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Effects of algal supplementation in feed to broiler breeders on transfer of nutrients and antibodies to chicks and quality of hatchlings

E. Ivarsson [a,*], H. Wall [a], M. Boyner [a], G. Cervin [b], H. Pavia [b], E. Wattrang [c]

[a] Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. 7024, SE 750 07 Uppsala, Sweden
[b] Department of Marine Sciences, Tjärnö, University of Gothenburg, SE 452 96 Strömstad, Sweden
[c] Department of Microbiology, National Veterinary Institute, SE 751 89 Uppsala, Sweden

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ABSTRACT

Breeder nutrition is an important factor for chick quality since the chick embryo relies on nutrients available in the egg for growth and development. In addition, the egg is providing the chick with important antibodies that are vital during the first weeks of life. Brown algae contains several bioactive compounds, and dietary supplementation with algal extracts have shown improved gut health and immune responses in both pigs and poultry. The aim of this study was to investigate if feeding the brown algae *Saccharina latissima,* intact or as an extract, to broiler breeders can affect breeder hens' antibody responses to vaccination, egg quality and transfer of antibodies and nutrients to the egg and thereby improve the quality of newly hatched chicks. Forty-five hens and nine roosters of the parent lines of the fast-growing broiler Ross 308 were included in the experiment where hens were 31 weeks at the start. The hens were housed individually and fed one of three dietary treatments for seven weeks; (a) control, (b) addition of 0.6% algal meal or (c) addition of 0.08% algal extract. The hens were given a booster vaccination against infectious bronchitis virus (**IBV**) 21 days after the start of experiment. During experimental days 32–42, hens were naturally mated every 5th day and hatching eggs were collected. A total of 255 chicks were hatched, and chick quality was assessed. Moreover, on chick day three, blood was collected from 48 focal chickens and total immunoglobulin Y levels and specific titres to IBV in serum were determined. The results showed that feeding the brown algae *Saccharina latissima,* intact or as an extract to broiler breeders did not affect egg production, egg quality, antibody responses to vaccination or transfer of antibodies from hen to chick. However, feeding intact algae significantly increased the levels of iodine and decreased the level of selenium in the eggs and resulted in a lower proportion of chicks with maximum quality score. Interestingly, algal feeding, both intact and as an extract, increased the abdominal fat pad in broiler breeders by about 17% without affecting BW. In conclusion, supplementation of broiler breeder diets with algal extract from *Saccharina latissima*, but not intact algal meal is a promising dietary strategy to increase the abdominal fat pad without causing any adverse effects on nutrient level in eggs or chick quality.

Implications

Feeding the brown algae *Saccharina latissima* as an extract to broiler breeders can be a promising strategy to increase the abdominal fat pad without affecting hen BW, nutrient level of eggs or chick quality. However, feeding intact algae meal increased the iodine and decreased the selenium level in eggs, and a negative impact on chick quality was observed. The high iodine level is limiting the use of intact algae meal in broiler breeder diets.

Introduction

Day-old chick quality is a term rather difficult to define and score but is an important parameter especially for hatcheries and farmers because a chick of high quality has a high viability and growth potential. A high-quality chick has thereby a high tolerance to initial challenges and great potential to reach the performance objectives and improving chick quality is therefore a key to optimise broiler flock performance and welfare (Tona et al., 2003; Willemsen et al., 2008; Chang et al., 2016). Several factors may influence day-old chick quality such as breeder age, incubation conditions, egg storage time and egg quality (Tona et al., 2003; 2004). In addition, breeder nutrition is an important parameter for chick quality (Chang et al., 2016) as the chick embryo relies

* Corresponding author.
E-mail address: Emma.Ivarsson@slu.se (E. Ivarsson).

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on nutrients available in the egg for growth and development (Surai, 2000). Within breeder nutrition, the levels of vitamins D and E, and the trace minerals selenium, zinc and manganese in the hens' diet seem to be of special importance for chick quality and viability (Chang et al., 2016). In addition to nutrients, the egg is also providing the chick with important antibodies and during the first weeks of life, the chick's humoral immunity is fully dependent on maternally derived antibodies. Avian immunoglobulin **(Ig)** Y is the functional equivalent to mammalian IgG and the transfer of maternal IgY from hen to chick is a two-step process where firstly IgY is transferred from the hen's bloodstream to the egg yolk and secondly, IgY is transferred from the egg yolk to the embryonic circulation (Ulmer-Franco et al., 2012). The main transfer from the hen circulation to the egg occurs 3–4 days before the egg is laid (Murai et al., 2020). The total level of IgY and the level of antigen-specific antibodies in the hen's blood has been shown to be in direct relation with the levels detected in the chick's circulation at day 3, with about a 30% transfer rate (Hamal et al., 2006). Therefore, finding means to optimise the antibody production in breeder hens could be a strategy to ensure that the newly hatched chick gets sufficient maternally derived antibodies during the first weeks of life when its own antibody production is not yet fully developed.

Brown algae contain several bioactive compounds that potentially could be used as health-promoting substances in animal feeds (Øverland et al., 2019). *Saccharina latissima* is a brown algae belonging to the family Laminariaceae that is rich in unique carbohydrates such as alginate, laminarin, and fucoidan (Sharma et al., 2018). Previous use of extracts from brown algae of *Laminaria* spp. rich in laminarin and fucoidan have shown beneficial effects on gut health and increased the expression of some immune genes in the intestine of broiler chickens (Sweeney et al., 2017). In addition, supplementation of a similar extract to sows during gestation and lactation have enhanced colostrum IgG and IgA concentration as well as the IgG concentration in serum of their piglets (Leonard et al., 2012). Øverland et al. (2019) stated that the positive health effects of using extracts from *Laminaria* spp. are well documented, while the number of studies evaluating the effects of using intact algae is few. Intact brown algae are good sources of minerals, carotenoids, vitamins, pigment and chelated micro-minerals (Evans and Critchley, 2014). It is clear that the mineral and vitamin content in the egg is highly influenced by the maternal intake and feeding organic forms of minerals to breeders have been found to be beneficial for chicken performance (Zudihof, 2015). However, to our best knowledge, no studies investigating the effect of feeding brown algal products to broiler breeders and the corresponding effect on egg and chick quality have been performed.

The major aim of this study was to investigate if feeding the brown algae *Saccharina latissima,* intact or as an extract, to broiler breeders can affect breeder hens' antibody responses to vaccination, egg quality and transfer of antibodies and nutrients to the egg and thereby improve the quality of newly hatched chicks. We hypothesised that algal supplementation, both intact and as an extract, would increase antibody responses to vaccination and transfer of antibodies to the egg and chick whereas algal supplementation with intact algae would increase nutrient transfer to the egg and improve egg and chick quality.

Material and methods

The animal experiment was conducted at the Swedish Livestock Research Centre, Uppsala, Sweden and was approved by the ethical committee of the Uppsala region, approval number Dnr. SLU ua 2019.4.1-4462. This study was part of a project where also 255 chicks hatched from eggs from the current breeders were monitored from hatch to 37 days of age (Ivarsson et al., manuscript in preparation). Results from serum antibody analyses (total IgY and infectious bronchitis virus (**IBV**) titers) from 48 focal chickens sampled on day 3 in that study were used for correlation analysis in the present study.

Broiler breeders

Housing and management

Forty-five hens and nine roosters of the parent lines of the fast-growing broiler Ross 308 were included in the experiment. The breeders were 28 weeks old when they arrived at the research centre from a conventional broiler breeder farm. The breeders had received a standard immunisation protocol for broiler breeders consisting of vaccination against Marek's disease, coccidiosis, IBV, infectious bursal disease (**IBD**/Gumboro), chicken anaemia virus (**CAV**) and avian encephalomyelitis (**AE**). After a three-week adaptation period, the hens were given three experimental diets with 15 hens per diet. A booster vaccination against IBV (Nobilis®, IB multi, Massachusettes D207/D274 serotypes) was given in the breast muscle to all hens 21 days after the introduction of the experimental diets.

The experiment was conducted when the hens were 31–37 weeks old, and the hens weighed on average 3711 g ± 397 g at the start of the experiment with no differences ($P > 0.05$) in start weight between treatments. The hens were housed in individual modules during the experiment. The modules were raised from the floor, with the size 1.5 × 0.75 m, and each module was provided with a laying nest, raised perch, nipple drinker, and wood shavings. The hens had visual and vocal contact with neighbouring hens. The light was turned on at 0800 in the morning, just prior to feeding and kept on for 13 h per day, the light intensity was 12 lux when measured at hen height (Mavolux electronic, Gossen). Hens were fed restrictively with 175 g pelleted feed/hen once a day, and feed residues were monitored daily. The hens had free access to water. Roosters were kept in the same room as the hens, in two groups of four and five individuals and fed restrictively once a day with a commercial feed for parent line roosters (Lantmännen, Sweden). The feed allowance for the roosters was adjusted according to body condition. During experimental days 32–42, hens were naturally mated every 5th day according to a schedule to ensure that each rooster got to mate a maximum of five hens per day. The pairing of hens and roosters was done randomly.

Experimental design and diets

Hens were divided into three different dietary treatments (n = 15) and fed the experimental diets for seven weeks. Antibody titres to IBV in hen serum were determined the day after the hens arrived at the research station and were accounted for when distributing the hens into dietary treatments. This was done by dividing the hens into three titre level groups; high, medium and low with 15 hens per titre group. Within each titre group, the three experimental diets were randomly distributed to obtain five hens per diet and titre group. The experimental diets were a control diet (**Control**) formulated according to the nutritional requirements of breeders stated by Aviagen (2016), one diet with the inclusion of 0.6% brown algal meal (**Algae**), and one diet with the inclusion of 0.08% of brown algal extract (**Algal Extract**; Table 1). The diets did not contain coccidostats. The brown algal meal and the extract were 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). The algal meal was dried and milled before mixed in the diet. Dried algae were also used as a substrate for the extract. Batches of 40 g of air dried and milled algae were mixed with 800 ml 0.3 M HCl and ultrasonicated for 30 min, with an end temperature of 73 °C to precipitate alginate.

Table 1
Composition of the experimental diets to broiler breeder hens and calculated apparent metabolisable energy (AMEn MJ/kg DM) and analysed proximate and amino acid composition (g/kg DM), selected trace elements (mg/kg DM) and selected vitamins (ug/kg DM).

Items	Experimental diets		
	Control	Algae	Algal extract
Ingredient composition for the experimental diets (% as fed)			
Wheat	60.58	60.58	60.58
Soybean meal	14.38	14.38	14.38
Oat	10.00	10.00	10.00
Limestone	7.80	7.80	7.80
Soy oil	2.43	2.43	2.43
Barley	2.00	2.00	2.00
Monocalcium phosphate	1.00	1.00	1.00
NaCl	0.27	0.27	0.27
Premix[1]	0.45		0.45
Premix[2]		0.45	
Methionine	0.17	0.17	0.17
Na_2CO_3	0.16	0.16	0.16
Threonine	0.06	0.06	0.06
Algae	.	0.60	.
Algal extract	.	.	0.08
Lysine- HCL	0.03	0.03	0.03
Analysed chemical composition (DM basis)			
AME_n[3]	12.3	12.3	12.3
DM	928.0	929.0	932.0
CP	179.0	176.5	172.3
Ash	128.5	123.0	117.0
Ether extract	51.6	52.8	52.1
Lysine	9.5	8.2	8.3
Methionine	4.4	4.0	4.4
Threonine	6.5	6.5	6.2
Iodine	1.4	12.9	1.8
Selenium	0.14	0.34	0.22
Vitamin A	2 478	2 164	2 264
Vitamin D_3	90.7	73.6	73.3

Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract.

[1] The premix provided per kg diet: 500 phytase units, 0,4 mg biotin, 2 mg folic acid, 20 mg pantothenic acid, 3.5 mg vitamin B, 0.03 mg vitamin B12, 12 mg vitamin B2 (5-phosphate), 60 mg vitamin B3 (niacin), 6 mg vitamin B6, 3 200 IE vitamin D3, 100 mg vitamin E premix, 4 mg vitamin K3, 10 000 international units vitamin A, 10 mg Cu-chelated, 5 mg $CuSO_4$, 60 mg $FeSO_4$, 2 mg $Ca(IO_3)_2$, 45 mg Mn-chelated, 100 mg $MnSO_4H_2O$, 0,2 Na_2SeO_3, 0.15 mg organic selenium, 45 mg Zn-chelated, 50 mg $ZnSO_4$, 5.5 mg Canthaxanthin, 2.5 mg carotenoid acid, 0.2 mg choline chloride.

[2] The premix provided the same as premix[2] except for 2 mg $Ca(IO_3)_2$ that was not included.

[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 (1989).

The samples were directly cooled on ice and centrifuged at 4000 rpm for 20 minutes. The supernatant containing the dissolved laminarin was saved and 70% EtOH was added. The samples were stored at 4 °C overnight to get a more efficient precipitation of laminarin, before they were decanted and centrifuged at 4000 rpm for 20 minutes on day two. The pellet was saved and was further washed in 90% EtOH to rinse out salts, and stored at 4 °C overnight; this was repeated on day three. On day four, the pellets were freeze dried after centrifugation. The dried algal meal contained 5.7% laminarin on DM basis and 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 32.2% in the algal meal and 14.5% in the algal extract on 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.

The Algae and Algal extract diets were then formulated to contain 300 ppm laminarin. The iodine content of the dried algae meal was analysed at Eurofins laboratory (EN 15111m:2007) prior to feed formulation and was 2300 mg/kg. The premix for the Algae diet was therefore adjusted and did not contain any added iodine.

Table 2
Experimental set-up for the current experiment with broiler breeders and their progenies.

Parameter/event	Which animals	Experimental day
Initial IBV titers in serum	Breeder hens	Pre-experiment
Start experimental feed	Breeder hens	Day 1
BW	Breeder hens	Days 1 and 49
Egg production	Breeder hens	Days 1–49
Boost vaccination against IBV	Breeder hens	Day 21
IBV and IgY level in serum	Breeder hens	Days 31 and 42
Vitamin and mineral content in eggs	Breeder hens	Days 26–31
Egg quality	Breeder hens	Days 33–34 and 47–48
IgY level in yolk	Breeder hens	Days 33, 34 and 47
IgA level in albumen	Breeder hens	Days 33, 34 and 47
Collection- hatching eggs	Breeder hens	Days 35–44
Abdominal fat pad and body condition	Breeder hens	Day 49
Chick quality	All hatched chicks	Incubation day 20–22
Chick serum IgY and IBV	2 focal chicks per module	Chick experimental day 3

Abbreviations: IBV = infectious bronchitis virus; Ig = immunoglobulin.

Measurements and sample collection

The BW of the hens was determined at arrival, start of feeding the experimental diet and at the end of the experiment. The number of laid eggs and eggs with visible cracks in the shells were registered daily. Eggs were collected and marked with hen identity and date, packaged with the tip down and stored at 4 °C until analysis or incubation as described in Table 2. Blood samples were drawn from the jugular vein the day after arrival at the research station and on experimental days 31 and 42. At the end of experiment, the hens were killed by an overdose of pentobarbital (Allfatal vet. 100 mg/ml, Omnidea AB, Stockholm, Sweden) and the abdominal fat pads were dissected and weighed.

Chicks

Hatching

Eggs were collected and labelled with mother hen identity and date between experimental days 35 and 44 for the purpose of hatching chickens. A total of 390 eggs were put in an incubator (J.Hernel Brutgerate GmbH & Co, Verl, Germany) on experimental day 45. The temperature in the incubator was maintained at 37.5 ± 0.5 °C and the humidity was kept at 55% ± 5%. The eggs were candled on incubation day 5, and 82% of the eggs were fertile. On incubation day 18, 301 eggs were moved to the rearing facility and placed in individual hatching compartments within the rearing pens to maintain information on mother hen identity of every hatched chick. The eggs were placed according to a randomised split-plot design with eggs from different maternal treatments in the same rearing pen. The hatched chicks then remained in the same rearing pens as they were placed in as eggs. The on-farm hatching occurred between incubation days 20 and 22, and 85% of the placed eggs were hatched within this time span. This gives a total of 46 unhatched eggs of which seven were from hens fed the Control, 19 from hens fed Algal extract and 20 from hens fed Algae. A recording of whether an egg had hatched or not was conducted every eight hour, and chicks hatched during the eight hour span got the same recorded hatching time, see example of the hatching protocol in Supplementary Table S1.

Chick quality

Throughout the hatching window, chick quality assessment was performed according to Tona et al. (2003) every eight hours on newly hatched chicks with dry down on its back. The following parameters were included: activity, down and appearance, retracted yolk, eyes, legs, navel, remaining membrane, and remaining yolk. In addition, the length and weight 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. The activity was assessed by placing the chick on its back and observe how quickly it returned to its feet. An immediate return to its feet was considered a good activity, while a delayed return to its feet was considered a weak activity. Regarding down and appearance, the chick should be dry and clean. The chick was placed on its back in the hand palm when the retracted yolk, navel, remaining membrane and remaining yolk were observed. Height of its abdomen and the consistency of the abdomen were estimated through palpation. A chick that had an abdomen containing a large yolk that was hard to touch was receiving zero points for this parameter. The navel should be completely closed and have the same colour as the skin around the navel for the chick to get a high navel score. Eyes were observed and should be open, alert and bright for a high scoring. Legs were assessed by putting the chick on its feet and observing whether it could stand easily or not. Toes should not be crooked. A total quality score of each chick was calculated based on scored physical appearance in accordance with Tona et al. (2003) with the total maximum sum of score as 100 (Supplementary Table S2). Chicks were individually marked with a distinct colour at different body parts to keep the individual identity of each chicken.

Housing, experimental diet and sampling

After scoring and marking, chicks were housed in groups of 10–13 individuals in the 24 rearing pens they were assigned to prior to hatching. The rearing pens provided access to feed and water immediately after completion of marking and quality scoring post-hatch. In half of the pens, chicks were fed a control diet and the remaining half were fed a diet with addition of the algal extract described previously in this paper, included to provide 290 ppm laminarin. Both chick diets were pelleted and formulated according to the nutrient requirement of ROSS 308 (Aviagen, 2019). At chick day three, calculated from the middle of the hatching window, blood samples were drawn from the jugular vein on two focal chicks per pen. Within pen, the focal chicks were selected according to a schedule based on hen treatment to ensure equal representations from all combinations of hen and chick treatment. This was the final sampling used for the present study.

Data management

To adjust for the effect of 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 first hatched chick was considered as an outlier as it was hatched 12 h before the second chick and was removed from the data set, the recorded hatching window was then in total 56 h, with a total of seven recording times occurring every eight hour. The early hatching group consisted of chicks hatched between 0 and 16 h from the start of hatch, according to the observations every eight hour. Chicks in the middle hatching group hatched between 16 and 24 h after the start of hatch and the late hatching group consisted of chicks that got a recorded hatching time in the time span of 24–56 h after the start of hatch. To get an equal number of chicks in each hatching group in accordance with Bergoug et al. (2015), 32 chicks were excluded from the experiment, 22 from the middle group and 11 from the late group. The excluded chicks were selected to get a similar distribution between treatments and hen treatment in each hatching group.

Analysis

Nutrient analysis

Eggs from all treatment groups were collected for nutrient analysis during experimental days 26–31. Eggs were stored for four weeks at 4 °C before ten hens per treatment were randomly selected among the hens with confirmed fertile eggs and five fresh eggs were collected from each selected hen. Eggs were sent to Eurofins laboratory for analysis of vitamin A (retinol; EN 12823-1 2014), vitamin D_3 (EN 12821: 2009-08), iodine (EN 15111m:2007) and selenium (EN 13805m:2014, EN ISO 17294m:2016). The experimental diets were also analysed for DM, CP and ether extract as described in Boyner et al. (2021). In addition, the amino acid composition of the experimental diets was analysed according to International Organization for Standardization (2005).

Egg quality

Eggs were collected for egg quality analysis during experimental days 33–34 and 47–48. Only eggs of normal size (no double yolks), without visible cracks or dirt, were selected and the eggs were stored for a maximum 5 days at 4 °C before the assessment. Exterior parameters included were egg weight, colour of the shell using an eggshell colour guide from Zinpro (Zinpro corporation, Minnesota, USA) ranging from 1 to 10, where 1 was white and 10 dark brown, shell breaking strength (Orka Food Technology, Bountiful, UT, USA), and shell thickness. The shell thickness was measured on three different spots around the equator of the egg using a micrometre and the three values were used to calculate a mean value. The dry shell weight with membranes was recorded after being dried at 103 °C overnight and put in a desiccator for 1 hour to adapt to room temperature. The internal parameters included were weight of the albumen, albumen height, Haugh units, weight of the yolk and yolk colour according to the La Roche colour fan scale.

The Haugh unit was calculated according to the following equation: $100 \times \log$ (albumen height (mm) $- 1.7 \times$ egg weight in grams$^{0.37}$ + 7.6).

Antibodies

Blood samples were collected into test tubes without additives, and samples were stored for 24 h at room temperature before centrifuging for 10 minutes at 10 000*g*. Serum was then collected and stored at −20 °C until antibody analyses with ELISA methodology. Analysis of IgA and IgY was made on eggs collected at days 32, 33, and 47 and prepared for analysis at storage day 4 ± 1. Eggs were cracked open, the yolk was separated from the albumen, and 2 ml of yolk was transferred into Eppendorf tubes. The albumen was homogenised, and 2 ml was transferred into Eppendorf tubes. The samples were centrifuged at 21 000*g* for 20 minutes, and the water phase was then transferred to Eppendorf tubes by using a 10–100 µl pipette. The samples were stored at +4 °C until analysis. IgY was analysed in samples from egg yolks and sera from hens and chickens using Chicken IgY ELISA kit from Immunology Consultants Laboratory (ICL, Inc, USA). In addition, IgA was analysed on albumen samples using Chicken IgA ELISA from Immunology Consultants Laboratory. Both ELISAs were set up according to the manufacturer's protocols. The assays were performed in flat-bottomed 96-well plates (MaxiSorp, Nunc™, ThermoFisher Scientific, https://www.thermofisher.com) and an in-house substrate buffer (1 mM 3,5,3′,5′-tetrametylbenzidine in 0.1 M potassium citrate, pH 4.2, with 0.007% H_2O_2) was used for visualisation of antibody binding. The colour reaction was stopped at a

Table 3
Results from the external and internal egg quality traits of eggs from broiler breeders. Least square means, and pooled SEM.

	Hen treatment[1]				
Egg Traits	Control	Algae	Algal extract	Pooled SEM	*P*-value
Egg weight (g)	64.13	63.82	64.24	0.546	0.811
Breaking strength (kgF)	3.81	3.67	3.84	0.101	0.362
Shell thickness (mm)	0.32	0.32	0.31	0.315	0.071
Shell weight (g)	5.62	5.64	5.53	0.06	0.296
Shell percentage (%)	0.09	0.09	0.09	0.000	0.209
Shell colour	2.92	3.00	2.70	0.535	0.554
Yolk weight (g)	19.72	19.6	19.79	0.273	0.810
Yolk colour	13.71	13.81	13.85	0.105	0.501
Yolk percentage %	0.31	0.31	0.31	0.002	0.8396
Albumen height (mm)	8.01	7.79	7.9	0.127	0.3767
Haught Unit	88.20	87.11	87.57	0.795	0.5327
Albumen weight (g)	35.23	34.99	35.35	0.34	0.6712
Albumen percentage (%)	0.55	0.55	0.55	0.003	0.5093

Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract.
[1] Hen treatment = Hen diet.

standardised time point with 2 M H_2SO_4, and the A_{450} was measured in an ELISA reader. The total concentration of IgY or IgA in the samples was calculated by linear regression from serial dilutions of the chicken IgY or IgA standards included in the kits, and the ELISAs' linear ranges of detection were between 25 and 200 ng IgY/ml and 50 and 400 ng IgA/ml.

Antibody titres to IBV were analysed in serum samples from both hens and chicks using the IDEXX IBV Ab Test kit (#99-0926; IDEXX Laboratories, Inc (USA) according to the manufacturer's protocol. Samples were tested in duplicate at a dilution of 1:500, and results were expressed as titres calculated according to kit instructions.

Statistical analysis

The SAS statistical software, ver. 9.4, was used for the analysis of egg quality, chick quality, egg production, level of nutrients and antibodies, and transfer of antibodies. The lay percentage, number of eggs laid by a hen per week/7, was analysed with Mixed procedure with treatment and age as fixed factors. The interaction between treatment × age was also included in the model, and the module was included as a random factor. The nutrient content of the eggs was analysed with GLM procedure with treatment as a fixed factor. For the analysis of egg quality parameters, the Mixed procedure was used and treatment, analyse day, and age of hen was used as fixed factors and module as a random factor. Since the residual plots of shell colour and percentage of eggs with cracked shell did not show a normal distribution, they were analysed by the Glimmix procedure in SAS, with treatment as a fixed factor, where a binary logistic model was used to evaluate if shell colour and crack in the shell was affected by the treatment. Prior to the analyses, scoring values of 1 and 2 (pale colour) were converted to binary value 1 and scoring values 4 and 5 (dark colour) to the binary value 0 for shell colour. The number of eggs with cracked shell per week (1 and 2) was converted to binary value 1, and no appearance was converted to 0. The Glimmix procedure was also used for chick quality score with hen treatment and hatching time as fixed effect, and module as a random factor; chicks with the maximum score of 100 were converted to binary value 1 and chicks with less than score 100 to binary score 0. Moreover, the Procedure Freq was used to assess the frequency of chicks with maximum scores for the different hen treatments and hatching time, respectively. For hatching weight and hatching length, the individual chick was used as an experimental unit and analysed with the Mixed procedure. The model included hen treatment and hatching time as fixed factors and module and egg number × hen treatment as random factors.

For statistical evaluation of antibodies in yolk, serum, and albumen, the antibodies levels were log transformed and analysed with Proc Mixed with treatment, sampling day and the interaction of sampling day and treatment included in the model. A repeated statement for hen with UNSTRUCTURED covariance was also included in the model. In addition, the transfer percentage of antibodies from hen serum to yolk, from yolk to chick and from hen to chick, was calculated by dividing the yolk, and the chick day 3-serum values with the hen's serum or chick values with egg yolk values, respectively, and multiplying the results by 100. The hen serum sample that was biologically most relevant in time to the laying date of the egg was selected, ideal 3–4 days before the egg was laid. The effect of treatment on antibody transfer was analysed with GLM procedure with treatment as a fixed factor.

Results

Body composition, egg production and egg quality

No feed residues were observed throughout the experimental period, and the feed intake was hence 175 g/hen and day regardless of dietary treatment. On the final day of the experiment, the BW of the hens was on average 4326 g ± 369 g and did not differ ($P > 0.05$) between treatments. The percentage of fat pad of total BW did differ between treatments ($P = 0.035$) and was higher for hens fed Algae (2.06%) and Algal extract (1.98%) compared to hens fed the Control (1.67%). There were no significant differences ($P > 0.05$) in lay percentage between the treatments, age or age × treatment, and the lay percentage was on average 93.3% ± 0.75 (SEM). The number of eggs with cracked shells detected by eye for the total period was nine (1.4% of total eggs) and did not differ between treatments ($P > 0.05$). There were no significant differences (>0.05) between treatments with regard to egg quality traits (Table 3). There was, however, a tendency (<0.1) to a difference in shell thickness, where hens supplemented with Algal extract had the lowest value.

Nutrient content in eggs

Table 4 shows the analysed nutrient content of fresh eggs where eggs from hens fed Algae had higher level ($P < 0.0001$) of iodine compared to eggs from hens fed Control and Algal extract. A difference ($P < 0.05$) was found in selenium, where Algae eggs

Table 4
Results from the analysis of iodine and selenium (mg/kg), as well as vitamin A and vitamin D3 (ug/kg) in fresh eggs from broiler breeders. Least square means and pooled SEM.

	Hen treatment[1]				
Nutrient	Control	Algae	Algal extract	Pooled SEM	*P*-value
Iodine	0.594[b]	4.630[a]	0.735[b]	0.157	<0.0001
Selenium	0.392[a]	0.363[b]	0.388[a]	0.008	0.0388
Vitamin A	2 340	2 180	2 480	8.865	0.0722
Vitamin D_3	14.49	12.62	12.75	0.990	0.3441

Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract.
[1] Hen treatment = Hen diet.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

had the lowest concentration. Also, a tendency ($P < 0.1$) to difference was found in vitamin A where Algal extract had the highest concentration and Algae the lowest.

Antibody levels

The IgY concentration was measured in yolk and serum from the hens (Fig. 1a). The hen serum IgY concentration was not affected by algal feeding ($P > 0.05$) but was significantly higher at day 42 (4.7 mg/l) than at day 31 (4.4 mg/ml). The yolk IgY concentration was approximately 2-fold higher than that in hen serum, on average 9.3 mg/ml. The yolk IgY concentration was not affected by algal feeding ($P > 0.05$) but was affected by sampling day with higher concentration on day 47 compared to day 34, no significant difference between day 33 and day 34 was observed. The IgA concentration was measured in albumen (Fig. 1b) and was not affected by algal feeding ($P > 0.05$) but differed between all sampling days ($P < 0.05$) with the highest values on day 34 and the lowest on day 47.

Specific antibody titers to IBV were measured in serum from hens on arrival at the animal facility (experimental day −20), and 10 (experimental day 31) and 21 days (experimental day 42), after IBV booster vaccination at experimental day 21; Fig. 1c). The IBV titers were not affected by algal feeding but differed due to sampling time ($P < 0.05$). IBV titers prior to vaccination were in mean 5800 and increased approximately 2.0-fold and 2.5-fold, respectively, 10 and 21 days after the booster vaccination for all groups.

The percentages of IgY transfer from hen serum to yolk and from yolk to chick serum day 3, as well as IgY and IBV transfer from hen serum to chick serum day 3, were not affected by treatment ($P > 0.05$; Fig. 1d).

Chick quality

Hatching time but not hen treatment had a significant effect ($P < 0.05$) on hatching weight with chicks that hatched late (**La**) having the lowest hatching weight when compared to the early (**E**) and middle (**M**) groups, no effect on chick length at hatch was observed (Table 5). Hatching time had also an effect on scored chick quality with chicks in the late hatching group having the lowest percentage of birds with a total score of 100. In addition, hens fed Algal extract had a higher percentage of chicks with a score of 100 than hens fed Algae (Table 6).

Discussion

Feeding the brown algae *Saccharina latissima,* intact or as an extract to broiler breeders did not affect egg production, egg quality, antibody responses to vaccination or transfer of antibodies from the hen to the chick. The lack of effect on antibody responses and antibody transfer is in disagreement with our hypothesis and to what previous studies have observed in sows and piglets while feeding extracts from *Laminaria* spp. (Leonard et al. 2012). However, similar to our observations, no effects on antibody responses were observed by Akinyemi and Adewole (2022) while feeding brown algal meal and extract from *Ascophyllum nodosum* to growing broilers. Differences in both algal species, extract composition and animal species do likely have an impact on the level and uptake of low molecular weight laminarin which previously have been linked to the immune modulatory effect of algal extracts (Øverland et al., 2019).

The egg production, measured as lay percentage, was on average 93.3%, which correspond to 6.5 eggs/hen and week, which is above what is expected in practical production for breeders of these weeks of production (production week: 7–14; Aviagen, 2021). Moreover, the eggs had few remarks such as cracks in shell and the average egg weight was well above the 50 g which is required to be considered as a hatching egg according to Aviagen (2021). Together, this indicates that a large proportion of the laid eggs were hatching eggs. Zhao et al. (2019) tested the effects of different pre- and probiotics on performance of broiler breeders and in accordance with the current study, no effect on laying rate was observed. However, the effects on egg quality with higher albumen height and Haught unit with the addition of prebiotic apple pectin were observed in that study.

In the current study, feeding intact algae significantly increased the levels of iodine in the eggs which was not surprising since it is known that brown algae have a high content of iodine (Roleda et al.,2018). This would result in high content of iodine in the algae diet although no iodine was used in the premix. The iodine level in the algae diet was 12 mg/kg feed, which is higher than the allowed upper limit for iodine in complete feed, namely, 5 mg/kg feed for laying hens and 10 mg/kg feed for other species (European Commission Regulation, 2015). Yalçin et al. (2004) showed that iodine supplementation with 12 and 24 mg/kg feed reduced egg weight, egg albumen index and egg Haugh units in laying hens compared to those supplemented with 3 and 6 mg/kg feed. Although no negative effects on egg quality were observed in the current study, the high iodine level might have had a negative impact on chick quality since hens fed algae had a lower proportion ($P < 0.05$) of chicks with maximum quality score than hens fed algal extract (37.3 vs 58.1%) and numerically lower than hens fed control (51.4%). This study shows that the high iodine level in intact brown algae limits brown algae use in broiler breeder feeds considering that only 0.6% of algal meal was included in the diet, and still, the allowed upper limit was exceeded. In addition to the high iodine level, hens fed algae had a lower level of selenium in their eggs although the algae diet had the numerically highest selenium level. This was not expected because selenium and iodine are considered synergistic, and simultaneous enrichment of selenium and iodine should hence be possible (Ponomarenko, 2015). However, Kavtarashvili et al. (2017) stated that there is a lot left to elucidate regarding the efficacy of transfer of iodine and selenium to the egg both regarding different sources and different doses.

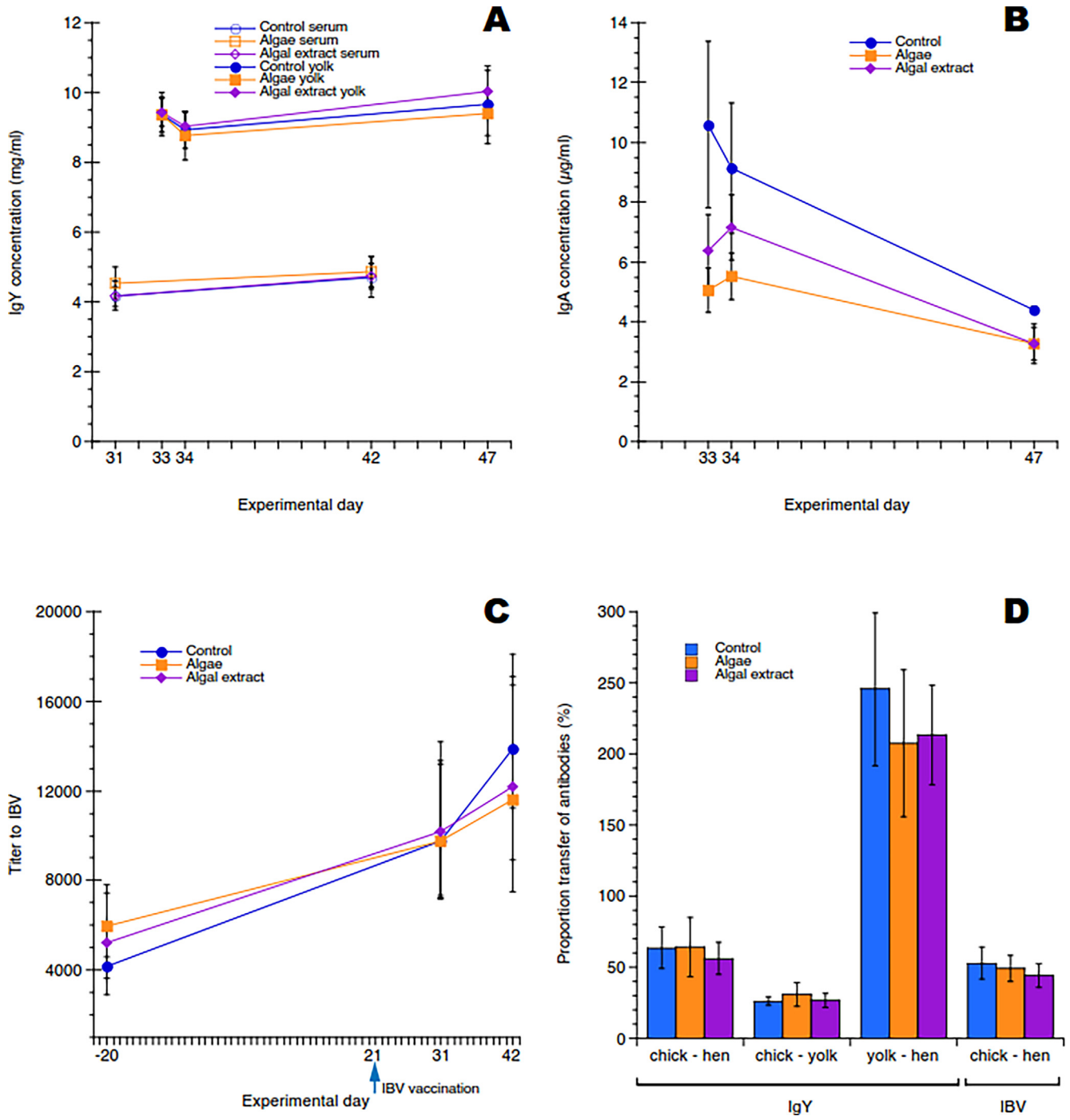

Fig. 1(a–d). Effects of feeding broiler breeders Control (blue colour), Algae (orange colour) or Algal extract (purple colour) on (A) the immunoglobulin (Ig)Y concentration in yolk and serum, (B) the IgA concentration in albumen and (C) titres to IBV in serum during the experiment. Samples were collected on the indicated experimental days and the infectious bronchitis virus (IBV) booster vaccination is indicated by an arrow in C. Results are shown as group mean values ± 1 SE. (D) Proportion transfer (%) of IgY from hen to chick, from hen to yolk and from yolk to chick and proportion transfer of specific antibodies to IBV from hen to chick. Results are shown as group mean values ±95% confidence interval. For details see material and methods. Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract.

Apart from hen dietary treatment, hatching time may affect initial chick parameters such as hatching weight, initial growth performance and chick quality (Bergoug et al. 2015; Van der Ven et al., 2011; Boyner et al., 2021). To consider this, hatching time was included in the statistical model while evaluating the effect of hen treatment. In addition, the results of hatching time itself were assessed and showed in accordance with Bergoug et al. (2015) that late-hatched chicks had lower chick quality. However, in contrast to Bergoug et al. (2015) and Boyner et al. (2021), the late-hatched chicks were lighter in the current study. One challenge in assessing the effect of hatching time is that the hatching windows may differ between hatches and studies and consequently, the definition of early, midterm and late-hatched chicks may differ and influence the results.

Table 5
Effects of hen treatment and hatching time on chick BW and length at hatch. Least square means and pooled SEM.

	Hen Treatment[1]					Hatching time[2]				
Parameter	Algae	Control	Algal extract	Pooled SEM	*P*-value	Early	Middle	Late	Pooled SEM	*P*-value
BW (g)	46.59	46.67	46.33	0.26	0.6063	46.84[a]	46.80[a]	45.96[b]	0.26	0.0496
Length (cm)	17.65	17.71	17.67	0.12	0.7413	17.69	17.65	17.69	0.12	0.8091

Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract. Early = chicks hatched between 0 and 16 h from the start of hatch; Middle = chicks hatched between 16 and 24 h from the start of hatch. Late = chicks hatched from 24 to 56 h from the start of hatch.
[1] Hen treatment = Hen diet.
[2] Hatching time = The hatching started at embryonic day 20 and occurred for 56 h and hatching time refers to when during this time span the chick was hatched.
[a,b] Values within hen treatment and hatching treatment, respectively, with different superscripts differ significantly at $P < 0.05$.

Table 6
Effect of hatching time and hen treatment on scored chick quality parameters. Percentage of chicks with score 100 and mean chick score ± SD for the different hen treatments and hatching times. Mean values of chick quality score for the different hen treatments and hatching times.

	Hen Treatment[1]				Hatching Time[2]			
Parameter	Algae	Control	lgal extract	*P*-value	Early	Middle	Late	*P*-value
Chicks with score 100	37.3[a]	51.4[ab]	58.1[b]	0.0463	56.2[a]	59.2[a]	32.4[b]	0.0031
Mean chick score	96.7 ± 4.29	97.3 ± 4.23	97.4 ± 4.12		97.9 ± 3.57	98.2 ± 3.11	95.3 ± 5.17	

Abbreviations: Control = Control diet; Algae = a diet supplemented with algal meal; Algal extract = a diet supplemented with Algal extract. Early = chicks hatched between 0 and 16 h from the start of hatch; Middle = chicks hatched between 16 and 24 h from the start of hatch. Late = chicks hatched from 24 to 56 h from the start of hatch.
[1] Hen treatment = Hen diet.
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[a,b] Values within hen treatment and hatching treatment, respectively, with different superscripts differ significantly at $P < 0.05$.

Interestingly, algal feeding, both intact and as an extract, increased the abdominal fat pad in broiler breeders with about 17% without affecting BW. The high selection for improved breast meat yields and FCR in the broilers have resulted in altered body composition in both broilers and breeders. Comparison of breeder lines from 1980 and 2000 shows that the abdominal fat pad has decreased from 5.38 to 2.65% of the BW during this period and that feed restriction is no longer needed to control body fat, though it may be important for other reproductive traits (Eitan et al., 2014). A threshold for minimum fat mass rather than lean mass for initiation of lay was suggested by van der Klein et al. (2018) showing that breeder hens that never commenced into lay only had 63% of the abdominal fat pad compared to those that laid an egg. The abdominal fat pad percentage was 1.67% in the control fed hens in the current study, which is rather close to the 1.5% reported by van der Klein et al. (2018) for the hens that did not commence into lay. Feeding strategies that increase the fat pad without increasing the BW such as algal supplementation in the current study could therefore be very interesting for the industry, and should be confirmed with further studies.

In conclusion, supplementation of broiler breeder diets with algal extract from *Saccharina latissima*, but not intact algal meal, is a promising dietary strategy to increase the abdominal fat pad without causing any adverse effects on nutrient level in eggs or chick quality.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101020.

Ethics approval

The experiment was approved by 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

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Author ORCIDs

E. Ivarsson: https://orcid.org/0000-0001-9813-6915.
H. Wall: https://orcid.org/0000-0002-4442-6826.
M. Boyner: https://orcid.org/0000-0001-5431-0408.
E. Wattrang: https://orcid.org/0000-0002-9804-169X.
H. Pavia: https://orcid.org/0000-0001-7834-6026.

Author contributions

E. Ivarsson: Conceptualisation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualisation, Validation, Writing – original draft, Writing – review and editing; **H. Wall:** Conceptualisation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review and editing. **M. Boyner:** Formal analysis, Methodology, Writing – review and editing. **G. Cervin:** Methodology, Validation, Resources, Writing – review and editing; H. Pavia, Funding acquisition, Resources, Writing – review and editing; **E. Wattrang:** Conceptualisation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Supervision, Writing – review and editing.

Declaration of interest

None.

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