in the control feed groups. Moreover, expression levels of the mannose receptor MRC1L-B on monocytes were statistically significantly higher on monocytes from chickens in the control feed group on day −11 ($p = 0.001$) and tended to be higher also on day −5 ($p = 0.06$) compared to chickens in the AE feed groups (Fig. 1D).

3.2. Clinical and parasitological outcome of the E. tenella infection

Half of the chickens in each feed treatment were infected with *E. tenella* oocysts on experimental day 0. Chickens were weighed and monitored for clinical signs of disease daily after the infection. On day 6 and 7 after infection small amounts of blood were observed in faeces from the infected chickens in both feed groups but not in faeces from uninfected chickens. No other clinical signs of disease were observed. For growth rates, results showed that the daily weight gain was statistically significantly reduced for uninfected chickens between days 3 and 4 ($p = 0.02$) and increased between days 4 and 5 ($p = 0.002$) after infection compared to that for infected chickens (Supplementary material figure 7 and supplementary material table 6). The body weights of infected chickens were statistically significantly lower on day 5 ($p = 0.009$) after infection compared to those of uninfected chickens. No significant effects of feed treatment on body weight or growth were observed.

Oocyst excretion was monitored in faeces collected from day 5 to day 9 after infection (Fig. 3 AB). Results showed that a low amount of oocyst were detected in faeces collected on day 6, subsequently oocyst excretion peaked at day 7 and thereafter progressively decreased. Both the kinetics of oocyst excretion and total amounts of oocysts excreted were similar for chickens in both feed groups. No oocysts were detected in faeces from uninfected chickens.

On day 7 after infection half of the chickens in both uninfected and infected feed groups were sacrificed and caeca were collected for lesion scoring and oocyst quantification (Fig. 3 CDE). Lesion scores were statistically significantly higher for infected chickens compared to uninfected chickens ($p < 0.0001$) and were on average slightly lower for infected chickens in the AE feed group compared to those in the infected control feed group, but this difference was not statistically significant (2.38 vs. 3.00, SEM 0.207; $p = 0.14$). Numbers of oocysts in caeca, both expressed as oocysts per bird and oocysts per g caeca, did not differ significantly between the two feed groups. No oocysts were detected in the caeca from uninfected chickens.

3.3. Leukocyte counts after E. tenella infection

Blood leukocyte counts were monitored on days 2 and 4 after the *E. tenella* infection (Figs. 1, 2 and supplementary material figure 8). Results showed that the numbers of circulating heterophils were statistically significantly increased for infected chickens ($p = 0.0002$) compared to uninfected chickens on day 2 after infection (Fig. 1A). On this day chickens in the AE feed groups also tended to have higher heterophil numbers ($p = 0.05$) compared to chickens in the control feed groups but interaction between infection and feed treatment was not statistically significant ($p = 0.98$). For the total population of lymphocytes as well as most lymphocyte subpopulations in general, numbers continued to increase during this period except for TCRγ/δ+CD8αβ and TCRγ/δ+CD8αα T-cells that showed progressively decreasing numbers. On day 4 after infection numbers of CD4-CD8αβ+ T-cells were

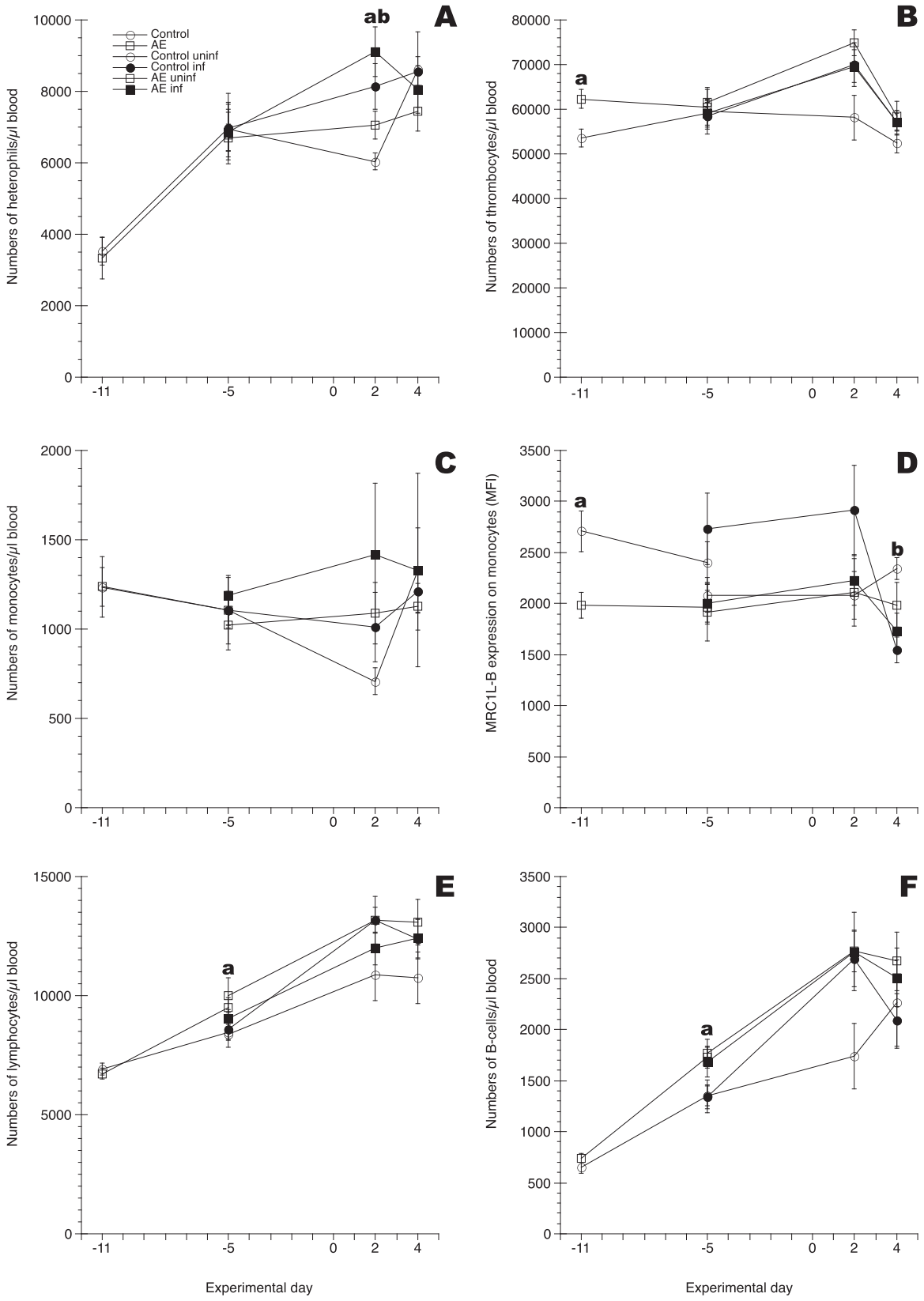

Fig. 1. Total numbers of A) heterophils, B) thrombocytes, C) monocytes, E) lymphocytes and F) B-cells in blood and D) MRC1L-B expression on monocytes. Blood was collected from chickens fed the control diet (control; circles) or the AE supplement (AE; squares) on the indicated experimental days. On experimental day 0, chickens in each feed group were either infected with 1000 live *E. tenella* oocysts (inf; filled symbols) or left uninfected (uninf; open symbols). Results are shown as group mean values± 1SE for the two feed groups (n = 32) on experimental days −11 and −5, and as group mean values± 1SE for the four treatment groups on days −5 (n = 16), 2 and 4 (n = 8). For details see Materials and Methods and Supplementary material Table 1. Letters above symbols indicate results where statistically significant differences ($p \leq 0.05$) between **a**) feed treatments and **b**) infected and uninfected chickens, respectively, were observed (full statistical analysis in Supplementary material table 6).

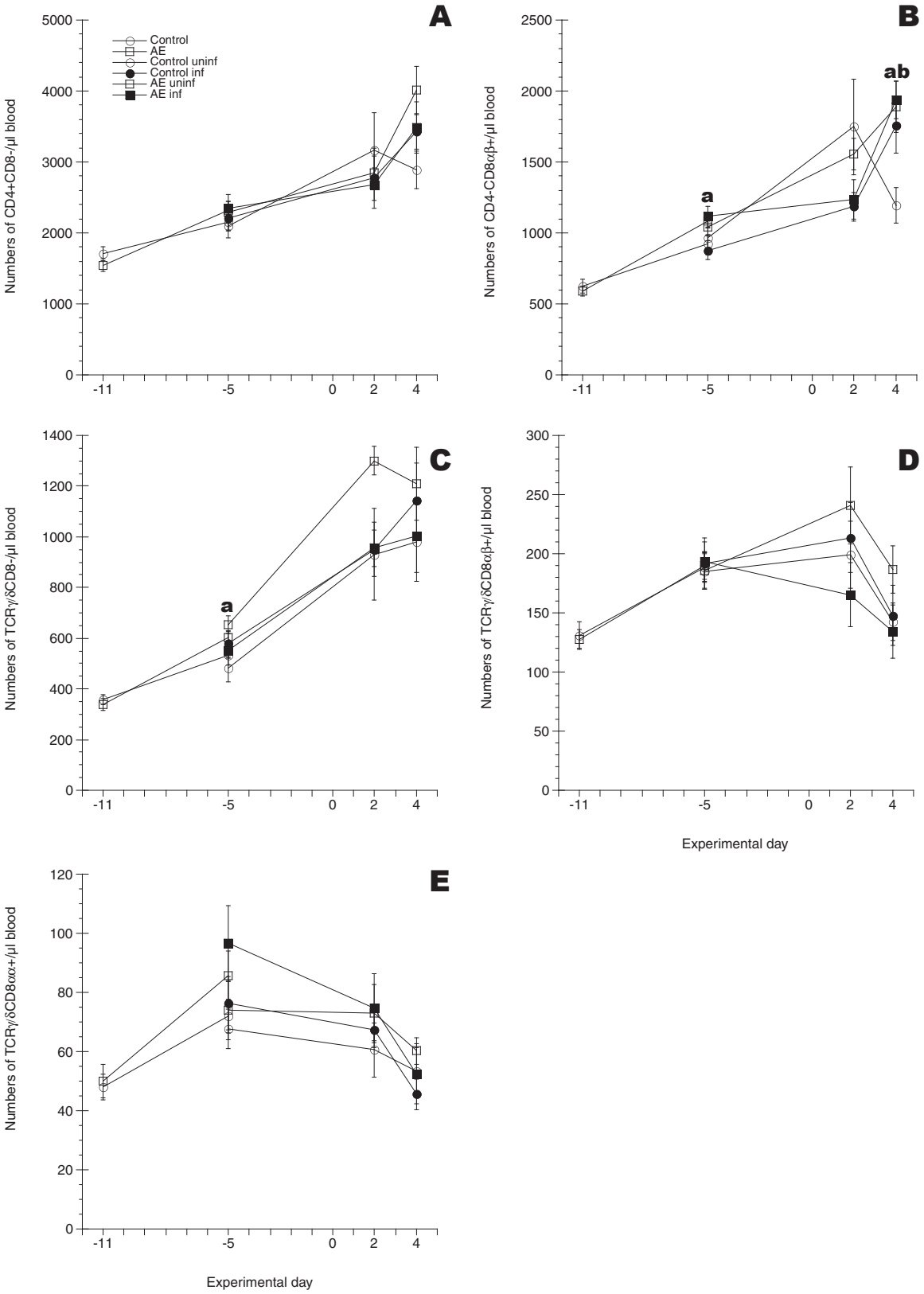

Fig. 2. Total numbers of A) CD4 +CD8-, B) CD4-CD8αβ+ , C) TCRγ/δ+CD8-, D) TCRγ/δ+CD8αβ+ and E) TCRγ/δ+CD8αα+ cells in blood. Blood was collected from chickens fed the control diet (control; circles) or the AE supplement (AE; squares) on the indicated experimental days. On experimental day 0, chickens in each feed group were either infected with 1000 live *E. tenella* oocysts (inf; filled symbols) or left uninfected (uninf; open symbols). Results are shown as group mean values ± 1SE for the two feed groups (n = 32) on experimental days −11 and −5, and as group mean values± 1SE for the four treatment groups on days −5 (n = 16), 2 and 4 (n = 8). For details see Materials and Methods and Supplementary material Table 1. Letters above symbols indicate results where statistically significant differences ($p \leq 0.05$) between **a**) feed treatments and **b**) infected and uninfected chickens, respectively, were observed (full statistical analysis in Supplementary material table 6).

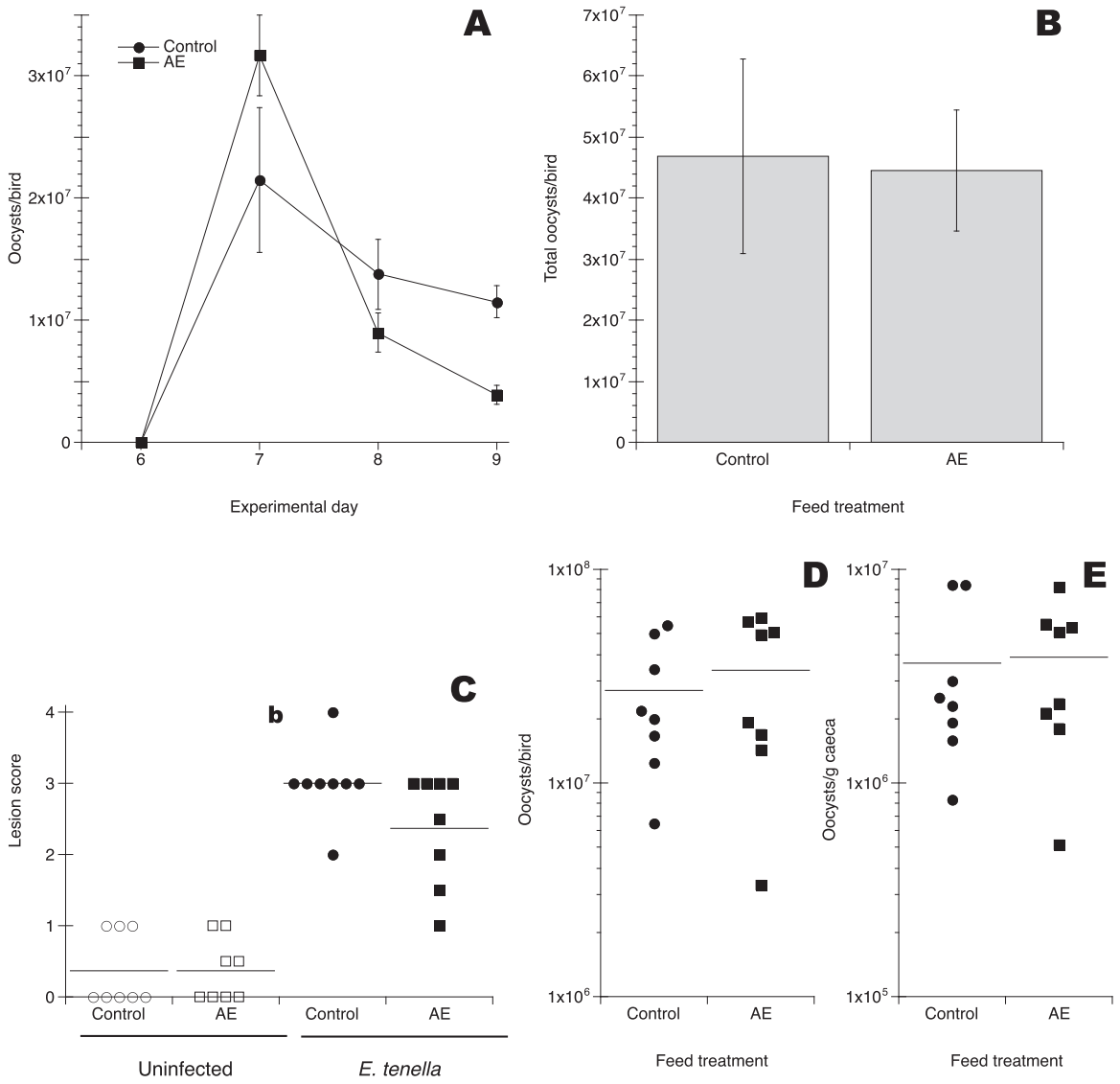

Fig. 3. A and B) Excretion of oocysts in faeces collected from experimental day 5 to day 9 after infection of chickens with 1000 live *E. tenella* oocysts on experimental day 0. A) Kinetics of oocyst excretion from day 5 to day 9 after infection in chickens fed the control diet (control; filled circles) or the AE supplement (AE; filled squares). Results are group mean values± 1SE (n = 6 replicate sample preparations) of oocysts excreted per bird from faeces collected for 24 h intervals. B) The total number of oocysts excreted per bird from day 5 to day 9 after infection. Results are mean values ± 95 % CI (n = 6 replicate sample preparations) for chickens fed the control diet (control) and chickens fed the AE supplement (AE). C) Caecal lesion scores for chickens sacrificed on experimental day 7. Chickens were fed the control diet (control; circles) or the AE supplement (AE; squares) and were either uninfected (open symbols) or infected with 1000 live *E. tenella* oocysts (*E. tenella*; filled symbols) on experimental day 0. Results are shown as individual values with a line indicating the group mean value. D and E) Oocysts in caeca collected on experimental day 7 from chickens infected with 1000 live *E. tenella* oocysts on experimental day 0. Chickens were fed the control diet (control; circles) or the AE supplement (AE; squares). Results are expressed as D) numbers of oocysts/bird or E) numbers of oocysts/g caeca and are shown as individual values with a line indicating the group mean value. For details see Materials and Methods. The letter **b** in panel C indicates a statistically significant difference ($p < 0.0001$) between results from uninfected and *E. tenella* infected chickens (full statistical analysis in Supplementary material table 6).

statistically significantly increased for infected chickens ($p = 0.04$) and chickens in the AE feed groups ($p = 0.02$) compared to those of uninfected and chickens in control feed, respectively (Fig. 2B), but no statistically significant interaction between infection and feed was observed ($p = 0.2$). Otherwise no statistically significant effects of the *E. tenella* infection or feed treatments were observed for numbers of lymphocyte subpopulations in the circulation. Circulating numbers of thrombocytes and monocytes seemed not to be influenced by the infection or the feed treatment. However, the expression of MRC1L-B on monocytes (Fig. 1D) was statistically significantly decreased ($p = 0.03$) for infected chickens and the interaction between infection and feed tended ($p = 0.05$) to be statistically significant at day 4 after the *E. tenella* infection.

3.4. Caecal microbiota after E. tenella infection

Samples of caecal content collected on experimental days 7 and 10 were subjected to 16 s rRNA community profiling. This resulted in between 79,000–195,000 reads per sample, with an average of 91,500, after filtering and trimming the number of reads per sample was between 34,000 and 96,000 with an average of 45,800. The taxonomic assignment showed 6544 observed taxa. The principal coordinate analysis (Fig. 4) showed clear differences between the infected and uninfected groups, whereas there were no major differences between the feed treatments or the sampling days (Supplementary material figure 9). The relative abundance of each taxon (order level), similarly to the principal coordinate analysis, showed clear differences between infected

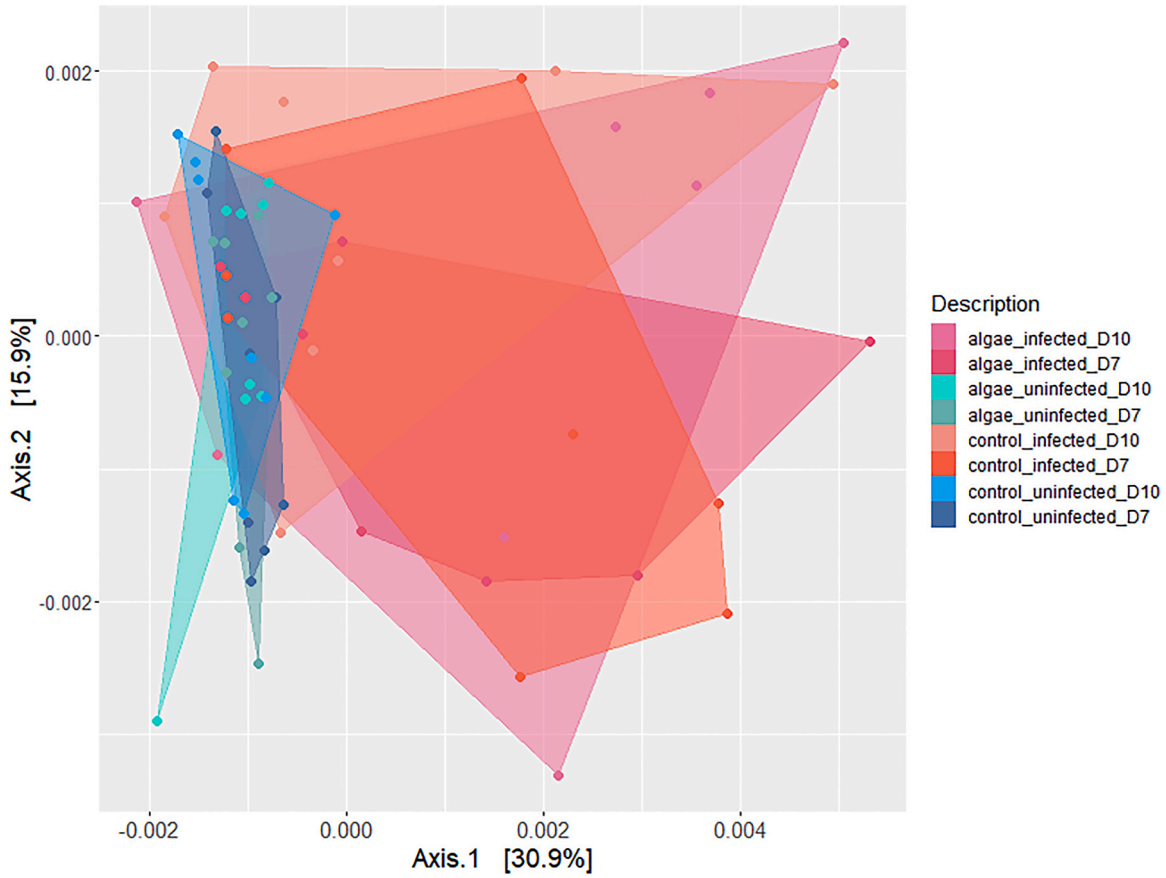

Fig. 4. Principal coordinate analysis based on unweighted UniFrac distance matrix between caecal microbiota of chickens infected with 1000 live *E. tenella* oocysts (infected) on experimental day 0 and caecal microbiota of uninfected chickens, fed control diet (control) or AE supplement (algae), sacrificed on experimental day 7 (D7) or day 10 (D10).

and uninfected groups, with Enterobacterales being more and Lactobacillales less abundant in infected samples (Fig. 5). For all conditions, Lachnospirales, Lactobacillales and Oscillospirales made up the majority of each sample. Comparison of abundance between conditions within families (Supplementary material figure 10) showed increases of approximately 3 LogFC of *Enterobacteriaceae* and 6 LogFC of (Oscillospirales) UCG-010 and an approximately 20 LogFC decrease of *Bifidobacteriaceae* in caecal microbiota from *E. tenella* infected chickens compared to that of uninfected chickens fed the control diet on both days 7 and 10 after infection. A similar pattern with increases of *Enterobacteriaceae* and (Oscillospirales) UCG-010 was observed for *E. tenella* infected chickens fed AE. In contrast, approximately 18 and 23 logFC, higher abundance of *Bifidobacteriaceae*, day 7 and day 10, respectively, in the caecal microbiota were observed for *E. tenella* infected chickens fed AE compared to infected chickens fed the control diet. In addition, on day 10 after infection an approximately 23 LogFC increase in *Clostridiaceae* was observed in caecal microbiota from *E. tenella* infected chickens compared to that of uninfected chickens fed the control diet. A lower, 5 LogFC, increase in *Clostridiaceae* was observed for *E. tenella* infected chickens fed AE compared to uninfected chickens fed AE and *E. tenella* infected chickens fed AE had approximately 2 LogFC lower *Clostridiaceae* compared to infected chickens fed the control diet. On day 10, higher *Clostridiaceae*, 16 logFC, were also observed for uninfected chickens fed AE compared to uninfected chickens fed the control diet.

Correlation analysis of the severity of ceacal lesion scores with caecal microbiota (family level) in *E. tenella* infected chickens on day 7 after infection (Supplementary material figure 11) showed that (Oscillospirales) UCG-101, *Anaerovoracaceae*, *Eggerhellaceae*, and *Acholeplasmatacea* showed a strong ($\geq$0.6) positive correlation and *Lactobacillaceae* a strong ($\leq$-0.6) negative correlation to higher lesion scores in chickens fed the control diet. In chickens fed AE *Enterobacteriaceae* showed a strong ($\geq$0.6) positive correlation to higher caecal lesion scores.

3.5. *In vitro induction of E. tenella-specific IFN-γ production and T-cell activation*

PBMC and spleen cells were purified from samples collected on experimental days 8 and 10, respectively, and IFN-γ producing cells were enumerated using ELISpot methodology after 18 h of culture in medium with *E. tenella* antigen or in medium alone. Spontaneous/"background" IFN-γ producing cells were detected in cultures incubated in medium alone, approximately 2 cells per well (means range from 1 to 6 cells/well for individual chickens) for PBMC cultures and approximately 200 cells per well (mean range from 1 to 1215 cells/well for individual chickens) for spleen cell cultures. The net number of IFN-γ producing cells in *E. tenella* antigen stimulated cultures (Fig. 6 AB) were statistically significantly higher for infected chickens compared to uninfected chickens ($p = 0.0005$ for PBMC; $p < 0.0001$ for spleen cells) while no significant effects of feed treatment ($p = 0.08$ for PBMC; $p = 0.3$ for spleen cells) or interactions between feed and infection were observed ($p = 0.2$ for PBMC; $p = 0.7$ for spleen cells).

Spleen cells purified from samples collected on experimental day 10 were also assessed for antigen specific activation of T-cell populations after 72 h of culture in medium with *E. tenella* antigen, in medium with positive control (anti-CD3) or in medium alone. In total eight different T-cell populations were identified: TCRα/β+ and TCRγ/δ+ , respectively analysed collectively, with TCRα/β+ cells further typed as CD4 +CD8-, CD4 +CD8αα+ and CD4-CD8aβ+ , and TCRγ/δ+ cells further typed as TCRγ/δ+CD8αβ+ , TCRγδ+CD8αα+ and TCRγδ+CD8-, that were

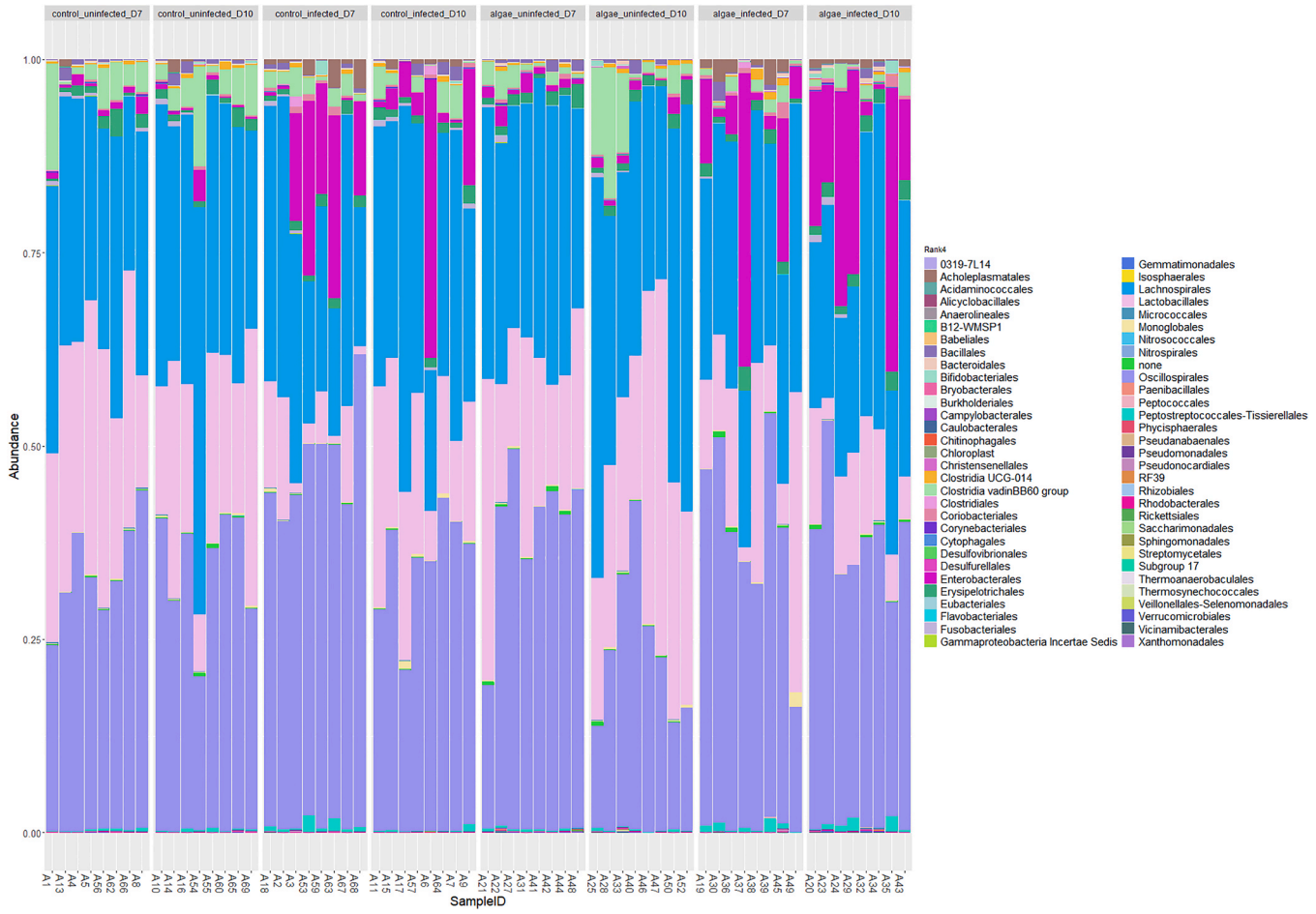

Fig. 5. Bar chart showing relative abundance (%) of bacterial orders in samples of caecal content from each chicken in the groups fed the control diet (control), in the groups fed AE supplement (algae), either uninfected (uninfected) or infected with 1000 live *E. tenella* oocysts on experimental day 0 (infected), sacrificed on experimental day 7 (D7) or day 10 (D10).

characterised with respect to blast transformation (high FSC and SSC) and expression of CD25 (Supplementary material Fig. 5). The proportions of the different T-cell populations out of the CD45 + cells in the spleen cell cultures from chickens in the different treatment groups is shown in Supplementary material figure 12. Results showed that overall TCRα/β+ cells dominated the T-cells in these cultures and among these CD4-CD8αβ+ and CD4 +CD8- cells were most common. Among TCRγ/δ+ cells, the TCRγδ+CD8αβ+ population was predominant. Stimulation with anti-CD3 seemed not to alter the proportion of CD4 +CD8- cells but generally seemed to increase the proportions of total TCRα/β+ cells and CD4 +CD8αα+ cells and decrease the proportions of all other T-cell populations in the cultures compared to *E. tenella* stimulated cultures and cultures in medium alone. No major alterations of the T-cell populations were observed after stimulation with *E. tenella* antigen compared to culture in medium alone. Some effects of the feed treatment and the *E. tenella* infection on proportions of the different T-cell populations were observed (supplementary figure 12, and supplementary material table 6). The most striking of these were for CD4-CD8αβ+ cells, in both medium and *E. tenella* antigen stimulated cultures where higher ($p < 0.05$) proportions were observed in cultures from chickens in AE feed groups compared to those from chickens in control feed groups and lower ($p < 0.05$) proportions were observed in cultures from chickens in the infected groups compared to those from chickens in the uninfected groups. Medium cultures from chickens in AE feed groups also had lower ($p = 0.02$) proportions of CD4 +CD8αα+ cells compared to cultures from chickens in control feed groups and *E. tenella* antigen stimulated cultures from chickens in the control feed groups had lower ($p = 0.01$) proportions of total TCRα/β+ cells compared to cultures from AE feed groups. Moreover, *E. tenella* antigen stimulated cultures from chickens in the infected groups had higher ($p < 0.05$) proportions of total TCRγ/δ+ , TCRγδ+CD8-, TCRγδ+CD8αα+ and CD4 +CD8αα+ cells compared to cultures from chickens in the uninfected groups.

The proportion of spontaneously blast transformed cells after 72 h of culture in growth medium alone varied between the different T-cell populations studied (Supplementary material figure 13A), where CD4+CD8αα+ and CD4+CD8-cells showed the highest and CD4-CD8aβ+ cells the lowest spontaneous blast transformation. In general, cultures from chickens in the AE feed groups tended to have lower proportions of spontaneous blast transformation among all T-cell populations compared to cultures from chickens in control feed groups and this was statistically significantly ($p < 0.05$) lower for total TCRα/β+ and CD4-CD8aβ+ cells. T-cells in cultures from chickens in infected groups tended to have higher spontaneous blast transformation compared to those from chickens in uninfected groups and this was statistically significantly ($p < 0.05$) higher for total TCRα/β+ and CD4-CD8aβ+ cells and for TCRα/β+ cells an interaction ($p < 0.05$) between feed and infection was observed.

For the *E. tenella* induced blast transformation, both TCRα/β+ and TCRγ/δ+ T-cells showed increased blast transformation in cultures from *E. tenella* infected chickens compared to those from uninfected chickens, except for TCRγδ+CD8αα+ cells that did not show any antigen specific *E. tenella* induced blast transformation (Fig. 6C-E). The antigen specific *E. tenella* induced blast transformation was statistically significant ($p \leq 0.01$) for cells from chickens in both feed groups, except for

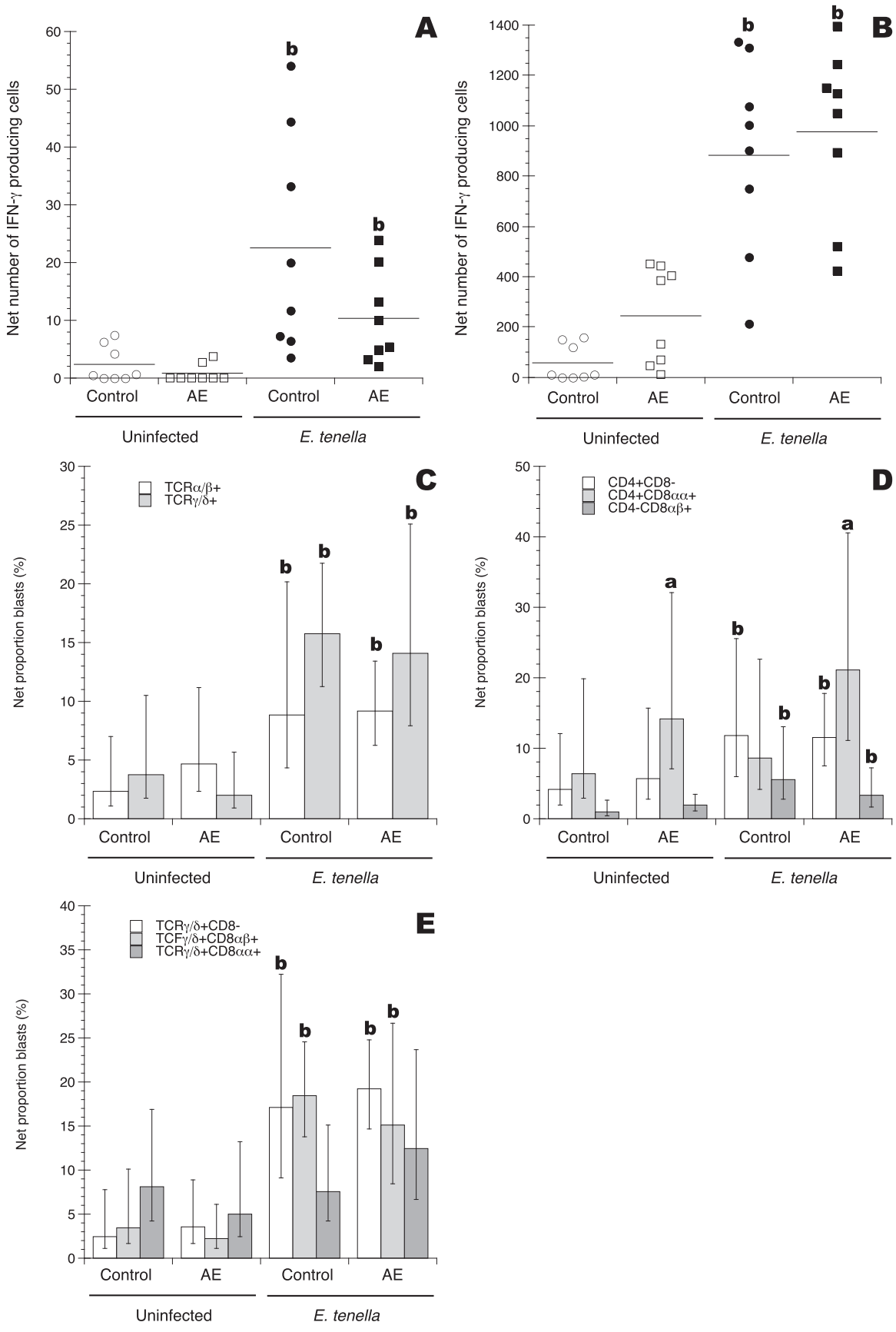

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Fig. 6. A and B) Net numbers of IFN-γ producing cells in cultures of A) PBMC, collected on experimental day 8, or B) spleen cells, collected on experimental day 10, stimulated for 18 h with *E. tenella* antigen. Cells were obtained from chickens fed the control diet (control; circles) or the AE supplement (AE; squares) and were either uninfected (open symbols) or infected with 1000 live *E. tenella* oocysts (*E. tenella*; filled symbols) on experimental day 0. Results are shown as individual values with a line indicating the group mean value. C, D and E) Net proportions of blast transformed cells in different T-cell populations upon *in vitro E. tenella*-antigen stimulation for 72 h in cultures of spleen cells obtained from uninfected or *E. tenella* infected (*E. tenella*) chickens on experimental day 10. Chickens were fed either the control diet (control) or the AE supplement (AE) and infected chickens were inoculated with 1000 live oocysts on day 0. C) TCRα/β+ (open bars) and TCRγ/δ+ (light grey bars) cells, D) TCRα/β+ sub populations: CD4 +CD8- (open bars), CD4 +CD8αα+ (light grey bars) and CD4-CD8αβ+ (dark grey bars), and E) TCRγ/δ+ subpopulations: TCRγ/δ+ CD8- (open bars), TCRγ/δ+CD8αβ+ (light grey bars) and TCRγ/δ+CD8αα+ (dark grey bars) in cultures of spleen cells. Values are group means ± 95 % CI, n = 8, note that CI for net proportions are asymmetrical. For details see Materials and Methods. Letters above symbols indicate results where statistically significant higher values ($p \leq 0.05$) were observed for **a**) chickens fed AE supplement compared to control diet and **b**) *E. tenella* infected chickens compared to uninfected chickens, respectively (full statistical analysis in Supplementary material table 6).

CD4 +CD8αα+ cells. Levels of *E. tenella* induced blast transformation were not influenced by the feed treatment except for CD4 +CD8αα+ cells where cells in cultures from chickens in the AE feed groups showed higher ($p = 0.04$) blast transformation compared to cells from chickens in the control feed groups.

The proportion of spontaneously CD25 expressing T-cells after 72 h of culture in growth medium alone (Supplementary material figure 13B) showed a similar pattern to the spontaneous blast transformation where CD4+CD8αα+ and CD4+CD8-cells showed the highest and CD4-CD8aβ+ cells the lowest spontaneous CD25 expression. However, the proportions of spontaneous CD25 + cells were in general higher than those for spontaneous blast transformation particularly for CD4+CD8-, TCRγδ+CD8−, and TCRγδ+CD8αα+ cells. In general, cultures from chickens in the AE feed groups tended to have lower proportions of spontaneous CD25 expression among the TCRα/β+ populations compared to cultures from chickens in control feed groups and this was statistically significantly ($p < 0.05$) lower for total TCRα/β+ and CD4-CD8aβ+ cells. TCRα/β+ cells in cultures from chickens in infected groups tended to have higher spontaneous CD25 compared to those from chickens in uninfected groups and this was statistically significantly ($p < 0.05$) higher for total TCRα/β+ , CD4 +CD8- and CD4-CD8aβ+ cells. There was no evidence of antigen specific induction of CD25 expression in any of the studied T-cell populations (Supplementary material figure 14).

The positive control for T-cell activation, anti-CD3, induced both blast transformation and CD25 expression with some variation between the different T-cell populations (Supplementary material figure 15). In general, anti-CD3 induced higher levels of CD25 expression than blast transformation. No differences between the treatment groups were observed for anti-CD3 induced blast transformation while total TCRα/β+ and CD4+CD8- cells in cultures from chickens in the infected groups showed lower ($p < 0.05$) anti-CD3 induced CD25 expression compared to those in cultures from chickens in the uninfected groups.

4. Discussion

The present study was carried out to test if in feed supplementation of laminarin rich AE could ameliorate the outcome of an *E. tenella* infection of chickens. We hypothesized that AE supplementation could alleviate disturbances to microbiota homeostasis triggered by *E. tenella* infection and/or improve chicken immune responses to the infection and thereby inhibit parasite replication more efficiently.

The present *E. tenella* infection was clearly established in the infected chickens with oocyst excretion comparable with our previous experiences with this infection model (Wattrang et al., 2023; Wattrang et al., 2016; Wattrang et al., 2019). The clinical impact of the infection on the chickens was characterised as mild, with mild clinical signs of disease, in average moderate lesion scores and no clear impact on body weight or daily weight gain. As expected, the *E. tenella* infection evoked substantial alterations of the caecal microbiota with prominent overall increases and decreased in the abundance of orders Enterobacterales and Lactobacillales, respectively. Comparisons in abundance within bacterial families also showed decreased abundance of *Bifidobacteriaceae* and increased abundance of *Clostridiaceae*. This pattern with increased abundance of *Enterobacteraceae* and *Clostridiaceae* and decreased abundance of *Lactobacillacaeae* and *Bifidobacteriaceae* in the caecal microbiota after *E. tenella* infection is consistent with previous studies of this parasite infection (Campos et al., 2022; Huang et al., 2018; Kimura et al., 1976; Macdonald et al., 2017).

Supplementation of AE to the feed of the current uninfected chickens, evoked only subtle alterations of the caecal microbiota compared to chickens fed the control diet. However, in *E. tenella* infected chickens AE supplementation increased the abundance of *Bifidobacteriaceae* and decreased the abundance of *Clostridiaceae* in the caecal microbiota compared to infected chickens fed the control diet. Hence, AE supplementation thereby reduced or even reverted some of the microbiota alterations induced by the *E. tenella* infection. In agreement with these observations, an increase of *Bifidobacterium* spp. in the caecal microbiota of healthy chickens in response to laminarin supplementation has been reported (Venardou et al., 2021). Hence, seems that the effects of laminarin on microbiota may not always be detectable in healthy chickens and may be more pronounced when the homeostasis is disturbed, such as in response to *Eimeria* infection. The mechanisms of the prebiotic effects exerted by laminarin have mainly been studied in mammals/mammalian models and comprise for instance increased formation of short chain fatty acids and preventing production of putrefactive compounds (Gotteland et al., 2020). It is thus likely that such mechanisms also were involved in the microbiota alterations observed in the current study. Although the AE supplementation did not affect the gross changes in caecal microbiota induced by *E. tenella* infection, the observed effects may nonetheless have an impact on chicken health. For example, increased susceptibility to infection with *Clostridium perfringens*, known as necrotic enteritis, is a well-known risk after *Eimeria* infection (Moore, 2016). Our results indicate that in feed inclusion of AE may reduce the expansion of *Clostridiaceae* after *Eimeria* infection, which potentially could be an aid in the prevention of necrotic enteritis. Further studies of the effects of AE on clostridial colonisation and other microbiota changes after *Eimeria* infection are thus justified.

In feed inclusion of AE clearly evoked an immune reaction in the present chickens that affected several of the immune parameters monitored in the present study, for instance the numbers of some lymphocyte populations in the circulation and in the spleen. However, despite the rather mild nature of the current *E. tenella* infection this immunomodulatory effect was not enough or not of an appropriate kind to influence immune responses to increased inhibition of parasite replication in a way that could be measured by lesion scoring and caecal oocyst burden. In comparison, in a study that reported lower lesion scores after a mixed *Eimeria* infection in chickens supplemented with yeast derived β-glucan (Cox et al., 2013), an average ceacal score of 0.6 was noted for untreated control chickens. The present *E. tenella* infection caused substantially higher scores; average score of 3. Hence, the present infection was more severe with respect to lesions, which could explain why significant reduction of lesion scores by the AE supplementation was not achieved.

The most prominent immunomodulatory effects of AE supplementation observed herein were increased numbers of TCRγ/δ+CD8-, B-cells and CD4-CD8αβ+ cells and lower numbers of CD4+CD8αα+ cells in blood and increased proportions of CD4-CD8αβ+ cells in the spleen. We

have previously noted higher numbers of TCRγ/δ+CD8- and CD4+CD8− cells and lower numbers of CD4+CD8αα+ and TCRγ/δ+CD8αβ+ cells for Ross 308 chickens when using the same level of AE in the feed (Ivarsson et al., Submitted). The observed influences on lymphocyte subpopulations could either reflect direct effects elicited by laminarin stimulation. Such effects may include innate receptor recognition by the cells in question, and/or indirect effects, through for instance cytokine production by other cells that have been activated through innate receptors. Interestingly, in both the current and our previous study (Ivarsson et al., submitted) we consistently observed increased numbers of TCRγ/δ+CD8- T-cells in the circulation of chickens fed AE. Moreover, activation of peripheral blood TCRγ/δ+ T-cells measured as increased MHCII expression was reported after a single dose of the polysaccharide ulvan to the drinking water of Ross 308 chickens (Guriec et al., 2021). Taken together, this might indicate that chicken TCRγ/δ+ T-cells could be a cell type directly activated by polysaccharide compounds such as laminarin. Our data also suggest that distinct TCRγ/δ+ subtypes respond differently to laminarin, which is consistent with evidence that CD8 expressing and CD8 negative TCRγ/δ+ subtypes may be of different lineages (Maxwell et al., 2024).

To assess the development of *E. tenella* specific T-cell responses we used *in vitro* antigen stimulation with enumeration of IFN-γ producing cells among PBMC and spleen cells as well as blast transformation and CD25 expression of T-cell subpopulations among spleen cells as readouts. By both methods parasite specific immune responses were indeed recorded upon *E. tenella* infection of chickens. However, no effects of the AE treatment were observed for the magnitude or T-cell subtype profile of these responses.

T-helper 1-type responses comprising IFN-γ producing T-cells are considered important in the development of protective immunity against *Eimeria* infections in chickens (Kim et al., 2019; Soutter et al., 2020) Early work, detecting cytokine activity after *in vitro* stimulation with sporozoite antigen using IFN and nitric oxide bioassays, showed the appearance of likely IFN-γ producing cells among PBMC one week after *E. tenella* infection of chickens (Breed et al., 1997). Using ELISpot methodology parasite specific IFN-γ producing cells were also detected upon *in vitro* oocyst antigen stimulation of PBMC collected 2 weeks after *E. tenella* infection (Yin et al., 2013). Our currents results confirm that IFN-γ producing cells can be detected in blood and also in the spleen after *E. tenella* infection. We found that the ELISpot methodology worked well with the sonicated *E. tenella* antigen that we previously have used to induce parasite specific T-cell activation (Wattrang et al., 2023; Wattrang et al., 2019) and it shows promise to be a useful tool to study immunity to *Eimeria* as well as other chicken pathogens.

Assessment of *E. tenella* specific T-cell activation as blast transformation and CD25 expression was carried out in spleen cell cultures, and we observed antigen specific blast transformation for several T-cell populations but no antigen specific induction of CD25 expression. We have previously set up and evaluated this methodology in a series of experimental *E. tenella* infections using leghorn layer type chickens (Wattrang et al., 2023). In those experiments we consistently detected antigen specific activation, both as blast transformation and CD25 expression, of CD4 +CD8-, CD4 +CD8αα+ and CD4-CD8αβ+ cells, that is, TCRα/β+ T-cells. For TCRγ/δ+ T-cells on the other hand, tendencies to antigen specific activation were observed for TCRγ/δ+CD8- and TCRγ/δ+CD8αβ+ cells but these responses did not reach statistical significance (Wattrang et al., 2023). In the present study however, the reverse situation was observed with very clear responses for TCRγ/δ+CD8- and TCRγ/δ+CD8αβ+ cells and lower responses for the TCRα/β+ T-cells. The role of TCRγ/δ+ T-cells in specific immunity is not yet fully understood but antigen specific TCRγ/δ+ T-cell responses have been identified in mammals (Baldwin and Telfer, 2015; Comeau et al., 2020) and we have earlier found evidence for *Erysipelothrix rhusiopathiae* specific responses of TCRγ/δ+CD8αβ+ cells in chickens (Wattrang et al., 2022). Hence, the present results suggest that antigen specific TCRγ/δ+ T-cells also are induced in chicken specific immunity to *Eimeria* infection. Moreover, factors such as chicken genetics, for example broiler vs. layer, may influence the magnitude of *E. tenella* recall responses of different T-cell subtypes. We also observed a higher background activation in spleen cell cultures in the present study compared to what we previously have observed with cells from layer hybrids (Wattrang et al., 2023), particularly for blast transformation of CD4 +CD8- cells and CD25 expression across T-cell subtypes. Because background responses are subtracted from responses in antigen stimulated cultures this may explain why low *E. tenella* induced CD4 +CD8- blast transformation and no *E. tenella* induced CD25 expression were recorded in the present study.

5. Conclusion

Inclusion of laminarin rich AE in the feed reduced and/or reverted some of the alterations in caecal microbiota evoked by *E. tenella* infection, for example reduced the contraction of *Bifidobacteriaceae* observed after the *E. tenella* infection. AE supplementation also elicited immunomodulatory effects on chickens, for instance by inducing alterations of lymphocyte subpopulations in blood and spleen. However, despite this the AE feed treatment did not alter the outcome of the *E. tenella* challenge with respect to parasite burden, lesions or the chickens' development of parasite specific immune responses.

Ethical standards

The experiment was approved by the Uppsala regional Ethical Committee for Animal Experiments, permit no. 5.8.18–01142/2022, and performed according to Swedish legislation and directives (SJVFS 2019:9 L 150) based on European Union legislation directive 2010/63/EU.

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CRediT authorship contribution statement

Robert Söderlund: Writing – review & editing, Visualization, Supervision, Formal analysis. **Gunnar Cervin:** Writing – review & editing, Resources. **Henrik Pavia:** Writing – review & editing, Resources. **Eva Wattrang:** Writing – original draft, Visualization, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Malin Boyner:** Writing – original draft, Investigation. **Emma Ivarsson:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Alma Hansen:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Anna Lundén:** Writing – review & editing, Software, Investigation. **Osama Ibrahim:** Writing – review & editing, Supervision, Investigation.

Declaration of Competing Interest

No competing interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110377.

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