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Effects of early access to feed and water in hatchers on growth performance in broiler chickens

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ABSTRACT

Conventional hatchers do not provide feed or water to chicks during hatch, which may negatively affect chick development, especially in early hatchlings subjected to prolonged feed deprivation due to biological variation in hatch time. This study aimed to evaluate the effects of early access to feed, water and a synbiotic product on performance, caecal microbiota development, organ development, intestinal morphology, total serum immunoglobulin (**Ig**) Y and antibody response to vaccination in Ross-308 chickens. A total of 330 chicks hatched in a specialised hatcher were divided into three hatch treatment groups: access to feed, water and synbiotic additive (**PS**); access to feed and water (**PosC**); no access to feed and water (**NegC**). Ten birds per hatch treatment were euthanised for organ sampling at placement, and the remaining 300 chicks were randomly allocated to 30 pens. All chicks received feed and water, and half the chicks in each treatment group received the synbiotic additive for 3 consecutive days (giving three hatching treatments and two postplacement treatments). All chicks were vaccinated against avian pneumovirus (**APV**) at 10 days of age. Blood sampling was performed weekly on three focal birds per pen for analysis of total serum IgY and antibodies to APV. Organ sampling was performed on days 11 and 32. Feed intake (**FI**) and BW were recorded weekly. The NegC group exhibited reduced early growth and lower FI throughout the study. At 25 days, they also demonstrated an inferior feed conversion ratio (**FCR**) compared with the other groups. At 4 and 25 days chickens that did not receive any postplacement treatment, None had superior FCR compared to those receiving PS also postplacement. There was also an effect of postplacement treatment where the None group weighed more compared to the PS group on almost all occasions. The NegC group had higher concentrations of IgY in serum compared to the PosC group at 3 days of age, an effect that remained a tendency until 25 days of age. No differences between treatments were found for antibody responses to APV vaccination. Some differences in relative weights of digestive organs between hatching groups were detected at the end of study, while no persistent effects on caecal microbiota composition were observed. In conclusion, delayed access to feed and water had adverse effects on productivity traits, lasting throughout the study. These findings warrant further validation in a practical context with higher stocking densities and pathogen loads.

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Implications

Conventional hatchers do not provide feed or water during hatch, potentially impairing chick development. This study investigated the effects of early access to feed, water, and a synbiotic additive. Delayed access resulted in long-lasting adverse effects on productivity traits. Additionally, birds given the synbiotic at placement showed lower BW, reduced feed intake, and inferior feed conversion ratio compared to none-supplemented groups. These findings suggest synbiotic supplementation cannot be assumed exclusively beneficial and may even have adverse effects in some conditions. The findings warrant further validation in a practical context before more substantiated recommendations to the broiler industry can be made.

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Introduction

The conventional method of hatching broiler chicks is in hatchers with no provision of feed or water. Due to biological variation, chicks hatch over a 'window' of 24–48 h (de Jong et al., 2017), which can result in hatchlings experiencing up to 72 h without access to feed and water before being moved to the rearing site (Willemsen et al., 2010). Many studies have shown that delayed access to feed and water posthatch has negative effects on broiler chickens. For example, a meta-study on the effects of posthatch food and water deprivation concluded that chicks which were deprived for 48 h posthatch showed lower BW and higher mortality, effects that persisted for up to 6 weeks of age (de Jong et al., 2017). Other studies have observed impaired skeletal muscle growth in early life in feed-deprived chicks (Halevy et al., 2000; Powell et al., 2016). The benefits of early access to feed include intensified development of the chick's immune system through different paths, e.g. Dibner et al. (1998) found higher bursal weight, earlier appearance of germinal centres and immunoglobulin (**Ig**) A (biliary) and improved resistance to disease challenge. Posthatch, immunity in the chick is dependent on maternally-derived antibodies that are present in the yolk sac in the abdominal cavity of the chick (Härtle et al., 2014). Rapid access to antibodies helps the chick withstand pathogens during the maturation of its own immune system (Härtle et al., 2014). Importantly, the secretion of yolk into the intestine increases with the presence of feed in the gastrointestinal tract (Noy and Sklan, 2001), which then would be another benefit of giving chicks early access to feed. However, recent studies have found residual yolk weights not to be affected by the presence of early feed (e.g. Hollemans, 2020). Although the embryo is not sterile and in fact inherits some of its microbiomes from the maternal side during embryogenesis (Ding et al., 2017), it diversifies its microbiome more and increases microbial abundance from the environment closer to hatch (Ding et al., 2017). The hatched chick is colonised naturally by microbiota from its mother hen and the surroundings, such as nest material (de Oliveira et al., 2014). In modern hatcheries, however, the chick is cut off from this microbiota transfer that can help establish a healthy microbiome to fend off disease (de Oliveira et al., 2014; Li et al., 2022). Early colonisation with beneficial bacteria blocks entry of pathogenic bacteria through competitive exclusion and is also important in stimulating the development of the immune system and, by extension, nutritional uptake through the establishment of a healthy gut (de Oliveira et al., 2014). To compensate for the lack of natural colonisation, probiotics are sometimes used as an additive to ensure that the chick is colonised artificially by beneficial microbiota (Seifi et al., 2017). Probiotics are defined by the Food and Agriculture Organization of the United Nations as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO, 2002). There are different commercial products available for poultry. If combined with substrate (fibre) for the bacteria, these are generally branded as synbiotic products.

The synbiotic additive used in this study has been shown to reduce caecal colonisation by *Campylobacter jejuni* (Ghareeb et al., 2012) and significantly limit the negative effects of *Clostridium perfringens* (McReynolds et al., 2009). The aim of the present study was to determine whether adaptation of posthatch management routines by providing them with access to feed and water together with synbiotics already in the hatcher could give the chicks a more solid start in life in terms of immune response and growth. This synbiotic treatment was later applied for 3 consecutive days in the growing facility. Parameters monitored during the study were feed intake, growth, total serum IgY level, vaccine-induced antibody response, gut microbiota development and intestinal development.

Material and methods

Procedure at the hatchery

A total of 330 Ross-308 chicks from a 37−week−old breeder flock were hatched at a hatchery in southern Sweden using the HatchCare™ system (HatchTech, Veenendaal, The Netherlands), which allows easy provision of feed in the hatcher baskets and water in water gutters on the side of the hatcher walls during the hatch procedure (for details on the HatchCare™ system see e.g. Van den Brand et al., 2023). Chicks were hatched in an illuminated environment. Temperature, humidity and CO_2 were set according to standard hatchery protocol. To minimise variation in hatching time, chicks with wet down were collected from hatcher baskets over a 3-h period at the peak of the hatching window and randomly assigned to one of three hatch treatment groups before being placed back in the hatcher. The treatments applied were: a negative control (**NegC**), where chicks received no feed or water during hatch; a positive control (**PosC**), where chicks received feed and water during hatch; and a synbiotic group (**PS**), where chicks besides feed were assigned water prepared with the *Lactobacillus*-based synbiotic additive PoultryStar (PoultryStar® sol^{EU}, Biomin Holding GmbH, Herzogenburg, Austria). After the replacement of all chicks in hatcher baskets according to the treatment schedule, chicks in the PS treatment received a freshly prepared water solution with PoultryStar every 4 h for a total of 12 h. Uncertainty in predicting water consumption led to a greater intake of PoultryStar per bird than originally planned. Due to this, the scheme for administration had to be changed so that chicks were only allowed access to PoultryStar for 12 h, instead of the 24 h originally intended. Before new solution was added, leftover solution was collected with a syringe, enabling calculation of consumption. Mean total consumption of PoultryStar was approximately 41 mg per bird during the hatch treatment. Chicks in the PosC and PS groups in the hatchery baskets were provided with a commercial corn-soybean meal–based prestarter feed that included a coccidiostat (Lantmännen, Falkenberg).

Transportation, placement and feed

After routine quality control at the hatchery, chicks were transported to the Swedish Livestock Research Center, Swedish University of Agricultural Sciences, Uppsala, Sweden. The hatchery's vehicles for transportation of day-olds were used for transporting most of the distance. Chicks were transported in darkness, and the hatchery's standard protocol for settings regarding temperature and relative humidity was used. Feed and water were made available to the chicks on transfer to pens at the research centre's growing facility, approximately 17 h after pull. The chicks hatched ~24–27 h prior to pull, resulting in NegC chicks being deprived of feed and water for approximately 40 h when placed in their pens. The PosC and PS groups were provided with drinking water only while awaiting loading (approximately 1 h) at the hatchery, hence, they were without access to feed and water for 16 and 15 h, respectively. Directly on arrival at the growing facility (before placement in pens), 10 chicks per hatch treatment group (n = 30) were immediately euthanised and dissected, and organ parameters were measured and gut samples collected. The remaining 100 chicks in each hatch treatment group (NegC, PosC and PS) were distributed between 10 pens. Of these, five replicate pens received PoultryStar in the drinking water for 3 consecutive days, while chicks in the remaining five pens were provided with drink-

ing water only. Chicks assigned to the PoultryStar postplacement treatment for 3 days in the growing facility had a mean consumption of 24 mg per day during those 3 days. Pen layout, temperature and light schedule were as described in Boyner et al. (2023). In brief, the pens (1.5 × 0.75 m) were bedded with wood shavings and were equipped with a feeder and three nipple drinkers. The PS postplacement treatment groups received water with PoultryStar in bell drinkers for the first 3 days. Indoor temperature was set initially to 33 °C and then gradually lowered between days 3 and 24 to a final level of 23 °C (note that day 0 in the study refers to embryonic day 20, when most of the chicks hatched). Constant light was provided at placement and the following day. From day 3, darkness was introduced for 1 h and was then successively prolonged with one more hour per night until 8 days. From then on, there was darkness between 2300 and 0500 h until the end of the study. To enable blood sampling of the same birds throughout the study, two focal birds per replicate pen were randomly chosen and wing-tagged. The chicks in all pens were given a commercial starter feed from 2 to 10 days of age, and this was replaced with a commercial grower feed from day 11. Both feed types were based on wheat and soybean meal and manufactured by Svenska Foder AB, Lidköping, and did not include any coccidiostats. Samples of both feeds were analysed for DM, CP, crude fibre, ether extract and ash content. DM content was analysed by drying samples at 103 °C for 16 h, while ash content was determined after incineration at 550 °C for 3 h (Jennische and Larsson, 1990). European Communities (1998) methodology was used for the analysis of ether extract and the Kjeldahl method (Nordic Committee on Feed Analysis, 2003) for the analysis of CP content (N × 6.25). DM content (as-is basis) was 91.1% in the starter feed and 89.6% in the grower feed. Other feed composition parameters were as follows: starter feed (g/kg DM): ash 74, CP 241, crude fibre 41 and ether extract 69; grower feed (g/kg DM): ash 52, CP 237, crude fibre 40 and ether extract 63. Calculated energy content (according to WPSA) was 12.8 ME MJ/kg DM for starter feed and 13.1 ME MJ/kg DM for grower feed.

Data collection

Feed conversion, growth and organ development

At 2 days of age (before placement in pens), 10 chicks per hatch treatment were euthanised and organ parameters were recorded. At 11 and 32 days of age, organ weight and (when relevant) organ length were determined in two euthanised chickens per replicate pen (i.e. 10 chickens per postplacement treatment group). Organs weighed were the yolk sac, heart, liver, spleen, small intestine (with content), proventriculus and gizzard (with contents ('full') and rinsed and dried ('empty')). BW, body length and length of the small intestine (with contents) were also recorded. Prior to dissection, the chickens were stunned by a blow to the head and killed by neck dislocation (2 and 11 days of age), or killed by an injection of pentobarbital sodium (100 mg/mL) administered intravenously in the wing vein (32 days of age). Feed consumption and chicken weight were recorded weekly and per pen. Mortality was recorded daily in every pen.

Histology

During organ sampling at 2 and 11 days of age, duodenal tissue was collected and prepared for morphological analysis (villi height, villi width, crypt depth). A 3 cm long section immediately distal to the duodenal loop was removed, cut open length-wise and fixed overnight with glutaraldehyde (2.5%, pH 7.2). The tissue was rinsed in phosphate buffer (1/15 M, 7.2 pH), trimmed into 2 mm thick slices and dehydrated in a gradually increasing concentration of ethanol. Tissue samples were embedded in water-soluble resin (Leica Historesin, Heidelberg, Germany), cut into 2–µm sections and stained with haematoxylin-eosin. All slides were coded and analysed by the same person, using a Nikon Microphot-FXA microscope, 4x objective lens (Bergström Instrument AB, Stockholm, Sweden). Care was taken to ensure that the villi measured had an undamaged *lamina propria* and a single epithelial cell layer (to exclude slanted cut villi). Villi with diffuse tip endings or scattered epithelial lining were excluded from the analysis, as were villi with indefinable crypts. For in-depth information regarding selection criteria, see Boyner et al. (2023).

Analysis of antibodies in serum and vaccination of chicks

Blood was drawn from the jugular vein of 15 focal birds per posthatch treatment group (n = 90) at 3, 11, 18, 25 and 31 days of age. After resting at room temperature for 24 h, the samples were centrifuged at 10 000 × g for 10 min. The serum fraction was collected and frozen at −20 °C pending antibody analyses. After the collection of blood samples at 11 days of age, all chicks were injected in the breast muscle with 0.5 mL of the commercial vaccine Nobilis RT Inac vet (MSD Animal Health) against avian pneumovirus (**APV**). Total amount of IgY was measured in serum samples from all sampling occasions, using the commercial Chicken IgG ELISA Quantitation Set (Cat. No. E30-10) kit from Bethyl Laboratories Inc. (USA) according to the product protocol, as described in detail in Boyner et al. (2023). To detect antibodies to APV, serum samples from days 11 (baseline) and 31 were analysed with the Avian Pneumovirus Antibody Test Kit (06-44300-04) from IDEXX Laboratories Inc. (USA) according to the manufacturer's protocol, with the exception of sample dilution and data evaluation as described in detail in Boyner et al. (2023). In brief, to increase sensitivity, sera were diluted 1:100 instead of the recommended 1:500 and the presence of antibodies to APV was assessed with reference to an absorbance cut-off value calculated as: mean absorbance value at 650 nm +2 SDs for all samples at day 11 (prevaccination, n = 117).

Gut microbiota

From the same birds used for organ sampling, caecal contents were collected as the pooled contents from both caeca of each individual at 2 days of age (n = 30). At later sampling occasions (11 and 32 days of age), caecal contents were collected from the two birds per replicate pen euthanised for organ sampling (n = 120). All these 150 samples were aseptically collected and frozen in liquid nitrogen instantly, before storage at −80 °C pending extraction.

DNA extraction and sequencing

DNA was extracted from 180-220 mg samples of caecal contents using QIAamp Fast DNA Stool Mini Kit (CatNo. 51604) made by Qiagen (Germany) according to the manufacturer's instructions with some minor modifications, e.g. adding bead beating to break down bacterial cell walls, as described in Boyner et al. (2023). Extracted DNA was stored at −20 °C for delivery to Novogene (Beijing, China). The sequencing library of the 16S rRNA gene was generated and sequenced at Novogene using the Illumina HiSeq 2500 platform. In brief, the 16S rRNA gene V3-V4 region was amplified with primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT). All PCR reactions were carried out using Phusion High-Fidelity PCR Master Mix (New England Biolabs).

Bioinformatic analysis

Processing of bioinformatics data was carried out using Quantitative Insights into Microbial Ecology 2 (Core 2020.02) (Bolyen et al., 2019). Primer and barcode sequences of raw demultiplexed reads were trimmed off. Further processing of trimmed reads was performed with DADA2 in order to merge pair–end reads, remove chimeras, de-noise and de-replicate reads (Callahan et al., 2016), with truncation length 221 bp used for both forward

and reverse reads. To build a phylogenetic tree, FastTree and MAFFT alignment were used (Katoh et al., 2002; Price et al., 2010). The SILVA SSU Ref NR 99 132 dataset was trained as a classify-sklearn taxonomy classifier (Pedregosa et al., 2011; Quast et al., 2013; Bokulich et al., 2018), after being trimmed to the corresponding primer region. The resulting classifier was used to assign taxonomy to amplicon sequence variants (**ASV**). After trimming and quality filtering, sequencing of the 16S rRNA gene yielded a total of 9 362 725 sequences from 149 samples. A minimum of 29 486 sequences per sample was used for rarefying the number of reads per sample (Weiss et al., 2017). To generate the generalized UniFrac distance matrix (alpha = 0.5) and alpha rarefaction, the QIIME2 diversity plugin was used (Chen et al., 2012; Bolyen et al., 2019). Raw sequencing data from this study have been deposited in the database of the National Center for Biotechnology Information (NCBI), under accession number PRJNA929198.

Statistical analysis

Performance parameters (growth, feed conversion ratio, feed intake), histology, organ data and log-transformed IgY titer values were tested for normality and homoscedasticity by use of diagnostic plots of residuals in the statistical software SAS (version 9.4). Data without apparent deviations from normality or homoscedasticity were analysed using mixed linear models in SAS, and the fixed part of the model included hatch treatment (three levels), posthatch treatment (two levels) and their interaction. Yolk sac residue on day 11, deviated from normal distribution, and was therefore subjected to logistic regression analyses with Proc Glimmix after conversion to a binary scale (presence of yolk sac residue = 1, absence = 0). The response to APV vaccination was assessed as chickens with APV-specific antibodies in serum on day 31 and was analysed using logistic regression analysis with Proc Glimmix. Chickens deemed seropositive for antibodies to APV (i.e. with absorbance values above the ELISA cut-off value calculated from prevaccination samples, see above), were considered responders, and those deemed seronegative were considered non-responders in this analysis. For all analyses, a *P*-value < 0.05 after Tukey-Kramer adjustment for multiple comparisons was considered statistically significant. All statistical analyses were performed on means per replicate pen.

Analysis of Composition of Microbiomes methodology (Mandal et al., 2015) was used to evaluate differences in microbial composition at phylum, class, order, family, genus and ASV level due to age and treatment. To investigate age-dependent microbial development, the rarefied ASV table was used to filter genera with relative abundance greater than 1.2%. Genera with lower abundance was grouped as a minor group. Unfortunately, due to technical reasons, one sample from the NegC group on day 2 was not included in the microbiota analysis.

Results

Organ development, BW and body length at placement

Analysis of the organ data obtained for 10 chicks per hatch group on arrival at the research centre revealed no differences between treatment groups with regard to yolk sac weight (absolute and relative) or relative weight of spleen, bursa, heart, liver, proventriculus and gizzard, joint (Table 1). There was a difference in relative body length, with NegC chicks being longer than PosC and PS chicks. BW and yolk-free body mass (**YFBM**) (both in grams) were lower in the NegC group than in the other groups. NegC chicks also had a relatively lighter and shorter small intestine than those in the other groups. In addition, there was a difference in relative gizzard weight (full) between the groups, with those from NegC chicks being lighter than those from PosC chicks, but gizzards from PS chicks did not differ from those in the two control groups. There was also a difference in relative gizzard weight (empty), which was lower for PosC chicks than for NegC and PS chicks (Table 1).

Performance and organ development during grow-out

Mortality was low with a total of seven birds being removed from the study. Across all ages, there was an effect of hatching group on BW as the NegC group weighed less compared with the PS and PosC group. Meanwhile, there was no difference between the PosC group and the PS group. At all ages except on days 11 and 18, there was a difference between postplacement treatments where the None group weighed more compared to the PS group. At 11 days of age, there was a tendency of higher weight in None group (Table 2). For feed intake, the was an effect of hatching group at all ages where the NegC group consumed less feed compared to the PS and PosC groups, meanwhile, there were no differences between the PosC group and the PS group. At 32 d of age, an effect

Table 1
Body and organ parameters at 2 days of age in chicks exposed to one of three different treatments in the hatcher: no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic supplement in the water (PS). Values are least square means.

Parameter	Hatchery treatment			SEM	*P*-value
	NegC n = 10	PosC n = 10	PS n = 10		Hatchery treatment
BW (g)	41.5[a]	44.1[b]	45.0[1b]	0.86	0.0206
Chick length (cm)	21.2	20.4	20.9	0.40	0.3441
Chick length (cm/kg BW)	512.4[a]	464.2[b]	465.8[b]	13.30	0.0250
Yolk sac (g)	1.69	1.76	1.45	0.184	0.4552
YFBM[1] (g)	39.8[a]	42.4[b]	43.6[b]	0.78	0.0068
Yolk sac (g/kg BW)	40.6	39.4	31.9	3.88	0.2461
Small intestine (g/kg BW)	60.3[a]	76.8[b]	78.6[b]	2.02	<0.0001
Small intestine (cm/kg BW)	1161	1317[b]	1316[b]	38.8	0.0111
Spleen (g/kg BW)	0.30	0.37	0.45	0.055	0.1925
Bursa (g/kg BW)	1.67	1.56	1.77	0.149	0.5833
Heart (g/kg BW)	8.9	9.3	9.4	0.21	0.2331
Liver (g/kg BW)	30.0	30.0	30.1	0.62	0.9961
Proventriculus + gizzard (g/kg BW)	73.3	81.6	78.6	2.77	0.1228
Gizzard full (g/kg BW)	61.3[a]	69.7[b]	66.7[ab]	2.20	0.0425
Gizzard empty (g/kg BW)	59.1[a]	53.9[b]	58.1[a]	1.23	0.0145

Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).
[1] Yolk-free body mass.

of postplacement treatment was detected where the None group had a greater feed intake compared to the PS group. An effect of the hatching group was also observed on feed conversion ratio (**FCR**) at 25 days, where the NegC group had inferior FCR compared to PosC and PS, meanwhile, there was no difference between the two latter groups. At 4 and 25 days of age, there was an effect of postplacement treatment where the None group had a superior FCR compared to the PS group. Tendencies for this effect were also detected at 18 and 32 d of age. At 25 days of age, there was an interaction effect between HxPPT treatments where the NegC hatching group that also received PS as a postplacement treatment had an inferior FCR compared to all other combinations of treatments.

An effect of hatching treatment was also apparent with regard to BW in the sample of chickens selected for organ sampling where the NegC group was lighter compared to the other two hatching groups at 11 and 32 days of age (Tables 3, 4). Moreover, relative heart weights were greater in NegC chicks compared to the PosC group at 11 days of age. At 32 days of age, NegC chicks tended to have the longest relative intestine lengths and PosC the shortest. Also, NegC chicks had higher relative weights of proventriculus and gizzard (joint weight), and relative gizzard weight (full and empty) compared to PosC. For the joint weight, NegC tended to have higher weights also compared to PS chicks and for the relative gizzard weight (empty), NegC gizzards weighed more also compared to PS chicks (Table 3). Regarding the effect of postplacement treatments at day 11, relative bursal weights tended to be greater in the PS group compared to the None–treated group while relative heart weights tended to be higher in the None–treated group compared to the PS group (Table 3). The relative spleen weights at 32 days were higher in the None group compared to the PS group. An interaction effect was observed at 32 days of age where the PosC group that were not receiving any PS had greater relative liver weights compared to the PosC × PS and PS × None group (Table 4).

Development of the small intestine

No treatment effects were observed with regard to villi height or width at 2 or 11 days of age (Table 5). For crypt depth, an effect was observed at 2 days of age, when NegC and PS samples had deeper crypts than PosC samples. A corresponding impact on the ratio between villi height and crypt depth was observed at 2 days of age. These effects were no longer apparent at 11 days of age (Table 5).

Total levels of immunoglobulin Y and Avian Pneumovirus-specific antibodies in serum

There was an effect of hatchery treatment on IgY concentration in serum where NegC chicks had higher values compared to the PosC chicks at day 3, an effect that remained as a tendency until 25 days of age (Fig. 1A, Table 6). The combined data from the five sampling occasions showed that in general, all chickens had their highest levels of IgY at 3 days of age, regardless of postplacement treatment group (Fig. 1AB). There was a pattern in all treatments for the concentration of maternally derived antibodies to decline rapidly during the first days of life, to reach the lowest observed levels (approximately 11% of day 3 levels) at 18 days of age. Serum IgY concentration then increased by 25 days of age (to approximately 23% of day three levels) and to 31 days of age (approximately 33% of day three levels) (Fig. 1, AB).

At 11 days of age, all chickens were injected with inactivated APV vaccine. Specific antibody levels to APV were recorded before vaccination on day 11 and at the end of the study (31 days of age, i.e. 20 days postvaccination) (Fig. 2AB). Based on the cut-off value (Abs_{650} 0.086; calculated using results obtained before APV vaccination), in total, 54% of the chickens tested positive for antibodies to APV after vaccination. However, fewer individual chickens showed any substantial antibody response, e.g. > Abs_{650} 0.3, to APV. No statistically significant effect of postplacement treatment was observed for the proportion of APV-positive chickens in the different groups (Hatching treatment (**H**): P = 0.3777; Postplacement treatment (**PPT**): P = 0.8800; HxPPT: P = 0.2382. Fig. 2AB).

Microbial development in the caecum

In total, 816 ASVs, representing 179 genera belonging to 77 taxonomic families, were recovered in 16 s rRNA sequencing. Observed ASV numbers at different ages plotted in rarefaction curves revealed an effect of age, with microbial diversity increasing from 54 at day 2 to 195 by day 11 and 250 at the end of the study (Fig. 3). A principal coordinate analysis (PCoA) plot of the generalized UniFrac distance matrix revealed an effect of age, but no effect of hatch treatment or postplacement treatment (Fig. 4). Two samples, one from NegC/PS and one from PS/- at day 32, were clustered together with the day 11 samples. This was most likely due to one of the samples being similar to day 11 samples, with higher levels of *Eisenbergiella* and *Lachnospiraceae NK4A136* group and lower levels of *Bacteroides*. The other sample was similar to day 11 samples with regard to *Lachnospiraceae NK4A136* group and higher levels of *Megamonas*.

Relative abundance of the 26 most abundant genera in each sample ranged from 87.5 to 99.4% (Fig. 5). The most evident age-related microbial shift was a decrease in *Escherichia-Shigella*, which was by far the most prominent genus at 2 days of age but had decreased considerably in abundance by days 11 and 32. *Clostridium sensu stricto 1* followed the same pattern. The opposite was seen for the genera *Bacteroides*, *Alistipes* and *Megamonas*, which increased in relative abundance with increasing age of the chickens. Several genera (*Eisenbergiella*, *Lachnospiraceae NK4A136* group, Unclassified *Lachnospiraceae*, *Clostridia vadinBB60* group and *Ruminococcus torques* group) were present in low abundance at 2 and 32 days of age and peaked mid-study, at 11 days of age. There was in general large individual variation in microbial composition within treatment groups and sampling occasions.

Discussion

This study examined whether chick robustness in early life in terms of immunological development and growth could be improved by modifying management routines to match the physiological needs of the birds. Feed, water and the synbiotic additive were therefore provided already in the hatcher baskets. In addition, the synbiotic additive was provided to pens of all hatch treatment groups for 3 consecutive days in the growing facility.

Hatching treatment affected all productivity traits where feed-deprived chicks weighed less and consumed less feed compared to both hatchery-fed treatment groups, with effects lasting throughout the study. In previous studies, some long-term effects such as lower weight at marketing age and poorer breast meat proportions have been observed in chicks feed-deprived at the hatchery than in fed chicks (Sklan et al., 2000; Ivarsson et al., 2022). For example, Wijnen et al. (2022) found early feeding regimens to result in higher growth until 35 days of age. There is a general consensus that prolonged time to first feed intake, e.g. 48 h (Juul-Madsen et al., 2004), negatively affects growth, immune function and broiler viability and in the meta-analysis by de Jong et al. (2017), negative effects on BW and mortality up to 6 weeks of age was observed after > 36–60 h. Whether or not long-term effects are found in studies are probably due to the duration of feed deprivation, e.g. in the study by Juul-Madsen et al. (2004), chicks feed-deprived for 24 h did not differ in BW at market age from

Table 2
BW, accumulated feed intake (FI, as-is basis) and feed conversion ratio (FCR) at six different ages in Ross-308 broiler chickens exposed to one of three different treatments in the hatcher (no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic product in the water (PS)) and one of two postplacement treatments (half of each group received the PS (in their bell drinkers) for 3 consecutive days after placement (PS) and the other half received regular drinking water (None)).

	Hatchery treatment				Postplacement treatment			Interactions							*P*-value		
Variable	NegC n = 10	PS n = 10	PosC n = 10	SEM	None n = 15	PS n = 15	SEM	NegC x None n = 5	NegC x PS n = 5	PS x None n = 5	PS x PS n = 5	PosC x None n = 5	PosC x PS n = 5	SEM	Hatchery treatment (H)	Postplacement treatment (PPT)	HxPPT
BW (g) at day:																	
2	40.2[b]	45.5[a]	45.3[a]	0.32	[2]44.1[a]	43.2[b]	0.26	40.5	39.9	46.2	44.8	45.6	44.9	0.45	<0.0001	0.0239	0.4536
4	70.4[b]	83.4[a]	84.1[a]	0.58	80.3[a]	78.3[b]	0.47	70.9	70.0	84.9	81.9	85.1	83.2	0.82	<0.0001	0.0080	0.4470
11	277.1[b]	312.7[a]	318.8[a]	3.02	306.1	299.6	2.47	277.9	276.3	318.3	307.2	322.2	315.4	4.27	<0.0001	0.0751	0.5389
18	669.6[b]	759.0[a]	783.3[a]	11.01	748.0	726.5	8.99	688.1	651.1	774.2	743.7	781.8	784.8	15.57	<0.0001	0.1042	0.4004
25	1244.8[b]	1381.4[a]	1429.6[a]	18.79	1374.4[a]	1329.4[b]	15.35	1283.4	1206.2	1412.0	1350.7	1427.8	1431.4	26.58	<0.0001	0.0491	0.2914
32	1966.6[b]	2113.6[a]	2205.3[a]	28.94	2136.4[a]	2054.0[b]	23.63	2027.2	1906.1	2187.4	2039.9	2194.6	2216.1	40.93	<0.0001	0.0213	0.1060
FI (g) at day:																	
4	32.5[b]	40.1[a]	40.5[a]	1.86	35.9	39.5	1.52	30.7	34.2	39.0	41.2	38.0	43.0	2.63	0.0082	0.1113	0.8674
11	275.9[b]	308.1[a]	312.9[a]	3.43	299.9	298.0	2.80	277.9	273.9	307.1	309.0	314.7	311.2	4.86	<0.0001	0.6389	0.8005
18	780.0[b]	862.9[a]	888.2[a]	9.50	850.5	836.8	7.75	791.2	768.7	874.3	851.5	886.1	890.2	13.43	<0.0001	0.2228	0.5256
25	1606.2[b]	1737.0[a]	1793.5[a]	19.53	1730.0	1694.4	15.94	1621.1	1591.3	1772.5	1701.5	1796.6	1790.5	27.62	<0.0001	0.1272	0.5031
32	2697.5[b]	2878.0[a]	2983.2[a]	31.2	2895.9[a]	2809.9[b]	25.44	2747.6	2647.4	2956.1	2799.9	2983.8	2982.5	44.06	<0.0001	0.0251	0.2253
FCR at day:																	
4	1.07	1.06	1.04	0.049	0.99[b]	1.13[a]	0.040	1.01	1.14	1.01	1.12	0.96	1.12	0.070	0.8919	0.0295	0.9338
11	1.17	1.16	1.15	0.012	1.15	1.17	0.010	1.17	1.16	1.14	1.18	1.14	1.17	0.017	0.7593	0.1582	0.2774
18	1.23	1.20	1.20	0.010	1.20	1.22	0.008	1.21	1.24	1.20	1.21	1.20	1.21	0.014	0.1720	0.0690	0.6461
25	1.32[b]	1.29[a]	1.29[a]	0.008	1.29[b]	1.31[a]	0.007	1.29[b]	1.35[a]	1.29[b]	1.29[b]	1.29[b]	1.29[b]	0.012	0.0210	0.0422	0.0302
32	1.39	1.38	1.38	0.008	1.38	1.39	0.006	1.37	1.41	1.37	1.39	1.38	1.37	0.011	0.3101	0.0730	0.0951

Values are group least square means.
Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).

Table 3
BW, yolk sac and organ weight at 11 days in Ross-308 broiler chicks exposed to one of three different treatments in the hatcher (no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic product in the water (PS)) and one of two postplacement treatments (half of each group received the PS (in their bell drinkers) for three consecutive days after placement (PS) and the other half received regular drinking water (None)).

Variable	Hatchery treatment				Postplacement treatment			Interactions							*P*-value		
	NegC n = 10	PS n = 10	PosC n = 10	SEM	None n = 15	PS n = 15	SEM	NegC x None n = 5	NegC x PS n = 5	PS x None n = 5	*PS* x PS n = 5	PosC x None n = 5	PosC x PS n = 5	SEM	Hatchery treatment (H)	Postplacement treatment (PPT)	HxPPT
BW (g)	298.7[b2]	322.2[a]	324.3[a]	6.64	316.7	313.4	5.42	300.7	296.6	322.7	321.6	326.6	321.9	9.39	0.0206	0.6709	0.9792
Intestine (g/kg BW)	84.6	84.3	84.4	1.74	84.4	84.4	1.42	84.8	84.5	83.0	85.5	85.6	83.2	2.46	0.9893	0.9788	0.6239
Intestine (cm/kg BW)	359.2	348.1	346.3	6.88	348.9	353.5	5.61	361.9	356.5	342.1	354.2	342.9	349.7	9.72	0.3708	0.5736	0.6567
Spleen (g/kg BW)	0.64	0.70	0.66	0.05	0.67	0.67	0.038	0.68	0.60	0.66	0.75	0.66	0.65	0.066	0.6147	0.9866	0.4621
Bursa (g/kg BW)	1.85	1.91	1.85	0.09	1.77	1.97	0.077	1.68	2.02	1.80	2.01	1.83	1.86	0.134	0.8914	0.0873	0.5234
Heart (g/kg BW)	8.5[a]	7.9[ab]	7.8[b]	0.19	8.3	7.9	0.16	9.0	8.0	7.8	8.0	8.0	7.6	0.27	0.0474	0.0894	0.1205
Liver (g/kg BW)	36.8	37.9	37.4	1.23	37.0	37.8	1.01	35.0	38.6	37.9	38.0	38.2	36.7	1.74	0.8111	0.6003	0.3401
Proventriculus & gizzard (g/kg BW)	47.3	47.3	47.6	1.30	47.4	47.3	1.06	46.4	48.1	50.2	44.4	45.7	49.5	1.84	0.9765	0.9319	0.0397*
Gizzard full (g/kg BW)	39.5	40.2	40.2	1.18	40.0	40.0	0.96	38.7	40.4	42.8	37.6	38.5	41.9	1.66	0.9041	0.9929	0.0404*
Gizzard empty (g/kg BW)	25.3	25.2	24.9	0.55	25.1	25.2	0.45	25.2	25.4	25.7	24.8	24.3	25.4	0.78	0.8238	0.8574	0.4390

Values are least square means.
Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).
* Effect was no longer apparent after adjustment with Tukey-Kramer's test.

Table 4
BW, yolk sac and organ weight at 32 days in Ross-308 broiler chicks exposed to one of three different treatments in the hatcher (no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic product in the water (PS)) and one of two postplacement treatments (half of each group received the PS (in their bell drinkers) for three consecutive days after placement (PS) and the other half received regular drinking water (None)).

Variable	Hatchery treatment				Postplacement treatment			Interactions							*P*-value		
	NegC n = 10	PS n = 10	PosC n = 10	SEM	None n = 15	PS n = 15	SEM	NegC x None n = 5	NegC x PS n = 5	PS x None n = 5	PS x PS n = 5	PosC x None n = 5	PosC x PS n = 5	SEM	Hatchery treatment (H)	Postplacement treatment (PPT)	HxPPT
BW (g)	1969.7[b2]	2064.0[a]	2186.6[a]	48.52	2096.0	2050.8	39.62	2005.2	1934.2	2139.9	1988.1	2143.0	2230.1	68.62	0.0151	0.4274	0.2290
Intestine (g/kg BW)	56.5	56.4	54.3	1.27	54.8	56.7	1.04	55.2	57.8	54.5	58.3	54.6	54.0	1.80	0.3898	0.1982	0.4827
Intestine (cm/kg BW)	88.8	84.4	82.2	2.05	83.3	86.9	1.68	88.4	89.1	79.1	89.7	82.5	82.0	2.91	0.0934	0.1432	0.1359
Spleen (g/kg BW)	0.96	0.86	0.98	0.056	1.00[a]	0.86[b]	0.045	1.05	0.87	0.91	0.81	1.06	0.897	0.079	0.2925	0.0371	0.8846
Bursa (g/kg BW)	2.16	1.98	1.89	0.106	2.07	1.95	0.087	2.32	2.00	1.97	1.98	1.92	1.86	0.151	0.2031	0.3398	0.5233
Heart (g/kg BW)	6.5	6.2	6.1	0.20	6.4	6.1	0.16	6.6	6.4	6.4	6.0	6.4	5.8	0.28	0.3124	0.1249	0.8613
Liver (g/kg BW)	26.6	25.1	26.7	0.57	26.5	25.8	0.47	26.7[ab]	26.5[ab]	23.9[b]	26.3[ab]	28.8[a]	24.6[b]	0.81	0.0982*	0.3181	0.0017
Proventriculus & gizzard (g/kg BW)	28.2[a]	25.2[ab]	24.1[b]	0.93	25.7	26.0	0.76	27.0	29.3	24.6	25.8	25.5	22.8	1.31	0.0147	0.8073	0.1460
Gizzard full (g/kg BW)	23.4[a]	20.8[ab]	20.0[b]	0.88	21.4	21.4	0.71	22.7	24.2	20.3	21.4	21.3	18.6	1.24	0.0254	0.9457	0.1934
Gizzard empty (g/kg BW)	14.7[a]	13.0[b]	12.4[b]	0.49	13.3	13.5	0.40	14.4	15.1	12.4	13.6	13.0	11.8	0.69	0.0062	0.6966	0.1753

Values are least square means.
Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).
* Effect was no longer apparent after adjustment with Tukey-Kramer's test.

Table 5
Villi height and length and crypt depth in intestinal segments sampled at 2 and 11 days of age in Ross-308 broiler chicks exposed to one of three different treatments in the hatcher (no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic product in the water (PS)) and one of two postplacement treatments (half of each group received the PS (in their bell drinkers) for three consecutive days after placement (PS) and the other half received regular drinking water (None)).

	Hatchery treatment				Postplacement treatment			Interactions							*P*-value		
Variable (μm)	NegC n(d2) = 5 n (d11) = 10	PS n(d2) = 5 n (d11) = 10	PosC n(d2) = 5 n (d11) = 10	SEM	None n = 15	PS n = 15	SEM	NegC x None n = 5	NegC x PS n = 5	PS x None n = 5	PS x PS n = 5	PosC x None n = 5	PosC x PS n = 5	SEM	Hatchery treatment (H)	Postplacement treatment (PPT)	HxPPT
Villi height At day:																	
2	699.5	711.2	585.5	51.79	[1] N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.2074	N/A	N/A
11	1140.4	1204.3	1207.7	66.96	1214.1	1154.2	54.67	1137.9	1142.9	1344.1	1064.5	1160.3	1255.2	94.70	0.7286	0.4466	0.1406
Villi width At day:																	
2	92.6	84.4	89.1	6.55	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.6812	N/A	N/A
11	120.0	127.5	128.8	7.47	126.6	124.3	6.10	116.5	123.5	128.9	126.0	134.2	123.4	10.56	0.6762	0.7956	0.7023
Crypt depth At day:																	
2	93.5[a4]	101.1[1]	61.7[2]	6.45	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.0023	N/A	N/A
11	132.0	134.5	145.1	6.27	140.0	134.4	5.12	126.9	137.1	144.8	124.2	148.1	142.0	8.8740	0.3137	0.4539	0.2426
Ratio[2]																	
2	27.5[1]	7.0[1]	9.9[2]	0.54	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.0065	N/A	N/A
11	8.7	9.0	8.4	0.48	8.8	8.6	0.39	9.1	8.3	9.4	8.6	7.9	8.9	0.67	0.6442	0.7197	0.2734

Values are least squares means.
Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).
[1] Not applicable.
[2] Villi height divided by crypt depth.

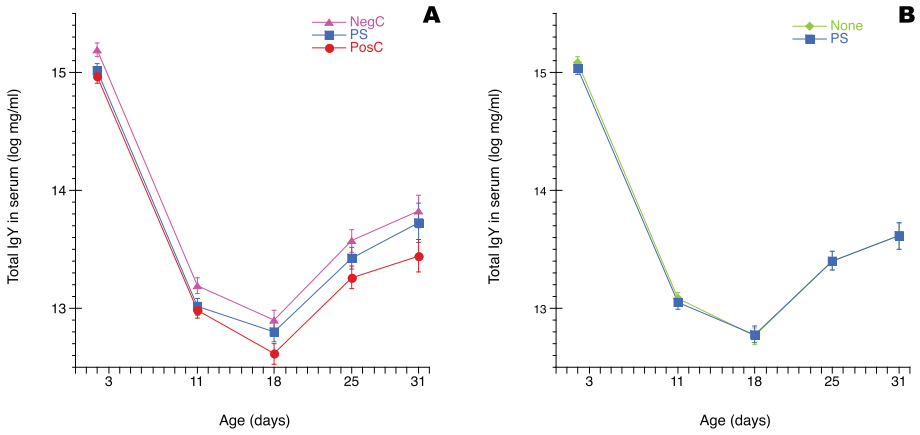

Fig. 1. Total concentration of Immunoglobulin Y (Ig Y) in serum collected from Ross-308 broiler chickens at 3, 11, 18, 25 and 31 days of age. Values are group mean ± SEM. (A) Hatch treatments: Chicks in the hatcher given: no access to feed and water in the hatcher (NegC: triangles); access to feed, water and a synbiotic product in water (PS: squares); or access to feed and water (PosC: circles). (B) Postplacement treatments: Chicks in the stable given: no supplement in drinking water at the research facility (None: diamonds) or supplement with synbiotic product in water at the research facility (PS: squares).

immediately fed chicks, whereas chicks feed-deprived for 48 h showed lower BW. In contrast to these results, Hollemans et al. (2018) found lower BW in chicks feed-deprived for 54 h posthatch at 21 d of age, but not at 35 days (Hollemans et al., 2018). Regarding the postplacement treatment, the None–supplemented chickens weighed more compared to the supplemented ones, and at the end of the study, they also consumed more feed. At 4 and 25 days of age, they also had a superior FCR compared to the supplemented chickens.

From these results, it appears that providing synbiotics at the growing facility had adverse effects on the productivity of the birds. A possible explanation for this, as discussed by Lan et al. (2005), is that an active microflora may increase the maintenance requirement of chickens, which would be an obvious disadvantage in chickens that have been feed-deprived.

In the present study, chickens in the NegC group had lower YFBM and relatively lighter and shorter intestine at 2 days of age. Delayed development of gut segments is commonly observed in feed-deprived chicks, especially during the first week of life (de Jong et al., 2017). Body length relative to BW was greater in NegC than in the other groups at placement, probably because the NegC chicks weighed less than the others.

Furthermore, the different treatments had no clear and persistent effects throughout the 32-day study on microbial development or on intestinal development. However, the relative gizzard weight (empty) was greater in NegC chicks at placement as well as at the end of the study when compared to the PosC group. One could speculate this was due to feed–deprived chickens assigning higher priority to anterior digestive organs' development during inadequate feed supply. Indeed, in a study using chicks feed-deprived for 48 h, Maiorka et al. (2003) suggested this phenomenon was a form of resource allocation. In their case, the joint weight of the gizzard and proventriculus was higher in chicks feed-deprived for 48 h (Maiorka et al., 2003). Demand organs are known to be downgraded priority-wise in favour of supply organs when the growth rate is high (Christensen, 2009). When gizzards were weighed full, the NegC group was lighter compared to PosC at hatch, while the opposite relationship was seen at 32 days. Why that is remains unknown and is not in line with for example de Jong et al. (2017) who in their meta-analysis concluded no long-term effects of feed deprivation of relative weights of, among other organs, gizzard (de Jong et al., 2017).

In general, results from organ measurements can be difficult to compare between studies, since some differences in relative organ weight may occur simply because BW gain differs between treatment groups, which may actually explain the differences in relative heart weight at 11 days of age in this study. On comparing absolute values, there were no differences in heart weight between any of the treatment groups. Moreover, sampling day is of great importance for intestinal development measurements because of the rapid development of the gastrointestinal tract in early life, which also adds to the difficulty in comparing results between studies (Ivarsson et al., 2022).

There was an effect of treatment on the ratio between villi height and crypt depth, deriving from deeper crypts in the intestine of chickens from the PS hatch group compared with the PosC control group at 2 days of age. These groups were provided with feed and water during hatch, so the synbiotic supplement could have resulted in a better-developed intestine. However, the negative control group was not different from the PS group concerning crypt depth, contradicting that theory. In addition, the differences observed were no longer apparent at day 32, supporting conclusions by de Jong et al. (2017) that differences in early organ development due to feed and water restriction are only short-term. At the end of the study, a difference in relative spleen weight arose between postplacement treatments, where the None–treated group had higher spleen weights. These results are not in line with previous research, because supplementation of probiotics has been found to stimulate the development of spleen and bursa, organs that are highly important for immune function (Karimi Torshizi et al., 2010).

In Sweden, most broiler producers are concentrated in the south of the country, geographically close to hatcheries and slaughter facilities. However, our research facility is located in Uppsala and the hatcher providing feed and water was located 670 km away in Lund, so the chicks in this study were subjected to a longer transport time with no access to feed and water than under Swedish commercial settings. Withdrawal of feed has been shown to be a very potent physiological stressor in broiler breeders that are routinely feed-restricted, e.g. domestic birds subjected to feed withdrawal for only 24 h may show increased and lasting levels of corticosterone (stress hormone) and adrenal hypertrophy (enlargement of the adrenal gland) (Mench, 2002). It may therefore be more stressful for chicks to receive feed and water and then have these withdrawn, leaving them with a hunger-signaling system that has been set in motion, than to not have any access to feed at the beginning of life. The lack of feed and water during

Table 6
Immunoglobulin Y (IgY) concentration in serum at days 2, 11, 17, 24 and 31 in Ross-308 broiler chicks exposed to one of three different treatments in the hatcher (no access to feed and water (NegC); access to feed and water (PosC); and access to feed, water and a synbiotic product in the water (PS)) and one of two postplacement treatments (half of each group received the PS (in their bell drinkers) for three consecutive days after placement (PS) and the other half received regular drinking water (None)).

Variable	Hatchery treatment			SEM	Postplacement treatment		SEM	Interactions						*P*-value			
	NegC n = 10	PS n = 10	PosC n = 10		None n = 15	PS n = 15		NegC x None n = 5	NegC x PS n = 5	PS x None n = 5	PS x PS n = 5	PosC x None n = 5	PosC x PS n = 5	SEM	Hatchery treatment (H)	Postplacement treatment (PPT)	HxPPT
IgY concentration in serum (mg/ml)*																	
Day 2	15.19[a1]	15.02[ab]	14.97[b]	0.058	15.09	15.03	0.048	15.20	15.18	15.02	15.02	15.04	14.90	0.082	0.0290	0.4340	0.6756
Day 11	13.19	13.01	12.99	0.069	13.08	13.05	0.056	13.24	13.14	12.99	13.04	13.01	12.96	0.097	0.0838	0.6599	0.7650
Day 17	12.90	12.80	12.61	0.087	12.77	12.78	0.071	12.75	13.05	12.88	12.73	12.67	12.56	0.122	0.0816	0.9083	0.1531
Day 24	13.57	13.43	13.26	0.094	13.40	13.44	0.077	13.43	13.71	13.47	13.38	13.31	13.21	0.134	0.0871	0.7756	0.3096
Day 31	13.82	13.72	13.44	0.136	13.61	13.72	0.111	13.67	13.98	13.75	13.70	13.42	13.47	0.193	0.1467	0.5211	0.6427

Least square mean values within rows with different superscript letters are significantly different ($P < 0.05$).
* Values were log–transformed before statistical analysis.

transport may have influenced the development of the gastrointestinal tract in our chicks.

In this study, the synbiotic was provided during hatch and also during the first 3 consecutive days at the growing facility, to provide the chicks with a healthy microbiota. However, this treatment had no effects on the caecal microbiota assessed by 16 s rRNA community profiling, which is in line with findings by Ballou et al. (2016) that probiotics may have only transient or minor effects when added in non-stressful conditions. Different studies on the use of probiotics have produced varying results, owing to e.g. lack of stressors (i.e. perfect conditions as regards nutrition and environment), inappropriate source of microorganisms, route of administration and dosage levels (Karimi Torshizi et al., 2010). Individual water consumption was not recorded in the present study, so uneven water consumption and thereby uneven intake of the synbiotic additive may have been overlooked, which could have influenced the results.

The microbial richness of the caecum is known to increase with age, with diversification generally starting at around 7 days of age (Oakley et al., 2014; Ballou et al., 2016). This was also the case in the present study, where the 2-day-old chicks showed low microbial diversity in the caecum, with *Enterobacteriaceae* as the dominant phylum (Fig. 3). From seven days of age, phylum *Firmicutes* generally increases in abundance in the chicken caecum (Kubasova et al., 2019), which was also observed in the present study (with the exception of *Clostridium sensu stricto 1*, which decreased with age). Overgrowth of *Clostridium sensu stricto 1* in combination with a decrease in *Lactobacillus* is associated with the development of necrotic enteritis in chicken (Yang et al., 2019; 2022). Later, at around 28 days of age, another compositional shift has been shown to occur, with the phylum *Bacteroidetes* increasing in abundance at the expense of *Firmicutes* (Kubasova et al., 2019). In the present study, this shift was displayed by the genera *Bacteroides* and *Alistipes,* which both increased in abundance by the end of the study. In conclusion, the microbial composition of the caecum seemed to follow the general trend of caecal microbial maturation with age described in the literature.

It has been suggested that feed deprivation of newly hatched chicks can have detrimental effects on their developing immune system (Dibner et al., 1998; Juul-Madsen et al., 2004; Bar Shira et al., 2005; Panda et al., 2010; 2015). Regarding the induction of antigen-specific antibody responses, one study found increased vaccine-induced responses in early-fed chicks compared with late-fed (Panda et al., 2010). To test for immune response to a novel antigen, we examined the induction of antigen-specific antibody production to an inactivated APV vaccine. Unfortunately, only 54% of the birds tested positive for APV-specific antibodies following vaccination, and many of those had low levels of antibodies to APV. Thus, we were unable to detect any significant treatment differences in antigen-specific antibody production and the overall low responsiveness made it impossible to draw any firm conclusions. In previous work, we have observed similarly poor APV-specific antibody responses to this vaccine in Ross-308 chickens (Ivarsson et al., 2022; Boyner et al., 2023). No clear reason for this has been identified, but it is possible that modern broiler chickens may generally have low immune responsiveness due to strong breeding selection over time for high growth rates (van der Most et al., 2011; Zuidhof et al., 2014). Use of a live vaccine might have been more effective, since these often have a higher stimulatory effect on the immune system (Aida et al., 2021). On the other hand, some studies have shown acceptable induction of antibody responses by inactivated vaccines in broiler chickens (Sharma, 1999; Juul-Madsen et al., 2004). Therefore, it is likely that a combination of factors such as chicken genetic make-up, vaccine antigen or adjuvant were involved in the poor vaccination outcome in the present study.

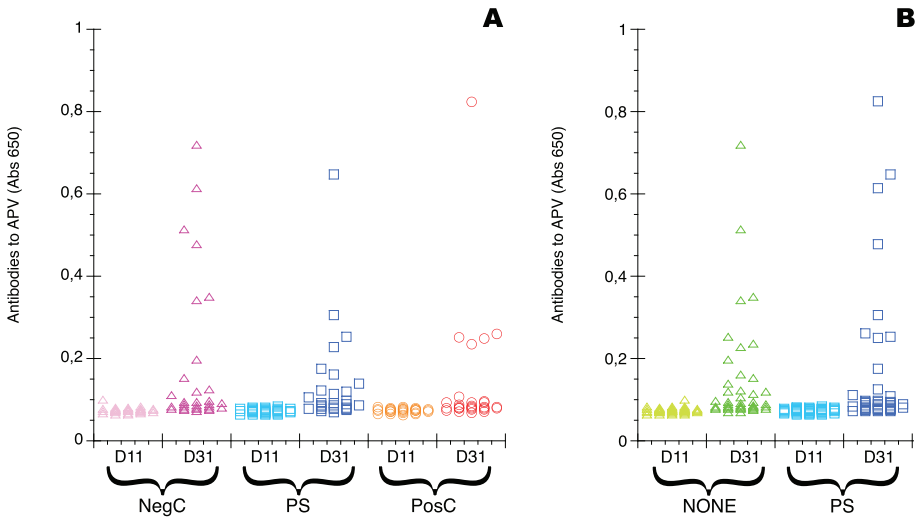

Fig. 2. Antibodies to avian pneumovirus (APV) in serum collected prevaccination at 11 days of age and 20 days after vaccination (i.e. at 31 days of age) from Ross-308 broiler chickens. Results are absorbance values at 650 nm (Abs_{650}) for individual chickens in the different treatment groups. A calculated cut-off value of Abs_{650} 0.086 was used to categorise samples as positive or negative to APV (for details, see Material and Methods). (A) Hatch treatments: Chicks in the hatcher given: no access to feed and water in the hatcher (NegC); access to feed, water and a synbiotic product in water (PS); or access to feed and water (PosC). (B). Postplacement treatments: Chicks in the stable given: no supplement in drinking water at the research facility (None) or supplement with synbiotic product in water at the research facility (PS).

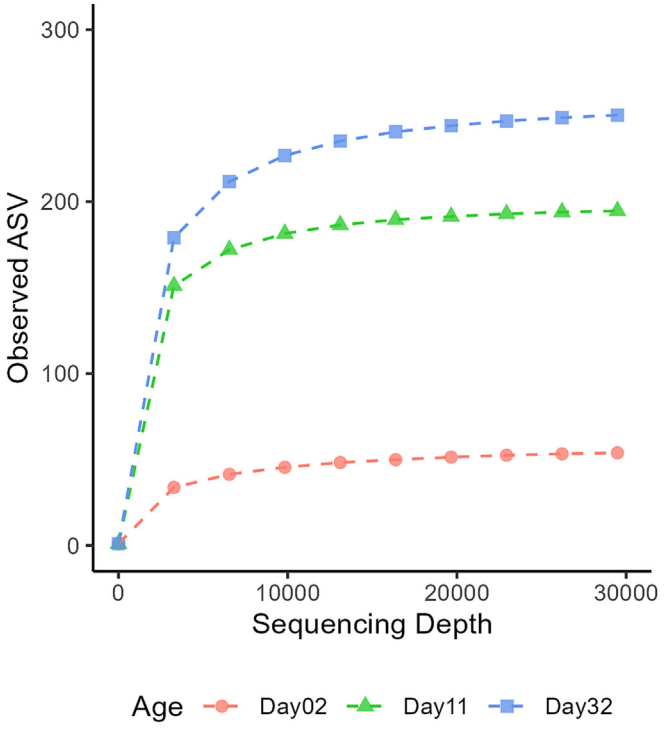

Fig. 3. Rarefaction curves of observed amplicon sequence variants (ASV) in caecal samples from Ross-308 broiler chickens at 2, 11 and 32 days of age.

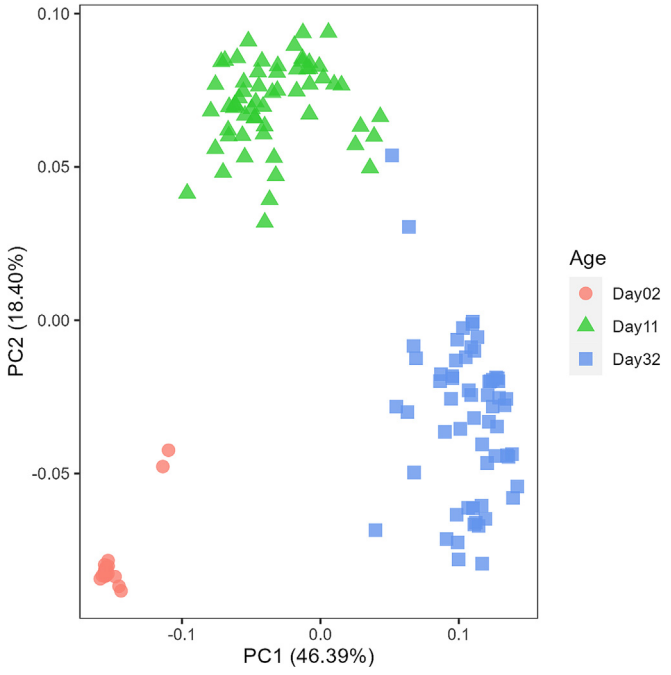

Fig. 4. Principal coordinate analysis (PCoA) plot showing differences in generalized UniFrac beta diversity of caecal microbiota of Ross-308 broiler chickens at 2, 11 and 32 days of age.

In addition to vaccine-induced antibody response, we also used total serum IgY to assess the immune status of the chickens. Overall, the chickens in all groups showed a similar pattern of change in serum IgY levels, with a rapid decrease in maternally derived IgY and a fairly slow onset of the chickens' own IgY production. However, there was a statistically significant difference in IgY concentration (log) values between the NegC group compared to the PosC group at placement, an effect that remained a tendency through to 25 days of age, for which we have no clear explanation. However, a genetic influence on immunisation-induced antibody production has previously been demonstrated in chickens (Minozzi et al., 2008; Zerjal et al., 2021). Moreover, in the present and previous work (Boyner et al., 2023), we found that chicks responding to the APV vaccination had higher serum levels of total IgY by the end of the study. It is therefore possible that the observed differences in serum IgY concentration between treatments may simply reflect an uneven distribution of chicks that were genetically high antibody producers and offspring of such mothers.

As discussed previously, a lack of stressors in the environment can lower the effect of microbial additives (Karimi Torshizi et al.,

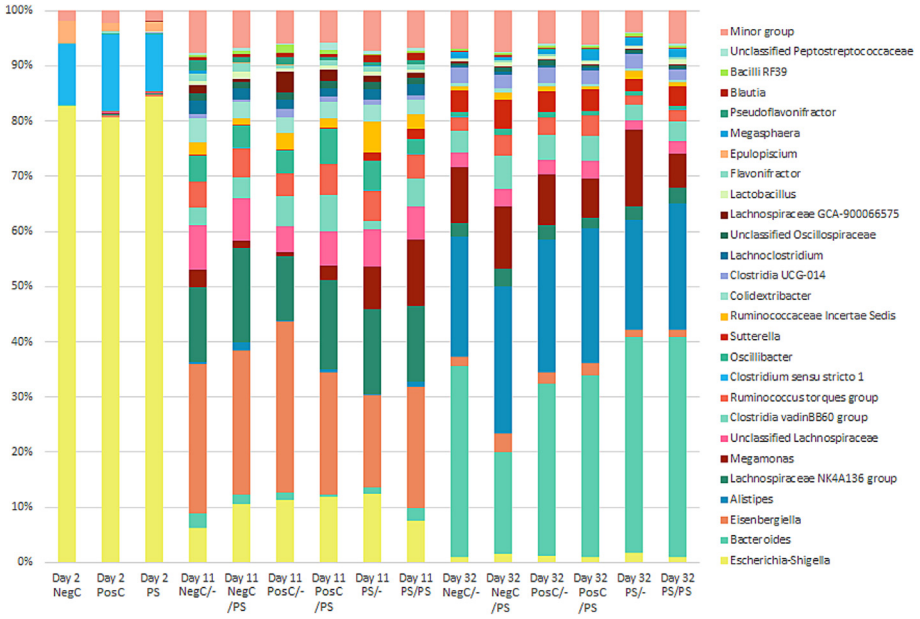

Fig. 5. Relative abundance (%) of genera in caecal samples of Ross-308 broiler chickens collected at 2, 11 and 32 days of age. Treatments given to chicks: access to feed and water in the hatcher and a synbiotic product in water in the growing facility for 3 consecutive days (PosC/PS); access to feed and water in the hatcher and no treatment in the growing facility (PosC/-); no access to feed or water in the hatcher and a synbiotic product in water in the growing facility for 3 consecutive days (NegC/PS); no access to feed or water in the hatcher and no treatment in the growing facility (NegC/-); feed, water and a synbiotic product in the hatcher and a synbiotic product in water in the growing facility for 3 consecutive days (PS/PS); or access to feed, water and a synbiotic treatment in the hatcher and no treatment in the growing facility (PS/-).

2010). In the present study, the birds were kept at a low stocking density in a research facility, with long fallow periods between studies. Biosecurity was high and pathogen pressure was presumably low, which probably posed less challenge to the birds' immune system than commercial rearing conditions. This effect has been described by Eckert et al. (2010), who had to almost double the number of animals kept in the rearing facility before the positive effects of probiotics on BW and FCR were noticeable. Feed deprivation for 48 h is suggested to be unfavourable for growth, immune function and viability in broilers, while a 24 h period of feed deprivation seems to give acceptable growth and immune function (Juul-Madsen et al., 2004). The chicks in the present study that were feed-deprived were so for 40 h, and all chicks had no access to feed and water during transport. They could possibly have showed similar negative effects on immunological parameters if they had been feed-deprived for slightly longer, especially since effects on productivity parameters emerged from the 40 h restriction.

Conclusion

In conclusion, delayed access to feed and water posthatch resulted in negative effects on productivity traits that lasted for the remainder of the study. Some effects were also observed for serum IgY concentration and relative organ weights while caecal microbiota development was unaffected by treatments. The synbiotic additive did not provide any performance-enhancing qualities when provided at hatch or for 3 days posthatch, suggesting that the costs of processing microbial additives in early life may outweigh the benefits for the chicks when the environmental pressure is low. Short exposure time and a higher dose of the synbiotic additive during hatch could also have influenced the outcomes of this study. An experimental set-up with conditions more closely resembling those in commercial production (higher stocking density, higher pathogen pressure) might have yielded different results. The long transport time between the hatchery and growing facility may also have lowered the treatment impact.

Ethics approval

This experiment was approved by the ethical committee of the Uppsala Animal Experiment Ethics Board (application number 5.8.18-07947/2017).

Data and model availability statement

Raw sequencing data from this study have been deposited in the database of the National Center for Biotechnology Information (NCBI), under accession number PRJNA929198 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA929198/). Models and data are available upon reasonable request.

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

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

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Declaration of interest

None.

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