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The development of the gastrointestinal tract in broilers

Effects of access to feed, water and probiotics in the hatcher

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The development of the gastrointestinal tract in broilers – effects of access to feed, water and probiotics in the hatcher

Magtarmkanalens utveckling hos slaktkyckling – effekter av tillgång till foder, vatten och probiotika i kläckaren

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Abstract

The study was performed at the research facility of SLU outside Uppsala, where the gastrointestinal tract development and production performance was evaluated in broilers without contra with access to feed, water and probiotics in the hatcher. After hatch, 450 chickens of Ross 308 were divided into five treatments. One treatment was not provided feed and water at hatch, one was provided with feed and water and the three remaining had probiotic addition of different characters. At arrival to the research facility, chickens of the unfed treatment, chickens of the fed treatment and one treatment with probiotic addition were split in two subgroups, where one of the two groups where provided one of the probiotics the first three days at the research facility. The remaining two treatments were not split nor supplemented with probiotics. Throughout the study data was collected in weights of chickens, feed and gastrointestinal organs. The two treatments without feed and water at hatch had lower weights up to 18 days of age compared to the majority of treatments with feed and water, although unfed chickens at hatch without probiotic supplementation in the research facility had compensated in weight the last day of the study. Unfed chickens at hatch with probiotic supplementation at the research facility were not able to compensate in weight until the end of the study. Differences in organ weights, feed conversion ratio, feed intake and chicken weights (after 18 days of age) were not exclusively linked to chickens of unfed treatments at hatch. Moreover, probiotic supplementation did not result in improved growth but was rather contributing to the opposite in three treatments. Goblet cells in the duodenum were ocularly studied in a light microscope of intestinal incisions. It appeared as if chickens in treatments with probiotic addition had a higher cell density compared to treatments without probiotic addition in two-day-old chickens. In addition, goblet cell sizes in two-day-old chickens seemed to be linked to unfed chickens at hatch, with smaller goblet cells in the duodenum than chickens in fed treatments at hatch. Unfed chickens without probiotic supplementation appeared most profitable and least time consuming from this study, however, more studies are required.

Sammanfattning

Studien utfördes vid SLU:s forskningsanläggning utanför Uppsala, där utvecklingen av mag-tarmkanalen samt produktionsresultat undersöktes hos slaktkyckling med eller utan tillgång till foder, vatten och probiotika i kläckaren. Efter kläckning delades 450 Ross 308-kycklingar in i fem behandlingar. En behandling hade inte tillgång till foder och vatten i kläckaren, en var försedd med foder och vatten och de tre resterande hade probiotikatillskott av olika varianter. Vid ankomst till forskningsanläggningen delades tre av kläckbehandlingsgrupperna in i två undergrupper. De behandlingsgrupperna var kycklingar utan tillgång till foder och vatten i kläckaren, kycklingar med tillgång till foder och vatten i kläckaren samt en av grupperna med probiotikatillskott. En av de två undergrupperna fick probiotikatillskott de tre första dagarna på forskningsanläggningen. Återstående två behandlingsgrupper delades inte upp och fick inte probiotikatillskott i forskningsanläggningen. Under studiens gång samlades data in av vikter från kycklingar och mag- tarm kanalens organ, samt foderintag. De två behandlingarna utan foder och vatten i kläckaren hade lägre vikter till 18 dagars ålder jämfört med majoriteten av behandlingar med tillgång till foder och vatten i kläckaren. Behandlingsgruppen utan foder och vatten i kläckaren utan probiotikatillskott i forskningsanläggningen hade kompenserat i vikt vid 32 dagars ålder. Behandlingsgruppen utan foder i kläckaren med probiotikatillskott hade däremot inte kompenserat i vikt till sista dagen. Skillnader mellan organvikter, foderomvandlingsförmåga, foderintag och kycklingvikt (efter 18 dagars ålder) var inte enbart kopplat till behandlingar utan tillgång till foder och vatten i kläckaren. Inte heller resulterade probiotikatillskott i bättre tillväxt utan bidrog snarare till motsatsen i tre behandlingar. Bägarceller från duodenum studerades okulärt från tarmsnitt i ett ljusmikroskop. Resultaten pekade mot att kycklingar i behandlingar med probiotikatillskott hade fler bägarceller jämfört med kycklingar i behandlingar utan probiotikatillskott, sett i duodenum från två dagar gamla kycklingar. Vidare verkade storleken på bägarceller i duodenum från två dagar gamla kycklingar kopplad till kycklingar utan foder och vatten i kläckaren, vilka hade mindre bägarceller än kycklingar i resterande behandlingar. Kycklingar utan tillgång till foder och vatten i kläckaren och utan probiotikatillskott verkade mest lönsamma och minst tidskrävande i denna studie, men fler studier behövs.

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Introduction

In traditional broiler production, chickens are hatched at a commercial hatchery, and the majority of hatcheries do not provide chickens with feed and water in the hatcher (Noy & Uni, 2010). Chickens without feed and water in the hatcher will have to wait until arriving at the rearing stable (Noy & Uni, 2010) which may take up to three days of time (Henderson *et al.*; 2008; Willemsen *et al.* 2010). One reason of this delay is that although all eggs are placed in the brooder at the same time, the chickens' hatch during a time span stretching from 24 to 48 hours before departure to the rearing stable and the time passed during hatching is known as the "hatch window" (Willemsen *et al.*, 2010). The hatch window or the delay in hatching is caused by biological differences (Ipek & Sozcu, 2014) such as egg size and diseases or temperature variability in the setters (Aviagen, 2009). When chickens eventually are removed from the hatcher there is additional time for quality sorting, packaging and transport (Bhaja *et al.*, 2009). The hatch window along with latter mentioned routines might lead to the earliest hatched chickens being deprived of feed and water for over 70 h (Henderson *et al.*; 2008; Willemsen *et al.* 2010; The Swedish Board of Agriculture [TSBA], 2015b).

Delaying access to feed and water results in a supressed weight gain and less heavy slaughter weights (Henderson *et al.*, 2008; Shafey *et al.*, 2011) and in addition, the risk of dehydrated chickens with a retarded development of the gastrointestinal tract increases (Bigot *et al.*, 2003; Noy & Uni, 2010). On the other hand, providing chickens with feed and water immediately after hatch is beneficial (Pinchasov & Noy, 1993; Maiorka *et al.*, 2003; Hendersson *et al.*, 2008; Noy & Uni, 2010). Early post-hatch feeding stimulate growth of the intestines and increase villi height, which in turn increases the ability of nutrient absorption (Noy & Uni, 2010) resulting in better feed conversion ratio and a shorter rearing period (Noy & Uni, 2010; Richards *et al.*, 2010; Mahapatra *et al.*, 2017). In addition, direct access to feed and water seems to improve chicken health, since chickens provided feed and water at hatch are more alert after being exposed to antigens, compared to corresponding control group without access to feed and water at hatch (Simon *et al.*, 2015). Moreover, additives such as probiotics have been of interest to investigate and benefits seen from probiotic addition are a decreased feed conversion ratio (Abdel-Hafeez *et al.*; 2017; Gao *et al.*, 2017), an increase in body weight gain (Nyamagonda *et al.*, 2011) and a higher immune response in form of increased serum IgG and intestinal secretory IgA (Gao *et al.*, 2017).

Further studies are important regarding effects of direct access to feed, water and probiotics in Swedish broilers. Wider and deeper knowledge may contribute to that more Swedish hatcheries introduce feed and water in the hatcher, which considering above-mentioned studies could lead to better growing chickens and possibly an increased animal welfare if chickens are stressed by being deprived of feed and water.

Aims

One aim of the present study was to investigate if access to feed and water immediately after hatch could result in a more developed gastrointestinal tract and a better production performance in broiler chicken, compared to corresponding control group without immediate access to feed and water after hatch. Another aim was to conclude if probiotic supplementation of different characters could affect the gastrointestinal tract and overall performance when provided during hatching and/or provided during the first three days after arriving at the research facility.

Literature review

Conventional broiler rearing in Sweden

In total, about 98 million broilers are reared every year in Sweden (The Swedish Poultry Meat Association [TSPMA], 2017a). In 2017 there were approximately 120 broiler farmers in Sweden where 99 percent were members of the trade organization "The Swedish Poultry Meat Association" (TSPMA, 2017b). Each breeder rears on average 85 000 chickens per batch and has seven or more batches per year (TSPMA, 2017b). Broiler chickens are always reared on floor covered with either wood shavings or chopped straw. Water and feed are at easy access for the chickens, and temperature, light and humidity thoroughly adjusted (TSPMA, 2017b).

Breeding and rearing

The most commonly used breeds in Sweden are the two genotypes Ross and Cobb (Sveriges Veterinärmedicinska Anstalt [SVA], 2016), which are bought in as day old breeding chickens from the UK and the USA and reared in Sweden (TSPMA, 2017c). These chickens are called grandparents (TSPMA, 2017c). The offspring's of the grandparents are called parents, which are both hatched and reared in Sweden (TSPMA, 2017c). The eggs of the parents are collected and hatched, with the chickens being transported to broiler producers throughout Sweden where they are reared as broilers (TSPMA, 2017c). Swedish broiler chickens are slaughtered at around five weeks of age and the stable is thoroughly cleaned between batches of chickens (SVA, 2016).

Regulations

Maximum animal density in conventional broiler rearing in Sweden is 36 kg per m^2, although it is not allowed to exceed 25 chickens per m^2 (TSBA, 2017). The weight regulation of 36 kg/m^2 is however only allowed for farmers with an approved control programme, otherwise there is a limit of 20 kg/m^2 (TSBA, 2017). Since nearly all broiler farmers in Sweden are members of TSPMA with an approved control programme, it means nearly all broiler chickens in Sweden are reared at a density of 36 kg/m^2 (TSPMA, 2017d). Regarding transport, Swedish newly hatched chickens are allowed to be transported for a maximum of 24 hours without feed and water, provided that the transport is ended at maximum 72 hours after hatch (TSBA, 2015b). Transport to slaughter must not exceed eight hours, exceptions up to twelve hours can only be made if the closest slaughterhouse is not within eight hours, in that case; transport must occur during dark hours in a vehicle with an adaptive system of ventilation and temperature (TSBA, 2015b).

Broiler production in Sweden is regulated by the animal protection law (SFS 1988:534), controlled by The Swedish Board of Agriculture (TSBA, 2017).

Feed and water

The Swedish Board of Agriculture is regulating poultry feed which must be of good quality with sufficient amount of nutrients and of proper structure (TSBA, 2015a). All ingredients for

broiler feed must be approved by TSBA and all feed has to be heat-treated (TSPMA, 2017e). Water of good quality must be provided at least twice a day, but free access is suggested (TSBA, 2015a).

Hatching

It takes approximately 20 days for the broiler chicken to develop inside the egg (Noiva *et al.*, 2014). During the first 17-18 days, the eggs are placed in setter trays in a brooder at the hatchery (Aviagen, 2015). Thereafter they are moved to hatcher baskets and placed in the hatcher for three more days (Aviagen, 2015). There is a slightly lower temperature in the hatcher compared to in the brooders, in order to reduce overheating of the hatched chickens (Cobb Vantress Inc. [CVI], 2013). Chickens stay in the hatcher until most chickens are hatched and dry (Aviagen, 2009; CVI, 2013). The chickens are thereafter taken out of the hatchers, separated from leftover shells and divided into first and second grade chickens. First grade chickens are for instance supposed to be well dried after hatch, have active eyes and an active appearance, have a completely healed navel and to be free from abnormalities such as cross beaks and crooked legs (CVI, 2013). First grade chickens will be reared as broiler chicken and the rest are culled (Aviagen, 2009; CVI, 2013).

Hatch window

The hatch window is the time that passes from when the first chicken hatch until the last chicken is hatched (Aviagen, 2009). The hatch window normally ranges between 24 and 48 hours before the chickens are removed from the hatcher (Willemsen *et al.*, 2010) for quality sorting, packaging and transport (Bhanja *et al.*, 2009). Differences in hatch time can depend on egg size, diseases and temperature variability in the setters (Aviagen, 2009). It may also be affected by age of the breeding flocks, where chickens from older hens hatch later than chickens from younger hens (Ipek & Sozcu, 2014). The aim in Ross production is for less than 1% of chickens to have hatched before 30 hours prior transport, since early-hatched chickens are at risk of dehydration, which in turn might impair growth (Aviagen, 2009).

The gastro-intestinal tract

Yolk sac

The yolk sac is attached to the small intestine of the chicken (Romanoff, 1944; Bagley, 2002) and contain nutrients which is taken up into the blood stream (Romanoff, 1944). The yolk slowly degrades during the development of the embryo (Bagley, 2002) and a few days after hatch (Romanoff, 1944). Close before hatch the yolk sac is retracted through the navel into the abdominal cavity (Bagley, 2002). At hatch, the yolk sac weighs around six grams (Bagley, 2002; Bhanja *et al.*, 2009). Since all yolk material is not completely absorbed by the time of hatch it can continue to provide the newly hatched chicken with nutrients, and the chicken can survive for a limited time after hatch even though there is no access to feed (Romanoff, 1944). Utilization of residual yolk sac is however faster in chickens fed immediately after hatch, compared to starved chickens (Romanoff, 1944; Bhanja *et al.*, 2009) and in addition; almost

70% of yolk sacs in unfed chickens rot within five days after hatch, suggesting that starving newly hatched chickens have a lower capacity of utilizing residual yolk sac (Romanoff, 1944).

Although newly hatched chickens are thought to survive for multiple days without feed or water considering the yolk sac is fully absorbed at approximately five days (120 h) after hatch (Romanoff, 1944; Noy & Sklan, 1998), there were in the 1940's proven that no chickens were able to survive without feed and water for five days of time (120 h) (Romanoff, 1944). Newer information of chicken survival without access to feed after hatch is lacking.

The small intestine

The small intestine in poultry is divided into three parts, first duodenum, followed by jejunum and ileum (Hodges, 1974; Bagley, 2002), although jejunum and ileum are not clearly separated from each other (Hodges, 1974). The small intestine is approximately 120 cm long in adult birds (Hodges, 1974). In mammals, the small intestine is the main area where nutrients and water are absorbed and the majority of nutrients and fluid is normally absorbed in the very beginning of the small intestine (Sjaastad, 2010). In chickens, the developmental phase of the small intestine immediately after hatch is of great importance for high growth performance (Noy & Uni, 2014).

Villi

The small intestine of birds is quite similar to the small intestine in mammals regarding digestion mechanisms and construction (Sjaastad *et al.*, 2010); therefore villi and cells are described from literature of both mammals and birds.

The small intestine is a great absorptive organ due to its large surface area (Sjaastad *et al.*, 2010). The surface area is characterised by a high number of folds, which are covered by numerous of outgrowths called "villi" (Figure 1) to extend the absorption area even more (Sjaastad *et al*., 2010). Every villus has a single layer of epithelial cells towards the intestinal lumen and these cells are covered by microvilli (Hodges, 1974; Sjaastad *et al.*, 2010). Microvilli appear on every epithelial cell of the villi and appear as hair like protuberances (Hodges, 1974; Sjaastad *et al.*, 2010). Between the villi at the very bottom close to the muscular mucosa (Figure 1) there are open ducts (Hodges, 1974). These ducts are called crypts or crypts of Lieberkuhn; here cells are produced by mitosis (Figure 1) (Hodges, 1974). In the middle of villus runs the Lamina propria, which mainly contains of smooth muscle bundles, connective tissue and capillaries (Hodges, 1974). The Lamina propria runs from the muscular mucosa into the villi (Hodges, 1974).

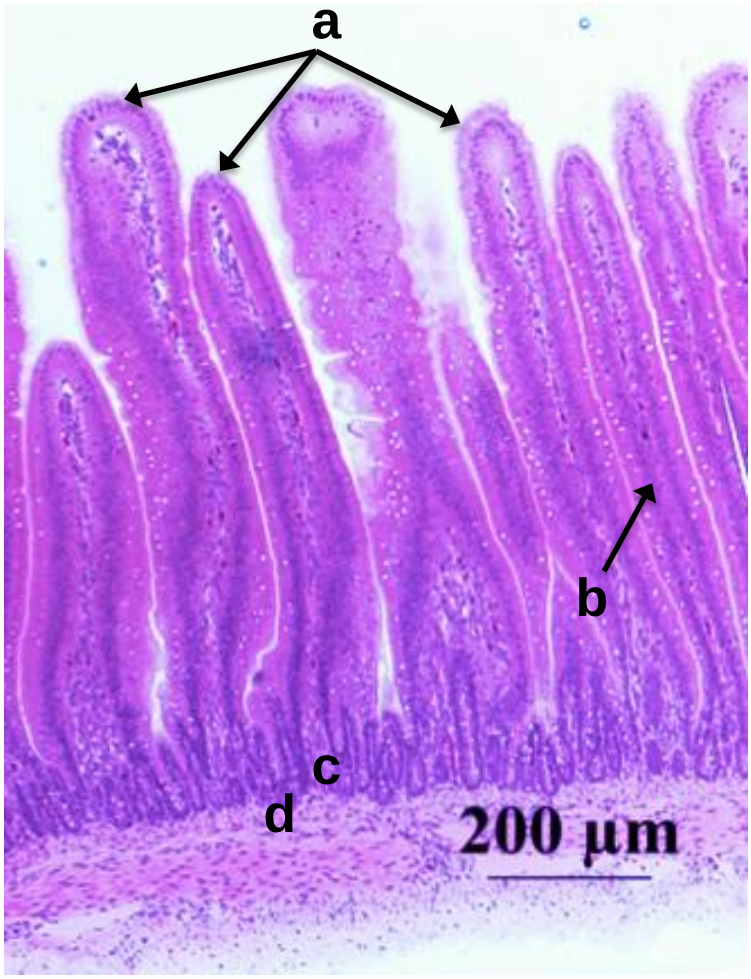

Figure 1. Villi (a) seen in duodenum of a chicken at two days of age (4x magnification). Goblet cells can be seen as white dots in the epithelium of the villi. Chief cells cover most part of the epithelium and the nuclei's are visible in lines of dark purple dots (b). At the base of the villi, crypts are seen (c) just above the muscular mucosa (d) (Hodges, 1974). Photo: Åsa Andersson.

Cells

Epithelial cells cover all villus (Hodges, 1974; Alberts *et al.*, 2014) (Figure 2). These cells are either absorptive brush-border cells (chief cells) or goblet cells producing mucus (Alberts *et al.*, 2014). The absorptive cells absorb nutrients and fluid from the intestinal tract (Sjaastad *et al.*, 2010), and cover the greatest area of the villus (Hodges, 1974; Alberts *et al.*, 2014). Secretory goblet cells are smaller in numbers and are scattered in between the absorptive cells (Hodges, 1974; Alberts *et al.*, 2014). The basal nucleus area of the goblet cell is narrow whilst the upper part is wide, appearing goblet shaped, hence the name "goblet cell" (Hodges, 1974). Goblet cell mucus in rats has an important role of protecting the small intestine surface from harmful immune complexes (Walker *et al.*, 1977). Mucus release of goblet cells is stimulated by immune complexes and work both as a shield for diffusion of the immune complexes and as a cleanser of attached ones (Walker *et al.*, 1977).

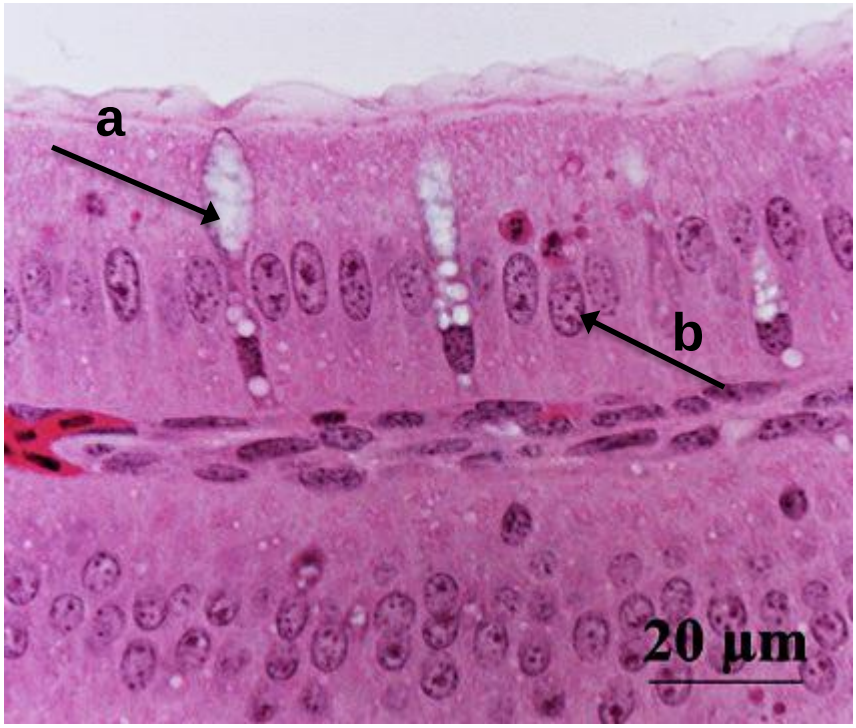

Figure 2. Section of villi in 60x magnification from a chicken at two days of age. Goblet cells are seen sparsely scattered between chief cells in the epithelium (a). Tall chief cells are lined up as the main part of the epithelium. The nuclei of chief cells can clearly be seen (b) (Hodges, 1974). Photo: Åsa Andersson.

All cells of the villi are produced by stem cells located at the bottom of the crypts (Hodges, 1974; Alberts *et al.*, 2014). The cells are undifferentiated and move upwards the villi and before exiting the crypt are differentiated into absorptive or secretory cells (Hodges, 1974; Alberts *et al.*, 2014). When the cells have wandered to the top of the villi they are released into the lumen and replaced by new epithelial cells (Hodges, 1974; Sjaastad *et al*., 2010; Alberts *et al.*, 2014). It takes three to six days for the epithelial cells to migrate from the crypts to the top of the villi (Alberts *et al*., 2014).

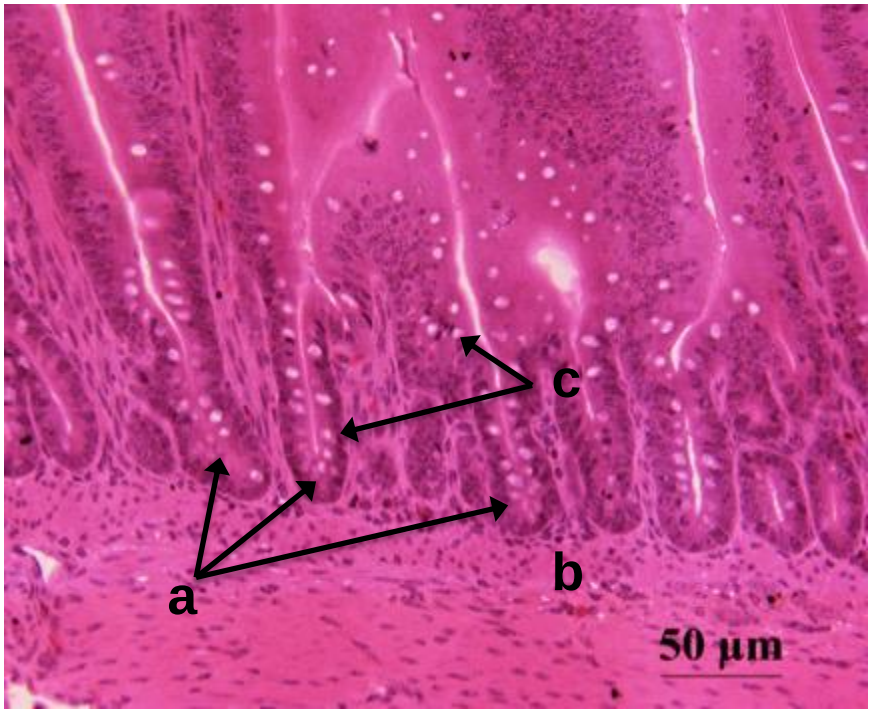

Figure 3. Crypts (a) just above the mucosal layer (b) in 20x magnification. The white dots visible are goblet cells (c) (Hodges, 1974).
Photo: Åsa Andersson.

Effects of fasting

Organ weight and size

Early post-hatch feed and water intake are directly connected to the development of the gastrointestinal tract in broilers (Maiorka *et al.*, 2003). Chickens deprived of feed the first 48 hours after hatch leads to a decreased length and width of the small intestine (Shafey *et al.* 2011). Similar results are seen in house sparrows, with lower intestine weights and liver weights after fasting for 34 hours (Funes *et al.* 2014). Fasting house sparrows also have decreased intestinal length/mass ratio and a decreased perimeter throughout the whole intestine (Funes *et al.*, 2014).

In broilers, direct access to feed and water at hatch result in both longer and heavier jejunum and ileum (Maiorka *et al.* 2003) and a higher weight/length ratio of jejunum and ileum in comparison to broilers with delayed access to feed and water (Lamot *et al.*, 2014). Combining feed and water is the most beneficial in two-day-old chickens, which have higher weights of jejunum and ileum than chickens provided only feed or water (Maiorka *et al.*, 2003). Small intestinal differences are seen in three-day-old chickens as well, were chickens receiving one of the three treatments (only feed, only water or both feed and water) have heavier and longer jejunum and ileum compared to the control group without access to feed or water within 24 h after hatch (Maiorka *et al.*, 2003).

Growth and feed intake

Providing feed and water at hatch increases weight gain of body mass in broilers, fed broilers have a greater slaughter weight than broilers with delayed feeding (Henderson *et al.*, 2008; Shafey *et al.*, 2011). In addition, feed conversion ratio increases, resulting in faster growing chickens with earlier reached market weight (Mahapatra *et al.*, 2017). Feed deprivation for between 48 and 72 h after hatch results in a supressed growth performance (Shafey *et al.*, 2011) and is seen up to 42 days of age (Gonzales *et al.*, 2003). In addition, mortality increases if chickens suffer from heat stress (Pinchasov & Noy, 1993).

Even though there are plenty results of lower slaughter weights in chickens unfed at hatch, other results prove lower body weights for merely up to two weeks after hatch (Bigot *et al.*, 2003; Uni & Sklan, 2003; Bhanja *et al.*, 2009; Lamot *et al.*, 2014). In fact, weights of fasted chickens at hatch are compensated before three weeks of age if feed deprivation remains less than 24 hours (Gonzales *et al.*, 2003; Bhanja *et al.*, 2009). And although many positive effects is seen regarding growth when provided feed and water immediately after hatch, no differences in feed conversion ratio between fasted chickens and fed chickens have been proven (Gonzales *et al.* 2003).

Feed intake is however affected by access to feed and water immediately after hatch, showing fed broilers with a higher feed intake at day seven (Lamot *et al.*, 2014) and day 33 of age

(Shafey *et al.*, 2011) in comparison to chickens deprived of feed and water for up to 48 hours after hatch.

Intestinal morphology

Broilers fasting for 30 and 48 hours respectively after hatch results in lower biometric values for length, weight and size of villi as well as crypt depth of the small intestine compared to fed broilers (Gonzales *et al.*, 2003). In fasting house sparrows, similar results can be seen, where villi height, villi width, lamina propria and enterocytes are significantly reduced (Funes *et al.*, 2014).

Duodenal goblet cell density increases in two-day-old chickens deprived of feed and water for 48 hours after hatch (Uni & Sklan. 2003) as well as in rats fasting for 72 h (Fernandez-Estivariz *et al.*, 2003). Furthermore, feed deprivation results in a change in goblet cell volume, where cells of fasted chickens are greater than in fed chickens (Uni & Sklan, 2003). The reason for an increase of goblet cell density and size after fasting is not concluded but might be a result of an impaired secretion of goblet cell or possibly an increase in mucin formation (Uni & Sklan, 2003). An increase in goblet cell density in the small intestine is in addition seen in broiler chickens infected by salmonella (Fasina *et al.*, 2010). In humans, a decrease in goblet cell density in the small intestine is a sign of immune system disease (Capuano *et al.*, 2011). Literature of normal density and size of intestinal goblet cells in broilers are lacking. Although, smaller sized goblet cells in humans indicates that goblet cell mucus has been released faster than normal due to intraluminal disturbances (Neutra & Schaeffer, 1977).

Immune system

The immune system of broilers is affected by immediate access to feed and water (Simon *et al.*, 2015). A higher prevalence of sickness behaviour (resting with eyes closed) caused by environmental antigens and an impaired performance are seen in chickens with 72 hours delay of feed after hatch compared to chickens given feed and water immediately (Simon *et al.* 2015). These results suggest that access to feed and water straight after hatch strengthens the immune system of the chicken.

Probiotic supplementation

Probiotics are live microorganisms that are supplemented in feed with the aim of improving gut health (Mizock, 2015). Probiotics help maintain equilibrium of intestinal microbes (Ştef *et al.* 2015) by colonizing the gastrointestinal tract and reducing the amount of pathogenic bacteria, a possible cause of diseases (Mizock, 2015). Improved health after probiotic supplementation has been seen in chickens (Ştef *et al.* 2015; Gao *et al.*, 2017) and in multiple studies of humans (Watson & Preedy, 2010). In broilers, a benefit of probiotic supplementation is for instance a strengthened immune system by a higher immunity response (higher expression levels of serum IgG and intestinal secretory IgA) compared to control groups without probiotic supplementation (Gao *et al.*, 2017). Probiotics are also seen prohibiting pathogen *Campylobacter jejuni* in broiler chicken, concluded from decreased goblet cell sizes and fewer leukocytes in the mucosal chorion (Ştef *et al.* 2015).

Regarding growth performance, chickens supplemented with probiotics are proven to have a lower feed conversion ratio compared to broilers without probiotic supplementation (Abdel-Hafeez *et al.*, 2017; Gao *et al.*, 2017) as well as an improved body weight gain after seven days of age when provided probiotics until 42 days of age (Nyamagonda *et al.*, 2011). Although in contradiction, other studies found no differences in feed conversion ratio or weight gain between broiler chicken supplemented with probiotics compared to broilers without probiotic supplementation (Nunes *et al.*, 2012; Ştef *et al.* 2015). Furthermore, slaughter weight (Nunes *et al.*, 2012; Abdel-Hafeez *et al.*, 2017) and feed intake (Ştef *et al.* 2015) is not seen affected by probiotic supplementation.

Materials and methods

Experimental design

A total of 450 broiler chickens of the hybrid Ross 308 were hatched according to five hatching treatments at a commercial hatchery in Lund. One treatment group did not have access to feed and water (-FW) and one treatment group had access to feed and water in the hatcher (FW). Two other treatment groups had access to feed and water in the hatcher as well as one of the two commercially used probiotic additives (A or B) in the drinking water for approximately 12 hours (ProA and ProB). The chickens of the final treatment had access to feed and water in the hatcher and were sprayed with the commercially used probiotic A in the down (ProC). The probiotic amounts were based on the manufactures recommendations (Table 1). The chickens were after hatch transported by car to the Swedish Livestock Research Centre of the Swedish University of Agricultural Sciences at Lövsta, outside Uppsala (Table 1).

Table 1. Treatments at the hatchery. A total of 450 chickens (Ross 308) divided into five different treatments at hatch. Three treatments had probiotic addition and the approximate amount of colony forming units (CFU) of probiotics consumed per chicken over 12 h of time in the hatcher is presented

Treatment	**Abbreviation**	**Number of animals**	**Probiotics consumed (CFU/chicken)**
No access to feed and water	-FW	110	-
Immediate access to feed and water	FW	110	-
Immediate access to feed and water Probiotic A in drinking water	ProA	110	$206.25x10^6$
Immediate access to feed and water Probiotic B in drinking water	ProB	60	$199.3x10^5$
Immediate access to feed and water Probiotic C sprayed on down	ProC	60	$100x10^5$*
Total number of chickens		450	

*The amount was sprayed on down, the actual amount consumed is not known

On arrival at the research facility the chickens were around 50 h of age and will further be referred to as two-day-old chicken. Shortly after arriving, treatments -FW, FW and ProA (Table 1) were divided into two new treatments each, where one had access to probiotic additive A for the first three days and the second treatment did not receive any probiotic supplementation. Treatments ProB and ProC were not divided in new treatments and did not get probiotic supplementation in the drinking water. This resulted in a total of eight treatments (Table 2) in the research facility. Ten chickens of each hatch treatment (50 in total) were randomly picked and euthanized for sampling; the remaining 400 chickens were distributed over the modules.

Table 2. Treatments and replicates in the research facility. A total of 400 chickens were placed in modules at arrival to the research facility. The hatchery treatment groups –FW, FW and ProA were each divided into two new sub groups which were either continuously provided with probiotic A (Yes)

($122x10^6$ CFU/chicken was consumed each day) or given no probiotics (No) the first three days after arrival. Treatments ProB and ProC did not get probiotic A after arrival. There were 50 chickens per treatment spread over five modules with ten animals in each

Treatment	Probiotic addition	Abbreviation	No. of animals	No. of modules	No. of animals per module	Probiotic consumed in three days (CFU/chicken)
-FW	Yes	-FW/Yes	50	5	10	$366x10^6$
-FW	No	-FW/No	50	5	10	-
FW	Yes	FW/Yes	50	5	10	$366x10^6$
FW	No	FW/No	50	5	10	-
ProA	Yes	ProA/Yes	50	5	10	$366x10^6$
ProA	No	ProA/No	50	5	10	-
ProB	No	ProB	50	5	10	-
ProC	No	ProC	50	5	10	-
		Total	400	40		

Chickens of treatments with immediate access to feed and water after hatch will further be referred to as “fed chickens” and chickens in treatments without immediate access to feed and water after hatch as “unfed chickens”.

Birds and housing

A total of forty modules were prepared for the arrival of the chickens. Each module held ten birds and was marked with numbers from one to forty. Each stable treatment and their replicas had been randomized throughout the modules. The modules measured 1.50x0.75m and the floor was covered with wood shavings. The temperature in the stable was 33°C at arrival. When all chickens were placed in the modules, a feeder and a bell drinker were placed in each module. All chickens had feed ad libitum from that moment. The bell drinker was only used during the first three days during the probiotic supplementation, thereafter the drinkers were removed and water nipples lowered in all modules.

Figure 4. Modules in the research facility, holding ten chickens in each. Photo: Åsa Andersson.

Feed, water and probiotics

When the chickens arrived at the research facility they were provided with a starter feed commonly used in commercial broiler rearing. The feed consisted of mini pellets with 22% crude protein and 12.3 mega joule (MJ) metabolisable energy (ME). At day ten all chickens were given a commercial grower diet consisting of a cut pellet with 20% crude protein and 12.6 MJ ME. No coccidiostats were used. Fifteen of the 40 modules from treatments –FW, FW and ProA were provided with probiotic A in the water for the first three days (Table 2). The probiotics was measured and mixed with water and at the end of each day the remaining amount of each tray were measured and registered.

Data collecting

Weight and length

The chickens of each module were weighed as a group the first day as well as once every week during the study. Length of chickens was measured only at day one in the research facility.

Figure 5. Measuring chicken length of a two-day-old chicken. Photo: Åsa Andersson.

Feed intake

The residual feed in each module were weighed once every week to register feed intake (FI). The feed intake was together with chicken weight used to calculate feed conversion ratio (FCR).

Organ data collecting

Euthanization of chickens was performed day one, day ten and day thirty-one for organ data collection. Day one and day ten the birds was stunned by a strike to the head and euthanized by decapitation. At day 31 the chickens were euthanized by an intravenous injection of pentobarbital sodium (100 mg/ml, Allfatal vet) to the wing vein. Ten birds from each treatment were euthanized each sampling day where heart, intestine (small intestine and colon including intestinal contents), liver, yolk sac, bursa and spleen was removed and weighed individually. Proventricilus and gizzard (proventricilus+gizzard) was also removed and weighed together, the gizzard was then weighed by itself. The gizzard was first weighed with feed left inside (full gizzard), then opened, emptied of food, cleansed with water, and weighed once again (empty gizzard).

The intestine was in addition measured in length, and every other intestine sample was prepared for histological analysis. Resulting in 50 intestines collected and 25 histology samples at day one. At day ten and at day 31 there were 80 intestines collected and 40 histology samples for each of the two collecting days.

Histology samples preparation

One piece (of approximately three cm) was cut of the small intestine directly after the duodenal loop. The piece was cut open and pinned to a piece of cork, fixed in 2.5% glutaraldehyde with a pH of 7.2. All jars were marked with an individual chicken number. The samples remained in the glutaraldehyde overnight and the following day rinsed three times in 1/15 M phosphate buffer with a pH of 7.2. After rinsing, the tissue was dehydrated four times in increasing concentration of ethanol (50%, 70%, 90% and absolute EtOH) of thirty minutes in each concentration. The tissue was then left in water-soluble Leica historesin overnight. Thereafter,

each intestine samples were cut two times diagonally (Figure 6) and placed in wells filled with a Leica historesin and hardener solution. All tissue was covered with a label presenting chicken number, thereafter left in room temperature to polymerize.

Figure 6. The figure illustrates a piece of open-cut intestinal sample, approximately three cm long. When prepared for embedding two cuts of the intestinal tissue was performed diagonally.

Sectioning histological intestine samples

After polymerization the tissue samples were mounted to a plastic adapter and sectioned on a microtome (Leica RM 2165, Leica Instruments, Germany) (Figure 7) with a thickness of two µm. The sections were placed on a glass slide and quick stained with azure blue followed by observation in a light microscope. If three or more villi attached to the muscular mucosa were observed; the definitive sections were cut. Eight glass slides were prepared for each sample with two sections on each, resulting in 16 sections per intestine sample.

Sectioning was only performed of intestines from two-day-old chickens and eleven-day-old chickens. Intestine samples from chickens of 32 days of age was not sectioned or evaluated in this study, due to lack of time.

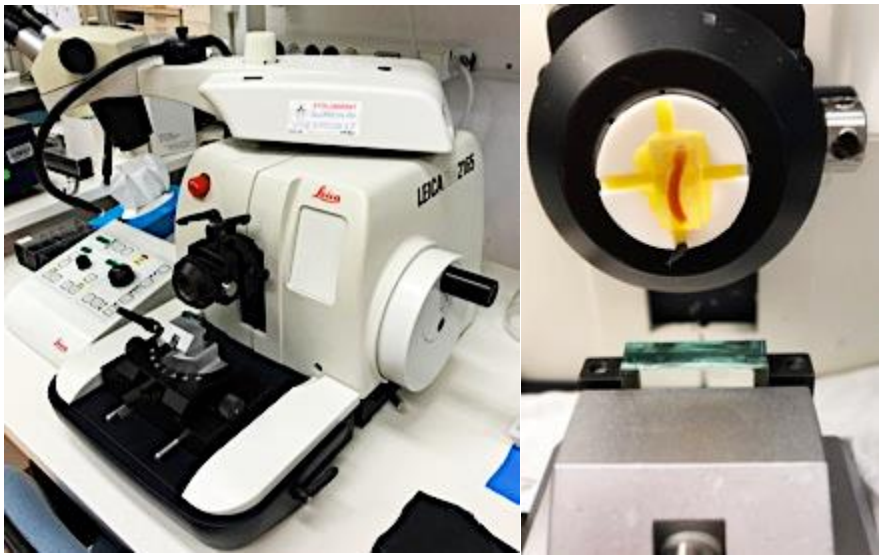

Figure 7. The Leica microtome used for sectioning chicken intestine seen as a whole to the left. To the right is a close-up of the glass knife. Just above the knife, the intestinal sample is seen embedded and mounted on a plastic adapter. Photo: Åsa Andersson.

Staining histological sections

The sections were stained in hematoxylin-solution followed by staining in eosin-floxin-solution. The glass slides were placed in hematoxylin and incubated in 60°C for one hour before dipped into cold water and rinsed under running water for 15 minutes, followed by one dip in MQ-H_2O. Thereafter sections were dried on a heat plate before placed in eosin-floxin for 3.5 minutes, followed by a dip in 95% EtOH and dried once more. The sections were covered with

glasses mounted by a drop of Agar100. The glass slides were once again placed on the heat plate until the Agar100 was fully spread, and afterwards dried in an incubator of 90°C for one hour.

Histological evaluation

The histology of all sections was subjectively studied in a light microscope and descriptively evaluated. In villi, the amount of goblet cells and the appearance of goblet cells (size and shape) was observed and described. Amounts of goblet cells were described as few, medium or many, while size was described as small, medium or large and shape was described as round or oval. The description of shape and size were made from the most frequently occurring shapes and sizes in the section (Table 3). Goblet cell amount was decided after seeing a deficiency of goblet cells (few) compared to an abundance of goblet cells (many); incisions in between were graded "medium many". Treatments were not known when observing and describing the goblet cells of the sections.

Table 3. Goblet cells in duodenal villi were evaluated and described according to the table. Amount of goblet cells in villi was described as few, medium or many; size of goblet cells as small, medium or large and shape of goblet cells as either round or oval

Goblet cells	**Amount of goblet cells in villi**	**Size of goblet cells**	**Shape of goblet cells**
Described as	Few	Small	Round
	Medium	Medium	Oval
	Many	Large	

Rating of goblet cells

Following figures presents the appearance of goblet cells in duodenal villi of chickens, this in term to get a better understanding of how the rating was performed. Appearances of few, medium or many goblet cells are presented in Figure 8 to 10. Appearances of different sizes (small, medium or large) of goblet cells are presented in Figure 11 to 13. Shape appearances (round and oval) are presented in Figure 14 and 15.

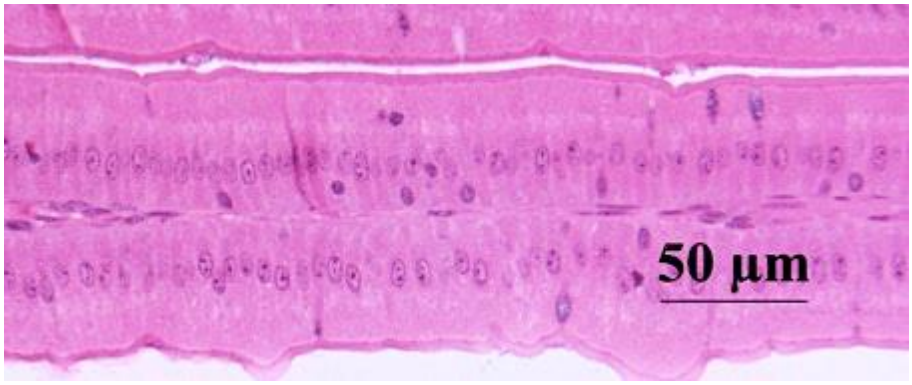

Figure 8. Few goblet cells in villi (20x magnification). Photo: Åsa Andersson.

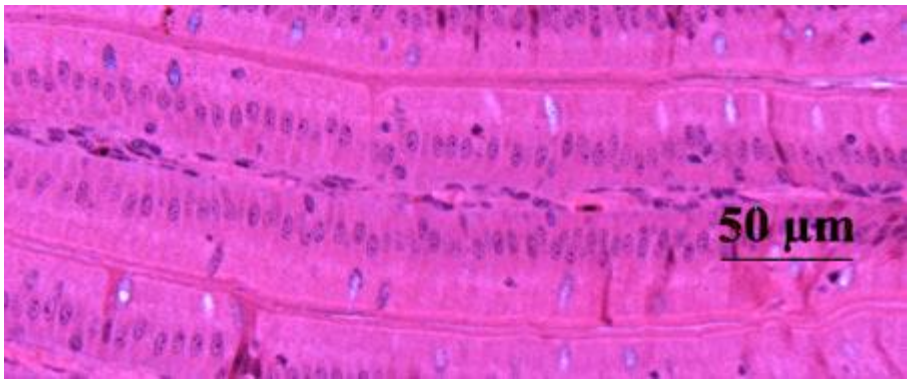

Figure 9. Medium many goblet cells in villi (20x magnification). Photo: Åsa Andersson.

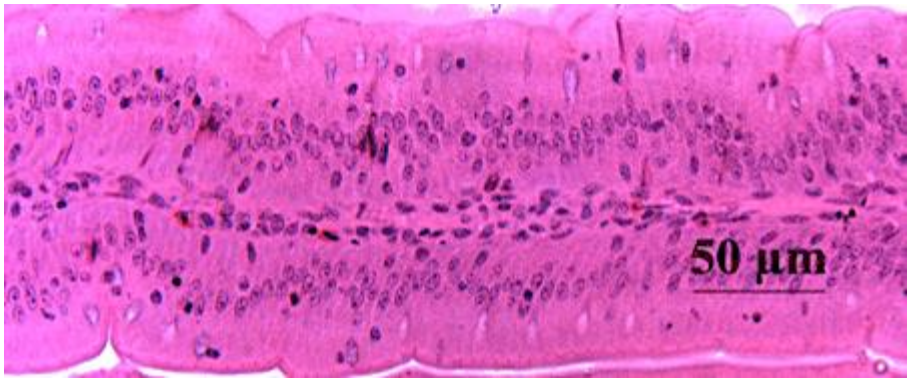

Figure 10. Many goblet cells in villi (20x magnification). Photo: Åsa Andersson.

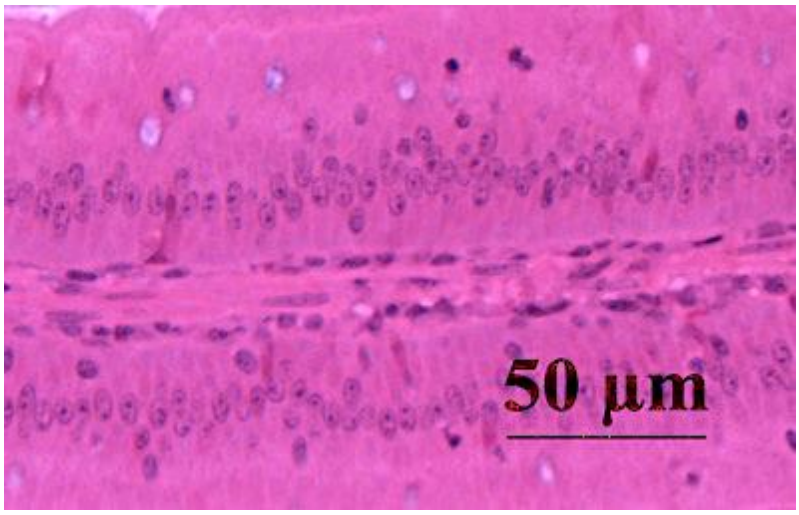

Figure 11. Small goblet cells in villi (20x magnification). Photo: Åsa Andersson.

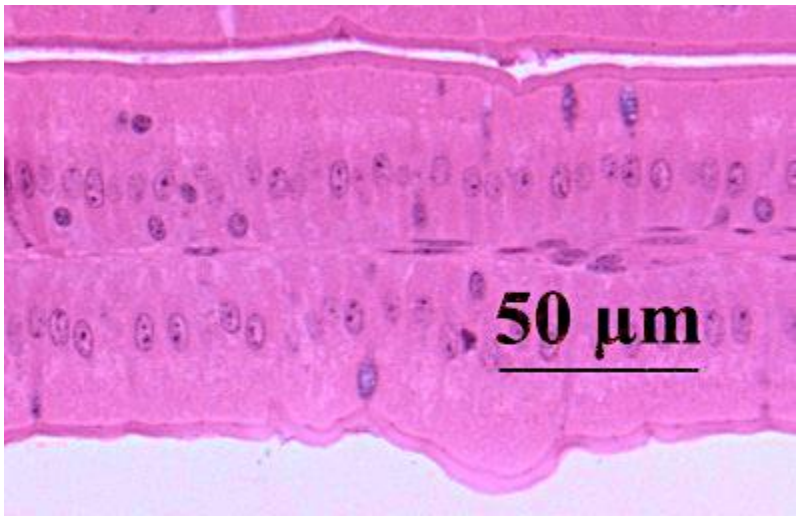

Figure 12. Medium large goblet cells in villi (20x magnification). Photo: Åsa Andersson.

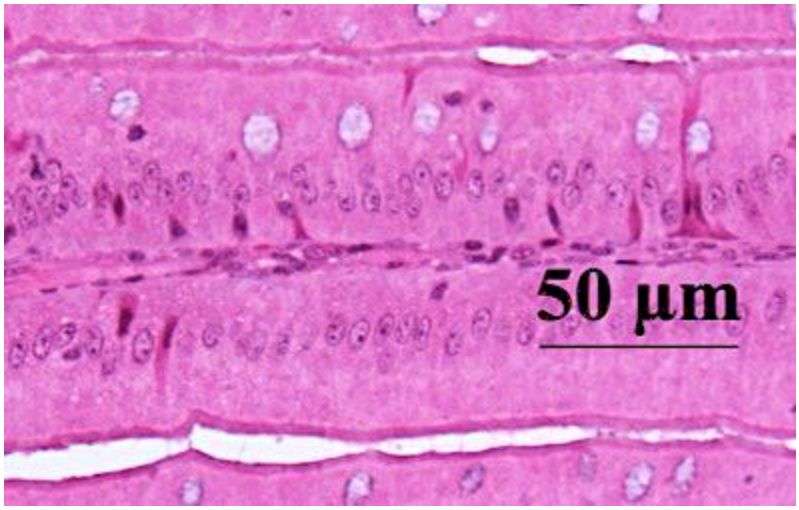

Figure 13. Large goblet cells in villi (20x magnification). Photo: Åsa Andersson.

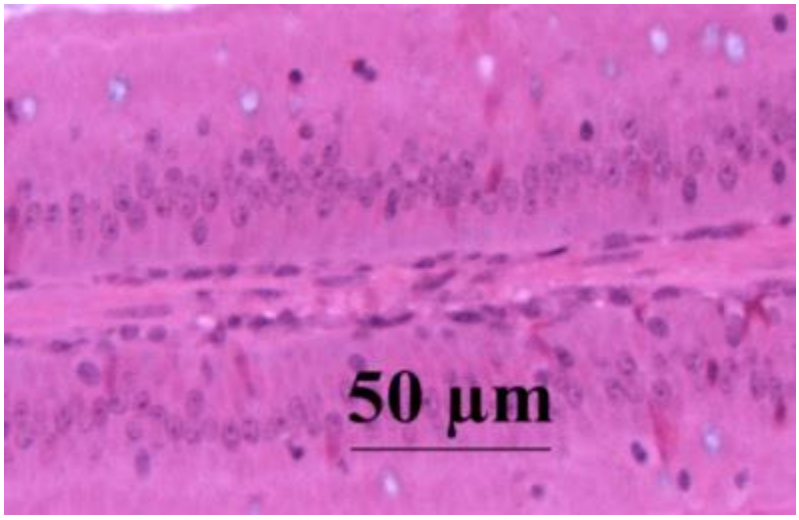

Figure 14. Round goblet cells in villi (20x magnification). Photo: Åsa Andersson.

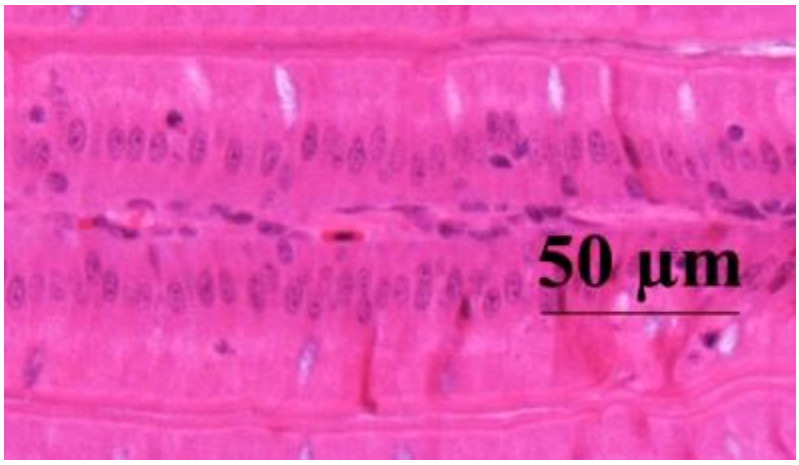

Figure 15. Oval goblet cells in villi (20x magnification). Photo: Åsa Andersson.

Statistical analysis

Estimates of feed intake, feed conversion ratio, organ weights and lengths of intestines was statistically analysed by using Statistical Analysis Software (SAS) (9.4). An analysis of variance (ANOVA) was performed with one fixed effect (treatments) and one random effect (module). This was performed with the help of SAS Procedure Mixed in order to prove significance between treatments. The start weight was corrected as covariant in the model. Differences between treatments were considered significant when $p \leq 0.05$.

Results

Organ development

Day one (two-day-old chickens)

Treatments during hatching affected two-day-old chickens with regard to chicken length ($p=0.0422$), intestinal weight ($p<0.0001$), intestinal length ($p=0.0336$) and weight of full gizzard ($p=0.0451$) as well as empty gizzard ($p=0.0021$). There was no effect between treatments on chicken weight and weights of yolk sac, cloacal bursa, heart, liver, spleen or proventricilus+gizzard when expressed as a proportion of the body weight (Table 4). This day significant results were mainly linked to unfed chickens.

Chicken length

Chickens of the unfed treatment (-FW) were longer than chickens of all fed treatments (Table 4).

Intestinal length and weight

The intestine was shorter and of lower weight in chickens of the unfed treatment (-FW) compared to all four treatments of fed chickens (Table 4). Moreover, the chickens of treatment ProB had higher intestinal weights compared to chickens in the FW treatment, both ProB and FW chickens were fed at hatch.

Gizzard weight

Weights of full gizzard in treatment unfed chickens (-FW) were lower compared to chickens in three out of four fed treatments; ProA, ProB and FW. Unfed chickens (–FW) as well as fed chickens of treatment ProA had heavier weights of emptied gizzards compared to the remaining three treatments of fed chickens (ProB, ProC and FW).

Table 4. Estimates of body weights, yolk sac weights, organ weights (intestine[1], heart, liver, spleen, proventricilus, gizzard, cloacal bursa) and intestinal lengths[1] of chickens from the five treatments at hatch. Data was collected at arrival to the research centre of two-day-old chickens. Results are considered significant when p≤0.05. Values are expressed as least squares means

	Treatment						
	ProA	**ProB**	**ProC**	**-FW**	**FW**	**Pooled SEM[2]**	**P-value**
Body weight (g)	45.04	44.3	44.5	41.52	44.12	0.94	0.1077
Body length[3]	0.47^{b}	0.47^{b}	0.48^{b}	0.51^{a}	0.46^{b}	0.012	0.0422
Yolc sac[4]	0.032	0.031	0.032	0.041	0.039	0.0038	0.2167
Intestine length[3]	1.32^{b}	1.3^{b}	1.33^{b}	1.16^{a}	1.32^{b}	0.041	0.0336
Intestine weight[4]	0.079^{ac}	0.084^{a}	0.078^{ac}	0.06^{b}	0.077^{c}	0.0021	<0.0001
Cloacal bursa[4]	0.0018	0.0014	0.0015	0.0017	0.0015	$1.2x10^{-4}$	0.3182
Heart[4]	0.0094	0.0092	0.0092	0.0089	0.0093	$3.0x10^{-4}$	0.4072
Liver[4]	0.03	0.03	0.032	0.03	0.03	$6.7x10^{-4}$	0.3801
Spleen[4]	$4.5x10^{-4}$	$4.7x10^{-4}$	$3.8x10^{-4}$	$3.0x10^{-4}$	$3.7x10^{-4}$	$5.2x10^{-5}$	0.1907
Proventricilus+ gizzard[4]	0.079	0.078	0.077	0.073	0.082	0.0027	0.2967
Gizzard, full[4]	0.067^{b}	0.066^{b}	0.065^{ab}	0.057^{a}	0.07^{b}	0.003	0.0451
Gizzard, empty[4]	0.058^{c}	0.053^{b}	0.054^{b}	0.059^{ac}	0.054^{b}	0.0013	0.0021

[1]Intestine = small intestine and colon

[2]Pooled SEM = Pooled standard of the mean

[3]Values are expressed as a proportion of the body weight (cm/g)

[4]Values are expressed as a proportion of the body weight (g/g)

Treatments with diverse letters are significantly separated from each other

Day ten (eleven-day-old chickens)

At day ten, there was only a difference between treatments in heart weights (p=0.0260) (Table 5). No significance was proven of chicken weights, intestinal weights, intestinal lengths, cloacal bursa weights, liver weights, spleen weights, proventricilus+gizzard weights, full gizzard weights or empty gizzard weights (Table 6).

Heart weight

The relative heart weight was heavier in unfed chickens with no probiotic supplementation after placement in modules (-FW/No), compared to heart weights of all other treatments except ProB (Table 5).

Day 31 (32-day-old chickens)

At day 31, differences were proven between treatments with regard to chicken weight (p=0.0281), liver weight (p=0.0385) and proventricilus+gizzard weight (p=0.0255) (Table 5 and 6). No differences were seen in intestinal weights and lengths or weights of cloacal bursa, heart, spleen, full gizzard and empty gizzard (Table 5 and 6).

Chicken weight

Body weights of unfed chickens with probiotic addition after placement in modules (-FW/Yes) were lower than of chickens in four fed treatments in chickens weighed individually before being euthanized for organ sampling (Table 6). Unfed chickens without probiotic addition after placement in modules (-FW/No) had lower body weights than merely one treatment of fed chickens (FW/Yes). In addition, chickens of treatment ProA/Yes had significantly lower body weights than chickens of treatment FW/Yes.

Liver

Chickens in the FW/No treatment had heavier livers compared to chickens in treatment ProC and FW/Yes. Liver weights of ProA/No treated chickens were significantly lower compared to –FW/No, -FW/Yes and FW/No treated chickens.

Proventricilus+gizzard

Chickens of –FW/Yes treatment had heavier proventricilus+gizzard weights compared to chickens in four treatments of fed chicken (Table 6). A similar trend was seen in the other treatment of unfed chickens as well (-FW/No), which had heavier proventricilus+gizzard weights than FW/Yes and ProC.

Table 5. Estimates for the eight stable treatments on body weights, intestinal lengths and weights[1], cloacal bursa weights and heart weights in chickens at day 10 respectively day 31. Results are considered significant when $p \leq 0.05$. Values are expressed as least squares means

	ProA/No	**ProA/Yes**	**ProB**	**ProC**	**-FW/No**	**-FW/Yes**	**FW/No**	**FW/Yes**	**Pooled SEM[2]**	**P-value**
Body weight (g)										
Day 10	322.7	320.78	332.89	313.1	300.7	296.61	326.6	321.9	9.3	0.0929
Day 31	2158.44^{b}	1988.1^{a}	2098.44^{ab}	2160.7^{b}	2005.2^{a}	1934.2^{a}	2143^{b}	2239.25^{b}	65.76	0.0281
Intestine length[3]										
Day 10	0.34	0.36	0.34	0.36	0.36	0.36	0.34	0.35	0.0092	0.6967
Day 31	0.078	0.089	0.087	0.085	0.087	0.088	0.082	0.082	0.0029	0.0833
Intestine weight[4]										
Day 10	0.084	0.088	0.087	0.088	0.086	0.086	0.087	0.085	0.0023	0.9284
Day 31	0.055	0.058	0.058	0.059	0.055	0.058	0.055	0.055	0.0019	0.4911
Cloacal bursa[4]										
Day 10	0.0018	0.0021	0.0019	0.0018	0.0017	0.002	0.0018	0.0019	0.00012	0.4451
Day 31	0.0019	0.0019	0.0021	0.0019	0.0023	0.002	0.002	0.0019	0.00016	0.4578
Heart[4]										
Day 10	0.0079^{b}	0.0082^{b}	0.0085^{ab}	0.0083^{b}	0.0091^{a}	0.0081^{b}	0.0081^{b}	0.0077^{b}	0.00029	0.0260
Day 31	0.0067	0.0064	0.0066	0.0066	0.007	0.0067	0.0066	0.0062	0.00031	0.6822

[1]Intestine = small intestine and colon

[2]Pooled SEM = Pooled standard of the mean

[3]Values are expressed as a proportion of the body weight (cm/g)

[4]Values are expressed as a proportion of the body weight (g/g)

Treatments with diverse letters are significantly separated from each other

Table 6. Estimates for the eight treatments after arriving to the research facility on chicken liver weights, spleen weights, proventricilus+gizzard weights, full gizzard weights and empty gizzard weights at day ten and day 31 respectively. Results are considered significant when $p \leq 0.05$. Values are expressed as least squares means

	ProA/No	**ProA/Yes**	**ProB**	**ProC**	**-FW/No**	**-FW/Yes**	**FW/No**	**FW/Yes**	**Pool SEM**	**P-value**
Liver (g)[2]										
Day 10	0.038	0.038	0.037	0.038	0.035	0.039	0.038	0.037	0.0014	0.7172
Day 31	0.024^{b}	0.026^{ab}	0.026^{ab}	0.026^{bc}	0.027^{ac}	0.027^{ac}	0.029^{a}	0.025^{b}	0.00093	0.0385
Spleen (g)[2]										
Day 10	0.00061	0.00064	0.0007	0.0007	0.00062	0.00054	0.00061	0.00059	0.000067	0.6359
Day 31	0.001	0.001	0.0011	0.001	0.0012	0.001	0.0012	0.001	0.000084	0.2721
Proventricilus + gizzard (g)[2]										
Day 10	0.052	0.047	0.047	0.048	0.049	0.05	0.047	0.052	0.0018	0.1105
Day 31	0.026^{ab}	0.027^{abc}	0.027^{abc}	0.025^{a}	0.028^{bc}	0.03^{c}	0.026^{ab}	0.024^{a}	0.0015	0.0255
Gizzard, full (g)[2]										
Day 10	0.044	0.039	0.039	0.04	0.04	0.042	0.039	0.043	0.0017	0.1574
Day 31	0.021	0.022	0.022	0.02	0.023	0.025	0.022	0.019	0.0014	0.0507
Gizzard, empty (g)[2]										
Day 10	0.026	0.025	0.024	0.025	0.025	0.026	0.024	0.026	0.00084	0.8789
Day 31	0.013	0.014	0.014	0.013	0.015	0.016	0.014	0.013	0.0018	0.0629

[1]Pooled SEM = Pooled standard of the mean.

[2]Values are expressed as a proportion of the body weight.

Treatments with diverse letters are significantly separated from each other

Body weight, feed intake and feed conversion ratio

Body weight (group-weighed chickens)

Differences in body weights between treatments were seen all days of weighing (day one, three, ten, 17, 24 and 31). Results and p-value for day one are presented in Table 7 for the five treatments at hatch. Results and p-values of the remaining days and eight treatments are presented in Table 8.

Day one (two-day-old chickens)

There were differences in body weights between treatments the first day at the research facility ($p<0.0001$). Unfed chickens (-FW) had lower body weights than all four treatments of fed chickens (Table 7). There were no differences in body weights between treatments of fed chickens (Table 7).

Table 7. Estimates of body weights for the five treatments day one. Results are considered significant when $p\leq0.05$. Values are expressed as least squares means

	ProA	**ProB**	**ProC**	**-FW**	**FW**	**Pool SEM**[1]	**P-value**
Body weight (g)	45.96[a]	45.39[a]	45.62[a]	40.71[b]	45.7[a]	0.534	<0.0001

[1]Pool SEM = Pooled standard of the mean

Treatments with diverse letters are significantly separated from each other

Day three (four-day-old chickens)

Day three had similar results as day one regarding body weights between treatments ($p=0.0002$). Both treatments of unfed chickens (-FW/No and –FW/Yes) had lower body weights than all six treatments of fed chickens (Table 8). In addition, chickens of treatment FW/No were significantly heavier than three treatments provided probiotics at hatch (ProA/Yes, ProB, ProC).

Day ten (eleven-day-old chickens)

The trend of unfed chicken being lighter remained also at day ten. The two unfed treatments (-FW/No and –FW/Yes) had lower weights than five out of six treatments of fed chickens (Table 8). The trend also remained for FW/No treated chickens from day three, which day ten was heavier than two treatments provided with probiotics at hatch (ProA/Yes and ProB).

Day 17 (18-day-old chickens)

At day 17 unfed chickens were still lighter than the majority of treatments of fed chicken (Table 8). Treatment ProB was the only of fed chickens that did not have higher weights than both treatments of unfed chickens; surprisingly, in addition they had lower weights than all of remaining five treatments of fed chickens except ProA/Yes (Table 8).

Day 24 (25-day-old chickens)

At day 24, the trend remained of unfed chickens with probiotic supplementation (-FW/Yes), which was still lower in weight than five fed treatments (Table 8). This was similar to treatment ProB that had lower weights than four treatments of fed chicken, only treatment ProA/Yes of fed treatments did not differ from ProB regarding weight. This day, chickens of unfed treatment –FW/No seemed to have caught up in growth and were merely lower in weights than one treatment of fed chickens (FW/Yes).

Day 31 (32-day-old chickens)

Differences in weights day 31 were mainly linked to fed treatments (Table 8). Chickens of unfed treatment –FW/No had completely compensated in weight day 31 and did not differ from any other treatment, although unfed chickens of treatment –FW/Yes still had lower weights than ProC and FW/Yes treated chicken. Chickens of fed treatment ProB were like day 24 lower in weights than chickens of all fed treatments except ProA/Yes. The chickens of ProA/Yes treatment had in addition lower body weights than three treatments of fed chicken (FW/Yes, FW/No and ProC).

Feed intake

Differences in feed intake (FI) were seen between treatments day 17 (p=0.0073) and day 31 (p=0.036). No differences in FI were seen day three, day ten and day 24 (Table 5).

Day 17 (18-day-old chickens)

At day 17, feed intake was especially connected to unfed chickens. Chickens without probiotic addition (-FW/No) had lower feed intake compared to chickens in five out of six treatments of fed chicken, and unfed chickens with probiotic addition (-FW/Yes) had a lower feed intake than chickens of all treatments of fed chickens (Table 8). Moreover, chickens in the ProB treatment had lower feed intake than both FW/Yes and FW/No treatments and chickens of treatment ProA/Yes had lower feed intake than FW/Yes-treated chickens.

Day 31 (32-day-old chickens)

At day 31, chickens of treatments ProA/Yes and –FW/Yes had lower feed intake than chickens of four fed treatments (ProA/No, ProC, FW/No and FW/Yes). Unfed chickens without access to probiotic supplementation (-FW/No) had this day lower feed intake than merely chickens of treatment FW/Yes.

Feed conversion ratio

The feed conversion ratio (FCR) did not differ between treatments at day three, day ten and day 17. There were however differences day 24 (p=0.0015) and day 31 (p=0.0063) and results are presented in Table 8.

Day 24 (25-day-old chickens)

At day 24, the –FW/No treated chickens had close to compensated in body weight, compared to the other treatment of unfed chickens –FW/Yes and treatment ProB which appeared to have

a suppression of body weight gain from day 17. This could also be seen in FCR since –FW/No had significantly better FCR than both -FW/Yes and ProB treated chickens at day 24. In addition, three treatments of fed chicken had better FCR than treatment -FW/Yes (Table 8). However, all other chickens of fed treatments had better FCR than ProB treated ones (Table 8).

Day 31 (32-day-old chickens)

The final day, chickens of treatment ProB had as day 24 poorer FCR than all other chickens of fed treatments and unfed treatment -FW/No (Table 8). Moreover, -FW/No had better FCR than the other treatment of unfed chickens (–FW/Yes).

Table 8. Estimates for the eight treatments after arriving to the research facility of feed intake (FI), feed conversion ratio (FCR) and body weights. Estimates are presented from day three, ten, 17, 24 and 31. Results are considered significant when p≤0.05. Values are expressed as least squares means

	Treatment									
	ProA/No	**ProA/Yes**	**ProB**	**ProC**	**-FW/No**	**-FW/Yes**	**FW/No**	**FW/Yes**	**Pool SEM[1]**	**P-value**
FI (g)										
Day 3	33.64	34.39	34.96	32.62	26.85	31.07	32.81	36.27	2.9	0.4102
Day 10	298.78	301.22	299.26	303.08	279.2	276.86	307.37	303.38	5.83	0.1023
Day 17	873.56^{ad}	851.27^{ae}	840.94^{ab}	876.04^{ad}	792.37^{bc}	770.04^{c}	885.57^{de}	889.91^{d}	15.82	0.0073
Day 24	1766.02	1699	1702.42	1763.05	1631.34	1603.46	1791.86	1787.83	34.55	0.0597
Day 31	2943.84^{ac}	2795.16^{b}	2847.92^{abc}	2958.6 ^{ac}	2767.24^{ab}	2670.63^{b}	2974.79^{ac}	2977.5^{c}	58.44	0.0409
FCR										
Day 3	0.89	0.95	0.98	0.89	0.89	1.02	0.84	0.96	0.08	0.4880
Day 10	1.1	1.14	1.15	1.12	1.15	1.14	1.11	1.13	0.02	0.3228
Day 17	1.2	1.21	1.26	1.21	1.21	1.24	1.2	1.21	0.02	0.0613
Day 24	1.29^{bc}	1.3^{b}	1.36^{a}	1.3^{bc}	1.29^{b}	1.35^{ac}	1.29^{b}	1.29^{b}	0.02	0.0015
Day 31	1.38^{bc}	1.4^{bc}	1.44^{a}	1.39^{bc}	1.36^{b}	1.39^{ac}	1.39^{bc}	1.37^{bc}	0.01	0.0063
Body weight (g)										
Day 3	82.07^{bc}	80.78^{b}	80.36^{b}	81.26^{b}	75.35^{a}	75.28^{a}	83.02^{c}	82.02^{bc}	0.73	0.0002
Day 10	313.46^{ac}	305.3^{ad}	300.89^{ab}	309.7^{ac}	285.63^{b}	285.51^{b}	318.6^{c}	313.4^{cd}	5.18	0.0293
Day 17	772.97^{b}	743.22^{bc}	703.46^{ac}	768.7^{b}	690.04^{ac}	653.48^{a}	780.84^{b}	784.29^{b}	19.09	0.0035
Day 24	1404.83^{bc}	1347.94^{bcd}	1309.26^{ad}	1395.54^{bc}	1294.91^{ac}	1219.78^{a}	1422.55^{bc}	1428.48^{b}	35.35	0.0245
Day 31	2162.13^{acd}	2030.20^{bd}	2021.80^{b}	2167.55^{a}	2067.50^{abcd}	1953.81^{bc}	2176.04^{ac}	2205.7^{a}	56.79	0.0364

[1]Pool SEM = Pooled standard of the mean

Treatments with diverse letters are significantly separated from each other

Histological evaluation of duodenum

Day one (two-day-old chickens)

At day one the amount of goblet cells in the small intestine seemed to be of greater quantity in chickens of the probiotic treatment groups (ProA, ProB, and ProC) compared to the amount of goblet cells in chickens with no addition of probiotics at hatch (FW and –FW). The size of the goblet cells seemed to differ between unfed chickens and fed chickens, where unfed (-FW) appeared to have smaller goblet cells in the duodenum compared to fed chickens (FW, ProA, ProB, ProC). In the duodenum of ProC treated chickens, 80% of the goblet cells were round shaped in comparison to chickens of the four other treatments (-FW, FW, ProA and ProB) where oval cells were most frequent.

Day ten (eleven-day-old chickens)

At ten days after arrival to the research facility the amount of goblet cells in the small intestine seemed to be fewer in chickens of fed treatment ProA/No and unfed treatment
–FW/No compared to amount of goblet cells found in chickens of the other treatments (ProA/Yes, ProB, ProC, -FW/Yes, FW/No and FW/Yes). A marginally higher frequency of oval shaped goblet cells in the duodenum was seen in chickens from treatment ProA/Yes and FW/No compared to the other groups, which had more equal amounts of both round and oval shaped goblet cells. The sizes of goblet cells appeared to vary within all groups and no distinct differences could be noticed.

Discussion

Chicken and organ development

Feed deprivation at hatch

Day three, ten, 17 and 31 unfed chickens at hatch did not have better or worse FCR than any treatments of fed chickens at hatch when comparing unfed and fed chickens without probiotic supplementation. These results corresponds quite well correspond to with Gonzales *et al.* (2003) who found no differences in FCR between fasted chickens and fed chickens at hatch. In contrast to FCR, significant results on body weights in both treatments of unfed chickens were seen until 18 days of age with lower weights of the unfed chicks than the majority of fed chickens. However, after day 17 those chicks started to catch up in weight and at day 31, one of the two unfed treatments (-FW/No) had completely compensated in weight and did not differ from any of the fed treatments, which were in accordance of studies by Uni & Sklan (2003), Bhanja *et al.* (2009) and Lamot *et al.* (2014). Their conclusions were that unfed chickens at hatch have lower body weights the first two weeks in life. Gonzales *et al.* (2003) and Bhanja *et al.* (2009) had similar conclusions with chicken weight being compensated before three weeks of age if feed deprivation lasted a shorter period than 24 h. The chickens of unfed treatments in this study were deprived for more than 48 h and the unfed treatment with probiotic supplementation (-FW/Yes) had not completely compensated in weight at the final day of sampling and had still lower body weight than two of the six treatments of fed chickens (ProC and FW/Yes). The suppressed growth performance was in accordance with Shafey *et al.* (2011), proving feed deprivation of more than 48 h after hatch to result in decreased growth.

Both treatments of unfed chickens of current study had a lower feed intake than the majority of chickens in fed treatments at day 17 and unfed chickens with probiotic addition had a lower feed intake than the majority of fed chickens also day 31, which explains the lower body weight observed in this group. These results are comparable with studies of Lamot *et al.* (2014) and Shafey *et al.* (2011) who found unfed chickens to have a lower feed intake at seven and 33 days of age. At day three and ten in the current study no differences ($p>0.05$) in feed intake between treatments were observed, and at day 24, only a tendency was observed ($p=0.06$) . The different results for the time points are hard to explain. An effect of feed depravation at hatch on organ weights could be detected in the two-day old chickens, where fed chickens had a longer and heavier intestine. However, from day ten and onwards the differences in organ weights were scattered between treatments of both fed, unfed and probiotic supplemented chickens and that merely unfed chickens at hatch (-FW/Yes and -FW/No) had a less developed gastrointestinal tract than fed chickens at hatch after day ten could not be concluded.

Probiotic treatments

Probiotic effects of body weight as well as FCR in current study were overall quite scattered between treatments and experimental days and therefore it was difficult to find specific connections to probiotic addition. The numerical highest weight at day 31 belonged to

probiotic-supplemented chickens (FW/Yes) but also the three lowest weights belonged to probiotic-supplemented chickens (ProA/Yes, ProB and –FW/Yes).. The availability of feed and water at hatch together with time point of probiotics supplementation seemed to have an effect on FCR day 24 and 31 in current study. Unfed chickens at hatch but supplemented with probiotic at arrival to the research facility (-FW/Yes) had poorer FCR than three treatments of fed chickens. These were also the chickens (-FW/Yes) that had not compensated in weight at day 31. In this case it seemed as supplementation of probiotics suppressed growth if chickens had been deprived of feed at hatch. Although, quite contrary at day 31 did unfed chickens at hatch without any probiotic addition (-FW/No) have a better FCR than one treatment of chickens fed in the hatcher supplemented with probiotic supplementation in the water (ProB). The ProB treated chickens had quite surprising results with a higher weight than unfed chickens at day one but then gradually lost growing pace throughout the study. In the end of the study, all treatments except –FW/Yes had higher weights and poorer FCR than chickens of ProB-treatment. Probiotic B fed to chickens in the hatcher would not be of recommendation before more studies are performed. However, for the other probiotic treatments supplemented in the hatcher (ProA and ProC) FCR did not differ from the groups without probiotics (-FW/No and FW/No). In addition, to continue the probiotic treatment in the research facility (ProA/Yes) did not give additional effect on FCR compared to just supplementing in the hatcher (ProA/No). The weight and FCR in current study thereby seem to be affected both by the time of supplementation and type of probiotic used. However, none of the treatments with probiotics had improved FCR or body weight at day 31 compared to the treatments without probiotics, which is in agreement with Nunes *et al.* (2012). A divergent trend of how probiotic affects FCR and weight is seen in literature with some studies in accordance to current study and some with different outcomes. Different outcomes were found by Abdel-Hafeez *et al.* (2017) and Gao *et al.* (2017), who saw probiotic supplementation to decrease FCR and Nyamagonda *et al.* (2011) found slaughter weight to increase in probiotic-supplemented chickens. However, the FCR in current study was overall good and all treatments had better FCR in comparison to the two studies mentioned above, which can explain the lack of improvement of production parameters.

Goblet cells in duodenum

The evaluation of goblet cells in the duodenum was performed subjectively and it might be wise to have in mind that the outcome could have turned out different if objectively studied. Literature for normal intestinal goblet cells in broilers in matter of size, shape and amount is missing and beneficial or unfavourable results are therefore hard to determine. More studies of goblet cell appearance of broiler intestines are needed. Sampling day 31 was never evaluated due to lack of time and eventual impacts of feed or probiotics of goblet cell appearance towards the end of a rearing period is consequently absent.

Feed deprivation at hatch

Unfed chickens at hatch had fewer goblet cells in the duodenum day one than fed chickens without probiotic addition, and two-day-old unfed chickens in current study had smaller goblet cells than chickens of the four fed treatments. This might be an indication that feed deprivation delays the development of goblet cells with a reduced mucus production. The results are

however in contradiction to studies performed by Uni & Sklan (2003) and Fernandez-Estivariz *et al.* (2003) who saw increased amounts of goblet cells in fasting animals (chickens and rats). The chickens in the study by Uni & Sklan (2003) were starved for two days from hatch as the chickens in current study, which makes time for sampling not a possible cause for our diverse results. The goblet cells in current study were not counted as in the two above-mentioned and that might be a possible reason. Speculations of Uni & Sklan (2003) regarding the larger sized goblet cells and a higher goblet cell density is that an increased amount of goblet cells is an outcome of feed deprivation where mucus release is impaired or that mucus production is increased and mucus in addition is stopped from being released. If that speculation is true; less mucus is secreted from the goblet cells, This is in agreement with. my own speculation that the unfed chickens in current study release less mucus into the intestinal lumen. In that case, regarding the importance of goblet cells and mucus secretion on immune response (Walker *et al.* 1977; Fasina *et al.* 2010) it might mean that unfed chickens in current study are in greater risk of intestinal damage than are chickens of fed treatments.

Probiotic treatments

Differences in goblet cell amount were seen both day one and day ten, where probiotic treated chickens had more goblet cells in their duodenums. Probiotic supplementation at hatch consequently seemed to increase goblet cell number in duodenums of chickens up to eleven days of age. If the goblet cells also release more mucus into the lumen it would be beneficial considering that they play an important role regarding immune responses of the small intestine (Walker *et al.*, 1977). However, an answer to that can not be given without analysing goblet cell secretion in relation to amount of goblet cells in a certain period of time, so that question is for future studies to answer.

Probiotics did not seem to have an effect of goblet cell size in two-day-old chickens of current study. The study by Ştef *et al.* (2015) found goblet cell sizes to decrease in broilers supplemented with probiotics, however, there were only three intestinal samples collected and the animals were at 42 days of age. In current study there were 25 samples from two-day-old chickens, which provides a more secure outcome, although the differences in age between the studies might play a role. There were however no clear differences in goblet cell sizes at day ten of current study, where unfed, fed and probiotic-supplemented chickens had approximately the same sizes of goblet cells. Since all treatments at day ten have had ad lib access to feed and water for a week it suggested that chickens provided feed and water produce goblet cells of similar sizes, regardless if supplemented with probiotics.

Goblet cell shape in two-day-old chickens of treatment ProC differed from all of the other treatments, with mostly round shaped goblet cells. If round goblet cells in contradiction to oval shaped goblet cells are positive is not known, although oval cells might be a result of a goblet cell squeezing together for mucus release. If this is the case, a speculation is that a mix of round cells and oval cells are preferable since normal mucus release of human goblet cells is slow but continuous. Neutra & Schaeffer, (1977), predicted that normal mucus release in broilers are the same. Further investigations of broiler chicken goblet cells are in any case needed to broaden

the knowledge and to get a better understanding of outcomes. Day ten of current study the shapes of goblet cells varied plenty between treatments. As in goblet cell size it seems as though chickens regardless fasted or fed at hatch or provided with probiotics in the hatcher/the first three days after placement have the same variance of round and oval shaped goblet cells when fed. These results strengthen the previous speculation regarding a mix of goblet cell shapes as preferable since the intestines might be considered as under normal conditions day ten.

Conclusion

High growth performance is not exclusively linked to chickens with immediate access to feed and water in the hatcher in broiler chickens after 18 days of age. Furthermore, probiotic supplementation in the hatcher or after arrival to the stable does not improve growth performance of either unfed or fed chickens at hatch. Based on the results of this study alone, chickens without feed and water in the hatcher and without probiotic supplementation are most profitable and least time consuming and growth performance is equally to fed chickens at hatch and chickens supplemented with probiotics. However, this is in experimental conditions of merely ten chickens per cage, which is not the common way of holding broiler chickens in Sweden. More studies are required to better understand the outcomes of probiotic supplementation and feed and water access at hatch before recommendations can be done.

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