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Effects of access to feed, water, and a competitive exclusion product in the hatcher on some immune traits and gut development in broiler chickens

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ABSTRACT

1. This study evaluated the effect of access to feed, water, and the competitive exclusion (CE) product Broilact®, administered in the hatcher, on broiler performance, caecal microbiota development, organ development, intestinal morphology, serum levels of IgY and vaccine-induced antibody responses.
2. In total, 250 chicks were hatched in a HatchCare™ hatcher and divided into four groups, given access to feed, water and the CE product sprayed on the chicks (CEs); access to feed, water, and the CE product in water (CEw); access to feed and water (Cpos); or no access to feed and water (Cneg) in the hatcher.
3. At the research facility, 10 chicks per hatching treatment were euthanised for organ measurements. The remaining 200 chicks were randomly distributed to 20 pens. On d 11, all birds were vaccinated against avian pneumovirus (APV). Three focal birds per pen were blood-sampled weekly for quantification of IgY and serum antibodies to APV. On d 11 and 32, two birds per replicate pen were euthanised for organ measurements and sample collection. Feed intake and body weight were recorded weekly.
4. Delayed access to feed and water reduced weight gain and feed intake early in life. At the end of the study, no differences in body weight remained.
5. There were some early effects on organs, with depressed intestinal development and higher relative gizzard weight for the Cneg group at placement. No treatment effects on the immune traits measured were detected.
6. The relative abundance of seven bacterial genera differed between treatment groups at d 11 of age. The results suggested that chickens are capable of compensating for 40 h feed and water deprival post-hatch. Provision of Broilact® did not have any persistent performance-enhancing properties, although different outcomes under rearing conditions closer to commercial production cannot be ruled out.

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Introduction

The majority of broiler chickens intended for meat production start their lives at a commercial hatchery. There, brooded eggs are inserted into the hatcher and, depending on factors such as egg storage time prior to brooding, broiler breeder age, and biological variation, each batch of eggs will hatch over a period of 24–48 h. This period is referred to as the 'hatching window' and may generate problems, if it is too long. In conventional practice, chicks are given their first access to feed and water at the rearing farm. After loading and transportation, particularly early-hatched chicks may be feed-deprived for up to 72 h post-hatch on arriving at the rearing farm (Willemsen et al. 2010).

Chicks that have been deprived of feed and water post-hatch have been shown to have lower utilisation rate of the yolk sac, which may have a negative impact on the uptake of maternal antibodies transferred from the mother hen to the chick *via* this temporary organ (Gonzales et al. 2003). The chick's immune system at hatch is still immature and the chick is therefore dependent on these maternal antibodies to withstand pathogens in the surrounding environment. Moreover, at hatch the gut is susceptible to bacteria, whether pathogenic or favourable (Lan et al. 2005). The early responsiveness of the gut makes it possible to colonise it artificially with bacteria that have been shown to be beneficial for chick gut health (Seifi et al. 2017). Favourable bacteria that are deliberately added to the diet are called probiotics and are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO 2002). Probiotic bacteria can act beneficially through several different activities. One of these is competitive exclusion, whereby the bacteria bind to receptor sites in the gut epithelium (Seifi et al. 2017), blocking harmful bacteria from colonising these sites and impacting the host. Supplementation with probiotics is well known to have immuno-modulatory effects and has been shown for example to increase the relative weight of the spleen and bursa, organs important for the immune response (Karimi Torshizi et al. 2010). Supplementation with *Lactobacilli* spp. has been shown to increase serum levels of immunoglobulin (Ig)Y and IgM in broiler chickens (Koenen et al. 2004). In Finland, most broiler chickens are given the competitive exclusion (CE) product Broilact® (Orion Corporation, Espoo, Finland), consisting of normal microflora of poultry derived from the caecum of healthy hens (Schneitz and Hakkinen

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2016). The main constituent bacterial groups of the product (at genus level) have been determined as *Escherichia* (named *Escherichia-Shigella*, 42.2–43.14%), *Enterococcus* (14.06–17.18%), *Bacteroides* (11.04–12.57%), and *Lactobacillus* (6.6–8.62%) spp. in a previous study (Such et al. 2021). For chicks hatched in hatcheries, the product may mimic the natural transfer of a healthy microbiota from mother hen to chick. Broilact® is provided in the drinking water or as an aerosol sprayed on the down of the chicks.

The aim of this study was to investigate whether adapted management routines immediately post-hatch can be beneficial for chicken immune response and growth. The effects of access to feed and water already in the hatcher, in combination with Broilact® supplied in the drinking water or as an aerosol of water sprayed on the down of the chicks, were studied. Variables of interest included growth, feed intake, serum levels of IgY, vaccine-induced antibody responses, intestinal development, and gut microbiota.

Materials and methods

Procedure at the hatchery

All chicks included in the study were hatched in a HatchCare™ hatcher manufactured by HatchTech. The baskets in a HatchCare™ hatcher have cavities into which feed is distributed prior to emergence. By stretching their heads out of the box, chicks can reach water in gutters lining the wall of the hatcher. The HatchCare™ system provides an illuminated environment for hatching in bright light. At the participating hatchery (located in southern Sweden), a total of 250 chicks with wet down were collected from the boxes during a period of 3 h, in order to reduce the variation in the hatching window. These chicks were randomly distributed to one of four treatments. Chicks were divided into baskets according to group where each treatment had their own separate water through. Treatment groups were: i) a negative control group that received neither feed nor water (Cneg); ii) a positive control group that received feed and water during hatch (Cpos); iii) a Broilact® in water group (CEw) that had access to feed and water with Broilact® added; and iv) a Broilact® spray group (CEs) that had access to feed from the beginning and received water when the droplets sprayed on the down had been consumed and/or dried. Chicks in the groups that were provided feed (Cpos, CEw and CEs) were given a commercial pre-starter feed including a coccidiostat (Lantmännen, Falkenberg) at the hatchery.

When all birds had been collected and placed in the hatcher according to the treatment schedule, fresh water with Broilact® added was provided in the water trough of the CEw group every 4 h for a total of 12 h. Before adding the fresh Broilact® solution, the residual solution in the water trough was drawn out using a syringe and consumption was calculated. Mean total consumption of Broilact® per bird was approximately 2 mg during the 12 h of supplementation. When the birds had started to drink in the boxes with immediate access to water, the 60 chicks in the CEs group were evenly sprayed with Broilact® solution (1 mg of Broilact® per 0.3 ml regeneration agent water solution per chick) at approximately 12 h after cease of placement, according to the dose recommendation protocol provided by Orion Corporation, using a handheld spray bottle.

Transportation, placement, and feed

After pull, sorting and standard hatchery quality control (approximately 1 h), the birds were transported (approximate transportation time 16 h) to the Swedish Livestock Research Centre at the Swedish University of Agricultural Sciences, Uppsala, where they were given access to feed and water 17 h after pull. The chickens in the study were hatched approximately 24–27 h prior to pull, meaning that chicks in the Cneg group had been without access to feed and water for approximately 40 h on arriving at the research facility. The remaining treatment groups were given continued access to regular water pending transportation but they had no access to feed after sorting and were thus without access to water and feed for about 15 and 16 h, respectively, post-pull. Immediately on arrival at the research facility, 10 birds per treatment group ($n = 40$ in total) were euthanised for dissection and organ excision and weighing. The remaining chicks from each hatchery treatment were randomly distributed to five replicate modules with 10 chicks in each, resulting in 200 chicks distributed over 20 modules. Three focal birds per replicate (module) were wing-tagged so that blood samples could be taken from the same birds throughout the study. Each module measured 1.5 m × 0.75 m and contained a feeder and three nipple drinkers and was bedded with wood shavings. On arrival, the temperature in the research facility was set to 33°C. After 3 d, the temperature was successively lowered to reach 23°C at 24 d and remained so for the rest of the study. Constant light was provided on the day of arrival and the following day. On the third day, the chicks were given 1 h of darkness between 11 pm and midnight. Thereafter, the chicks were provided with 1 h of extra darkness per night until d 8. From this point until the end of the study, the lights were off between 11 pm and 5 am. Day 0 was defined as the time when most chicks hatched, *i.e.*, during embryonic d 20 (ED20). From day of placement (d 2) until d 10, the chicks were provided with a commercial starter feed. On d 11, the starter feed was replaced with a commercial grower feed (both feeds Svenska Foder AB, Lidköping, Sweden). No coccidiostats were included in the feed given at the research facility. Feed samples were analysed for dry matter, crude protein, crude fibre, ash, and fat (as ether extract). Sub-samples were dried for 16 h at 103°C for analysis of dry matter (DM). Ash was analysed according to Jennische and Larsson (1990), after incineration for 3 h at 550°C. The European Community (1998) methodology was used for analysis of ether extract, while crude protein content (Nx6.25) was measured according to the Kjeldahl method (Nordic Committee on Food Analysis 2003). Analysed chemical composition of the starter feed was ash 74 g/kg DM, crude protein 241 g/kg DM, crude fibre 41 g/kg DM, and ether extract 69 g/kg DM, and that of the grower feed was ash 52 g/kg DM, crude protein 237 g/kg DM, crude fibre 40 g/kg DM, and ether extract 63 g/kg DM. The calculated metabolisable energy content was 12.8 ME MJ/kg DM for the starter feed and 13.1 ME MJ/kg DM for the grower feed.

Growth, feed intake and organ development

Organ weight (yolk sac, small intestine with content, spleen, bursa, heart, liver, proventriculus, gizzard with contents and rinsed gizzard) and length (body length, small intestine with contents) were recorded from the 10 euthanised birds per

treatment (two birds per replicate) at 2 (prior to placement at the research facility), 11 and 32 d of age. Birds at 2 and 11 d of age were euthanised by neck dislocation following stunning with a blow to the head. Birds at 32 d of age were euthanised by a 100 mg/ml intravenous injection of pentobarbital sodium in the wing vein. Chicken weight and feed consumption per module were recorded weekly. Mortality was recorded daily.

Histology: villi height, villi width, and crypt depth in duodenum

From the chicks sacrificed for organ measurements at two and 11 d of age, the small intestine was rapidly removed and a 3-cm-long piece of duodenum, distal to the duodenal loop, was excised. The tissue was cut open, pinned to a small rectangle of cork to minimise distortion and fixed in glutaraldehyde (2.5%, pH 7.2) overnight. It was then rinsed in phosphate buffer (1/15 M, 7.2 pH) and trimmed into 2 mm thick transverse slices, which were dehydrated in increasing concentrations of ethanol and embedded in water-soluble resin (Leica Historesin, Heidelberg, Germany). Sections (2 µm) of resin-embedded duodenum were stained with haematoxylin-eosin for evaluation by light microscopy. Before evaluation, all slides were coded, to avoid bias due to the observer, and digital images of duodenum sections were taken with a Nikon Microphot-FXA microscope using a 4× objective lens (Bergström Instrument AB, Stockholm, Sweden). Five consecutive villi per sample were measured. The villi chosen had to have an intact lamina propria and a single epithelial cell layer, to avoid including samples that could have been cut askew. Only representative villi that were judged not to have been affected by preparation and that were free from artefacts were chosen. Villi where the tip ends were diffuse or those with invisible crypts were not selected for analysis. Crypts were measured in the same direction as the villi base, from the branching to the start of the 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 to the tip. Villi width was measured perpendicular to the tip (Figure 1).

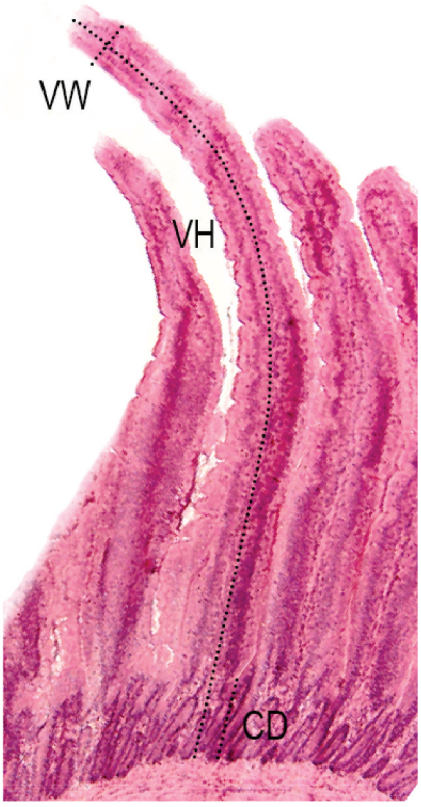

Figure 1. Histological image of the duodenum. Villi height (VH) was measured from the tip of the villus to the start of the muscularis mucosa. Crypt depth (CD) was measured in the same direction as the villi base, from the branching to the start of the muscularis mucosa. Villi width (VW) was measured beneath the villi tip where the epithelial cell nuclei had straightened out and were no longer at an angle to the tip. Villi width was measured perpendicular to the tip.

Quantification of serum levels of IgY and vaccine-induced antibody responses

Blood samples from 15 focal birds per treatment were collected from the jugular vein into test tubes without additive at 3, 11, 18, 25 and 31 d of age. These samples were stored for 24 h at room temperature before centrifuging for 10 min at 10 000 × g. Serum was collected and stored at −20°C prior to analysis with ELISA methodology.

The total amount of IgY in serum from all sampling occasions was analysed using the Chicken IgG ELISA Quantitation Set (Cat. No. E30–10) manufactured by Bethyl Laboratories Inc. (U.S.A) and the ELISA assay was set up according to the manufacturer's protocol. The assay was performed in flat-bottomed 96-well plates (MaxiSorp, Nunc™, ThermoFisher Scientific, www.thermofisher.com). 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 with 2 M H_2SO_4 at a standardised time point and the A_{450} value was measured in an ELISA reader. The total IgY concentration in the samples was calculated by linear regression from serial dilutions of the chicken IgY standard included in the kit. The linear range of detection of the ELISA assay was between 25 and 200 ng IgY/ml.

After blood sampling on d 11, all birds were vaccinated with commercial vaccine Nobilis RT Inac vet (MSD Animal Health) against avian pneumovirus (APV). All birds were injected intramuscularly with 0.5 ml vaccine into the breast muscle. Serum samples from d 11 and 31 were analysed for antibodies to APV, using the Avian Pneumovirus Antibody Test Kit (06-44 300-04) manufactured by IDEXX Laboratories Inc. (U.S.A) according to the manufacturer's protocol. Samples were tested in duplicate and, to increase the detection limit, serum was diluted 1:100, rather than the recommended 1:500. Results were expressed as absorbance values at 650 nm and a cut-off value for samples deemed positive for antibodies to APV was calculated as the mean absorbance value +2 standard deviations for all pre-vaccination samples collected at d 11 ($n = 117$).

Gut microbiota

At d 2, the contents from both caeca were collected from 10 birds per treatment. On d 11 and 32, samples were collected from two birds per replicate euthanised for organ sampling. In total, 120 samples were collected with an aseptic procedure, immediately frozen in liquid nitrogen, and thereafter stored at −80°C until extraction.

DNA extraction and sequencing

DNA was extracted from 180 to 220 mg caecal contents from 120 samples in total (four treatments, three ages, and 10 replicates per treatment at each age) using a QIAamp Fast DNA Stool Mini Kit (CatNo. 51604, Qiagen, Germany). Due to technical reasons, one sample from Cneg at d 2 and one sample from CEs at d 32 were missing in the analysis. The kit was used according to the manufacturer's instructions with some minor changes, including use of bead beating to break down bacterial cell walls. In brief, 0.3 g sterilised 0.1 mm zirconia/silica beads (BioSpec Products, Bartlesville, U.S.A) and 1 ml InhibitEX Buffer from the QIAamp Fast DNA Stool Mini Kit were added to each sample and homogenised by vortexing for 1 min. The suspension was heated for 5 min at 70°C to lyse cells. Samples were then cooled on ice before running in the Precellys24 sample homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France) at 1 × 60 s at 60 × *g* for two rounds, with 5 min on ice in between. Samples were then centrifuged for 5 min at 9600 × g to pellet particles. The supernatant (700 µl) was pipetted to new 1.5 ml tubes and centrifuged again for 5 minutes at 17 000 × *g*. Thereafter 400 µl of the supernatant were mixed with 30 µl proteinase K and 400 µl AL buffer and vortexed for 15 s, followed by incubation at 70°C for 10 min. A further 400 µl 99.5% ethanol were then added before vortexing again. Lysate was added (2 × 600 µl) to clean QIAamp spin columns and centrifuged at full speed (21 100 × *g*) for 3 min. Each QIAamp spin column was placed in a new collection tube, AWI buffer (500 µl) was added, and the tube was centrifuged again for 1 min at full speed. The column was then moved to a new collection tube and AW2 buffer (500 µl) was added, followed by centrifuging for 3 min at full speed. The columns were placed in clean collection tubes and centrifuged empty for 1 min before being moved to Eppendorf tubes. The DNA was eluted with 100 µl buffer and stored at −20°C for delivery to Novogene (Beijing, China). The library of 16S rRNA gene was constructed and sequenced at Novogene using the Illumina HiSeq 2500 platform. In brief, the V3-V4 region of 16S rRNA gene was amplified with primers 341F (CCTAYGGGRBGCASCAG) and 806 R (GGACTACNN GGGTATCTAAT). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs).

Bioinformatic analysis

The raw sequencing data have been deposited in database of the National Centre for Biotechnology Information (NCBI), under accession number PRJNA813981. Bioinformatic data processing was performed using Quantitative Insights into Microbial Ecology 2 (Core 2019.04; Bolyen et al. 2019). The barcode and primer sequence of raw demultiplexed reads were trimmed off. The trimmed reads were further processed using DADA2 to denoise, dereplicate reads, merge pair end reads, and remove chimeras (Callahan et al. 2016), using truncation length of 221 bp for both forward and reverse reads. A phylogenetic tree was built using FastTree and MAFFT alignment (Katoh et al. 2002; Price et al. 2010). The SILVA SSU Ref NR 99 132 dataset was first trimmed to the corresponding primer region and trained as classify-sklearn taxonomy classifier (Pedregosa et al. 2011; Quast et al. 2013; Bokulich et al. 2018). The amplicon sequence variants (ASV) were then assigned taxonomy using the resulting classifier. After trimming and quality filtering, the sequencing of 16S rRNA gene yielded a total of 7,793,838 sequences from 118 samples. A minimum of 27 311 sequences per sample was used for rarefying the number of reads per sample (Weiss et al. 2017). The generalised UniFrac distance matrix (alpha = 0.5) and alpha rarefaction were generated using the QIIME2 diversity plugin (Chen et al. 2012; Bolyen et al. 2019).

Statistical analyses

Analysis of data on growth, feed conversion ratio (FCR), feed intake, organ weight, and histology was performed using the statistical program SAS (version 9.4). All data were analysed using the procedure mixed (PROC MIXED) statement, with hatching treatment as fixed factor and module as random factor. At d 2, module was not included in the random statement for organ and histology data, because the chicks were yet to be assigned to modules. Organ weights (d 11 and 32) were analysed with age as an additional fixed factor and a repeated statement. The unstructured UN covariance structure was primarily used and replaced with the first order autoregressive AR (1) when needed. Antibody data were presented as mean values with 95% confidence intervals. Mean values with non-overlapping confidence interval were treated as rejecting the null hypothesis of no difference. Fisher's exact test was used to investigate whether there were significant differences in proportions of positive and negative responders to APV between groups. Microbial differences due to hatching treatment and age at the phylum, class, order, family, genus and ASV level were analysed with ANCOM methodology (Mandal et al. 2015). To investigate the microbial difference between hatching treatment at genus level on d 11, the rarefied ASV table was used to select the genera that had a relative abundance (RA) higher than 1%. The selected genera were analysed with quasi-Poisson generalised linear models using R (https://r-project.org).

Results

Body weight, length and organ development at placement

On arrival at the research facility on d 2, the organ data collected from 10 chicks per hatching treatment group revealed no differences in body weight, chick length, or yolk sac weight (in g or as proportion of body weight; Table 1). There was a tendency for a difference in yolk-free body mass (YFBM), *i.e.*, body weight excluding yolk weight, with lower weight in the Cneg group compared with all other groups. There were no differences in the relative weight of spleen, bursa, heart, liver, or of proventriculus and gizzard when weighed together. However, there was a difference in intestinal weight (expressed as a proportion of total body weight), with the CEw, CEs, and Cpos groups having heavier intestines than the Cneg group. The CEw group had greater relative intestine weight than the Cpos group. In addition, there was a difference in absolute numerical terms (data not shown), with the Cneg group having lighter intestines ($P < 0.0001$; 2.5 g) than the Cpos (3.39 g), CEw (3.71 g) and CEs (3.45 g) groups. Regarding intestine length, the Cneg group had shorter intestines than all other groups. The Cneg group also had shorter intestines in numerical terms (48.1 cm, $P < 0.0001$) than Cpos (57.95 cm), CEw (57.4 cm), and CEs (58.25 cm). Moreover,

Table 1. Body, yolk sac, and organ weight at 2 d of age in chicks subjected to four different treatments in the hatcher: no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion product (CE) provided in the water (CEw), or access to feed, water, and a CE product sprayed on the down of the newly hatched chicks (CEs). Values shown are based on measurements on individual birds.

	Hatching treatment					P-value
Variable	Cneg n=10	Cpos n=10	CEw n=10	CEs n=10	SEM	Hatching treatment
Body weight (g)	[1]41.5	44.1	44.3	44.0	0.98	0.1588
Chick length (cm)	21.2	20.4	20.8	20.9	0.36	0.4272
YFBM (g)	39.8	42.4	42.9	42.6	0.94	0.0925
Yolk sac (g)	1.69	1.76	1.35	1.43	0.18	0.2999
Yolk sac (g/kg bw)	40.6	39.4	30.7	32.5	3.86	0.1947
Small intestine (g/kg bw)	[a]60.3[c]	76.8[b]	83.8[a]	78.4[ab]	1.88	<.0001
Small intestine (cm/kg bw)	1161[b]	1317[a]	1298[a]	1327[a]	36.6	0.0091
Spleen (g/kg bw)	0.30	0.37	0.47	0.38	0.047	0.1350
Bursa (g/kg bw)	1.67	1.55	1.43	1.51	0.115	0.5312
Heart (g/kg bw)	8.91	9.30	9.20	9.19	0.327	0.8480
Liver (g/kg bw)	30.0	30.0	30.4	31.7	0.682	0.2902
Proventriculus & gizzard (g/kg bw)	73.3	81.6	77.9	76.7	2.58	0.1770
Gizzard full (g/kg bw)	61.3	69.7	66.4	65.0	2.22	0.0895
Gizzard empty (g/kg bw)	59.1[a]	53.9[b]	53.0[b]	53.7[b]	1.2	0.0039

[1]Values are least squares means (LSM).
[2]LSM values within rows lacking a common superscript are significantly different ($P<0.05$).
***$P<0.001$; **$P<0.01$; *$P<0.05$; ns $P>0.05$.

the Cneg group had the heaviest gizzard, when weighed emptied and rinsed, of all the groups (Table 1).

There were no differences between treatments in absolute numerical gizzard weight when weighed emptied and rinsed ($P < 0.7678$; Cneg: 2.46 g; Cpos: 2.38 g; CEw: 2.35 g, CEs: 2.37 g). However, there was a tendency ($P = 0.0895$) for a difference in full relative gizzard weight, where Cpos had the numerically highest and Cneg the numerically lowest weight.

Growth, FCR, feed intake, and organ development during the growing phase

The Cneg group had lower body weight than all other groups from 2 to 11 d (Table 2). At 18 d, Cneg still had lower body weight compared with CEs and Cpos. Moreover, CEw had lower body weight compared with the CEs and Cpos groups. At 25 d, the difference in body weight persisted only between Cneg and Cpos. At 32 d of age, there were only slight differences between treatments, and these were no longer significant after adjustment using the Tukey's test.

At 4 d of age, the Cneg group had lower feed intake (FI) than the CEw group. At 11 and 18 d of age, Cneg had lower FI than all other groups, while at 25 and 32 d of age Cneg had lower FI than the Cpos and CEs groups.

There were some differences in FCR during the grow-out period. At 18 d of age, the CEw group had inferior FCR to the Cpos group, while at 25 and 32 d of age the CEw group had poorer FCR compared to the other groups (Table 2).

No effects of hatching treatment on organ weight and length were observed during the grow-out period (Table 3). However, there was an effect of age, with YFBM (g) and relative weight of spleen and bursa increasing with age. Moreover, a decrease in proportional weight or length was

Table 2. Body weight, accumulated feed intake (FI), and feed conversion ratio (FCR) at six different ages in chickens subjected to four different treatments in the hatcher: no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water and a CE product sprayed on the down of the newly hatched chicks (CEs). Values are hatching group mean values.

	Hatching Treatment					
	Cneg n = 5	Cpos n = 5	CE_W n = 5	CE_S n = 5	SEM	*P-value*
Body weight (g)						
2	[1]40.7[b]	45.7[a]	45.2[a]	[2]45.2[a]	0.66	<.0001
4	70.9[b]	85.1[a]	81.7[a]	82.6[a]	1.07	<.0001
11	278[b]	322[a]	303[a]	312[a]	4.77	<.0001
18	688[b]	782[a]	704[b]	769[a]	15.4	<.0011
25	1283[b]	1428[a]	1313[ab]	1399[ab]	29.3	0.0095
32	2027	2195	2034	2180	48.0	0.0396[3]
FI (G)						
4	26.5[b]	34.9[ab]	36.7[a]	35.1[ab]	2.88	0.0191
11	273[b]	311[a]	302[a]	306[a]	6.24	0.0003
18	791[b]	886[a]	841[a]	876[a]	14.0	0.0010
25	1621[b]	1797[a]	1706[ab]	1766[a]	28.6	0.0030
32	2748[b]	2984[a]	2854[ab]	2965[a]	49.5	0.0145
FCR						
4	0.92	0.89	1.02	0.95	0.082	0.5258
11	1.14	1.12	1.16	1.14	0.019	0.1904
18	1.21[ab]	1.20[b]	1.26[a]	1.21[ab]	0.013	0.0166
25	1.31[b]	1.31[b]	1.37[a]	1.32[b]	0.015	0.0028
32	1.37[b]	1.38[b]	1.44[a]	1.39[b]	0.012	0.0083

[1]Values are least squares means (LSM).
[2]Values within rows lacking a common superscript are significantly different ($P < 0.05$).
[c]No statistically significant differences between treatments could be demonstrated using Tukey's test.
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.

Table 3. Body and organ weight at 11 and 32 d of age in chickens subjected to four different treatments in the hatcher: no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water and a CE product sprayed on the down of the newly hatched chicks (CEs). Values are based on measurements on individual birds.

	Hatching group					Age			P-value		
Variable	Cneg n = 5	Cpos n = 5	CE_W n = 5	CE_S n = 5	SEM	11 n = 20	32 n = 20	SEM	Hatching treatment	Age	Hatching treatment *age
Body weight (g)	[1]1153	1235	1220	1237	25.7	[2]18[b]	2105[a]	14.5	0.1100	<.0001	0.2038
YFBM (g)	1153	1235	1220	1236	25.8	317[b]	2104[a]	14.5	0.1125	<.0001	0.1994
Intestine (g/kg bw)	70.0	70.1	71.9	72.0	0.97	85.4[a]	56.6[b]	0.83	0.3030	<.0001	0.7146
Intestine (cm/kg bw)	225.2	212.7	218.8	221.8	6.04	353.2[a]	86.0[b]	3.35	0.5282	<.0001	0.8263
Spleen (g/kg bw)	0.86	0.86	0.90	0.79	0.054	0.71[b]	1.00[a]	0.036	0.5699	<.0001	0.1046
Bursa (g/kg bw)	2.00	1.87	1.98	1.88	0.112	1.79[b]	2.08[a]	0.070	0.7860	0.0036	0.1452
Heart (g/kg bw)	7.79	7.20	7.33	7.20	0.202	8.38[a]	6.38[b]	0.160	0.1682	<.0001	0.8272
Liver (g/kg bw)	30.9	33.5	31.4	31.7	1.157	36.8[a]	26.9[b]	0.736	0.4324	<.0001	0.7171
Proventriculus & gizzard (g/kg bw)	36.7	35.6	35.3	34.5	0.724	45.6[a]	25.4[b]	0.583	0.2094	<.0001	0.6443
Gizzard full (g/kg bw)	30.7	29.9	29.5	28.9	0.655	38.4[a]	21.2[b]	0.539	0.3086	<.0001	0.4043
Gizzard empty (g/kg bw)	19.8	18.7	18.6	18.8	0.395	24.8[a]	13.2[b]	0.305	0.1496	<.0001	0.3230

[1]Values are least squares means (LSM).
[2]LSM values within rows lacking a common superscript are significantly different ($P < 0.05$).
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.

observed when considering intestinal weight, intestinal length, heart, liver, proventriculus, and gizzard weighed together, as well as gizzard alone, either with contents or emptied and rinsed (Table 3).

Intestinal development

There were no differences between hatching treatments with regard to villi height, width, crypt depth or the ratio between villi height and crypt depth at 2 or 11 d of age (Table 4). There was a tendency ($P = 0.0654$) for a difference in crypt depth at 2 d of age, with Cpos having numerically more shallow crypts. A corresponding tendency ($P = 0.0978$) in the ratio between villi height and crypt depth was recorded at the same age.

Total levels of IgY in serum and vaccine-induced antibody responses

Total concentration of IgY in serum was monitored throughout the experiment (Figure 2). The results showed that in general, all chicks had the highest observed levels of IgY in serum on d 3. The serum levels of maternally derived antibodies then rapidly declined and serum IgY showed the lowest observed levels on d 18 (approximately 10% of d 3 levels). Thereafter, serum IgY levels were found to be slightly increased on d 25 and 31 (to approximately 20% of d 3 levels). To reduce the influence of variation between individuals, individual IgY levels relative to d 3 values were also calculated (data not shown). However, no differences in total IgY levels, either as actual or relative amounts, were observed between treatments during the experiment.

All chicks were vaccinated with an inactivated APV vaccine at 11 d of age and specific antibody levels to APV were recorded on d 11, prior to vaccination, and on d 31, 20 d after vaccination (Figure 3). Based on pre-vaccination serum values, a technical cut-off value for detection of antibodies to APV was calculated as Abs_{650} 0.086. Based on this definition, 44% of the chicks responded with antibody production after vaccination, although substantial antibody responses were observed for fewer individuals (Figure 3). No difference in APV antibody levels or in the proportion of responding individuals was observed between the treatment groups (CEs 47%, CEw 36%, Cpos 33%, Cneg 60%). Overall, chickens that were deemed positive for vaccine-induced antibody production also showed higher total serum IgY levels (1.131 ± 0.174

Table 4. Villi height and length and crypt depth in intestinal sections sampled at 2 and 11 d of age in chicks given four different treatments in the hatcher: no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water, and a CE product sprayed on the down of the newly hatched chicks (CEs). Values are based on measurements on individual birds.

	Hatching treatment					P-value
Variable (µm)	Cneg n = 5	Cpos n = 5	CE_W n = 5	CE_S n = 5	SEM	Hatching group
Villi height						
2d	[1]699.5	585.5	669.6	704.6	48.0	0.3034
11d	1138	1160	1276	1265	81.2	0.5327
Villi width						
2d	92.6	89.1	97.1	100.5	5.63	0.5152
11d	116.5	134.2	124.5	134.5	10.8	0.6046
Crypt depth						
2d	93.5	61.7	85.7	92.2	8.64	0.0654
11d	126.9	148.1	154.9	134.2	9.49	0.1864
Ratio[2]						
2 d	7.51	9.86	8.14	7.81	0.666	0.0978
11 d	9.12	7.85	8.23	9.67	0.628	0.1991

[1]Values are least squares means (LSM).
[2]Ratio is defined as villi height divided by crypt depth.

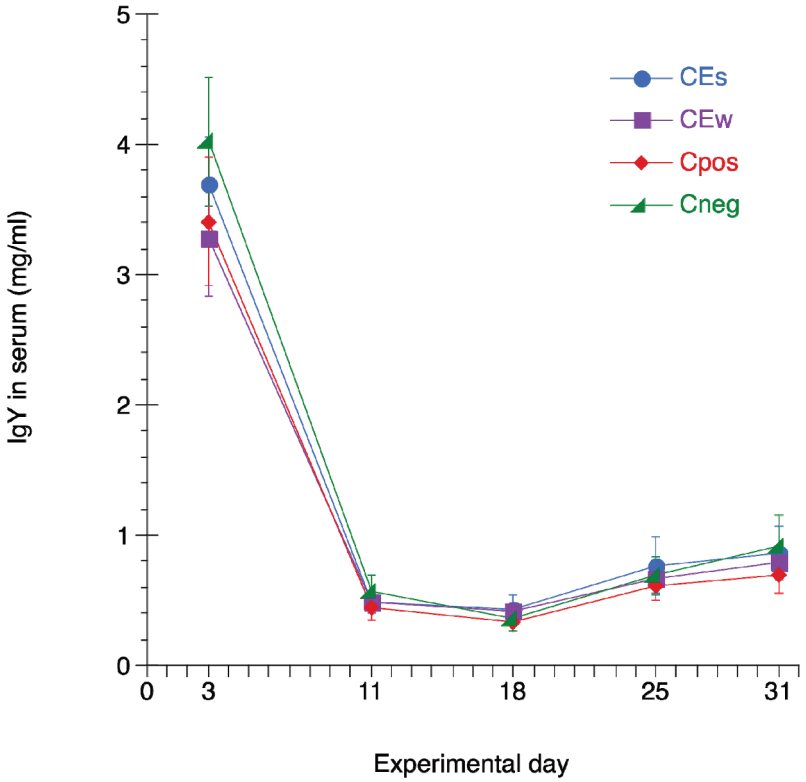

Figure 2. Total amounts of IgY in serum collected from chickens at 3, 11, 18, 25 and 31 d of age. Values are group mean ±95% confidence interval. Treatments: chicks in the hatcher were given access to feed, water, and a competitive exclusion (CE) product sprayed on the down of the newly hatched chicks (CEs; circles), access to feed, water, and a CE product provided in the water (CEw; squares), access to feed and water only (Cpos; diamonds), or no access to feed and water (Cneg; triangles).

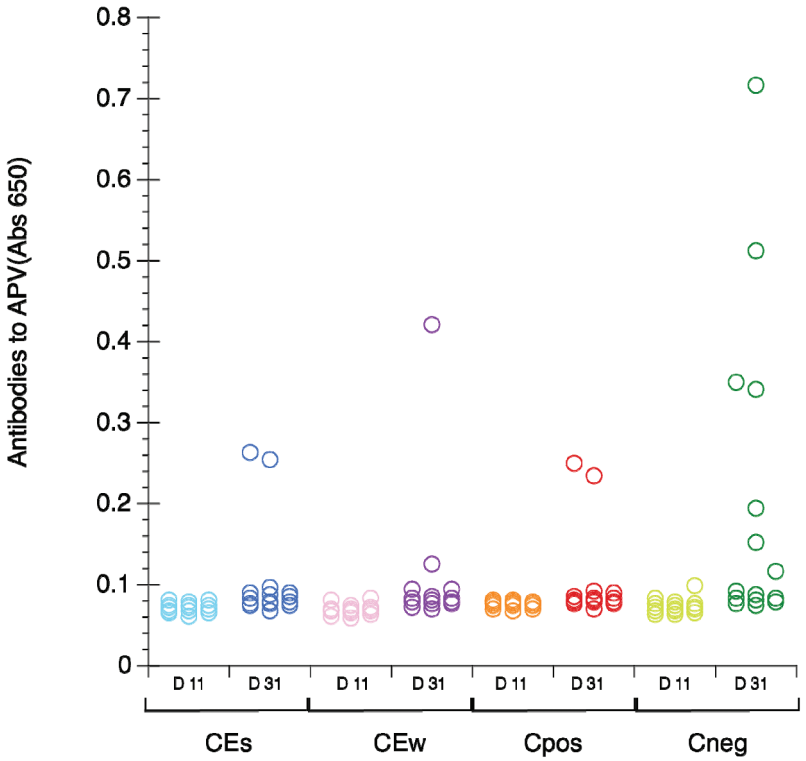

Figure 3. Antibodies to avian pneumovirus (APV) in serum samples collected before vaccination against APV at d 11 and 20 after vaccination at d 31. Results shown are absorbance 650 nm values for individual chickens in the four treatment groups. The cut-off value for samples testing positive for antibodies to APV was calculated to be 0.086 (for details, see Materials and Methods). Treatments: chicks in the hatcher were given access to feed, water, and a competitive exclusion (CE) product sprayed on the down of the newly hatched chicks (CEs), access to feed, water, and a CE product provided in the water (CEw), access to feed and water only (Cpos), or no access to feed and water (Cneg).

mg IgY/mL serum) at d 31 compared with negative chickens (0.700 ± .083 mg IgY/mL serum) (mean ±95% confidence interval; $n = 57$ and $n = 59$, respectively).

Microbial populations

The 16s rRNA gene sequences were distributed in 807 amplicon sequence variants (ASV), representing 91 taxonomic families and 179 genera. The rarefaction curves of observed ASV revealed an effect of age (Figure 4(a)). As the age of chicks increased, the average number of observed ASV increased from 53 at d 2 to 187 at d 11 and 258 at d 32. A principal coordinate analysis (Poi) plot of generalised UniFrac distance matrix revealed an effect of age, whereas treatments did not show clear effects (Figure 4(b)). Four samples from the CEw group and one sample from CEs at d 11 were clustered closer to d 32, and one sample from CEw at d 32 was closer to d 11. The relative abundance of *Bacteroides* and *Alistipes* most likely explained this clustering pattern, with higher levels of these two genera associated with samples at d 32.

An effect of treatment on the microbial composition at genus level was observed at d 11 (Figure 5). Seven bacterial genera where differences were apparent could be distinguished (Table 5). *Megamonas* spp. were more abundant in the CE groups compared to both control groups. *Eisenbergiella* spp. were more abundant in Cpos compared to CEs, while *Escherichia* spp. were more abundant in Cpos compared to both CE groups. Unclassified *Lachnospiraceae* spp. were more abundant in the Cneg group compared to all other groups. *Colidextribacter* and *Pseudoflavonifractor* spp. were both more abundant in the Cneg group compared to both control groups. *Clostridia vadinBB60* group had a higher abundance in the Cpos group compared to the CEw group.

The top 10 most dominant genera were present in a relative abundance ranging from 47.8% to 98.9% within

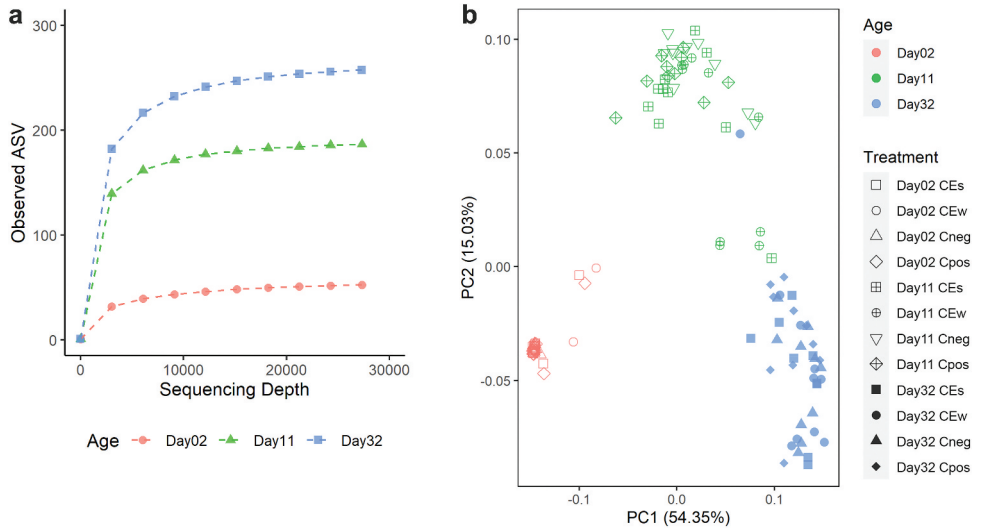

Figure 4. (a) Rarefaction curves of observed amplicon sequence variants (ASV) in caecal samples of different ages and (b) principal coordinate analysis (PCoA) plot showing differences in generalised UniFrac beta diversity at different treatments and ages. Treatments: chicks in the hatcher were given no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water and a CE product sprayed on the down of the newly hatched chicks (CEs). Different treatments are indicated by symbols, ages are indicated by colours.

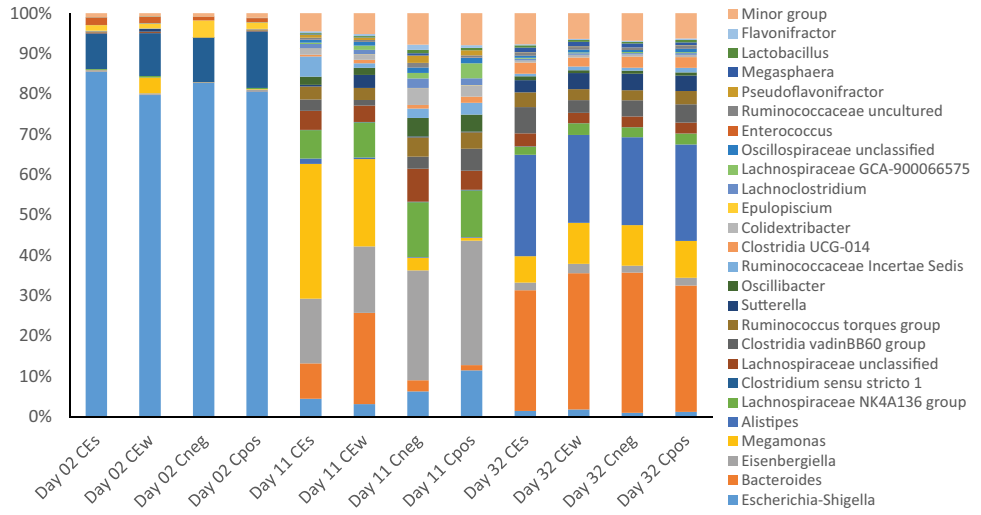

Figure 5. The relative abundance (%) of genera in caecal samples at three different ages (2, 11 and 32 d of age) in chickens given one of the following four treatments at hatch: chicks in the hatcher were given no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water and a CE product sprayed on the down of the newly hatched chicks (CEs).

Table 5. Estimated marginal means (± SE) of genus level sequencing counts differing between hatching treatments at d 11.

	CEs	CEw	Cneg	Cpos
Eisenbergiella	[1]8.38 ± 0.206[a2]	8.41 ± 0.203[ab]	8.91 ± 0.158[ab]	9.04 ± 0.148[b]
Megamonas	9.12 ± 0.149[b]	8.68 ± 0.186[b]	6.76 ± 0.486[a]	5.33 ± 0.992[a]
Escherichia-Shigella	7.1 ± 0.24[a]	6.74 ± 0.287[a]	7.44 ± 0.202[ab]	8.05 ± 0.149[b]
Unclassified *Lachnospiraceae*	7.16 ± 0.134[a]	7.01 ± 0.144[a]	7.71 ± 0.102[b]	7.16 ± 0.134[a]
Clostridia vadinBB60 group	6.66 ± 0.293[ab]	5.94 ± 0.42[a]	6.72 ± 0.284[ab]	7.3 ± 0.212[b]
Colidextribacter	6.04 ± 0.21[a]	5.95 ± 0.22[a]	7.04 ± 0.127[b]	6.63 ± 0.156[ab]
Pseudoflavonifractor	5.24 ± 0.308[a]	5.05 ± 0.34[a]	6.2 ± 0.191[b]	5.86 ± 0.226[ab]

[1]Values are estimated marginal means ± standard error. Results are given on the log scale.
[2]Values within rows lacking a common superscript are significantly different ($P < 0.05$).

Table 6. Mean relative abundance of the top 10 genera detected in caecal samples from chicks at 2, 11 and 32 d of age.

Genera	Day 2 (%)	SD	Day 11 (%)	SD	Day 32 (%)	SD
Escherichia-Shigella	82.14	7.59	6.30	5.85	1.33	1.68
Bacteroides	0.07	0.04	8.87	18.08	32.50	21.37
Eisenbergiella	0.27	0.94	22.64	13.88	1.96	1.23
Megamonas	1.07	4.44	14.74	18.65	9.04	6.39
Alistipes	0.00	0.00	0.52	1.38	23.10	11.94
Lachnospiraceae NK4A136 group	0.22	0.74	10.22	7.35	2.51	1.83
Clostridium sensu stricto 1	11.12	6.21	0.10	0.13	0.04	0.08
Unclassified *Lachnospiraceae*	0.16	0.42	5.42	2.61	2.79	0.94
Clostridia vadinBB60 group	0.02	0.06	3.17	3.33	4.49	4.01
Ruminococcus torques group	0.12	0.42	3.77	2.08	3.03	1.59

each sample (Table 6). The age-related change was clearly apparent in the most obvious microbial shift, where *Escherichia* spp. and *Clostridium sensu stricto 1* were the two most dominant genera at the beginning of the chick's life (d 2) and decreased considerably, to the advantage of other species, by d 11 and 32. In contrast, *Bacteroides* spp. presented at very low levels on d 2, but increased by d 11 and became the most dominant genus by d 32. *Alistipes* spp. were present at very low levels on both d 2 and 11, but became the second most dominant genus by d 32. *Eisenbergiella*, *Megamonas*, and *Lachnospiraceae NK4A136* spp. were present at low levels on d 2, became dominant by d 11, but eventually decreased in relative abundance by d 32. An unclassified *Lachnospiraceae* spp. together with *Clostridia vadinBB60* and *Ruminococcus torques* were all present at low levels on d 2 but increased by d 11 and maintained the same levels to d 32. Despite the general trend observed over the age of the birds, there was great variation in microbiota composition of individual birds within the same treatment group at same age.

Discussion

The aim of this study was to determine whether adapted management routines immediately post-hatch can improve the development of immune response and growth in broiler chicks. To the authors' knowledge, this is the first longitudinal scientific study providing results on a broad spectrum of variables such as immunological responses, organ development and productivity in chickens that have received feed, water and a CE product already in the hatcher. No effects of hatching treatments on antibody traits, gut microbiome development, organ development or intestinal morphology that lasted throughout the study were found. However, delayed access to feed and water reduced weight gain and feed intake early in the growth period. Physiological differences due to time to feed intake post-hatch have been investigated in many studies (Noy and Sklan 1999; Juul-Madsen et al. 2004; Van de Ven et al. 2013), and such disadvantages associated with prolonged time to feed access have been thoroughly reviewed (Willemsen et al. 2010;Powell et al. 2016).

In the present study, some effects were observed when the chicks were not allowed initial access to feed and water, mostly with regard to early organ growth and body weight gain. Early fed chicks generally prioritised the development of the gastrointestinal tract. In previous studies, increased length and weight of the ileum and jejunum have been observed in chicks fed early post-hatch (Maiorka et al. 2003). This corresponded with the findings in the present study, where, at d 2, feed-deprived chicks had both shorter and lighter intestines in relation to body weight and in absolute terms, than all other treatment groups. However, the intestines were weighed with digesta in this study, which may have biased the results due to *e.g.*, timing of sampling in relation to feed intake.

It has been reported that amino acids derived from yolk protein and most of the general energy in the yolk are spent on gastrointestinal development in fed and feed-deprived birds (Noy and Sklan 1999). The non-fed chicks in the present study tended to have lower relative gizzard weight when gizzards were not emptied and rinsed before weighing, which was logical due to the lack of feed. However, non-fed chicks had higher relative empty gizzard weight than all other groups. This suggested that non-fed chicks may have given priority to digestive organs located higher up in the digestive tract (*e.g.*, gizzard), to prepare for efficient feed digestion, and that they prioritised lower GIT development (small intestine) later, when feed was available. In agreement with our findings, in a meta-analysis, De Jong et al. (2017) found relatively shorter and lighter gut segments in the first week of life in feed- and water-restricted chicks. Conversely, Lamot et al. (2014) found proportionally longer intestines in feed-restricted chicks. However, De Jong et al. (2017) found lower villus height and crypt depth, particularly during the first week of life, which suggested that differences in organ development due to feed and water restriction ($\geq$36–60 h) may only be short-term. Therefore, sampling at 2 and 11 d in the present study might not have been optimal for detection

of differences in intestinal development, including morphology. Because differences in gut development seem to be highly dependent on sampling day in early life, which made comparisons between studies difficult (Ivarsson et al. 2022). It is likely that some differences in relative organ weight may be due to differences in body weight gain between treatment groups, and not organ development per se, which makes results difficult to compare between studies. However, in this study, differences in small intestine length and weight between fed and non-fed chicks were apparent when comparing absolute values.

At 2 d of age, the CEw group had significantly greater relative intestinal weight than both control groups, which indicated that the CE product supported intestinal development in early life. This effect did however not persist throughout the study and did not generate any other beneficial effects. Similarly, O'Dea et al. (2006) did not find any differences at the end of the grow-out period regarding body weight, feed conversion, or mortality in chicks provided with probiotics through four different administration routes at hatch. Relative weight of intestine, heart, liver, proventriculus, and gizzard decreased with age in this study, as did relative length of intestine, corresponding well with previous findings (Boyner et al. 2020; Ivarsson et al. 2022). Relative weights of spleen and bursa increased with age, which agreed with Kaiser and Balic's (2014) description of the bursa reaching its maximum size at approximately eight weeks of age and thereafter regenerating. The relative weight of the bursa was greater at 20 d of age than at 6, 10, and 34 d of age in the study by Boyner et al. (2020), whereas there was no effect of ageing with regard to bursal weight in the study by Ivarsson et al. (2022). However, the latter observed that relative spleen weight increased with age (Ivarsson et al. 2022), as found in the present study.

Unsurprisingly, the Cneg group experienced a disadvantage in body weight gain compared with all other groups in early life (d 2, 4, and 11). Lower body weight has been shown to persist for up to six weeks in chicks kept feed- and water-restricted for 48 h (De Jong et al. 2017). However, this was not the case in the present study, possibly because the chicks were only subjected to feed and water restriction for 40 h. Unfortunately, all treatments were constrained by lack of feed and water during transportation. These conditions were probably not in favour of GIT development or other traits, which may have made the results less comparable to those in other studies. Another risk of withdrawal of feed and water during transportation after it has been offered is that a slightly more developed intestine (as in the case with the CEw group) can signal hunger, which may cause the chickens more stress during transport.

Vertical transmission of gut microbiota from the mother hen to her offspring via the oviduct (Shterzer et al. 2020) is a mechanism facilitated by the embedding of microbiota (beneficial or pathogenic) in the developing egg and has been known for some time. In addition, under natural conditions, the hatching chick comes into contact with environmental and conspecific microbes already when its egg tooth hits the shell. In terms of microbiota development, the chick would likely benefit from close contact with the hen, gaining a commensal healthy microbiota. In modern production systems, this natural step in microbial transfer is not available to the chicks, making them more vulnerable to possible pathogenic microbes colonising their gut instead (Carrasco et al. 2019). However, some phyla of microbiota important to the chick have been discovered which are not primarily obtained from the mother hen. When chicks were hatched together with a hen in one study, donor hens did not seem sufficient as a source of *Firmicutes* spp. (Kubasova et al. 2019). This suggests that *e.g.*, *Lachnospiraceae* and *Ruminococcaceae* spp. originate from the surrounding environment rather than from adult birds (Kubasova et al. 2019). On the other hand, the *Firmicutes* phylum has been highlighted as one of the most easily transmitted phyla between hen and offspring (Aruwa et al. 2021).

In the present study, *Megamonas* spp of the phylum *Firmicutes* was the only genus more abundant in the CE groups compared to both control groups at 11 d of age. Moreover, *Firmicutes* is the second most abundant phylum in Broilact® (Such et al. 2021). *Megamonas* spp. has been speculated to be highly abundant in chicken caeca due to its hydrogen removing capacity, which is thought to benefit other microbes. This might have an indirect beneficial effect on the host, by improved energy recovery from feed (Sergeant et al. 2014). Unclassified *Lachnospiraceae*, *Colidextribacter* and *Pseudoflavonifractor* spp., all members of *Firmicutes* phylum, were all significantly more abundant in the Cneg group compared to CE groups. One explanation for this could be that these three genera were unable to compete in the same place as *Megamonas* spp., hence they could increase in the Cneg group instead. However, why *Colidextribacter* and *Pseudoflavonifractor* spp, were not more abundant in the Cpos group remained unclear. Although being the most abundant genus in Broilact®, *Escherichia* spp. was more abundant in the Cpos group compared to both CE groups. As concluded by Ballou et al. (2016), addition of bacterial cultures, such as probiotics, when chickens are reared under non-stressful conditions seem only to have small or transient effects on the microbiome's function and activity. However, the same authors highlighted the important effect of age on the microbial composition and diversity and pointed out age-dependent shifts in dominant phyla during the chicks' life, as reported in the current study.

The rarefaction curves of ASVs in the present study clearly demonstrated increased richness in microbiota with age (Figure 4(a)). Increased caecal microbiota richness and diversity with increasing age has been reported in many previous studies (Oakley et al. 2014; Ballou et al. 2016). The microbiota of the young chick typically has low diversity and is dominated by *Enterobacteriaceae* spp. (Ballou et al. 2016). Microbial diversity starts to increase around 7 d of age, when the phylum *Firmicutes* increases in abundance (Ballou et al. 2016). This corresponded well with findings in the present study on relative abundance of the top 10 genera, where *Escherichia* spp. (*Enterobacteriaceae*) was dominant at 2 d of age. Moreover, there was an obvious shift towards genera within the *Firmicutes* phylum at 11 d of age (*Clostridium sensu stricto 1* being the exception), which agreed with previous findings (Kubasova et al. 2019). Overgrowth of *Clostridium sensu stricto 1* in combination with a decrease in *Lactobacillus* spp. in the jejunum has been correlated with the development of necrotic enteritis in chickens (Yang et al. 2019). At approximately four weeks of age, there is another compositional shift where *Firmicutes* spp. are generally accompanied by *Bacteroidetes* spp. (Kubasova et al. 2019). This was the case in the present

study, where the genera *Bacteroides* and *Alstipes* spp. showed higher abundance at 32 d of age. Development of the caecal microbiota seemed to follow the normal maturation pattern in this study, with corresponding shifts in ageing modern broiler caecum, and differences in microbial composition between hatching treatment groups at d 11 were no longer apparent at d 32. The inferior FCR observed in the CEw group was therefore presumably not due to any microbiota-related differences. However, in a study by Such et al. (2021), some changes in microbiota composition due to Broilact® were observed in chicks at 7 d of age, but not at later time points It was concluded that differences in microbiota composition are determined mostly by sampling site and time point (Such et al. 2021). On the other hand, Broilact® treatment has been found to increase resistance towards colonisation of *Salmonella enterica* by competitive exclusion (Schneitz et al. 2016). In a field study, Broilact® supplementation was associated with positive, but non-significant effects on *Clostridium perfringens*-associated lesions and performance traits (Kaldhusdal et al. 2001). In the present study, individual water consumption was not recorded and chicks in the CEs group were sprayed manually with a handheld spray bottle, so it was possible that the Broilact® solution was unevenly consumed by the chicks. Moreover, under commercial settings, Broilact® is not sprayed manually, but in an automatic cabinet. These circumstances may have affected the results obtained for intestinal microbiota.

In order to assess responses to a novel antigen, the birds in the present study were vaccinated with an inactivated virus vaccine. As the read-out for the vaccine-induced immune responses, antibody production to APV was used. However, only 44% of the birds developed antibodies to APV and birds that tested positive generally had low antibody levels to this antigen. Hence, it was difficult to identify any putative effects of the experimental treatments on this trait. This low responsiveness to vaccination was unexpected and no clear explanation was identified. In Sweden, broiler chickens are not routinely vaccinated post-hatch, but, internationally, broiler-type chickens are regularly subjected to vaccination programs comprising vaccines against several infectious diseases (Sharma 1999; Landman 2012). The vaccines used in these programs are often live, which are generally considered more potent as immune activators (Aida et al. 2021). Thus, a live vaccine might have induced more prominent antibody responses in the birds in this study. However, some inactivated vaccines are used for broilers (Sharma 1999) and Juul-Madsen et al. (2004) observed clear antibody responses in Ross 208 chickens after administration of an inactivated vaccine against infectious bursal disease virus at 10 d of age. Hence, it seems unlikely that the choice of an inactivated vaccine was the sole reason for the poor responses in the present study. Genetic background has an influence on immune response and it has been shown that antibody production upon immunisation may be influenced by selective breeding of chickens (Minozzi et al. 2008; Zerjal et al. 2021).

In the present study there was a correlation between birds responding to the APV vaccination and higher serum levels of total IgY at d 31. This indicated that chickens responding to the vaccination also produced more antibodies in general, *i.e.*, could potentially be identified as high antibody responders, which suggested that antibody production may have been influenced by genetic factors in the experimental birds. Moreover, concerns have been raised that the modern broiler chicken may have generally low immune responsiveness due to potentially heavily biased selection for increased growth (Van der Most et al. 2011), which may have contributed to the poor vaccine-induced responses observed in the present study. Consequently, it seemed likely that several factors contributed to the observed low vaccine-induced antibody responses.

Other factors that may have contributed to the relatively low immune responsiveness and limited effects of the experimental treatments may have included the low stocking density during the experiment and high biosecurity at the research facility. The university research facility used in this study may not have provided the same challenge to the birds' immune system as those encountered under commercial conditions, where more birds are kept in the same pen at higher stocking densities. Moreover, the research facility has no birds for long periods between studies, which may have resulted in lower pathogenic pressure compared to a commercial set-up. This hypothesis was supported by findings reported by Eckert et al. (2010), who did not detect any differences in body weight or FCR in chickens provided with probiotics in the drinking water until the stocking density was increased to simulate commercial conditions. In fact, those researchers had to almost double the number of chickens in the rearing facility before they observed increased body weight and lowered FCR in probiotic-fed birds (Eckert et al. 2010).

A more potent infectious or inflammatory challenge to the chickens in the present study might have revealed greater impacts of the experimental treatments. In a study by Van den Brand et al. (2009) where the challenge was a cocktail of lipopolysaccharide/human serum albumen (as a model for lung infection), chickens that were kept for 24, 48 or 72 h post-hatch without access to feed and water showed significantly lower body weight gain post-challenge than birds fed directly post-hatch. Those authors concluded that directly fed birds can withstand immunological challenges better, although this was not confirmed by the mean values of different antibody titers (Van den Brand et al. 2009).

In the present study, early measures were taken in the hatcher, in terms of provision of feed, water and a CE product, to strengthen the immune response and prerequisites for growth of the newly hatched chicks. For the observed early differences between hatching groups, only the difference in feed intake between control groups and the increased FCR in CEw birds persisted throughout the study. Moreover, no new differences appeared, which suggested that modern broiler chickens are capable of compensating for 40 h of feed and water deprival post-hatch.

Provision of Broilact® did not have any persistent performance-enhancing properties with the set-up tested, although an experimental set-up allowing chicks to continue their respective treatments (especially water access for relevant groups) during transportation might have given a different outcome. As mentioned by Ballou et al. (2016), probiotics may have only small effects on the microbiome when chickens are reared under non-stressful conditions. An experimental environment closer to that in commercial production, mimicking more fairly the pathogen pressure

and stocking densities, might also have given a different outcome.

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Disclosure statement

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