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Effects of algal supplementation on broiler chicken growth performance, gut development, blood leukocyte counts and antibody levels

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ABSTRACT

Brown macroalgae contains complex polysaccharides including laminarins that have shown prebiotic potential. The aim of this study was to investigate if feeding algal products from *Saccharina latissima* to either broiler breeders or directly to their chickens could affect growth performance, gut- and immune development in the chickens. A total of 45 hens of the parent line of Ross 308 were used to obtain fertilised eggs. The hens were fed one of three experimental diets, a control, the addition of 0.6% algal meal or addition of 0.08% algal extract. The progenies of those hens were followed in an experiment using a split-plot design where eggs from the three hen treatments were distributed into 24 modules. Half of the modules were assigned a control diet, and half of the modules were a diet supplemented with 725 ppm algal extract. A total of 255 chicks remained after hatching and individual marking, they were weighed at hatch, on days 3, 7, 14, and 37 and blood samples for determination of leukocyte counts and serum antibody levels were drawn on days 3, 7 and 12. Chickens were killed to assess organ development at days 7, 14 and 37, and histological examination of ileal tissue was performed on day 7. The results showed that chicks fed the algal extract diet had higher ($P < 0.05$) BW on days 3, 7 and 37, a higher proportion of serum immunoglobulin Y (**IgY**) and a lower proportion of maternal antibodies to infectious bronchitis virus on day 12 ($P < 0.05$). Chicks fed algal extract showed higher numbers ($P < 0.05$) of CD4+CD8- helper T-cells and total T-cell receptor (**TCR**)γ/δ+ T-cells, and among the TCRγ/δ+ T-cell subpopulations, the TCRγ/δ+CD8- T-cells were increased, and lower ($P < 0.05$) numbers of cluster of differentiation (**CD**)4+CD8αα+ and TCRγ/δ+CD8αβ+ T-cells in the circulation. Feeding algal extract to the breeders resulted in higher chick BW on day 7, and the villus height–to–crypt depth ratio was higher ($P < 0.05$) for chicks from hens fed algal extract than for chicks from hens fed algal meal. In conclusion, feeding algal extract from *Saccharina latissima* directly to the chicken improved growth performance throughout the growing period and altered the composition of T-cell populations in the circulation and may have enhanced the chicks' IgY production. Maternal supplementation of algal extract to breeder hens had positive effects on the chickens' early growth performance and gut architecture. However, no synergistic effects of both maternal feeding and direct supplementation to the chicken were found.

Implications

Algal extract from *Saccharina latissima* rich in laminarin that has growth and immune stimulating properties was tested as a bioactive component in poultry diets. Feeding the algal extract directly to broiler chickens increased the BW at days 3, 7 and 37, while feeding the extract to breeder hens improved their chickens' BW at day 7 and gut architecture in the chickens. However, feeding the algal extract both to the hen and the chicken did not result in any further additional benefits. These findings implicate that supplementation of algal extract in feed to chickens may improve their lifetime growth performance.

Introduction

The intestinal development of newly hatched chicks is known to have a major impact on both short- and long-term growth performance and a healthy and well-developed intestine is a key to maintain a healthy gut and to obtain optimal growth, feed

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conversion and overall health (Uni et al., 1999). However, although its importance is well described, gut health is a complex term that combines nutrition, microbiology, immunology, and physiology of the animals (Jha et al., 2019). Understanding and improving gut health by different means is an important and current topic in monogastric nutrition (Jha et al., 2019). One way to address this and support the gut and immune development is to use different feed additives such as prebiotics as it has been recognised as one alternative strategy for maximising growth and immunity in poultry (Kim and Lillehoj, 2019).

Brown macroalgae contains several complex polysaccharides including laminarins that have shown prebiotic potential (Cherry et al., 2019). Furthermore, immunomodulatory properties of algal laminarins on both innate and adaptive immune functions have been identified in mammals where also potential use in e.g. vaccines and cancer therapy have been identified (Goodridge et al., 2009; Bonfim-Mendonça et al., 2017). Thus, algal polysaccharides appear as promising candidates to explore as prebiotic and immunomodulating compounds also for chickens. Indeed, a recent study in broiler chicks fed 300 ppm laminarin obtained from the extract of *Laminaria* spp. showed promising effects with improved growth performance, upregulation of genes involved in nutrient absorption, intestinal integrity and immune responses as well as beneficial effects on the microbial profile (Venardou et al., 2021). In addition, Sweeney et al. (2017) found improved growth performance, villus architecture and upregulation of proinflammatory immune genes in the intestine when feeding broiler chickens 250 ppm laminarin using extract from *Laminaria Digitata*. However, we (Ivarsson et al., 2022) did not find any effects on growth performance or gut health in broiler chickens by supplementing starter feeds with 250 or 500 ppm laminarin obtained from algal extract from *Laminaria digitata*. A common consensus within research using different products to improve gut health is that there often is a lack of consistency between studies and the results vary from farm to farm which likely is due to that the mode of action is poorly understood (Kim and Lillehoj, 2019).

Saccharina latissima is a brown algae belonging to the family Laminariaceae that is rich in complex polysaccharides such as alginate, laminarin, and fucoidan (Sharma et al., 2018). We recently showed that broiler breeders fed algal products from *Saccharina latissima* had increased abdominal fat pads, indicating an altered nutrient utilisation (Ivarsson et al., 2023). Furthermore, in the same study, the progenies of the breeders were used for chick quality assessment when newly hatched. The study did not show any effect of algal supplementation in the breeder diet on chick quality; however, the hatch time of the chicks influenced both chick quality scores and hatching weights, with poorer performance in late –hatched chicks. Interestingly, feeding algal extract to sows has shown altered microbial composition, improved gut architecture and growth performance in their piglets (Leonard et al., 2011). But to our best knowledge, no previous studies on progeny development posthatch when breeder hens were fed brown algal products have been performed. The major aim of the current study was hence to investigate if feeding algal products from *Saccharina latissima* to either broiler breeders or directly to broiler chickens would affect growth performance, gut and immune traits in the chickens.

Material and methods

The animal experiment was conducted at the Swedish Livestock Research Centre, Uppsala, Sweden and was approved by the ethical committee of Uppsala region, approval number Dnr. 2019.4.1-4462. This study was part of a larger project where in the first part, the effect of feeding brown algae, intact or as an extract, to broiler breeder hens on hen antibody responses, egg quality as well as their progeny chick quality was evaluated (Ivarsson et al., 2023). The progenies from the breeder hens used in Ivarsson et al. (2023) were further monitored in the present study that focuses mainly on gut and immune development until day 14. Subsequently, when the chickens used in the current study were 17 days of age, they were intraoesophageally inoculated with *Campylobacter jejuni*. A more detailed description of that infection study and results on the microbial composition is provided in Eliasson et al. (2024). However, the results of these chickens' growth performances until day 37 as well as organ development are included in the present study.

Hatching

Fertilised eggs from the parent line of the fast-growing broiler Ross 308 were incubated at the Swedish Livestock Research Centre, Uppsala, Sweden using an incubator (J.Hernel Brutgerate GmbH & Co, Verl, Germany). A total of 45 individually housed breeder hens were used and fed one of three experimental diets (15 hens/diet) for 5 weeks prior to a ten-day egg collection period for incubation. The hen dietary treatments were a control diet (**Control**) formulated according to Aviagen (2016), one diet with the inclusion of 0.6% brown algae meal (**Algae**), and one diet with the inclusion of 0.08% brown algal extract (**Algal extract**). The inclusion levels were set to provide 300 ppm laminarin. A more detailed description of breeder diets and management is provided in Ivarsson et al. (2023). On day 18 of incubation, 301 eggs were moved from the incubator to the poultry facility and placed in individual hatching compartments for on-farm hatching. The hatching occurred between incubation h 474–530, and 85% of placed eggs were hatched during this time. Day 0 was set to the peak of the hatching window, namely 494 h of incubation. The hatch time of each chick was registered every 8 h and chicks were individually colour marked to maintain identity. In addition, the chick quality was assessed and the lengths and weights of the chicks were monitored. The length was measured by laying the chick over a ruler and measuring from the tip of the beak to the longest toe. A detailed description of the hatching procedure and registrations is provided in Ivarsson et al. (2023).

Data management

To include hatching time in the statistical analysis, the chicks were divided into three hatching groups, early, middle and late with 73 chicks in each group. The procedure for dividing the hatching groups is described in detail in Ivarsson et al. (2023). Briefly, the hatching time was recorded every 8 h during the 56 h hatching window. The early hatching group (**Early**) consisted of chicks hatched at 474–490 h of incubation. Chicks in the middle hatching group (**Middle**) hatched at 491–498 h of incubation and the late hatching group (**Late**) consisted of chicks that hatched at 499–530 of incubation.

Chick housing and management

After hatching, a total of 255 mix-sexed Ross 308 chicks (n = 255) were kept in groups of 10–13 chickens in 24 rearing modules. The rearing module was (1.5 x 0.75 m) with solid floors and bedded with wood shavings and equipped with a feeder and three nipple drinkers. The house temperature was maintained at 34 °C during hatching and 33 °C for the first 3 days and thereafter gradually reduced according to age until reaching 23 °C on day 24, remaining the same for the rest of the period. Light was provided 24 h/day during the first 2 days and was then reduced by 1 h every

day until day eight, giving 18 h of light per day during the remaining period. Chicks were weighed individually at hatch, on days 3, 7, 14 and at the end of the experiment, day 37. On day 7, the colour identifications were changed to 1 cm x 1 cm laminated number tags attached in the neck subcutaneously with a plastic strap. Feed intake and group weights of chickens were registered weekly, mortality was registered daily, and the weight of dead chickens was corrected for during feed conversion ratio (**FCR**) calculations.

Study design and diets

The experiment was designed according to a randomised split-plot model where eggs from the three hen treatments were placed in the same rearing module (Supplementary Table S1). A total of 24 experimental modules with initially 12–13 eggs per module were used. The distribution of hen treatments in the rearing modules was determined before hatch when the chicks still were in the egg. To maintain the identity of the eggs, individual hatching compartments were placed in the rearing modules, and after hatch, the chicks were colour marked before they were included in groups in the rearing modules. The rearing modules were divided into two chick treatment groups: a control diet formulated according to the nutrient requirement of ROSS 308 (Aviagen, 2019; Control) and a control diet supplemented with 725 ppm algal extract from *Saccharina latissima* (Algal extract), optimised to contain 290 ppm laminarin. The ingredient and chemical composition of the experimental chick diets are presented in Table 1. The apparent metabolisable energy$_n$ was calculated based on the sum of included feedstuffs energy value according to the European Federation of Branches of the World's Poultry Science Association (1989).

The experimental diets were analysed for DM, ash, CP, ether extracts and amino acids as described by Valečková et al. (2020). The algae were cultivated at sea on longlines in the Koster archipelago outside Tjärnö Marine Laboratory at the Swedish West Coast (Thomas et al., 2022), and dried algae were used as a substrate for the extract and obtained as described in Ivarsson et al. (2023). The algal extract contained 41.6% laminarin, determined enzymatically by measuring the β 1,3/1,6-glucan content (K-YBGL 12/16, Megazyme). In addition, the ash content was determined to be 14.5% in the algal extract on a DM basis and the non-starch polysaccharide content of the algal extract was determined by the Uppsala method (Theander et al., 1995), and was in total 45.7% on DM-basis of which: 31% was glucose 2.2% fucose, 3.0% mannose, 1.8% galactose, 0.7% xylose and 0.5% arabinose.

Table 1
Composition of experimental chicken diets (% as fed), calculated apparent metabolisable energy (AME$_n$; MJ/kg DM) and analysed chemical composition (g/kg DM) of the experimental diets.

	Chicken diets[1]	
Items	Control	Algal extract
Ingredient composition of experimental diets (% as fed)		
Wheat	64.95	64.87
Soy protein concentrate	27.22	27.22
Rapeseed oil	2.70	2.70
Limestone	1.91	1.91
Monocalcium phosphate	1.00	1.00
Lysine-HCl	0.48	0.48
Sodium bicarbonate	0.38	0.38
Methionine-DL	0.37	0.37
Premix[2]	0.25	0.25
Threonine	0.23	0.23
NaCl	0.10	0.10
Valine	0.01	0.01
Algae extract	.	0.075
Analysed chemical composition (DM-basis)		
Apparent Metabolisable Energy$_n$[3]	13.3	13.3
DM	916.0	915.0
Ash	60.3	64.7
CP	232.0	234.6
Ether extract	50.1	48.8
Arginine	13.0	13.6
Histidine	5.5	5.6
Leucine	16.2	16.6
Lysine	15.2	15.7
Methionine	6.8	7.1
Phenylalanine	11.5	11.6
Threonine	9.7	10.5
Valine	10.1	10.3
Alanine	9.0	9.4
Aspartic acid	20.2	21.5
Cystine	4.2	4.5
Glutamic acid	48.9	51.8
Glycine	9.4	9.8
Proline	14.8	14.9
Serine	10.8	11.3

[1] Control = chicken control diet; Algal extract = chicken diet supplemented with algal extract.
[2] The premix provided per kg diet: Vitamin A: 10 000 IU; Vitamin D_3: 5 000 IU; Vitamin E: 100 mg; Vitamin K_3: 4 mg, Vitamin B_1: 4 mg, Vitamin B_2: 10 mg; Vitamin B_3: 80 mg; Vitamin B_5: 20 mg; Vitamin B_7: 0.4 mg; Vitamin B_9: 2 mg; Choline: 1 371 mg; 150 mg; Fe: 20 mg; Cu:20 mg; Mn: 170 mg; Zn: 119 mg; Se: 0.4 mg; I: 1.4 mg.
[3] Calculated based on the sum of included feedstuffs energy value according to the European Federation of Branches of the World's Poultry Science Association.

Organ development

To assess organ development two chickens per module were selected and killed on days 7, 14 and 37 posthatch. Chickens for organ sampling were chosen based on hen and chick treatment, to achieve an equal number of chickens from all combinations. The selected chickens were stunned and killed by cervical dislocation on days 7 and 14 posthatch and by intravenous injection of sodium pentobarbital (Allfatal vet. 100 mg/ml, Omnidea AB, Stockholm, Sweden) in the wing vein on day 37.

On each sampling day, individual BW, intestinal length (cm), intestinal weight with content (full; g), bursa of Fabricius weight (g) proventriculus weight (g) gizzard weight with content, (full; g) gizzard weight without content, (empty; g) were measured. The intestine was defined as the proximal tip of the duodenum to the ileocecal junction. 'Empty gizzard' referred to each gizzard that has been split longitudinally with a surgical blade to remove the contents without peeling off the inner lining, rinsed in water and wiped dry with tissue paper.

Histological measurement

On day 7, ileal tissues were collected from approximately 2 cm distal to Meckel's diverticulum for histological assessment. The tissue was cut open and pinned to a small rectangle of cork to minimise tissue distortion. Tissues were fixed in glutaraldehyde (2.5%, pH 7.2) overnight before rinsed in phosphate buffer (1/15 M, 7.2 pH). Tissues were trimmed into 2 mm thick transverse slices and were thereafter dehydrated in increasing concentrations of ethanol and embedded in water-soluble resin (Leica Historesin, Heidelberg, Germany). Sections (2 μm) of resin-embedded ileum were stained with hematoxylin-eosin for evaluation in light microscopy. Before evaluation, all slides were coded to avoid bias due to the observer knowing the treatments and digital images of ileum sections were taken with a Nikon Microphot-FXA microscope using the 4x objective lens (Bergström Instrument AB, Stockholm, Sweden). Five consecutive villi per sample were measured. Villi chosen should have an intact Lamina Propria and a single epithelial cell

layer to avoid including villi that could have been cut askew. Only representative villi that could be assumed not to have been affected by preparation or with artefacts were chosen. Villi, where the tip ends were diffuse or those with invisible crypts, were not chosen for analysis. Crypts were measured in the same direction as the villi base from the branching to the start of muscularis mucosa. Villi width was measured beneath the villi tip where the epithelial cell nuclei had straightened out and were no longer at an angle towards the tip. Villi width was measured perpendicular to the tip.

Quantification of immunoglobulin Y concentration and antibody titers to infectious bronchitis virus in serum

Two focal birds per module, in total 48 chickens, were sampled for the antibody parameters. The focal birds were selected based on chick treatment (n = 24/ chick treatment) and hen treatment. Blood samples were drawn from the jugular vein on days 3, 7 and 12 and were collected in test tubes without additives; samples were stored for 24 h in room temperature before centrifuging for 10 min at 10 000 × g. Serum was subsequently collected and stored at −20 °C prior to antibody analyses. The immunoglobulin Y (**IgY**) concentration was determined using Chicken IgY ELISA kit from Immunology Consultants Laboratory (ICL, INC, 7150 SW Sandburg Street, Portland, OR, USA 9722), and titers to infectious bronchitis virus (**IBV**) were quantified using IDEXX IBV Ab Test kit (#99-0926; IDEXX Laboratories, Inc., USA). Both tests were set up according to the manufacturers' instructions as described in detail in Ivarsson et al. (2023).

Blood leukocyte counts

Two or three focal birds per module (different from the antibody focal birds), in total 60 chickens (20/hen treatment), were used for the leukocyte counts. For this analysis, blood was collected into tubes with 1.0 mg EDTA K_2 as an additive (#363706, BD Microtainer ® MAP). Absolute counts of different leukocyte populations in whole blood samples were determined using a no-lyse, no-wash flow cytometry-based methodology described in Wattrang et al. (2020). The antibody panels used (Supplementary Table S2) were expanded to include phenotyping of different lymphocyte sub-populations in addition to heterophilic granulocytes, monocytes and thrombocytes as originally described (Wattrang et al., 2020). Twenty-five µl of EDTA stabilised blood was diluted 25-fold in FACS-buffer, i.e. phosphate–buffered saline supplemented with 0.2% bovine serum albumin (Sigma-Aldrich), 0.2% sodium azide, 0.05% normal horse serum (Sigma-Aldrich) and 2 mM EDTA. Antibody labelling, paraformaldehyde fixing and addition of fluorescent counting beads (123count eBeads, #01-1234-42, Invitrogen, Thermo Scientific) were performed as earlier described (Wattrang et al., 2020). Flow cytometry data were recorded for 1 min, and the gating strategies to define different leukocyte populations are shown in Supplementary Figs. S1 and S2. The number of events counted in the bead gate was according to the manufacturers' recommendations at least 1 000 and was used to determine the volume of blood sample analysed and calculate the absolute numbers of the leukocyte populations. Flow cytometry was performed using a BD FACSVerse™ (BD Biosciences), equipped with 488 nm blue, 633 nm red and 405 nm violet lasers, and results were analysed using the FACSDiva (BD Biosciences) software. Single-stained compensation controls and fluorescence minus one (FMO) negative controls were included in the assays. Titrations of all antibodies were performed to determine optimal labelling conditions prior to the experiment.

Statistical analysis

Data were analysed using SAS (version 9.4 SAS Inst. Inc., Cary, NC) and tested for normality and homoscedasticity using the diagnostic plots of residuals in SAS. All data presented in the tables are expressed as least square means and SEM. The *P* value, which denotes statistical significance, was $P < 0.05$, and *P*-values were Tukey adjusted for pairwise comparisons. Codes for the statistical models used are presented in Supplementary Material S1. The weekly BW, feed intake and FCR were registered and analysed on a module basis using the Procedure Mixed with chick treatment and hatching weight as fixed factors and module as a random factor. For hatching weight and hatching length of the chickens, the individual chicken was used as an experimental unit and analysed with Procedure Mixed. The model included hen treatment and hatching time as fixed factors and module and hen identity × hen treatment as random factors. For individual weights, organ weight, histology parameters and blood data, the individual chicken was used as an experimental unit and analysed with Procedure Mixed. The model included chick treatment, hen treatment, hatching time and hen treatment × chick treatment as fixed factors and module and hen identity × hen treatment as random factors. For the final BW, sex was also included as a fixed factor in the model and for the leukocyte population data as well as the antibody data, a repeated statement with UNSTRUCTURED covariance was included in the model and the interaction between sampling day and chick treatment was tested. The antibody data were log −transformed before analysis.

Results

Growth performance

Supplementation with algal extract in the chick diet resulted in higher ($P < 0.05$) BW on days 3, 7 and 37 posthatch (Table 2). An effect of hatching time was observed on BW on days 3, 7 and 14. At days 3 and 7, a gradual decrease in BW was observed with later hatching times, whereas at day 14, only the late–hatched chicks had a lower ($P < 0.05$) BW than early hatched chicks. In addition, at day 7, an effect of hen treatment on chick BW was observed with higher weights ($P < 0.05$) in chicks from hens who were fed algal extract than from hens fed the control. The final BW was affected by sex ($P < 0.001$) and was higher for male compared to female chickens (3209.2 vs 2725.8 g ± 57.35).

Because the feed intake was measured on a group basis, feed intake, FCR and weekly growth performance were calculated on a group basis. None of these parameters were affected ($P > 0.05$) by algal supplementation (Supplementary Table S3). However, on days 6, 27 and 34, birds in the algal extract group tended ($P < 0.01$) to have a higher BW and on day 6, they tended to have a higher feed intake. On days 34 and 37, chicks fed algal extract tended ($P < 0.01$) to have improved FCR.

Organ development

Chickens fed algal extract had a lower ($P < 0.05$) relative bursa of Fabricius weight compared to chicks fed the control at day 14. No other effects of chick treatment, hen treatment or hatching time on organ weights were observed (Table 3). Hen treatment but not chick treatment nor hatching time influenced ileal crypt depth (Table 4). Chicks from hens that were fed algal extract had shallower crypts ($P < 0.05$) than those from hens that were fed algae. The ratio of villus height to crypt depth was lower ($P < 0.05$) for chicks from hens fed algae than chicks from hens supplemented with algal extract. A tendency ($P = 0.084$) to deeper crypts in chicks fed algal extracts than in chicks fed control was also observed.

Table 2
Accumulated chicken BW (g/bird) based on individual weighing from days 3, 7, 14 and 37 of broiler chickens. Least square means, pooled SEM and *P*-value for hen treatment, chick treatment and hatching time are presented.

	Hen treatment[1]					Chick treatment[2]				Hatching time[3]				
Items	Control	Algae	Algal extract	SEM	*P*-value	Control	Algal extract	SEM	*P*-value	Early	Middle	Late	SEM	*P*-value
3 d														
n	75	72	65			102	110			72	69	71		
BW	73.2	74.2	75.8	1.128	0.263	72.4[b]	76.5[a]	0.929	0.002	82.5[a]	74.0[b]	66.7[c]	1.135	<0.001
7d														
n	57	50	58			79	86			63	49	53		
BW	176.9[b]	181.1[ab]	187.8[a]	3.080	0.040	177.3[b]	186.6[a]	2.516	0.011	194.4[a]	182.8[b]	168.7[c]	3.092	<0.001
14d														
n	55	49	58			79	83			61	48	53		
BW	412.1	414.6	430.8	14.447	0.085	413.1	425.2	13.970	0.117	438.8[a]	419.4[ab]	399.3[b]	14.473	<0.001
37d														
n	29	24	29			37	45			29	26	27		
BW	2980.0	2926.6	3036.4	95.354	0.782	2925.4[b]	3036.6[a]	60.054	0.029	3040.4	2980.6	2921.8	64.849	0.151

Abbreviations: Control= Control diet; Alage = diet supplemented with algal meal; Algal extract = diet supplemented with Algal extract; Early = chicks hatched at 474–490 h of incubation; Middle = chicks hatched at 491–498 h of incubation; Late = chicks hatched at 499–530 h of incubation.
[1] Hen treatment= Hen diet.
[2] Chick treatment= Chicken diet.
[3] Hatching time" The hatching started at 474 h of incubation and lasted for 56 h and hatching time refers to when during this time span the chicks were hatched.
[a–c] Values within treatment[1,2,3] and age, respectively, with different superscripts differ significantly at $P < 0.05$.

Table 3
Effect of chick treatment, hen treatment and hatching time on relative organ lengths (cm/g) and weights (g/kg) of broiler chickens at days 7, 14 and 37. Least square means and pooled SEM are presented.

	Chick treatment[1]			*P*-value		
	Control n = 24	Algal extract n = 24	SEM	Chick treatment	Hen treatment	Hatching time
Day 7						
Intestine length	595	628	27.04	0.402	0.092	0.287
Intestine weight	83.7	84.4	2.276	0.829	0.311	0.418
Bursa	1.42	1.45	0.068	0.811	0.888	0.821
Proventriculus	9.91	9.98	0.364	0.901	0.629	0.583
Gizzard (full)	50.1	47.7	2.557	0.516	0.597	0.067
Gizzard (empty)	32.3	33.2	1.380	0.627	0.953	0.110
Day 14						
Intestine length	221	221	5.277	0.933	0.145	0.322
Intestine weight	60.6	61.0	1.829	0.863	0.321	0.773
Bursa	1.82[a]	1.56[b]	0.089	0.046	0.265	0.288
Proventriculus	5.87	5.88	0.173	0.960	0.270	0.906
Gizzard (full)	29.5	28.4	1.795	0.652	0.642	0.417
Gizzard (empty)	19.5	19.0	0.850	0.679	0.840	0.824
Day 37						
Intestine length	65.6	62.8	1.348	0.153	0.194	0.750
Intestine weight	41.1	40.8	1.342	0.904	0.715	0.425
Bursa	1.58	1.55	0.105	0.826	0.472	0.198
Proventriculus	3.56	3.48	0.144	0.695	0.228	0.115
Gizzard (full)	13.6	13.4	0.733	0.839	0.914	0.111
Gizzard (empty)	10.5	9.84	0.456	0.352	0.991	0.329

Abbreviations: Control = control diet; Algal extract = diet supplemented with algal extract.
[1] Chick treatment= Chicken diet.
[a,b] Values within treatment and age, respectively, with different superscripts differ significantly at $P < 0.05$.

Antibody responses

Antibody parameters, i.e. total amount of IgY and antibody titers to IBV, were quantified in serum samples from focal birds (n = 24/ chick treatment) collected on days 3, 7 and 12 (Fig. 1). On day 3, the total levels of IgY were overall approximately 2.5 mg/ml serum with a large variation between individuals, and subsequently declined ($P < 0.05$) throughout the experimental period for all chickens (Fig. 1A). No effects ($P > 0.05$) of chick or hen treatment were found on total IgY levels. To reduce the effects of individual variation in IgY levels, the relative change in IgY levels was calculated within chicken as the proportion of total IgY on day 3 (Fig. 1B). This analysis showed that the total IgY levels were in general reduced ($P < 0.05$) to approximately 34 and 14% of day 3 levels on days 7 and 12, respectively. Moreover, effects ($P < 0.05$) of both hen and chick treatments were found with higher ($P < 0.05$) overall (i.e. over both days 7 and 12) proportion of relative IgY left in chicks fed algal extract (26%) than the control (21%), and chicks from hens fed the algal meal had lower proportion relative IgY amount left (20%) than chicks from hens fed the control (26%).

The antibody titers to IBV also declined ($P < 0.05$) for all chickens throughout the experimental period and were overall higher

Table 4
Effects of chick treatment and hen treatment on intestinal villi histological parameters (μm) of broiler chicks on day 7. Least square means and pooled SEM are presented.

Item	Hen treatment[1]					Chick treatment[2]			
	Control n = 15	Algae n = 16	Algal extract n = 17	SEM	*P*-value	Control n = 24	Algal extract n = 24	SEM	*P*-value
μm									
Villus Height	562.5	563.4	590.4	21.70	0.5619	565.0	579.2	18.19	0.5818
Villus width	115.1	112.5	106.2	17.40	0.8959	113.1	109.5	14.33	0.6039
Crypt depth	94.8[ab]	104.9[a]	90.9[b]	8.89	0.0444	92.6	101.1	8.61	0.0841
Villus height/crypt depth	6.1[ab]	5.6[b]	6.8[a]	0.62	0.0139	6.3	6.0	0.60	0.4111
Villus height/Villus width	4.8	4.6	5.4	0.78	0.7105	4.8	5.1	0.60	0.4670

Abbreviations: Control= Control diet; A = diet supplemented with algal meal; Algal extract = diet supplemented with algal extract.
[1] Hen treatment= Hen diet.
[2] Chick treatment= Chicken diet.
[a,b] Values within hen treatment and treatment, respectively, with different superscripts differ significantly at $P < 0.05$.

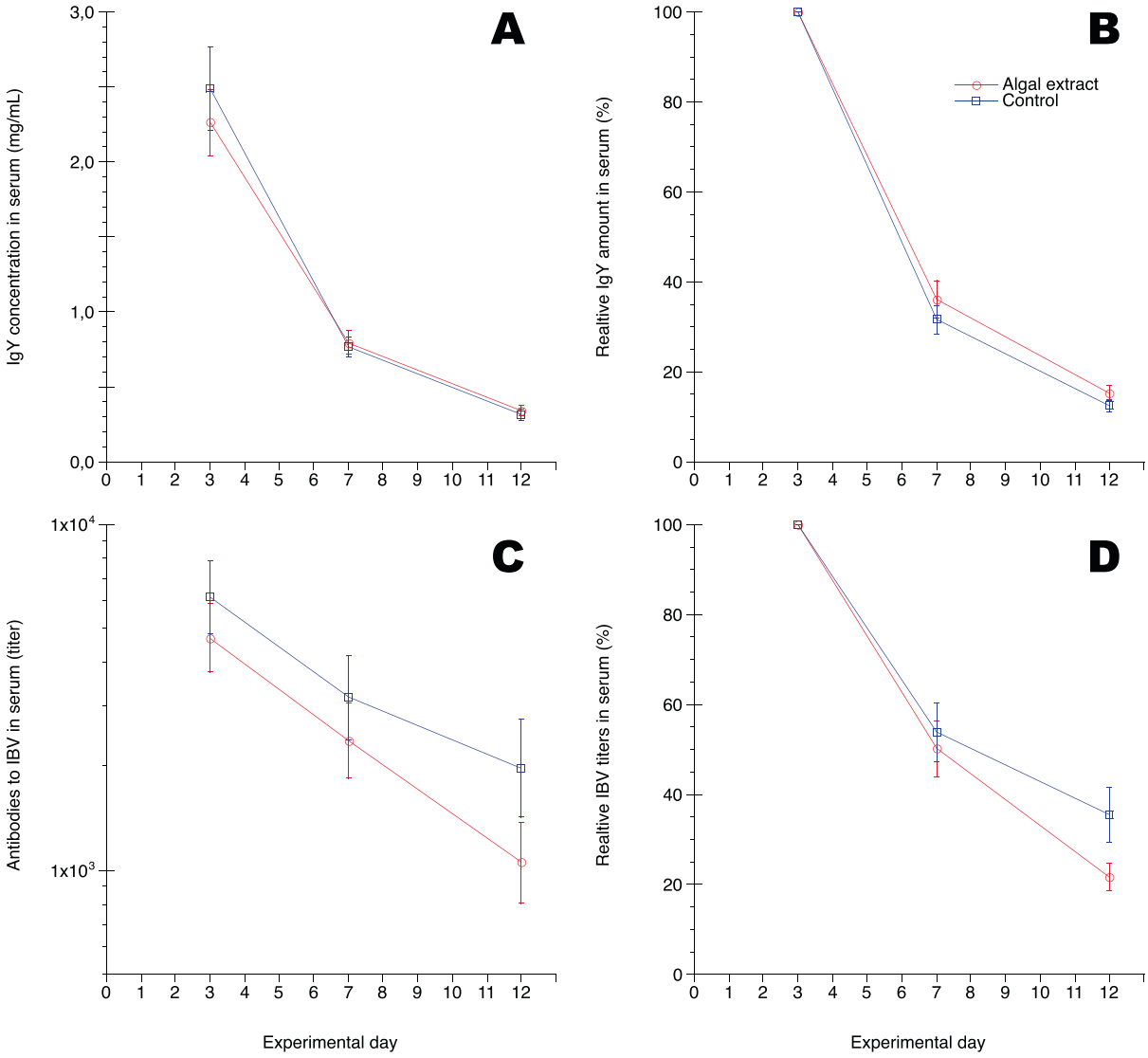

Fig. 1. (A) Total immunoglobulin Y (IgY) concentration (mg/mL), (B) relative IgY levels within chick calculated as proportion of IgY on day 3 (%), (C) antibody titers to infectious bronchitis virus (IBV), and (D) relative IBV titers within chick calculated as proportion of IgY on day 3 (%) in serum samples collected on the indicated days from chickens fed a diet supplemented with algal extract (red circles) or a control diet (blue squares). Results are presented as arithmetic, A, B and D, or geometric, C, group mean values ± 95% confidence intervals.

($P < 0.05$) in sera from chicks fed the control diet compared to sera from the chicks fed algal extract (Fig. 1C). Moreover, an effect ($P < 0.05$) of hen treatment was found with about 1.2 times higher values in chicks from hens fed the control than both algal treatments. The IBV titers similar to the IgY levels showed a large variation between individuals, and relative IBV titers were likewise calculated. The results showed that within chicken, IBV titers on day 7 were reduced to approximately 55% of those on day 3

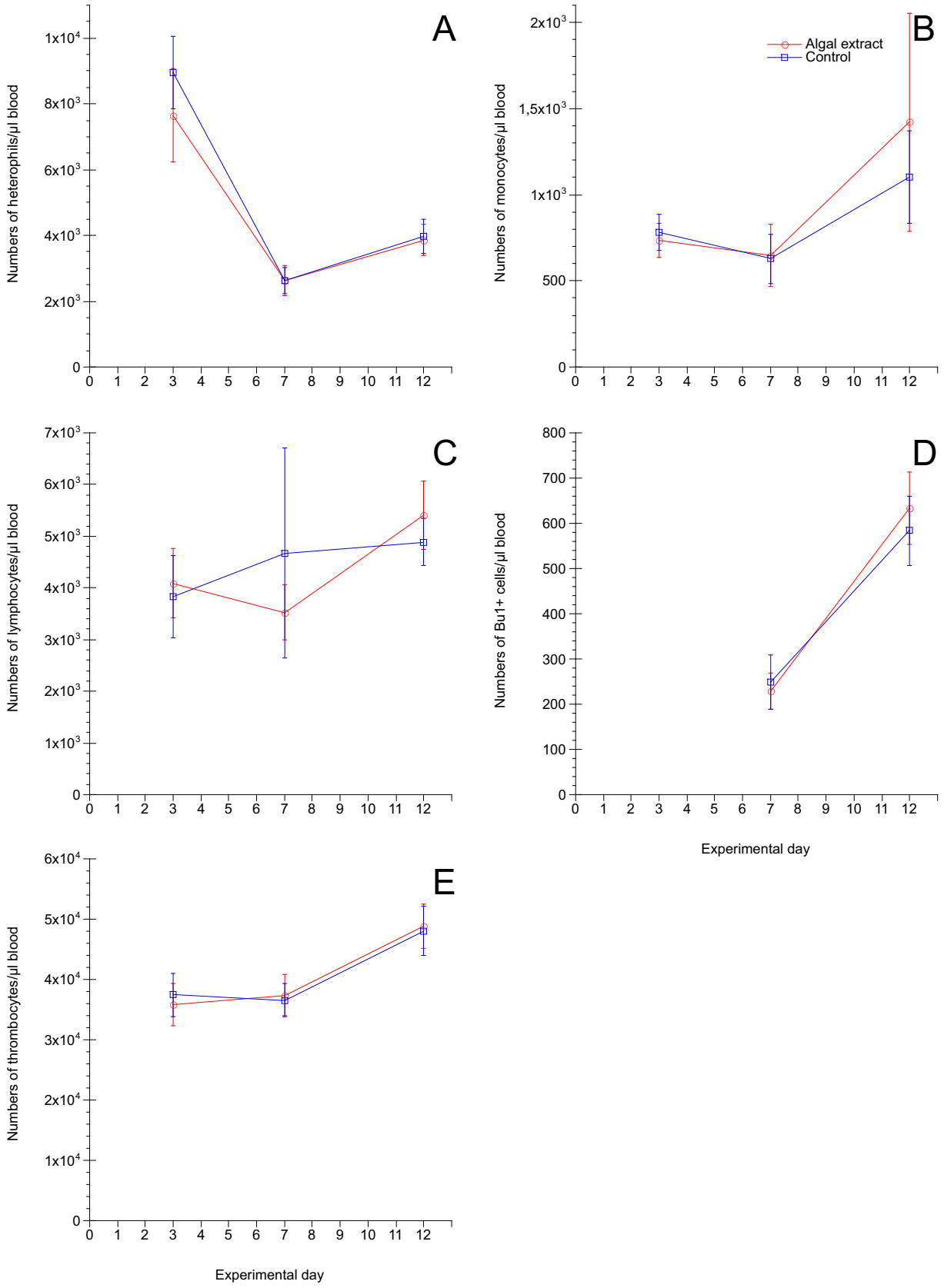

Fig. 2. Total numbers of (A) heterophils, (B) monocytes, (C) lymphocytes, (D) Bu1+ cells (B-cells) and E) thrombocytes in blood samples collected on the indicated days from chickens fed a diet supplemented with algal extract (red circles) or a control diet (blue squares). Due to technical reasons results on Bu1+ cells on day 3 are missing. Results are presented as arithmetic group mean values ± 95% confidence intervals.

(Fig. 1D) and were further reduced ($P < 0.05$) to 37% on day 12. An overall effect of chick treatment was found with a higher proportion of relative IBV titers ($P < 0.05$) left in chicks fed the control (46%) compared to chicks fed the algal extract (38%). In addition, an interaction between chick treatment and day was found with a higher proportion ($P < 0.05$) of relative IBV titers left in control –fed chicks on day 12 (37%) compared to chicks–fed algal extract (24%).

Hatch time did not affect ($P > 0.05$) the measured antibody parameters in the current study.

Blood leukocyte counts

Absolute numbers of different leukocyte populations were determined in whole blood with flow cytometry-based methodology. Using antibody panel 1 (Supplementary Table S2 and Supplementary Fig. S2), numbers of heterophilic granulocytes, monocytes, thrombocytes, total lymphocytes and B-cells were determined (Fig. 2). Overall, circulating numbers of these leukocyte populations showed differing kinetics over time but were in general similar within leukocyte populations, for all chickens regardless of diet. Heterophil numbers showed a pronounced decrease between day 3 and day 7 and subsequently increased slightly on day 12 compared to day 7 (Fig. 2A). For monocytes, similar numbers were observed in blood samples from days 3 and 7 and numbers then subsequently increased on day 12 and also showed a higher variation between individuals on this occasion compared to the previous samplings (Fig. 2B). The total number of lymphocytes showed a large variation between individuals and group mean numbers varied without a distinct trend during the experiment (Fig. 2C). Due to technical reasons, B-cell numbers were not recorded on day 3. However, between day 7 and day 12, blood B-cell numbers showed a pronounced increase (Fig. 2D). Numbers of thrombocytes were similar in blood collected on days 3 and 7 and subsequently increased on day 12 (Fig. 2E).

The levels of cluster of differentiation (**CD**)45 expression were assessed on each of the leukocyte populations defined by panel 1 (Supplementary Fig. S3). Results showed that overall, the kinetics of CD45 expression differed between leukocyte populations but were in general similar within leukocyte populations, for all chick-

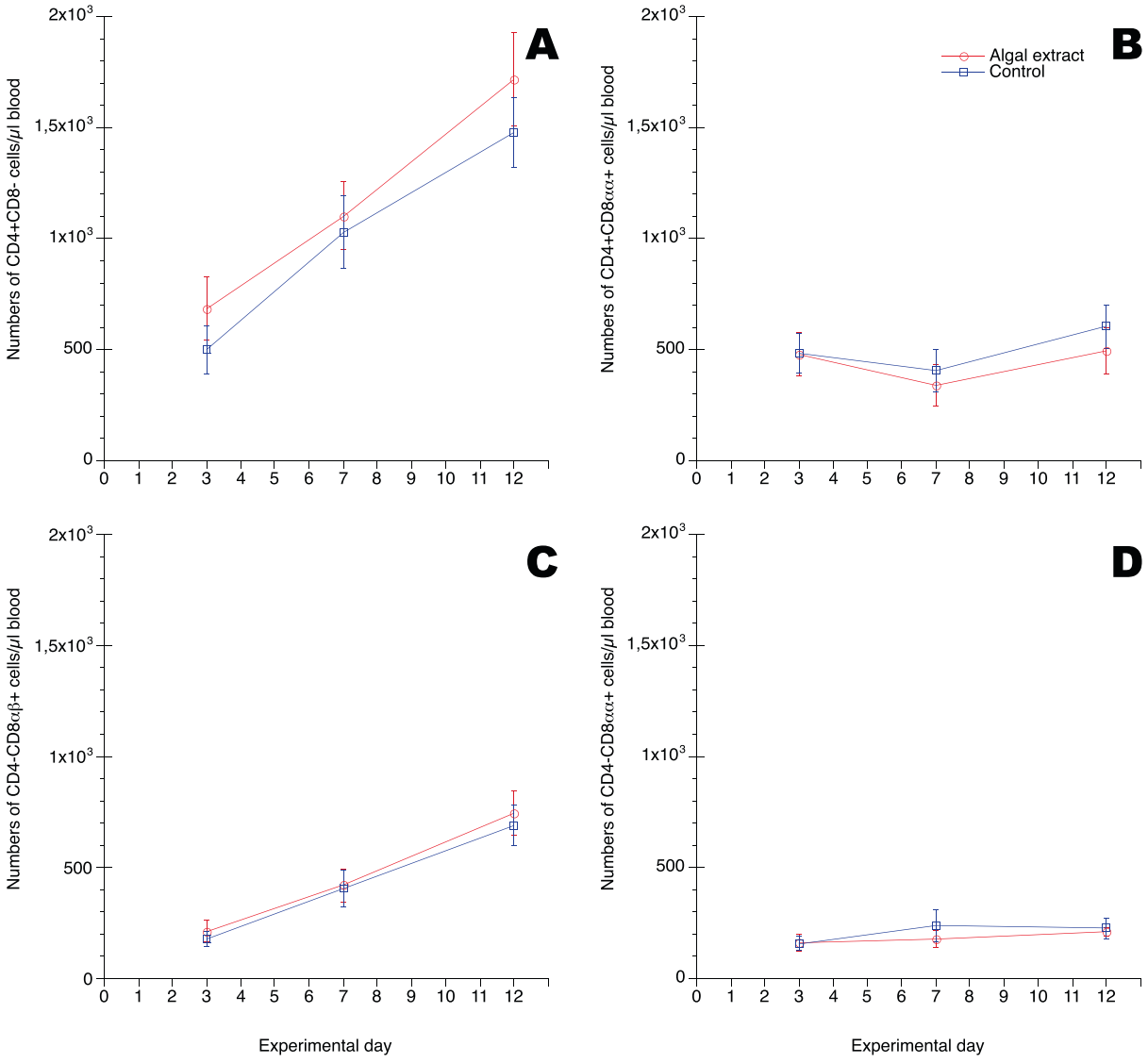

Fig. 3. Total numbers of TCRγ/δ – lymphocyte subpopulations (A) CD4+CD8- cells, (B) CD4+CD8αα+ cells, (C) CD4-CD8αβ+ cells, and (D) CD4-CD8αα+ cells in blood samples collected on the indicated days from chickens fed a diet supplemented with algal extract (red circles) or a control diet (blue squares). Results are presented as arithmetic group mean values ± 95% confidence intervals. Abbreviations: TCR = T-cell receptor; CD = cluster of differentiation.

ens regardless of diet. For heterophils, a transiently increased CD45 expression on day 7 compared to days 3 and 12 was observed (Supplementary Fig. S3A). For monocytes, a pronounced decrease in CD45 expression was observed on day 7 compared to days 3 and 12 (Supplementary Fig. S3B). For lymphocytes in total, CD45 expression was lowest on day 3 and subsequently increased on day 7 and remained at this level on day 12 (Supplementary Fig. S3C). For B-cells (days 7 and 12) and thrombocytes, the levels of CD45 expression remained similar during the experiment (Supplementary Figs. S3D and E). The chicken mannose receptor MRC1L-B is exclusively expressed in monocytes among leukocytes, and the levels of its expression were monitored during the experiment (Supplementary figure S3F). Results showed similar levels of MRC1L-B expression on monocytes during the experiment with high variation in expression between individuals.

Absolute numbers of some lymphocyte subpopulations with respect to cell surface expression of T-cell receptor (**TCR**)γ/δ, CD4, CD8α and CD8β, respectively, (Figs. 3 and 4) were determined using antibody panel 2 (Supplementary Table S1 and Supplementary Fig. S3). Results showed that among these subpopulations, the CD4+CD8-helper T-cells were the most numerous and their numbers increased constantly during the experiment (Fig. 3A). The CD4+CD8αα+ helper T-cells were present at similar levels as the CD4+CD8- cells in blood collected on day 3 and remained at these levels throughout the experiment (Fig. 3B). Correspondingly, numbers of CD4-CD8αβ+ cells, i.e. cytotoxic T-cells, constantly increased during the experiment (Fig. 3C) while CD4-CD8αα+ cells remained at numbers similar to those on day 3 throughout the experiment (Fig. 3D). The TCRγ/δ+ T-cells were analysed in total as well as divided into subpopulations (Fig. 4) based on the expression of CD8α and CD8β, respectively (as described in Supplementary Fig. S3). The total numbers of TCRγ/δ+ T-cells were relatively stable between day 3 and day 7 and subsequently increased to day 12 (Fig. 4A). A similar pattern was observed for TCRγ/δ+CD8- cells (Fig. 4B) that also constituted the largest subpopulation of the TCRγ/δ+ T-cells, approximately 85% of the TCRγ/δ+ T-cells on day 3 and approximately 75 and 70% on day 7 and day 12, respectively. The TCRγ/δ+CD8αβ+ and TCRγ/δ+CD8αα+ cells constituted

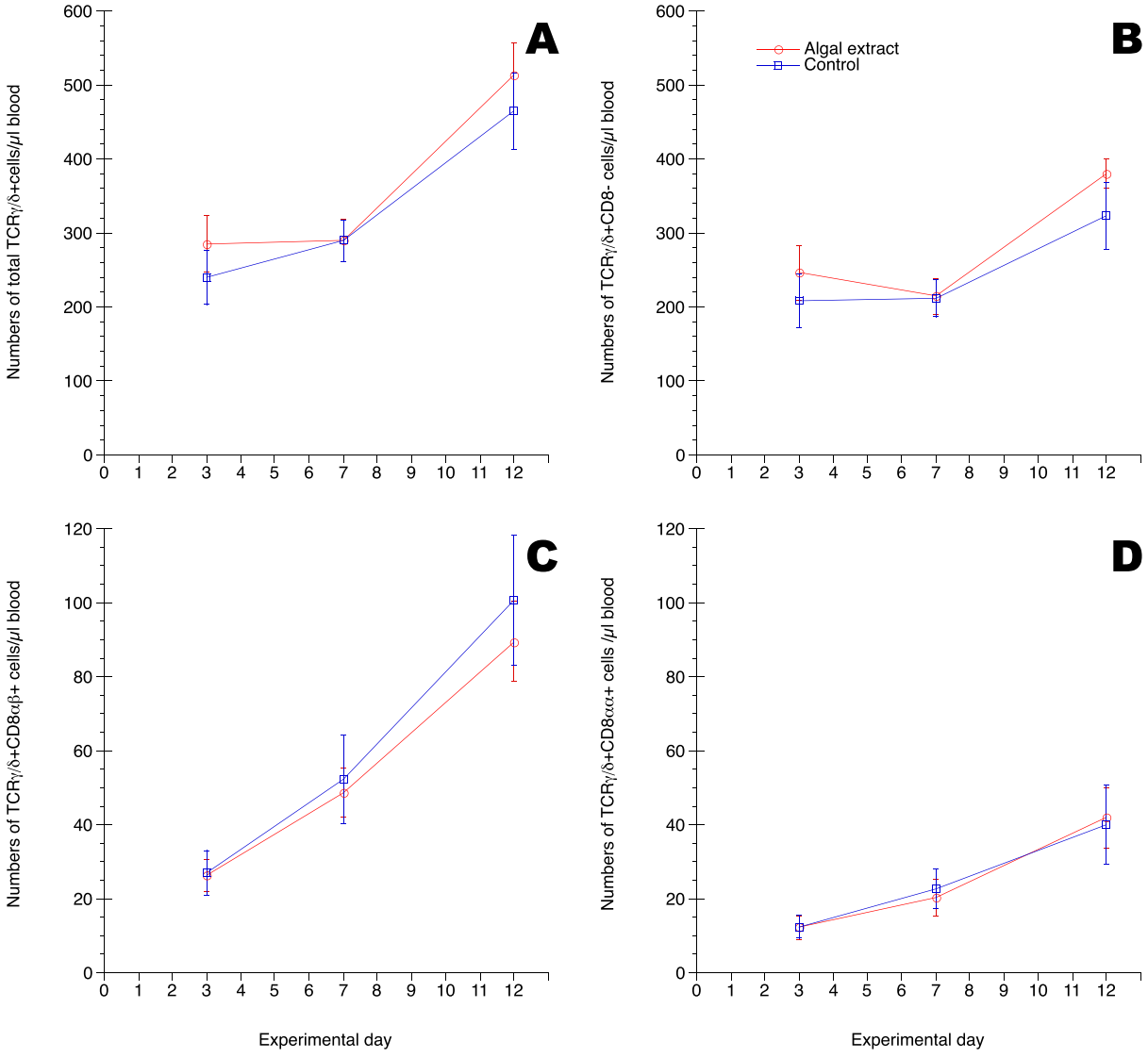

Fig. 4. Total numbers of TCRγ/δ+ lymphocyte subpopulations (A) total TCRγ/δ+ cells, (B) TCRγ/δ+CD8- cells, (C) TCRγ/δ+CD8αβ+ cells, and (D) TCRγ/δ+CD8αα+ cells in blood samples collected on the indicated days from chickens fed a diet supplemented with algal extract (red circles) or a control diet (blue squares). Results are presented as arithmetic group mean values ± 95% confidence interval. Abbreviations: TCR = T-cell receptor; CD = cluster of differentiation.

the second and third largest of the TCRγ/δ+ T-cell subpopulations and both populations showed a continuous increase in cell numbers during the experiment (Fig. 4C and D). Also, the proportions of these cell populations among TCRγ/δ+ T-cells increased, for TCRγ/δ+CD8αβ+ cells from approximately 10% on day 3 to approximately 20% on day 12 and for TCRγ/δ+CD8αα+ cells from approximately 5% day 3 to approximately 8% day 12.

Statistical analysis of the potential effects of chick treatment on the different cell populations is shown in Table 5. The analysis showed that chickens fed algal extract had higher numbers ($P < 0.05$) of CD4+CD8-helper T-cells and total TCRγ/δ+ T-cells, and among the TCRγ/δ+ T-cell subpopulations, the TCRγ/δ+CD8- T-cells were increased compared to chickens fed the control diet throughout the experiment. In addition, chicks fed the control diet had higher numbers ($P < 0.05$) of TCRγ/δ+CD8αβ+ cells and CD4+ CD8αα+ helper T-cells in blood compared to chickens fed algal extract throughout the experiment.

In addition, the effects on hatching time and age are shown in Supplementary Tables S4 and S5. As expected from the observed kinetics for numbers of the different leukocyte populations in blood, the experimental day significantly affected all tested populations (Supplementary Table S5). Statistically significant effects of hatching time were observed for the total numbers of TCRγ/δ+ T-cells, numbers of TCRγ/δ+CD8αα+ cells, CD4-CD8αβ+ cells, and CD4+CD8- helper T-cells with higher numbers/μl blood ($P < 0.05$) in early than middle and late hatched groups. Furthermore, the numbers of CD4-CD8αα+ cells were higher in blood from late compared to early hatched chickens, and the numbers of lymphocytes were higher in late compared to middle hatched chicks.

Effects of hen treatment were also analysed, but they did not show any statistically significant effect ($P > 0.05$) on any of the cell populations and the results are therefore not shown in the table. In addition, the interactions between sampling day and treatment were tested and for all parameters except for two parameters, no effects were found. The parameters where a significant interaction was found were for TCRγ/δ+CD8αβ +/μl blood and total lymphocytes /μl blood where for both parameters, higher levels were found in chicks fed control compared to chicks fed algal extract on day 7.

Discussion

Feeding algal extract rich in laminarin from *Saccharina latissima* to broiler chicks improved growth performance throughout the study. This result is in agreement with those obtained by Venardou et al. (2021) and Sweeney et al. (2017) when feeding laminarin-rich extracts to broiler chicks. A higher feed intake with laminarin supplementation was also reported in both studies. No significant effect on feed intake was however found in the current study, although a tendency for higher feed intake in the algal extract group was observed at day 6. Venardou et al. (2021) also found an upregulation of a protein-transporter gene and they suggested that the improved performance was linked to an increased feed intake and improved absorption of protein. Contrary, Sweeney et al. (2017) did not find any effects on nutrient transporter genes, however, they found a positive effect on gut architecture and thereby increased absorptive capacity. In the current study, the gut architecture was not affected by feeding algal extract directly to the chick. However, positive effects of feeding algal extract to the mother hen were found both on gut architecture and on growth performance at day 7. Interestingly, Leonard et al. (2011) observed increased villus height and villus height: crypt depth ratio in jejunum and ileum in piglets of sows fed a seaweed extract from *Laminaria* spp. They speculated about a mammary uptake and secretion of laminarin and thereby an early exposure of laminarin to the gastrointestinal tract of the piglets. This theory has not yet been proven, but it has been shown that when rodents were fed laminarin orally, about 20% of the administered dose was found in the serum and further bound to intesti-

Table 5
Effect of chick treatment on blood leukocyte counts and cell surface expression of CD45 on different leukocyte populations, and cell surface expression of the chicken mannose receptor MRC1L-B on monocytes in blood from broiler chickens. Least square means and SEM are presented.

Items	Chick treatment[1]		SEM	*P*-value
	Control n = 29	Algal extract n = 31		
Cell population (numbers of cells/μl blood)				
Total TCRγ/δ +	334.83[b]	369.68[a]	24.426	0.0345
TCRγ/δ+CD8αβ +	52.87[a]	38.26[b]	6.317	0.0025
TCRγ/δ+CD8–	277.27[b]	317.47[a]	30.272	0.0064
TCRγ/δ+CD8αα +	25.64	25.77	3.378	0.9614
CD4-CD8αβ +	440.07	478.64	69.142	0.2240
CD4-CD8αα+	150.70	125.35	29.045	0.1390
CD4+CD8αα+	421.32[a]	336.17[b]	49.768	0.0350
CD4+CD8-	796.03[b]	952.23[a]	70.351	0.0091
Heterophils	5122.58	4730.43	435.335	0.2456
Thrombocytes	42031.0	42091.0	2230.22	0.9672
Monocytes	752.32	837.72	235.540	0.5202
Lymphocytes	5254.29	5309.81	724.090	0.8890
B-cells	387.09	405.34	47.011	0.5802
Cell surface expression (MFI)				
CD45 on heterophils	13 646	14 256	405.6	0.0628
CD45 on lymphocytes	10 189	10 294	1126.4	0.8577
CD45 on B-cells	17 162	17 237	868.3	0.9141
CD45 on monocytes	15 671	16 146	475.1	0.4065
CD45 on thrombocytes	4703.5	4886.8	154.58	0.1312
MRC1L-B on monocytes	2406.3	2683.8	368.43	0.3515

Abbreviations; Control = control diet; Algal extract = diet supplemented with algal extract TCR = T-cell receptor; CD = cluster of differentiation; MRC = mannose receptor; MFI = mean fluorescence intensity.
[1] Chick treatment= Chicken diet.
[a,b] Values within row with different superscripts differ significantly at $P < 0.05$.

nal cells and gut-associated lymphoid tissue (Rice et al., 2005). It could therefore be discussed that the observed effect of hen treatment in current study could be due to a transfer of laminarin from the hen serum to the egg and thereby an early exposure to the chick gastrointestinal tract. This was however not assessed in current study and needs to be further researched and proven. Nonetheless, it is known that the chick intestinal development undergoes rapid changes already during the embryonic development (Uni et al., 2003), and previous studies have shown that early exposure by in ovo supplementation with mannan-oligosaccharide 3 days before hatch stimulates the development of gut morphology (Cheled-Shoval et al., 2011). Bednarczyk et al. (2016) tested in ovo injection of different prebiotics including an extract from *Laminaria* spp. containing laminarin and fucoidan and found improved early growth rate in broiler chicks. They selected prebiotics based on the solubility in physiological saline since only prebiotics that are fully solved can be correctly injected into the egg and pass the egg membrane into the bloodstream and the guts of the embryo. Bednarczyk et al. (2016) also tested the effects of supplementing the same algal extract in water during the first week of life and found beneficial effects on early growth of both in ovo and in water administration. However, no additive effect of both in ovo and in water treatment was found. Similarly, in the current study, no interactions between hen treatment and chick treatments were found and no additive effect of feeding both the hen and the chick could be observed.

In addition to the effect of algal feeding, hatching time is known to affect initial growth performance (van der Ven et al., 2011). To consider this, the effect of hatching time was included in the statistical model while evaluating the effect of algal feeding on BW. The late–hatched chicks showed lower BW until day 14, however, on day 37, they were able to catch up. This is in agreement with van der Ven et al. (2011) showing lower BW in late–hatched chicks until day 21 while this difference did not remain at slaughter on day 45.

In the current study, the relative bursal weights were affected by treatment on day 14 with higher relative weights in control–fed chickens. As a primary lymphoid organ, bursal development is strictly controlled by preprogrammed intrinsic factors of the young chicken's developing immune system (Fellah et al., 2014). Hence, these inter-study differences in relative bursal weight are most likely due to differences in growth rate and BW of the chickens that are probably more easily influenced by external factors such as feed and environmental stressors than the bursa development.

Among the leukocyte populations monitored in the present study, higher numbers of CD4+CD8- and TCRγ/δ+CD8- T-cells and lower numbers of CD4+CD8αα+ and TCRγ/δ+CD8αβ+ T-cells were observed in blood from chicks fed algal extract compared to chickens fed the control diet. In mammals, it is known that laminarin is recognised by certain innate immune receptors of which Dectin-1 is the most well recognised (Goodridge et al., 2009), that elicits various immune reactions including e.g. proinflammatory cytokine production. A receptor recognising laminarin in chickens has not yet been identified. Nonetheless, immune stimulation induced by the in-feed addition of laminarin to broiler chickens has been reported as increased intestinal mRNA expression of cytokines interleukin-17A (Venardou et al., 2021) and tumour necrosis factor-α (Sweeney et al., 2017) and innate receptors Toll-like receptor (TLR) 2 (Venardou et al., 2021) and TLR 4 (Sweeney et al., 2017). Hence, our results further support that the chicken immune system responds to laminarin exposure. The observed effects on circulating T-cell populations could be due to either direct, by innate receptor recognition, and/or indirect, through e.g. cytokine activation, effects elicited by laminarin stimulation. Interestingly, we have observed differential expression of some transcription factors that may indicate that chicken CD8 expressing and non-CD8 expressing TCRγ/δ+ T-cells are of different lineages (Maxwell et al., 2024), which could explain the opposite responses to laminarin of the TCRγ/δ+CD8- compared to TCRγ/δ+CD8αβ+ T-cells.

The present results from the antibody parameters monitored showed that chicks fed algal extract had higher relative serum levels of total IgY, but lower relative levels of antibodies to IBV at the end of the experiment compared to chicks fed the control diet. Because these chicks were not exposed to IBV, the antibodies to IBV were solely of maternal origin. In contrast, total serum IgY in addition to maternally derived IgY also includes antibodies produced by the chick, particularly at the end of the experiment. Hence, the results show that chicks fed algal extract used their maternal antibodies faster than chicks fed the control diet, which likely is linked to their higher growth performance and/or higher rate of metabolism. Moreover, it seems that chicks fed algal extract produced more IgY and/or commenced their IgY production earlier than chicks fed the control diet, which may be a consequence of the immune–stimulating effects of the algal extract. We also observed an effect of hen treatment on the amount of maternal antibodies, where offspring of hens fed the control diet showed higher levels of antibodies to IBV. Although not statistically significant, hens in the control feed group showed the highest levels of antibodies to IBV in serum compared to the other feed groups when the eggs for the current study were laid (Ivarsson et al., 2023) which probably explains this result. In the studies by Venardou et al. (2021) and Sweeney et al. (2017), the laminarin extract that was used was obtained from *Laminaria* spp. It is known that the structure and bioactivity of macroalgal polysaccharides differ between seaweed species as well as extraction methods (Garcia-Vaquero et al., 2017). The current study used algal extract obtained from *Saccharina latissima*, a species within the Laminariaceae family. However, the results from the current study indicate that the used algal extract indeed had immunomodulating properties and can be used as a bioactive component in poultry diets.

In addition to the effects induced by the algal extract treatment, we also observed some effects of age and hatch on the blood leukocyte populations monitored. In general, the numbers of the different lymphocyte subpopulations in the circulation increased over time, which likely reflects the concurrent maturation of the immune system during this time in the chicken's life (Fellah et al., 2014). Moreover, the numbers of several of the lymphocyte subpopulations were also influenced by hatching time. Lymphocyte development is intense during late embryonic life (Fellah et al., 2014), and a possible explanation to these hatch effects may thus be that the level of immune maturation reached at hatch continues to affect the development of the immune system during the early life of the chick. In the current study, a striking decrease in circulating heterophil numbers from day 3 to day 7 was also noted for all of the chickens. We have previously observed a similar decrease in heterophil numbers from newly hatched chickens to the first week of life when monitoring leukocyte counts of Ross 308 chickens throughout the rearing period using the same methodology (manuscript in preparation). Several studies have similarly reported high heterophil/lymphocyte ratios of newly hatched chicks that progressively decrease during the days/weeks after hatch (Burton and Harrison, 1969; Zulkifli and Siegel, 1994; Maxwell et al., 1997; Gonzales et al., 2003; Oznurlu et al., 2010; Wijnen et al., 2020, Madej et al., 2024). However, any conclusive explanation for this observation has to date not been put forward.

In conclusion, feeding algal extract from *Saccharina latissima* directly to the chicken improved growth performance throughout the growing period and altered the composition of T-cell populations in the circulation and may have enhanced the chicks' IgY production. Maternal supplementation of algal extract to breeder hens had positive effects on the chickens' early growth performance and gut architecture. However, no synergistic effects of both maternal feeding and direct supplementation to the chicken were found.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101560) can be found at the foot of the online page, in the Appendix section.

Ethics approval

The experiment was approved by the Uppsala Animal Experiment Ethics Board (application reference number: SLU ua 2019.4.1-4462).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings and models are available from authors upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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