Linköping Studies in Science and Technology
Dissertation No. 2005

Intermittent fasting in chickens

PHYSIOLOGICAL MECHANISMS
AND WELFARE IMPLICATIONS
FOR BROILER BREEDERS

Caroline Lindholm

Linköping Studies in Science and Technology
Dissertation No. 2005

Intermittent fasting in chickens

Physiological mechanisms and welfare implications for broiler breeders

Caroline Lindholm

IFM Biology
Department of Physics, Chemistry and Biology
Linköping University, SE-581 83 Linköping, Sweden
Linköping 2019

Intermittent fasting in chickens
Physiological mechanisms and welfare implications for broiler breeders

Linköping Studies in Science and Technology
Dissertation No. 2005
ISBN: 978-91-7685-018-3
ISSN: 0345-7524

Front cover: Ross 308 broiler chickens on a Swedish farm
Photo: Caroline Lindholm

© Caroline Lindholm
Printed by LiU-Tryck, Linköping 2019

Abstract

Broiler breeder chickens are the parent stock used to produce broiler chickens raised for human consumption and have long been caught in a welfare dilemma. The immensely successful selection of fast-growing meat-type chickens over the last 80 years has created a remarkably efficient meat-producer and given us access to cheap chicken meat but has also created health problems for the animals. These problems especially affect the breeder generation, which must be raised with strict feed restriction to limit weight gain and thus maintain physical health and normal fertility. At the same time, however, feed restriction causes chronic hunger and feeding frustration with reduced animal welfare as a result. In the EU alone 60 million breeders are affected annually and although the problem is well-known within the industry, there is financial incentive to keep selecting for even higher growth potential in broilers, further increasing the problems for broiler breeders.

Many strategies for reducing the impact of growth restriction on breeder welfare have been suggested and are usually aimed at somehow increasing the amount of feed given at feeding times. This can be done either through adding bulky fibers to the diet or by reducing feeding frequency. In this thesis, focus is on the latter as we explore the effects of intermittent fasting (IF) on the welfare of young broiler breeders as well as wild-type Red Junglefowl. Intermittent fasting, or “skip-a-day” feeding, is supposedly the most common feeding strategy for broiler breeders worldwide but is perceived as welfare-reducing and thus illegal in Sweden and several other European countries. In spite of this, the scientific knowledge of how this type of feeding affects chicken welfare is scarce.

Assessing the overall effect of IF on breeder welfare is complicated by large variations in both physiological and behavioral parameters between feeding and fasting days, but it does appear that chicken welfare is improved at least on the feeding days of IF regimens. It also seems that some of the health benefits reported from mammalian studies of IF may apply to chickens as well, although behavioral indicators still point to welfare issues unless the level of feed restriction can be relaxed. In comparison with daily feed restriction, IF appears to increase the motivation for feed consumption but to reduce the motivation for appetitive behaviors such as foraging, which may explain why birds fed on this type of schedule are often reported as calmer.

Populärvetenskaplig sammanfattning

Kyckling har hyllats som en resurseffektiv och klimatsmart källa till protein och ett mer hållbart alternativ till andra typer av kött. Produktionen av klimatsmart kyckling är dock inte oproblematisk, och problemen handlar främst om en bristfällig djurvelfärd. Dagens slaktkycklingar, eller broilers, är resultatet av ca 80 års fokuserad avel, och produktionstiden – alltså tiden det tar för en nykläckt kyckling på ca 40 g att växa till en slaktvikt på 2,0-2,5 kg – har under den perioden kortats från 16 veckor på 1940-talet till bara 35 dagar idag. Denna extrema utveckling har fört med sig en del nya hälsoproblem för slaktkycklingarna, där främst immunförsvaret, skelettet och hjärt-kärlsystemet har fått problem med exempelvis lamhet och ascites (en form av hjärtsvikt med vätskefylld bukhåla). Genom anpassningar i aveln, djurens uppväxtmiljö och tack vare deras korta levnadstid har kycklingindustrin ändå överlag kunnat hantera dessa problem väl.

Kycklingindustrins mindre kända och mer besvärliga välfärdsproblematik handlar dock inte om slaktkycklingarna själva, utan om deras föräldrar. Slaktkycklingföräldrarna, s k broiler breeders, har samma enorma aptit och höga tillväxtpotential som sina avkommor men lever ett helt annat liv. Dessa djur lever i ca 15 månader och behöver vara både fysiskt friska och fertila för att uppfylla sina roller i produktionskedjan. Eftersom det är välkänt att fri tillgång till mat för dessa djur inte bara leder till problemen som nämnts ovan utan också till ägglossningsstörningar, måste föräldradjuret växa upp under strikt foderbegränsning. En breeder-höna får därför ca 30% av den mängd mat som hennes avkomma äter vid samma ålder (eller ca 42% av vad slaktkycklingen äter vid samma kroppsvikt) och tar fyra gånger så lång tid på sig att nå slaktkycklingens slutvikt på drygt 2 kg.

Till följd av foderbegränsningen växer föräldradjuret upp med ständig hunger och frustration kring den upplevda bristen på mat. Problematiken blir värre i djurgrupper med stor variation i kroppsstorlek, och därför valde vi att titta närmare på varför djuren växer i olika takt och hur de påverkas av detta i de första två artiklarna i denna avhandling. Våra resultat pekar på att variation i tillväxt är något som etableras under hönornas första dagar i livet, innan deras fodertillgång har begränsats (artikel 2). Tidigare har det ofta föreslagits att ojämn tillväxt i en grupp skulle vara ett resultat av just foderbegränsningen, men våra resultat stödjer alltså inte detta. Vid 4 veckors ålder kunde vi konstatera att de största och minsta hönorna i en flock var både fysiologiskt och beteendemässigt olika, men kunde inte se att detta skulle ha någon tydlig effekt på djurens välfärd (artikel 1).

Flera olika strategier för att minska föräldradjurens hungerstress har föreslagits, och täcker hela spannet från förändringar i aveln (exempelvis användning av dvärghönor) till inblandning av näringsfattigt fiber i fodret för att på så vis öka fodergivan. I den här avhandlingen har vi särskilt fokuserat på användning av intermitterant fasta.

Intermittent fasta innebär att djur (eller människor) avstår från mat under 12–24 h (eller tillräckligt lång tid för att förbruka kroppens kolhydratreserver) i taget enligt ett återkommande schema. Studier på däggdjur har visat på många positiva hälsoeffekter med denna typ av diet, däribland minskad viktuppgång utan kaloribegränsning och förbättrad kognitiv förmåga. För människor har denna typ av diet populärt spridits som exempelvis 5:2-dieten, men globalt sett är den också en vanlig strategi för utfodring av slaktkycklingföräldrar. Fördelen med denna typ av utfodringsschema, inom kycklingvärlden ofta kallad ”skip-a-day”, är att djuren kan få en större mängd foder när de väl blir utfodrade vilket skapar en lugnare matsituation med mindre konkurrens mellan individer, en ökad mättnadskänsla och en minskad risk för att enstaka djur får för lite mat.

I Sverige och flera andra europeiska länder är utfodring med fastedagar olaglig då lagen kräver att lantbruksdjur ska utfodras dagligen, men det har egentligen inte funnits tillräckligt vetenskapligt underlag för att avgöra om daglig utfodring verkligen är att föredra ur välfärdssynpunkt för dessa djur. I denna avhandling har vi därför studerat hur intermittent fasta påverkar både broiler breeders (artikel 6-7) och den ursprungliga hönstypen röd djungelhöna (artikel 3-5). Sammantaget visar kycklingarna god kapacitet för att fysiologiskt anpassa sig till intermittent fasta, där broilerdjurens förmåga att äta mer mat än de behöver gör dem särskilt väl förberedda för att hantera fastedagar. Trots detta är det svårt att uttala sig om hur djurens välfärd påverkas, särskilt på de stora variationerna som kycklingen upplever mellan utfodringsdagar och fastedagar under intermittent fasta (artikel 6). Att de intermittently fastande djuren upplever en förbättrad välfärd under utfodrade dagar jämfört med dagligen utfodrade djur (som ju alltså får mindre portioner vid varje utfodring) är dock ett återkommande tema. Även om de hälsofördelar av intermittent fasta som rapporterats i däggdjur också kan vara aktuella för slaktkycklingföräldrarna skulle de behöva växa upp med en mindre strikt foderbegränsning för att uppnå en acceptabel djurvälfärd (artikel 7).

List of publications

Theme 1: Early growth in broiler breeders

- I. C Lindholm, A Calais, J Jönsson N Yngwe, E Berndtson, E Hult & J Altimiras (2015). Slow and steady wins the race? No signs of reduced welfare in smaller broiler breeder hens at four weeks of age. *Animal Welfare* 24: 447-454. doi:10.7120/09627286.24.4.447
- II. C Lindholm, J Jönsson, A Calais, A Middelkoop, N Yngwe, E Berndtson, JJ Lees, E Hult & J Altimiras (2017). Growth heterogeneity in broiler breeder pullets is settled before the onset of feed restriction but is not predicted by size at hatch. *Journal of Animal Science* 95: 182-193. doi:10.2527/jas.2016.0929

Theme 2: Intermittent fasting in Red Junglefowl

- III. JJ Lees, C Lindholm, P Batakis, M Busscher & J Altimiras (2017). The physiological and neuroendocrine correlates of hunger in the Red Junglefowl (*Gallus gallus*). *Scientific Reports* 7: 17984. doi:10.1038/s41598-017-17922-w
- IV. C Lindholm, J Altimiras & J Lees (2019). Measuring ketones in the field: Rapid and reliable measures of β -hydroxybutyrate in birds. *Ibis* 161: 205-210. doi:10.1111/ibi.12643
- V. C Lindholm, P Batakis, J Altimiras & J Lees. Intermittent fasting induces chronic changes in the hepatic gene expression of Red Junglefowl (*Gallus gallus*). *Submitted manuscript*.

Theme 3: Intermittent fasting in broiler breeders

- VI. C Lindholm, A Johansson, A Middelkoop, JJ Lees, N Yngwe, E Berndtson, G Cooper & J Altimiras (2018). The quest for welfare-friendly feeding of broiler breeders: Effects of daily vs 5:2 feed restriction schedules. *Poultry Science* 97: 368-377. doi:10.3382/ps/pex326
- VII. C Lindholm & J Altimiras. Disentangling the effects of intermittent fasting and caloric restriction in meat-type poultry. *Manuscript*.

List of publications not included in thesis

- C Lindholm & J Altimiras (2016). Point-of-care devices for physiological measurements in field conditions. A smorgasbord of instruments and validation procedures. *Comparative Biochemistry and Physiology, Part A* 202: 99-111. doi:10.1016/j.cbpa.2016.04.009
- C Lindholm, L Karlsson, A Johansson & J Altimiras (2017). Higher fear of predators does not decrease outdoor range use in free-range Rowan Ranger broiler chickens. *Acta Agriculturae Scandinavica, Section A – Animal Science* 66(4): 231-238. doi:10.1080/09064702.2017.1337214

Paper I is re-printed with the permission of the Universities Federation for Animal Welfare.
Papers II and VI are re-printed with the permission of Oxford University Press.
Paper III is re-printed under a Creative Commons Attribution 4.0 International License.
Paper IV is re-printed with the permission of John Wiley and Sons.

Table of contents

Background	11
Rise of the modern broiler	11
Overview of broiler production	12
Health problems in broilers: leg health	14
Health problems in broilers: cardiovascular disorders	15
Health problems in broilers: immunocompetence	17
The need for feed restriction in broiler breeder rearing.....	18
Eating behavior and appetite in meat-type chickens	20
Improving the breeder situation: breeding goals	23
Improving the breeder situation: precision feeding.....	23
Improving the breeder situation: qualitative feed restriction.....	25
Improving the breeder situation: intermittent fasting.....	26
The physiology of fasting	29
Therapeutic fasting and the beneficial ketosis	32
Welfare in feed-restricted chickens	34
Paper summaries	38
Theme 1: Early growth in feed-restricted broiler breeders	38
I. Slow and steady wins the race?	38
II. Growth heterogeneity is settled before feed restriction	38
Theme 2: Intermittent fasting in Red Junglefowl	38
III. Physiological and neuroendocrine correlates of hunger	38
IV. On-site measurements of whole-blood ketones	38
V. Intermittent fasting chronically changes gene expression	39
Theme 3: Intermittent fasting in broiler breeders	39
VI. Effects of daily vs 5:2 feed restriction in broiler breeders.....	39
VII. Disentangling effects of intermittent fasting and caloric restriction	39
Discussion	40
Theme 1: Early growth in feed-restricted broiler breeders	40
Theme 2: Intermittent fasting in Red Junglefowl	41
Theme 3: Intermittent fasting in broiler breeders	43
Conclusions.....	46
Acknowledgments.....	47
References.....	48

Background

Rise of the modern broiler

The story of the modern meat-type chicken, or broiler, begins during the first half of the 20th century. A variety of chicken breeds were already available at the turn of the century, but because chicks could not be sexed until around 7 weeks of age surplus males were raised for meat and chicken meat was largely a by-product of egg production (Griffin and Goddard, 1994). In the early 1930s the Japanese method for sexing newly hatched chicks by the appearance of their cloaca (“vent-sexing”) was introduced in the US, which for the first time allowed the rearing of separate egg- and meat-producing flocks. The White Rock and Cornish breeds were the fastest-growing breeds available, and birds from these lines became the foundation of the modern broiler (Griffin and Goddard, 1994).

The selection of large, fast-growing chicks specifically bred for meat production really took off in the 1950s and since then these chickens have undergone an astonishing increase in growth rate and efficiency. The time it takes a broiler hatchling of approximately 40 g to grow to its slaughter weight of 2.0-2.5 kg has been reduced from 16 weeks in the 1940s to only 35 days today (Griffin and Goddard, 1994; Zuidhof et al., 2014; **Figure 1**). This corresponds to a consistent decrease in production time of 1 day/year (Weeks et al., 2000).

While the size of the newly hatched chick is constrained by egg size (Griffin and Goddard, 1994) and has not changed much over the last 60 years (Zuidhof et al., 2014), selection for rapid growth has had profound effects on broiler chickens.

Figure 1. Scale drawing of broiler chickens from representative 1957 and 2005 commercial strains raised under the same conditions. While hatchling size is constrained by egg size and has therefore seen limited change, the growth rate to and body composition at 8 weeks of age has undergone a tremendous transformation. Adapted from Zuidhof et al., 2014 (Fig 1).

Modern broiler embryos grow faster than layer embryos and have a higher number of muscle fibers at hatch compared to chicks from egg-laying strains (Griffin and Goddard, 1994). These birds also show higher muscular activity of ornithine decarboxylase, an enzyme involved in the regulation of cell proliferation, starting from just a few days after hatch (Griffin and Goddard, 1994).

The genetic potential for growth in modern broilers corresponds to an approximately 100-fold increase in body mass during the first eight weeks of life (Zuidhof et al., 2014) and will reach an adult body mass of 5-6 kg compared to the typical laying hen weighing in at 1.5-2 kg (Griffin and Goddard, 1994). Although 85-90% of this increase in growth is explained by the effects of genetic selection (Havenstein et al., 2003), current broiler growth is so rapid that concentrated diets and increased day lengths are now necessary for caloric intake to match the growth potential of the animals (Bessei, 2006). With consumer demands changing from whole birds to mostly parts and processed meats (Griffin and Goddard, 1994), selection for increased breast meat percentage has further changed broiler growth so that breast muscle now increases twice as fast as overall body mass (Griffin and Goddard, 1994) with profound effects on bird posture (Zuidhof et al., 2014). Despite the large effects on growth, the shape of the broiler growth curve has changed relatively little (Griffin and Goddard, 1994), showing linear growth until around 18 weeks of age and 4.6 kg where the birds voluntarily start to decrease their food intake (Katanbaf et al., 1989).

As an example of the power of artificial selection, the development of broiler chickens has been an extraordinary success, but it has not been without its problems. The mortality of *ad libitum*-fed broilers reaches 50% within 360 days (Katanbaf et al., 1989), a problem that particularly affects the broiler breeders. Broiler breeders are the parent stock of broiler chickens and are expected to live for 60-70 weeks rather than just five. In addition to the high mortality rates, *ad libitum*-fed breeder hens reach sexual maturity too early and too heavy, requiring feed restriction from an early age both to remain physically healthy and to produce acceptable numbers of settable eggs (Bartov et al., 1988; Bruggeman et al., 1999). At the same time, the level of feed restriction required to maintain healthy breeding stock promotes hunger and frustration which creates an animal welfare trade-off known as the broiler breeder paradox (Decuyper et al., 2010).

Overview of broiler production

Over 60 billion chickens are produced yearly around the world (CIWF, 2019), and the large majority of them come from just a handful of breeding companies (EFSA Panel on Animal Health and Welfare, 2010; Griffin and Goddard, 1994). In Sweden, the conventional (i.e. non-organic) chicken meat producers rely entirely on two chicken lines: the Ross 308 broiler supplied by Aviagen internationally and SweChick and SweHatch nationally, and the Cobb 500 broiler supplied by Cobb Vantress internationally and Blenta AB nationally. Regardless of supplier choice, the logistics

of supplying broiler chicks to farmers are very similar. The international companies run breeding centers where they maintain many genetic pedigree lines, typically with a focus on high growth in male lines and efficient egg production in female lines, used for the continuous breeding of great-grandparental lines (Griffin and Goddard, 1994; Pollock, 1999). Young chicks from these great grandparents are then imported to Sweden and other countries where they are raised at grandparent facilities (SweChick AB and Blenta AB respectively), typically keeping one genetic line to produce male parents and two to produce female parents (Pollock, 1999). Eggs produced by the grandparents then become the penultimate broiler breeder generation (raised by SweHatch AB and Blenta AB) which finally produces the eggs that will hatch to become the actual broilers. It is expected that all generations in this production except for the final broilers require some form of feed restriction to maintain physical health, however the broiler breeder generation is the one attracting most research interest for several reasons:

- 1) It is reasonably accessible to researchers. Grandparental and great-grandparental lines are usually kept under much stricter control and a certain level of secrecy to protect proprietary breeding information.
- 2) The number of birds in each generation increases, meaning a lot more broiler breeders than broiler grandparents abound. Any welfare issue faced by broiler breeders thus affects more individual animals than issues faced by previous generations. For the same reason, the welfare of broiler breeder hens have generally attracted more attention than the welfare of the parental roosters, who typically make up only 6-9% of the breeding birds (EFSA Panel on Animal Health and Welfare, 2010).
- 3) Being the penultimate step in the broiler production chain, broiler breeders must be the most similar to the actual broilers compared to previous breeding generations and can therefore be expected to experience the worst animal welfare as far as feed restriction is concerned.

The life of a broiler breeder proceeds as follows, with grandparents and broilers following similar trajectories. Eggs are incubated and hatched at a generation-specific hatchery. The breeder hatchlings are then sex-sorted either by vent or by wing feathers to keep only the relevant sex within each genetic line. The unwanted sex can be raised as broilers and is not necessarily discarded, unlike the situation for laying-type chicks (EFSA Panel on Animal Health and Welfare, 2010). The day-old chicks are then packed in crates and transported by truck to a rearing farm. Although hatchery solutions with immediate access to feed and water have been suggested and are implemented in some parts of the world (van de Ven et al., 2009), Swedish breeder (and broiler) chicks will typically encounter their first feed and water upon arrival to the rearing farm. At the rearing farm, chicks are provided with plenty of feed and kept in well-lit stables for the first week of life to promote early growth and

organ development. It is well-established that feed and water intake during the first few days of a chick's life are crucial to e.g. intestinal maturation (Lilburn and Loeffler, 2015; Noy and Sklan, 1999) and we have seen that individual variations in early feeding efficiency can have long-term effects in broiler breeders (Lindholm et al., 2017, 2015). After the first week, feed restriction is gradually imposed, and light is gradually reduced to minimize aggression as feeding frustration increases. Around 18 weeks of age hens are photostimulated by an increase in lighting to promote egg laying and at 20 weeks the animals are moved to a laying farm where they are introduced to the opposite sex at a ratio of about 10 hens to 1 rooster.

In contrast with the increasing feed restriction and reduced lighting in breeder rearing, the ultimate broiler generation is presented with abundant feed and long, bright days for all of their five weeks of life.

Health problems in broilers: leg health

Of the health problems that come with the extreme growth of *ad libitum*-fed broilers, impaired leg health is often recognized as the most important welfare problem (Pedersen and Forkman, 2019; Weeks et al., 2000). Leg health in broilers is often monitored in terms of gait scores, where a score of 0 describes an agile bird with no signs of gait issues, and a gait score of 5 represents a bird that is unable to walk (Weeks et al., 2000). Gait scores 4-5 are associated with reduced growth rates (Weeks et al., 2000), but a score of 3 already appears to be painful to the animal as these birds show improved gaits under analgesic treatment and tend to self-select analgesic-laden feed in food preference tests (Bessei, 2006). The prevalence of high gait scores can vary a lot depending on breed, production company, farm and season (Knowles et al., 2008) but gait scores of 3 or higher are typically found in 3-30% of broilers in Europe (Bessei, 2006; Knowles et al., 2008).

Poor leg health in broilers can be the result of several conditions, ranging from skeletal deformations such as twisted legs and tibial dyschondroplasia (TD) via joint inflammations, to contact dermatitis including hock burns and foot pad lesions (Bessei, 2006; Pedersen and Forkman, 2019). Skeletal deformations can be reduced through genetic selection (Bessei, 2006), while dermatitis is more of a management-related problem. These leg (and breast) sores increased in broilers in the late 1900s and Swedish surveys found on average 5-10% of animals to be affected (Bessei, 2006). The increase may be an indirect result of increased selection for improved feed conversion ratios (FCRs), as the amount of time spent lying also increased in broilers during the 1900s, with modern broilers spending the major part of their day (76-86%) lying down even though they sleep less than both laying hens and wild-type junglefowl chickens (Weeks et al., 2000). Contact dermatitis increases with high stocking density (Pedersen and Forkman, 2019), but probably mostly as a secondary effect of poorer litter quality with higher temperature and humidity (Bessei, 2006).

A lot of research on how to improve the leg health of broilers has focused on the improvement of gait scores. These generally improve with factors that are associated with reduced body weight such as early slaughter, more fibrous feed, longer scotoperiods, increased physical activity, feed restriction and poorly pelleted feed (Knowles et al., 2008). Lower stocking densities have also been associated with improved gait scores, although again probably through effects on environmental parameters (Bessei, 2006). As for length of scotoperiod, providing broilers with at least 1 h of darkness daily significantly improves their leg health although it should be noted that the EU requires a minimum of 6 h of darkness daily for broilers of which 4 h must be consecutive (Council Directive, 2007).

Increased activity levels also have positive effects on broiler leg health, but generally the need is biggest in the heaviest birds which are also the individuals that are the least likely to interact with activity-promoting forms of enrichment such as perches (Bessei, 2006). Neither perches nor high light intensity, both expected to increase activity levels, appear to have actual positive effects on leg health in broilers (Bessei, 2006; Pedersen and Forkman, 2019), but the provision of straw bales and separation of resources (i.e. by using obstacles to increase the activity needed to move between water and feed) have shown promising results in a few studies (Pedersen and Forkman, 2019).

Health problems in broilers: cardiovascular disorders

Although leg health problems may be the biggest welfare concern in broiler chickens, cardiovascular problems such as sudden death syndrome (SDS) and ascites are the main causes of mortality associated with rapid growth (Bessei, 2006). Long-term monitoring of broiler flocks has suggested that 3-6% of the birds are lost to SDS (Olkowski et al., 2008) with an expected suffering time (i.e. time from first sign of ill health until death) of around 1 min (Bessei, 2006). In comparison, ascites or pulmonary arterial hypertension (PAH) syndrome is a slowly progressing disease which is expected to have a larger effect on animal welfare (Bessei, 2006).

Growing broilers have relatively small lung volumes for their body weight compared to other chickens, and as a result they need to keep almost all pulmonary blood vessels continuously filled with very little reserve capacity available (Wideman et al., 2013). This can quickly become a serious health problem if vascular resistance increases even slightly in the lungs, for example as a result of hypoxia, respiratory damage or disease, or simply from a mild stress-induced release of the adrenergic neurotransmitters adrenaline and noradrenaline (Wideman et al., 2013). If the increased resistance cannot be attenuated, which is often the case in ascites-susceptible individuals, the right ventricle of the heart will eventually increase pressure leading to both ventricular hypertrophy and impaired gas exchange as pulmonary blood flow rates increase too much (Wideman et al., 2013). The resulting hypoxemia will both stimulate increased erythropoiesis, leading to increased blood

viscosity and flow resistance, and increased dilation of systemic arteries. The latter increases blood flow through the body and leads to a higher rate of return to the right ventricle, which then attempts to compensate by increasing its output further in a vicious circle of escalating pulmonary hypertension and ventricular hypertrophy (Wideman et al., 2013). If this is allowed to continue, pressure in the right ventricle will eventually reach a point where the atrioventricular valve can no longer prevent blood from flowing back into the atrium, marking the onset of congestive heart failure. This flowback will also increase venous pressures, which is a problem further exacerbated by the kidneys attempting to address the arterial hypotension by retaining more sodium and water. Poor venous blood flow and undersaturation of oxygen will also affect the flow of portal blood from the intestines to the liver, which results in hepatic cell death and scarring. Both the over-filled veins and the necrotic liver will thus start leaking plasma into the abdominal cavity, which has given ascites its nickname “water-belly” (Wideman et al., 2013). The final cause of death in chickens with untreated ascites can be a combination of hypoxemia, congestive heart failure, starvation and respiratory distress as the ascitic fluid compresses the air sacs in the abdomen (Wideman et al., 2013).

Luckily, ascites is reversible even at the point when fluid has started to accumulate in the abdomen as a combination of feed restriction and diuretic medication can restore clinical health in these birds (Wideman et al., 2013). Prevalence of the disease is also responsive to changes in diet, with high levels of dietary tryptophan increasing the risk and high levels of L-arginine reducing the risk under certain conditions (Wideman et al., 2013). Susceptibility to ascites syndrome is also largely genetically controlled, with three different genetic regions associated with the phenotype across several breeding lines and a rapid response to selection in experimental models (Wideman et al., 2013). In one study, selection over 14 generations for ascites-susceptible and -resistant chickens respectively led to a disease mortality of 90% at 31 days of age in the susceptible line as compared with 2% at the same age in the resistant chickens (Wideman et al., 2013). Although ascites-resistant genotypes typically come with reduced growth rates and/or less favorable FCRs, the prevalence of ascites in commercial broiler flocks has been dramatically reduced since breeding companies started using routine oximetry measurements to remove individuals prone to hypoxemia from the breeding stock in the 1990s (Wideman et al., 2013).

Although SDS only leads to mortality in a low percentage of broilers, 17-35% show cardiac arrhythmia under normal circumstances and the same study reported a 92% prevalence of arrhythmia during an experimental stress test (Olkowski et al., 2008). For 18% of the birds the stress-induced arrhythmia was considered severe and life-threatening and although all birds survived the test, five of the 28 birds in this category died within 3 days (Olkowski et al., 2008). The pathology is not very well understood, but SDS is most common in growth-selected breeds with more deaths occurring in conjunction with stressful events such as catching, weighing, loading and

transportation (Olkowski et al., 2008). Microscopical examination of hearts from the birds that had undergone the stress test mentioned above revealed that birds with severe arrhythmias had myocardial lesions, in some cases fibrosis, and signs of cell death in both ventricular cardiomyocytes and Purkinje cells. None of these changes were seen in healthy broilers (Olkowski et al., 2008).

Health problems in broilers: immunocompetence

Reduced immunocompetence in broiler chickens has long been suggested by reports of, for example, high susceptibility to Marek's disease (Han and Smyth, 1972) and relatively high mortalities from common diseases, both infectious and metabolic in origin (Yunis et al., 2000). While broilers rarely display behavioral signs of ill health, gastrointestinal inflammation due to subclinical coccidiosis, necrotic enteritis and poor gut microflora are also considered important welfare issues (Berghman, 2016).

Cheema et al. (2003) raised the modern Ross 308 broiler together with a random-bred broiler line representative of a broiler in the 1950s on commercially recommended diets from both 1957 and 2001. Regardless of diet, the modern broilers grew consistently heavier but with relatively smaller (measured as % of body weight) bursas, spleens and cecal tonsils. Although the thymus was not significantly smaller, they concluded that lymphoid organ growth has been reduced by selection for fast growth and suggested that this is the result of a larger proportion of resources being allocated to growth processes (Cheema et al., 2003).

Measurements of actual immune parameters in modern broilers compared with other chickens do, however, show a more complicated picture than suggested from just lymphoid organ masses. Injection of the T-cell mitogen phytohemagglutinin (PHA) into the toe web of Ross 308 broilers produced more swelling and higher phagocytic activity compared to the same treatment in the 1950s line (Cheema et al., 2003), and injection of both PHA and concanavalin A (con A, another T-cell mitogen) into the spleen invoked a higher response in broilers compared to layer-type chickens (Leshchinsky and Klasing, 2001). In contrast, the broilers in the latter study did not develop fever – and sometimes even developed hypothermia instead – in response to an intraperitoneal lipopolysaccharide (LPS, primarily a B-cell mitogen) injection. The layer chickens all developed some level of fever within 4-12 h of injection, and also showed a greater response to splenic LPS injection, both in terms of immune cell proliferation and in the form of cytokine mRNA expression (Leshchinsky and Klasing, 2001). Broiler chickens thus appear to have a reduced inflammatory response compared to layers, but may instead be relying heavily on T-cell mediated immunity (Leshchinsky and Klasing, 2001).

Cheema et al. (2003) also tested the capacity for antibody production by immunization to sheep red blood cells and found that the 1950s strain generally produced higher levels of IgM, IgG and total antibodies in response. They concluded

that modern broilers have a reduced adaptive immunocompetence (Cheema et al., 2003). The reduced inflammatory responses of broilers are likely a direct result of selection for rapid growth, as inflammatory cytokines bind to receptors on the vagus nerve and induce effects such as anorexia and sleepiness while depleting energy stores (Berghman, 2016; Leshchinsky and Klasing, 2001), but a suppressed inflammatory response can also increase mortality risks and prolong recovery times (Leshchinsky and Klasing, 2001). A reduced antibody response may also lead to a reduced ability to defend against bacterial infections, further explaining why broilers show high susceptibility to certain infections (Cheema et al., 2003).

The need for feed restriction in broiler breeder rearing

All of the health problems discussed above are connected to selection for rapid growth in broilers, and all of them can be alleviated by feed restriction. The broiler breeder paradox stems from the fact that both *ad libitum* feeding and feed restriction lead to welfare impairments in meat-type chickens (Decuypere et al., 2010), as restrictive feeding induces high levels of hunger-related stress and feeding frustration. Studies carried out in the last 25 years suggest that feed restriction to around 50% of *ad libitum* intake appears to have overall positive effects on breeder welfare (Hocking et al., 1996), with more intense restriction levels resulting in signs of psychological stress (de Jong et al., 2003). In the early 90s, further restriction was not yet required for optimal fertility in breeder hens but was already routinely used in commercial practice (Hocking, 1993; Hocking et al., 1989). In 1993, Hocking reported that the harshest restriction in commercial rearing was 30% of *ad libitum* comparing by age (at 8 weeks) or 42% comparing by body weight (at 1.5 kg) (**Figure 2**). Compared to a commercial laying strain, broiler breeders ate 68% of *ad libitum* laying hen intake at 8 weeks of age when the two strains were similar in body weight

Figure 2. Feed intakes of restricted broiler breeders (○), *ad libitum*-fed broilers (●) and *ad libitum*-fed layer chickens (■) as experimentally determined by Hocking, 1993 and visualized compared by body weight (left) or age (right). Adapted from Hocking, 1993 (Fig 1, Table 1 and Table 3).

(Hocking, 1993). Since then restriction levels are expected to have kept increasing to match the improvements in broiler growth potential (Decuypere et al., 2010), with body weights of 6-week-old breeder hens corresponding to approximately 27% of that of age-matched broilers in 2005 as compared with 52% in 1979 (Renema et al., 2007). Few studies have experimentally determined the level of feed restriction, but commonly cited numbers are feed rations at 25-33% of *ad libitum* (by age) in rearing and 50-90% in lay (Van Krimpen and De Jong, 2014). The applicability of these numbers was confirmed in a study by Dunn et al. (2013) where broiler breeder hens feed-restricted according to commercial guidelines ate 27% of the feed intake of age-matched *ad libitum*-fed hens.

Although most research on broiler breeders, including this thesis, have focused on the hens, roosters are also reared and maintained on restricted diets. The main reason for this is that the growth of males cannot diverge too much from the females to achieve optimal mating and minimal levels of aggression towards the hens during the laying phase (Decuypere et al., 2010). Feed restriction also has beneficial effects on the fertility of male breeders with heavy males associated with lower fertility and hatchability of eggs (Arrazola et al., 2019b) and some, but not all, studies have reported improved semen quality in restricted males (Decuypere et al., 2010; Griffin and Goddard, 1994).

From a fertility perspective, the goal of feed restriction is to ensure that the hens reach an optimal body weight for sexual maturation just a few weeks before the onset of laying. Growth rate, adiposity and lean body mass all affect sexual maturity, as does photostimulation and chronological age (Decuypere et al., 2010). The negative correlation between rapid growth and reproductive efficiency in chickens was identified already in the 1960s (Renema and Robinson, 2004) and has been robustly reported since then (Decuypere et al., 2010). It is important to note that this is not just the result of reallocation of resources in a zero-sum game, but that there are endocrinological and mechanistic links between traits selected for in broiler breeding stock and reproductive disorders (Decuypere et al., 2010). Bruggeman et al. (1999) divided the rearing period into three different phases and found that feed restriction during the 7-15-week period had both the largest effect on growth and the largest effect on total egg production as well as the number of settable eggs. The importance of this time period is probably the result of early development of the hypothalamus-pituitary-gonadal (HPG) axis which is a key regulator of reproduction (Decuypere et al., 2010). For example, feed restriction is known to increase the sensitivity of the pituitary gland (hypophysis) to luteinizing hormone (LH)-releasing hormone (LHRH) as well as to ovarian feedback factors and to increase the ratio of LH to follicle-stimulating hormone (FSH) (Decuypere et al., 2010). Feed restriction also reduces the sensitivity of ovarian follicles to FSH while increasing their sensitivity to local growth factors and gonadotropins which improves ovarian steroid production (Decuypere et al., 2010). While *ad libitum*-fed breeders produce few and poorly

settable eggs, the problem at the level of the ovary is actually that multiple ovulations occur in too close succession (within the same day), often leading to internal ovulations and eggs laid with defective or soft shells as shell production becomes rushed (Griffin and Goddard, 1994; Renema and Robinson, 2004). Feed restriction in broiler breeders thus restores the normal follicular hierarchy, where F1 and F2 follicles respond with different levels of progesterone production in response to LH (Decuypere et al., 2010), leading to a decrease in multiple ovulations. In addition to the endocrine problems that occur under *ad libitum* feeding conditions, the increased adiposity that comes with rapid weight gain further exacerbates the problem as excessive triglycerides end up in non-adipocytes causing cell death through lipotoxicity and thereby ovarian dysfunction (Chen et al., 2006).

Concerns have been raised that increased selection for FCR would reduce fatness while increasing growth and lead to problems with further delays in sexual maturation (Decuypere et al., 2010). However, these problems may be partly counteracted by an increased responsiveness to feed restriction on ovarian function in FCR-selected breeders (Decuypere et al., 2010). Similarly, Zuidhof et al. (1995) reported that breeders with improved FCRs became more productive layers. In the study by Bruggeman et al. (1999), the thresholds found for optimal fertility were an ovary weight of at least 1.7% of total body mass at the time of first oviposition, corresponding to a maximum body weight of 2.8 kg at 18 weeks, however these numbers may have changed with further selection in the 20 years since then.

Eating behavior and appetite in meat-type chickens

Selection for rapid growth has also affected the eating and appetite regulation of broiler chickens, with no apparent lower limit for hunger behaviors and the upper limit for eating only reached at maximal gut capacity (Barbato et al., 1984; Bokkers and Koene, 2003). In contrast with other chicken types who eat until their nutritional demands are met, the eating behavior of broilers appears relatively insensitive to circulating nutrient levels (Van Krimpen and De Jong, 2014). In addition, our understanding of how peripheral energy stores and nutrient availabilities are communicated to central systems for appetite control in birds is generally poor. In mammals, the main regulators appear to be leptin, released from adipose tissue, and ghrelin, expressed in the stomach, which repress and stimulate eating respectively. Although birds do produce ghrelin in the proventriculus with circulating levels increasing in fasting and decreasing with feeding, both central and peripheral injection of the hormone appears to decrease feeding behavior (Boswell and Dunn, 2017). The leptin gene is poorly conserved between mammals and birds and shows minimal expression in bird adipose tissue. It has been suggested that leptin is more of a paracrine and/or autocrine agent than a hormone in birds (Seroussi et al., 2016) and it does not appear to be expressed in any of the hypothalamic nuclei typically involved in the regulation of appetite (Yi et al., 2017). Circulating levels of insulin are correlated with food intake in chickens but are also a poor candidate for the role as a

main peripheral energy signal as fasting plasma concentrations do not differ between lines selected for fat and lean body types. Central insulin injection also does not reduce food intake in laying-type chickens and has variable effects on the expression of orexigenic peptides (Boswell and Dunn, 2017).

Although peripheral energy sensing systems do not appear to be conserved between mammals and birds, central appetite regulation seems similar. Five hypothalamic nuclei are considered crucial for appetite regulation: the lateral hypothalamus (LHA), the paraventricular nucleus (PVN), the ventromedial hypothalamus (VMH), the dorsomedial nucleus (DMN) and the arcuate nucleus (ARC) (Yi et al., 2017). The VMH is typically considered the primary hypothalamic satiety center and fasting has been shown to increase expression of the neuronal activation marker c-Fos in the VMH of chickens (Yi et al., 2017). Most studies of central appetite regulation have however focused on the ARC, historically known as the “infundibular nucleus” (Boswell and Dunn, 2017). The ARC is well established as a center for energy balance regulation in mammals, regulating behaviors such as wheel running activity, overall energy expenditure and food intake in rodents (Boswell and Dunn, 2017; Thomas and Xue, 2018). Similar functions of the ARC have been observed in birds, although these studies are mostly based on gene expression data and little is known about actual peptide levels (Boswell and Dunn, 2017).

The effects of the ARC on energy intake and expenditure are mainly the result of the opposing activities of orexigenic AgRP/NPY-synthesizing neurons and anorexigenic POMC/CART-synthesizing neurons (Boswell and Dunn, 2017). The expression of AgRP in the ARC is known to increase after 24 h of food withdrawal in adult Japanese quail and within 48 h of food deprivation in young broiler chicks, while effects on POMC expression are small to non-existent (Boswell and Dunn, 2017). In feed-restricted broiler breeders, both AgRP and NPY were elevated although the effect on AgRP was much larger (167- vs 8-fold increase compared to *ad libitum*), but neither POMC nor CART expression was affected (Dunn et al., 2013). It would be tempting to assume that the ARC is essentially keeping a simple tally of energy intake and expenditure in relation to each other, or to some sort of central setpoint, and studies showing that males express more AgRP than females and that high-growth individuals also display higher AgRP levels would support this (Boswell and Dunn, 2017). However, several studies suggest a more complex role for this system. For example, incubating junglefowl hens abstain from food while brooding but show elevated levels of AgRP compared to pair-fed non-incubating hens, and AgRP levels quickly decreased in feed-restricted broiler breeders when they were given *ad libitum* access to feed even though their body weights had not had time to recover (Boswell and Dunn, 2017).

A more complex role of the ARC is also suggested by studies on mammals prone to food hoarding, such as humans and Siberian hamsters (*Phodopus sungorus*). In both

of these species food restriction causes a short-lasting increase in food intake, similar to what has been reported in chickens (Robinson et al., 1993), but a prolonged increase in food hoarding activity that lasts for around a week. A similar effect is seen for a similar amount of time following an injection with AgRP in these animals, suggesting it is an important food hoarding signal. This signal is probably at least partly the result of diminishing adipose reserves as surgical lipectomy also affects food hoarding but not food intake for an extended period of time (Thomas and Xue, 2018). It has been suggested that hoarding food is actually a more effective way of avoiding future problems with energy balance than immediate overeating (Thomas and Xue, 2018), although extrapolating this to birds is somewhat complicated by the fact that they can keep food stored in their crops which makes hoarding and overeating overlap.

AgRP also appears to be involved in driving the increase in locomotory behaviors that is typical of feed-restricted animals in anticipation of food (Thomas and Xue, 2018). This behavior is independent of the circadian rhythm control in the suprachiasmatic nucleus (Thomas and Xue, 2018) and is also well documented in chickens (D'Eath et al., 2009; Kostal et al., 1992; Lindholm et al., 2018; Savory and Lariviere, 2000). The appetitive effects of AgRP are further supported by studies showing that presentation of food, especially if it is palatable, to a calorie-deficient rodent reduces AgRP and increases POMC expression within seconds. If food is removed before the animal has a chance to eat it, the effect is entirely reversible suggesting this is a mechanism of inhibiting foraging behaviors to promote actual eating (Thomas and Xue, 2018). NPY signaling outside of the PVN is probably also involved in driving foraging behaviors, while NPY signaling to the PVN promotes immediate feed intake. The different effects of NPY also seem to be linked to different receptors, with NPY receptor Y1 promoting hoarding behaviors and the Y5 receptor promoting food intake (Thomas and Xue, 2018). Interestingly, fasting in chickens actually down-regulates expression of the NPY receptor Y5 in the PVN specifically and this is in line with results from rodent studies (Yi et al., 2017). In animals that continue to hoard food even when food access is restored, this effect is probably mediated through AgRP continuing to act on downstream melanocortin receptors (Thomas and Xue, 2018).

In conclusion, hypothalamic appetite control is complicated as the exact effects of each peptide at a given time depends not only on its concentration but also the connections of the neuron expressing it, the type of receptor receiving it and interaction with other appetite-signaling peptides. In some cases, one of these signals may override the others, or they may be overridden by other signals not originating in the hypothalamus (Boswell and Dunn, 2017; Yi et al., 2017). When the expression levels of several of these peptides are reported together however, the expectation is that we get a relatively good indication of an individual's overall appetite.

Improving the breeder situation: breeding goals

It is tempting to think that the most obvious way to improve the welfare of broiler breeders would be to choose breeding goals specifically aimed at promoting the well-being of the longer-lived generations. It has also been suggested that broilers could realistically be bred for high juvenile growth with reduced disease burden so that breeders could be kept healthy without or with only minimal feed restriction (Dawkins and Layton, 2012). The case for prioritizing the breeders' situation is further emphasized by the fact that the meat output per breeder rather than per chick is considered the ultimate performance measure in the broiler industry since the early 1990s (Decuypere et al., 2010). Unfortunately for the breeders, they are only consuming around 5% of the total feed eaten by the two ultimate generations, and for this reason the financial incentives to invest in breeder welfare are small (Decuypere et al., 2010). Even though improved breeder welfare could theoretically result in improved productivity, a 1% increase in egg production only results in a 0.23% increase in profits, whereas a 1% increase in feed conversion or breast meat percentage of the broiler increases profits by 1.21% and 3.10% respectively (Decuypere et al., 2010). Because of this, further improvements in broiler breeding stock cannot be expected to lead to welfare improvements for the breeding generation unless more value is assigned to their situation.

An already existing alternative to current breeder hens with the potential of improving breeder welfare would be to rely on dwarf hens, as suggested by Jones et al. (2004) and many others. Because dwarf breeders naturally eat and grow less, they can be reared with less intense feed restriction without compromising their health and reproductive capacity (Decuypere et al., 2010; Jones et al., 2004). As long as the dwarf hens are mated with normal-size roosters they will produce normal-size offspring (Decuypere et al., 2010), however the mating of males and females that are very different in size can be problematic and may lead to a need for artificial insemination (Decuypere et al., 2010). Dwarf breeder hens also show higher adiposity than regular breeders, a trait which they pass on to their offspring, and generally produce fewer eggs (Leeson and Summers, 1985). A less drastic alternative is crossing broiler roosters with hens from large-bodied but healthier breeds. This is a common approach in France, where so called Label Rouge hens are often used as breeders producing broilers that need 10-12 extra days to reach slaughter weight (Decuypere et al., 2010). This shift to a slower broiler production is often considered a niche-market and financially unviable but makes up approximately 85% of the chicken meat market in France (Van Krimpen and De Jong, 2014), highlighting that when consumers see a value in this type of production it becomes viable.

Improving the breeder situation: precision feeding

A novel suggestion for improving the situation of broiler breeders is the use of a precision feeding system. In this system, young birds are taught to enter feeding

cubicles to gain access to an individualized meal based on RFID identification at the entrance, weighing in the cubicle and a target growth curve updated in real time (Zuidhof et al., 2017). It has long been suggested that access to many small meals during the day may represent a more natural eating pattern for chickens and could thus have positive welfare implications (Jones et al., 2004). The exact eating pattern allowed by the precision feeding system is a combination of competition for entry into the cubicle and limitations imposed on eating time by the experimenters. Because early trials allowing for longer eating times (up to 10 min) showed that feed intake rates decrease logarithmically over time, subsequent experiments have used a maximal meal time of 1 min (Girard et al., 2017a; Zuidhof et al., 2017; Zuidhof, 2018). With this setup, precision-fed birds eat approximately 8-10 meals per day with an average meal size of around 8-10 g, although the total number of visits to the feeder was much higher and averaged 51 visits (full range: 28-138 visits) per day and bird (Girard et al., 2017a; Zuidhof et al., 2017; Zuidhof, 2018).

While precision feeding has proven extremely good at producing homogeneous flocks, with reported coefficients of variance (CVs) in the single digits and frequently below 2% as compared with CVs in the 11-14% range in conventionally fed control groups (Zuidhof, 2018; Zuidhof et al., 2017), improvements in other parameters of welfare and productivity have been miniscule at best. The frequent feedings in this system appear to reduce FCRs somewhat (from 4.89 g feed/g bird to 4.06 g feed/g bird during the 10-23-week period; Hadinia et al., 2018), but total egg production was higher in conventionally fed controls (Zuidhof, 2018).

While a previous study comparing the effect of two daily meals with the conventional one meal/day schedule found that the division of feed into two smaller meals increased walking in the birds, a possible sign of restlessness and frustration (de Jong et al., 2005), precision-fed pullets exhibit less foraging and more resting behaviors (Girard et al., 2017b). This could indicate less feeding frustration in these birds, but on the other hand they also exhibited increased object pecking which is often interpreted as a sign of unfulfilled foraging needs (Girard et al., 2017b). From 12 weeks of age, precision-fed pullets also demonstrated more aggressive behaviors and threats, which was probably the result of a high degree of competition for entering the cubicle (Girard et al., 2017a). Feather pecking was technically less common in the precision-fed group, but made up less than 1% of behaviors in both groups and was not correlated with feather scores, suggesting this was mostly performed as “gentle feather pecking” which is not a welfare concern (Girard et al., 2017b).

Although the research on precision feeding systems has so far failed to show relevant improvements for production settings, the extreme homogeneity produced and the possibility of keeping several feeding treatments together in a common garden experiment (Zuidhof et al., 2017) suggests it can be of high value to researchers.

Improving the breeder situation: qualitative feed restriction

A common suggestion for reducing or even completely replacing the need for quantitative feed restriction in broiler breeder rearing is the use of more nutritionally dilute feeds. In comparison, dry sows are also routinely feed-restricted to around 50% of *ad libitum* intake to prevent over-eating, and since 2001 EU legislation requires that they are provided with bulky feed to allow them to perform their natural feeding behaviors even when restricted (D'Eath et al., 2009). Since the natural feeding pattern for chickens seems to consist of eating several small meals spread out over the day (and the night!) with consumption peaks just after dawn and before dusk (Jones et al., 2004), using bulkier food could be a strategy to improve broiler breeder welfare as well.

Qualitative feed restriction can come in many forms, such as inclusion of soluble or insoluble fiber, using appetite-suppressing feed additives or combinations of the two. While calcium propionate (CaP), the most commonly used appetite suppressant, has shown promising results in some studies, it is worth noting that it is suspected both of having low palatability for the hens and to induce unpleasant side effects (Nielsen et al., 2011; Savory et al., 1996). In a recent study by Arrazola and Torrey (2019), pullets' preferences for either a placebo or an incapsulated CaP pill were examined in a conditioned place preference test. Even though the incapsulation was expected to hide the poor taste of the substance, the hens still showed a significant preference for the placebo (Arrazola and Torrey, 2019), and it has been questioned whether we can ever consider bird welfare to be improved under any feeding scheme that includes appetite suppressants (Van Krimpen and De Jong, 2014).

Nutrient dilution is usually accomplished by adding either soluble or insoluble fibers to the diet, although it is important to recognize that fiber is not entirely inert for poultry who can ferment them into short-chain fatty acids (Kiarie et al., 2017, 2014). Fibrous diets are often assumed to decrease digestability (Jones et al., 2004), but inclusion of just 2-3% insoluble fibers actually improves the nutrient utilization of chickens (Van Krimpen and De Jong, 2014). Insoluble dietary fibers tend to accumulate in the gizzard until they've been ground to an appropriate size to enter the intestine, and this prolonged gizzard fill is expected to trigger a temporary satiety (Van Krimpen and De Jong, 2014). Some studies have suggested that soluble fibers are even more effective in increasing satiety as they bind more water, thus increasing distention of the gastrointestinal tract but signs of discomfort have also been observed in these birds, possibly as a result of increasing moisture in the litter. Insoluble fibers have shown promising results on welfare indicators such as object pecking and dust bathing (Van Krimpen and De Jong, 2014) and are usually preferred over soluble fibers. An alternative to fiber inclusion is the use of low-protein diets, however protein-restricted birds show behavioral signs of nutrient deficiency during the most intense growth phase around 6-12 weeks as well as an

increased prevalence of feather pecking which coincided with reduced feather scores (Li et al., 2019).

In two studies, Sandilands et al. (2006, 2005) managed to rear broiler breeders to target body weights using *ad libitum* feeding of diets diluted with 30-40% oat hulls and supplemented with around 5% CaP. The treatments had no effect on flock growth uniformity, or heterophil to lymphocyte ratios (HLR), circulating corticosterone levels (Sandilands et al., 2006, 2005) or other white blood cell counts (Sandilands et al., 2006) which were the physiological stress markers used. Object pecking was typically lower in the flocks receiving the quantitatively restricting diets, but the total amount of oral behaviors did not differ significantly between treatments (Sandilands et al 2005, 2006). Hocking (2006) also found no effects on HLR, corticosterone levels or antibody titers when birds were fed diets with 20% or 40% oat hulls, suggesting that dilute diets are not perceived as stressful by the animals.

Some studies have shown decreased feed intakes using fiber inclusion (Jones et al., 2004; Leung et al., 2018; Van Krimpen and De Jong, 2014), although it's unclear whether this was the result of increased satiety or of reaching maximal gut fill more quickly (Leung et al., 2018). Few studies so far have looked at the expression of appetite-regulating peptides in qualitatively restricted birds, but broiler breeders fed an *ad libitum* diet diluted with ispaghula husks showed no change in the expression of AgRP and POMC in the ARC compared to birds on regular quantitative restriction (Boswell and Dunn, 2017). Behavioral studies of hunger and frustration in qualitative restriction have produced highly variable results (Van Krimpen and De Jong, 2014).

In general, qualitatively restricted diets do not reliably limit body weight gain and often lead to problems with flock uniformity (Arrazola et al., 2019a), as well as increased mortality (Sandilands et al., 2006). For example, Zuidhof et al. (1995) found that broiler breeders fed a diet with 15% fiber inclusion showed signs of improved satiety with better flock uniformity as well as higher egg and chick production, but these effects were lost at 30% fiber inclusion. More recent studies have shown that qualitatively restrictive diets can lead to improvements in feather coverage and feather quality with improved flock uniformity and reduced feeding motivation, however these effects were not unique to dilute feeds but were seen in all treatments that allowed at least 50% extra feed on some days which also included intermittent fasting schedules (Arrazola et al., 2019a, 2019b). In these studies, no effect was seen on total egg production to 64 weeks of age (Arrazola et al., 2019b).

Improving the breeder situation: intermittent fasting

The use of intermittent fasting (IF), or “skip-a-day”, in broiler breeder rearing is supposedly the most common feed restriction regimen used globally in chicken production and is widely acknowledged to increase flock uniformity by increasing portion sizes, and thus feeding times, which reduces the amount of competition over

Figure 3. Crop fill estimates from an on-farm sampling of both daily-fed (●) and intermittently fasted (○) broiler breeders at 12 weeks of age. Grey background indicates dark hours, with feeding taking place approximately 10 min after lights on. Data collected as part of the sampling for Paper VI, but previously unpublished. A slight increase in crop fill in unfed birds may be the result of accidental ingestion of wood shavings while foraging (Jones et al., 2004).

feed (Bartov et al., 1988; Bennett and Leeson, 1989; de Beer et al., 2007; Mench, 2002; Morrissey et al., 2014). Intermittent fasting has mostly been used in the form of alternate-day fasting, although fewer fasting days per week have been suggested from a welfare perspective (Renema and Robinson, 2004) and the use of two consecutive fasting days has also been tested (Bartov et al., 1988; Katanbaf et al., 1989). Generally speaking, however, IF in this context refers to fasting for no more than one consecutive day 1-4 times per week. Regardless of which schedule is used, it is worth noting that IF of chickens is illegal in Sweden, Denmark, Norway and the United Kingdom due to animal welfare concerns (Morrissey et al., 2014; Statens jordbruksverk, 2010).

Although the regular practice of daily restricted feeding leaves the animals without feed for at least 23 h, which would constitute a daily fasting challenge for mammals, chickens retain some food in their crop for at least 16 h after feeding (de Beer et al., 2008; Jones et al., 2004; **Figure 3**) and are thus not truly fasting in daily feeding regimens. Intermittent fasting leads to slower growth than daily feeding using equivalent feed amounts, and this has generally been attributed to inefficiencies in the metabolic processes of storing and re-mobilizing nutrients (Bartov et al., 1988; de Beer and Coon, 2007; Leeson and Summers, 1985). This can be seen either as a drawback of IF as it leads to increased feeding costs to achieve target growth rates, or as a benefit as it can allow for a slight relaxation of feed restriction and may thus increase access to nutrients. We have observed accelerated feathering in IF-fed

pullets when fed to the same growth curves as daily-fed animals (unpublished data) and Arrazola et al. (2019a) recently reported better feather coverage and improved re-growth of feathers (both heavier and longer) in IF-fed breeders, which may be the result of increased availability of amino acids on feeding days.

Although most experimental studies have failed to show significant effects of IF on flock uniformity, this may simply be an artefact of the small animal groups typically kept in lab facilities (10-35 animals/pen) simply not achieving feed competition similar to what is found in commercial-size flocks (2,500-4,000 animals per pen). It has been reported that broiler breeders fed every other day, and thus given twice the amount of feed, take 2.5 times as long to finish their meals which would support the idea of reduced feed competition (Bennett and Leeson, 1989). Lower numbers of feather fault bars (Arrazola et al., 2019a) and reduced anticipatory behavior before feeding time (Lindholm et al., 2018) reported in IF-fed breeders are also suggestive of reduced competition stress in these animals. While these regimens have sometimes been criticized for imposing high stress levels on fasting days (Renema and Robinson, 2004), others have concluded that IF regimens do not increase overall stress levels compared to daily feeding as assessed by HLRs and behavioral observations (Skinner-Noble and Teeter, 2009).

Behavioral studies on IF-fed compared to daily-fed breeder pullets show a complicated picture, with more feather pecking in young IF pullets (≤ 11 weeks) but no effect on object pecking (Morrissey et al., 2014). The fact that feather pecking decreased and eventually disappeared entirely with age (Morrissey et al., 2014) may suggest that this was the result of a nutritional deficiency or of hunger-induced aggression before animals fully habituated to the harsh restriction level imposed at this age. The low levels of aggression seen in IF-fed breeders in the afternoon of feeding days when hunger and feeding frustration are expected to be high (Girard et al., 2017a) as well as the observation that environmental enrichments do not appear to reduce aggression in broiler breeders (Van Krimpen and De Jong, 2014) also suggest that aggression in these animals is highly concentrated to situations of competition for feed.

Although the slower growth of IF pullets delays the onset of laying, total egg production is usually not affected (Arrazola et al., 2019b; Decuyper et al., 2010; Powell and Gehle, 1976). One study even found that breeder hens fed on an IF schedule with gradual introduction of fasting days laid 7.7% more eggs over their lifetime compared to daily-fed controls (Arrazola et al., 2019b), although this may simply have been the effect of compensatory growth experienced by these birds when switched to a daily feeding schedule around the time of photostimulation (Arrazola et al., 2019a). While IF led to the laying of slightly lighter eggs in that study, there was no difference in hatchling weight (Arrazola et al., 2019a).

The physiology of fasting

As important as feeding is, a large part of the day of any animal will be made up of activities other than eating. At any such time, when the animal is abstaining from food more or less voluntarily, it is technically fasting. Wild and domestic animals fast under a range of circumstances, for birds these typically include times of migration, incubation and molting. The physiological response to fasting is especially well described in penguins that regularly fast for anything from a couple of days (*Eudyptula minor*) to 4 months in incubating male emperor penguins (*Aptenodytes forsteri*) and up to 5 months in king penguin chicks (*Aptenodytes patagonicus*) during their first winter (Groscolas and Robin, 2001).

An extended fast such as the ones endured by many penguins typically progresses through three phases. Phase I is a relatively short phase where the animal goes through rapid metabolic changes to adapt to fasting. Phase II makes up the bulk of time in a prolonged fast and is characterized by slow weight loss as the animal actively copes with the fasting challenge. At some point however, the animal will reach a critical condition where it can no longer maintain important physiological and behavioral functioning without food and enters Phase III as the body uses up its final reserves before eventually dying of starvation unless food can be obtained.

The first phase of fasting is characterized by an initially rapid weight loss that gradually slows down towards Phase II. At the start of the fast, stored glycogen provides most of the needed energy and glucose but these stores will not last for more than a few days. Obtaining physiological measurements from penguins in Phase I has proven difficult as adult penguins are already in Phase II when they return to shore (Groscolas and Robin, 2001). In young king penguin chicks, who are still downy and thus spend their first winter on land, Phase I has been shown to include endocrine changes geared towards sparing protein and catabolizing fat (Le Ninan et al., 1988a). Such changes include reduced circulating levels of corticosterone and insulin which are accompanied by decreasing concentrations of protein metabolism markers alanine, urea and uric acid, and increasing concentrations of fat catabolites free fatty acids and β -hydroxybutyrate (β HB) (**Figure 4**). At the same time, reduced levels of thyroid hormones T₃ (in domestic fowl) and/or T₄ (in king penguins) reduce resting metabolic rate to conserve energy (Le Ninan et al., 1988a), leading to a 62% drop in resting metabolic rate (from 2.43 ± 0.06 W/kg to 1.81 ± 0.04 W/kg in Duchamp et al. (1989)). In mammals, Phase I also brings about marked hypoglycemia, but this is not the case in birds (Le Ninan et al., 1988a). Although Cherel and Le Maho (1985) saw a drop in glycemic levels throughout fasting in king penguin chicks, those results were not reproduced in a later study which reported a stable glycemic level in the 12.5-13.0 mM range during the first 20 days of fasting (Le Ninan et al., 1988a).

In fasting-resilient species such as large penguins, Phase II constitutes the bulk of fasting time and its length is dependent on the amount of accumulated fat stores. In

Figure 4. Body mass loss (A) and metabolites (B-F) in fasting king penguin chicks during Phases I-III. β HB and free fatty acids are indicators of fat catabolism, while urea and alanine indicate protein catabolism. Datapoints are a composite of values reported by Cherel and Le Maho 1985 (CLM85, ●), Le Ninan et al 1988a (LN88a, ■) and Le Ninan et al 1988b (LN88b, □). Dashed vertical lines indicate the transition between phases as reported in CLM85 (day 8 and 128 respectively), but similar values were reported by LN88a (day 7 and 132 respectively). CLM85 ended their experiment after 149 days but estimated that the animals could have survived until approx. 3 kg. ● in (A) represent expected values based on mass loss rates reported by CLM85 and Cherel et al 1987. LN88b did not report body weights other than at the start and end of their experiment, which were 13.1 ± 0.3 kg at capture and 3.43 ± 0.1 kg after 150 ± 8 days of fasting.

king penguin chicks it is typically reported to last for about 3 months (Cherel and Le Maho, 1985; Le Ninan et al., 1988b). This phase is characterized by slow weight loss, high dependency on lipid catabolism as seen by elevated levels of β HB and free fatty acids in the blood and low levels of glucocorticoids to prevent proteolysis (Cherel and Le Maho, 1985; Le Ninan et al., 1988a, 1988b). While most parameters are highly stable during Phase II, levels of glucose and β HB tend to show a slow decrease and increase respectively, as a growing proportion of central energy demands are fulfilled by ketones rather than glucose.

Phase III sets in when the animal reaches approx. 20% remaining lipid stores and constitutes a period of progressive failure to cope with the fasting challenge, although the animal can still be rescued by refeeding (Robin et al., 1988). As fat stores approach depletion, β HB levels start to fall and glucocorticoid and glucagon levels increase to meet energy demands from proteolysis instead (Le Ninan et al., 1988b). Although lipolysis could theoretically be maintained for a while longer, the shift into Phase III may also serve as a metabolic signal to break the fast in e.g. incubating adults that can choose to abandon the nest to prioritize re-feeding (Groscolas, 1986). With the increase in protein catabolism, circulating levels of urea, uric acid and alanine start to increase again (Cherel and Le Maho, 1985; Le Ninan et al., 1988b). With the loss of protein comes an accelerated rate of weight loss, with reports of rates increasing from around 6 g/kg daily at the end of Phase II to around 22 g/kg on day 150 of fasting and finally peaked around 35 g/kg in chicks that starved to death (Cherel et al., 1987; Cherel and Le Maho, 1985; Le Ninan et al., 1988b). In wild king penguin colonies, it has been estimated that 49% of chicks die during their first winter season (Cherel et al., 1987).

Figure 5. Blood glucose and β HB measurements from 4-week-old Red Junglefowl and 12-week-old broiler breeders taken during a 40 and 48 h fast respectively clearly shows the higher fasting resilience in the broiler breeders.

Unlike penguins, chickens normally consume several small meals throughout their days (Jones et al., 2004) and are not usually considered a fasting-resilient species. Exactly how long a chicken can survive while fasting is not known, but studies fasting chickens for up to 72 h have shown that plasma glucose levels remain high as expected, while plasma β HB and alanine levels both increase within 24 h but then stabilize (Belo et al., 1976). The increase rather than decrease in free alanine suggests an inability to spare protein while fasting. Some habituation to fasting appears to take place under IF regimens, although mostly in the form of increased hepatic glycogen stores in preparation for future fasts (Lees et al., 2017). The time a chicken spends in Phase I largely depends on its hepatic energy reserves (muscle glycogen does not appear to contribute much), with *ad libitum*-fed broilers depleting their liver glycogen reserves in around 6 h (Warriss et al., 1988), but time courses can vary widely between different chicken strains and feeding strategies. For example, we have seen an approximately 9-fold increase in β HB after 40 h of fasting in 4-week-old Red Junglefowl (Lindholm et al., 2019), while 12-week-old broiler breeders only exhibited a 1.6-fold increase in the same time (**Figure 5**).

Therapeutic fasting and the beneficial ketosis

Therapeutic fasting in humans is far from a new concept but has gained a lot of attention in recent years. Popular diet schemes involving fasting include whole-day IF schedules such as 5:2 or alternate-day fasting or temporally restricted fasting where an individual eats daily but abstains from food for periods of at least 12 h consecutively (Anton et al., 2018). These diets have sometimes been promoted as easier to adhere to compared to traditional restrictive diets, but so far the evidence that dropout rates are actually lower in fasting diets is lacking (Anton et al., 2018). Key to the beneficial effects of therapeutic fasting is the switch from glucose to ketones as the preferred energy substrate for both brain and body (Anton et al., 2018). In humans, liver glycogen reserves typically last for 10–14 h without exercise (Mattson et al., 2018), and the “metabolic switch” is reported to occur 12–36 h after the last meal (Anton et al., 2018). This switch is equivalent to the Phase I – Phase II transition previously described, and just as in the fasting penguins the switch to ketones helps preserve muscle which is seen as improved retention of lean mass in weight loss studies employing IF schemes as well as prevention of age-related muscle mass loss in mice (Anton et al., 2018).

Ketogenesis occurs primarily in the mitochondrial matrix of hepatocytes, although a few other cell types (e.g. astrocytes) have been reported to have ketogenic capacity (Anton et al., 2018; Puchalska and Crawford, 2017). Ketone bodies are released into the blood stream from the liver and transported down a concentration gradient into other tissues, with the heart, skeletal muscle and brain being the most important consumers of ketone bodies. Liver cells themselves are incapable of oxidizing ketones (Puchalska and Crawford, 2017). The ketone body β HB is the only known endogenous ligand for the GPR109A receptor, which provides a negative feedback

loop on ketogenesis as it inhibits lipolysis in adipose tissue under ketotic conditions. In prolonged ketosis, this feedback mechanism appears to be overridden by sympathetic stimulation of lipolysis (Puchalska and Crawford, 2017).

Ketones have traditionally been viewed as something of byproducts of intense β -oxidation, but more recent observations suggest that ketones are important signaling molecules even when carbohydrates are available (Puchalska and Crawford, 2017) and β HB appears to be the key signal for therapeutic effects of fasting (Mattson et al., 2018). For example, β HB upregulates GABAergic tone in the nervous system which protects against seizures and is important for the seizure reductions commonly seen in children with epilepsy when treated with ketogenic diets (Mattson et al., 2018; Puchalska and Crawford, 2017). Anti-inflammatory effects of β HB have been reported both through down-regulation of the NLRP3 inflammasome (Puchalska and Crawford, 2017) and of proinflammatory cytokines (Mattson et al., 2018). The GPR109A receptor is abundantly expressed on macrophages and monocytes and β HB probably also confers anti-inflammatory effects through this receptor as GPR109A ligands are known to have anti-inflammatory effects in pathologies such as atherosclerosis, obesity, irritable bowel syndrome, neurological disease and cancer. β HB is previously known to counteract inflammation induced by $\text{TNF}\alpha$ and LPS challenges, to scavenge reactive oxygen species and to inhibit class I histone deacetylases leading to higher expression of antioxidant genes (Puchalska and Crawford, 2017). In contrast, chronic ketosis in type 1 diabetes is a proinflammatory state. This effect may be mediated by another ketone body, acetoacetate, which is known to trigger inflammatory responses in high concentrations (Puchalska and Crawford, 2017). Other therapeutic effects of intermittent ketosis include increased cellular resistance to stress and disease, reduced resting heart rate and blood pressure, increased mitochondrial biogenesis and synaptic plasticity in neurons, reduced fasting glucose in prediabetes, reduced diabetes risk and improved cardiovascular health (Anton et al., 2018; Mattson et al., 2018; Puchalska and Crawford, 2017). Some of these effects are probably mediated through the energy-sensing enzyme AMPK which is also known to confer short-term improvements in neuronal plasticity, to stimulate mitochondrial biogenesis and autophagy in muscle and to promote insulin sensitivity (Anton et al., 2018; Mattson et al., 2018).

Interestingly, β HB has also been gaining increasing interest as a metabolic signal affecting appetite and eating behavior. One meta-analysis examining studies of very-low-energy diets and ketogenic low-carbohydrate diets, both of which induce varying levels of ketosis, showed that although participants in these studies typically consumed less energy than normal and lost substantial amounts of weight, they reported slightly reduced levels of hunger (Gibson et al., 2015). Similarly, mice fed the non-toxic alcohol R/S-1,3-butanediol, which is hepatically oxidized to β HB, show lower levels of feed intake (Carpenter and Grossman, 1983) and IF-fed broiler breeders show reduced feeding motivation (Arrazola et al., 2019a). β -hydroxy-

butyrate also appears to have important signaling effects for food-anticipatory behavior, with diurnal increases in plasma β HB seemingly mechanistically linked to increased activity, body temperature and plasma corticosterone levels before meal-time in feed-restricted mice (Chavan et al., 2016).

Welfare in feed-restricted chickens

Animal welfare is by and large an intuitive concept and finding good scientific definitions of it has proven challenging (Duncan, 2002; Korte et al., 2007). In the words of Decuyper et al. (2010) there is simply “no such thing as a universal golden standard to objectively measure welfare” and animal welfare science has sometimes been criticized for focusing more on questions that are well suited for scientific exploration than what is truly relevant for welfare applications (Rushen, 2003). Part of the confusion stems from different ideas of what forms the basis of animal welfare, with some researchers and schools of thought focusing almost exclusively on health parameters while others argue that the emotional status of the animal should take precedence (Duncan, 2002). In this thesis, we have taken the more recent approach of considering good animal welfare as a situation where animals are able to react appropriately to challenges in their environment, meaning that animals should not be exposed to situations outside of their coping abilities (Korte et al., 2007). To assess the welfare of animals under this school of thought, many parameters of animal well-being, ideally a combination of physiologically and behaviorally relevant ones, must be integrated and analyzed together (Rushen, 2003). While few parameters have been properly validated for use in animal welfare assessments (Rushen, 2003), and the widely adopted Welfare Quality Assessment Protocol for Poultry does not include broiler breeders (Butterworth et al., 2009), there are many commonly used welfare parameters used in broiler breeders.

Glucocorticoid secretion is the classical physiological response to stress in general, and plasma corticosterone (CORT) levels have been shown to be elevated in feed-restricted chickens for at least 10 weeks after the introduction of feed restriction (Mench, 2002), and especially in feed-restricted broiler breeder pullets at the ages of 6-18 weeks when restriction is the most intense (Savory et al., 1996). Higher levels have also been reported in intermittently fasted broiler breeders on off-feed days, and Renema and Robinson (2004) suggested this as a reason for pursuing IF schedules with fewer fasting days to improve animal welfare. However, an important function of glucocorticoids is to regulate glucose metabolism which is a functional response under calorie restriction and not necessarily a good indicator of psychological stress (Veissier and Boissy, 2007). There is also evidence that chickens habituate to feed restriction after a number of weeks, and then produce lower levels of CORT in response to a fasting challenge although they still respond normally to other stressors or injections of the CORT-releasing hormone ACTH (Rees et al., 1985).

Another common physiological stress marker in chickens is the heterophil to

Figure 6. Welfare parameters showing the welfare optimum of broiler breeders to be $\geq 50\%$ of *ad libitum* intake (dashed line). On the left, circulating CORT levels as reported by Hocking et al., 1996 (●) in 12-week-old birds and by de Jong et al., 2003 (■) in 7-week-old birds. On the right, basophil counts (○) and heterophil to lymphocyte ratios (▲) as reported by Hocking et al., 1996. All trend lines shown are third-grade hyperbolic relationships as suggested by de Jong et al., 2003.

lymphocyte ratio (HLR). This ratio is actually a long-term indicator of circulating CORT levels, but may be more relevant for welfare both because elevated CORT levels over a prolonged time may indicate a failure to cope with a challenge, and because it represents a physiological effect of CORT on immune cells. HLRs typically increase in response to stressful situations including feed restriction (**Figure 6**). For example, a study comparing feed-restricted broiler breeder hens to slower-growing Label Rouge chickens showed increasing HLRs in the intensely restricted broiler breeders at 5-10 weeks of age, but stable levels in the Label hens (Jones et al., 2004). Interestingly, a recent study examining various forms of feed restriction in broiler breeders found higher HLRs on fed compared to fasted days in a complex IF schedule (Arrazola et al., 2019a). Quantifications of other leukocytes and antibody titers are also sometimes performed as part of welfare studies. In the already mentioned study including broiler breeders and Label Rouge chickens, no differences in eosinophil, basophil or monocyte counts were seen between breeds or feeding treatments, while feed-restricted birds showed increased antibody titers in response to vaccinations against infectious bronchitis virus and infectious bursal disease, but not Newcastle disease virus (Jones et al., 2004).

On the behavior side, over-drinking (polydipsia) has received a lot of attention in feed-restricted chickens. Over-drinking in these birds is typically a redirected foraging behavior and limiting water access to feeding times does not appear to reduce welfare of these animals (Hocking, 1993; Jones et al., 2004). In unrestricted Label Rouge chickens drinking behavior generally decreases with increasing age, while feed-restricted broiler breeders show marked increases in drinking or drinker-directed behavior during the most intense feed restriction period (Jones et al., 2004).

Other studies have reported that frustrated behaviors, such as preening, and non-eating oral behaviors, such as object pecking, initially increase in feed-restricted chickens but tend to disappear with habituation rather than relaxation of the restriction level (Savory et al., 1996). Sometimes these oral behaviors can become aggressive in nature, but this also seems to be a transitional effect and they are later redirected into competing for feed (Mench, 2002; Van Krimpen and De Jong, 2014). Pacing is also commonly reported in feed-restricted broiler breeders, especially around feeding times (Van Krimpen and De Jong, 2014), which is expected as a form of feed-anticipatory behavior (Chavan et al., 2016).

It is not surprising that feed restriction of broiler breeders leads to increased oral behaviors and stereotypies. While we generally cannot define the natural state of broiler breeders (Renema and Robinson, 2004), we know that both junglefowl and laying hens spend 50-60% of their time eating and foraging while broiler breeders finish their daily ration within 15 min (Van Krimpen and De Jong, 2014). As a result, 8-week-old broiler breeder hens have been reported to spend about half their day pecking at empty feeders (Van Krimpen and De Jong, 2014). Feed-restricted birds are also more motivated to consume feed during compensatory feeding tests, and the compensatory feed intake is linearly correlated with the level of previous restriction (Van Krimpen and De Jong, 2014). When restriction levels are relaxed, the birds also tend to spend more of their time resting which has been suggested to indicate increased satiety (Jones et al., 2004).

A general problem in assessing animal welfare is the effect of management practices and their interaction with what is being examined (Rushen, 2003). These practices include everything from lighting and ventilation to beak trimming and routine use of antibiotics (both of which are illegal in Sweden and are not used in any of our studies). Development of a circadian rhythm is one example of a welfare indicator which is intrinsically related to management practices. The minimal scotoperiod length needed for broilers to develop a circadian pattern is not known but is somewhere in the range of 1-8 h (Bessei, 2006). For broiler breeders this is not a problem as a daylight schedule of 8 h daylight and 16 h darkness is commonly used. This is suggested to reduce the level of perceived restriction as *ad libitum*-fed meat-type chickens grow the slowest on this daylight schedule (Griffin et al., 2005). While many people may find the large group sizes that broilers and broiler breeders are often kept in intuitively off-putting, broiler behavior does not appear to be affected by large group sizes (Weeks et al., 2000), and high stocking densities (>30 kg/m²) only seem to be a problem when they cause high litter temperatures and humidities (Bessei, 2006).

Although understanding animal welfare in feed-restricted broiler breeders is a complex task, many studies relying on a wide range of quantitative parameters appear to give a reasonably coherent picture. For example, several welfare indices

have shown reduced welfare in feed-restricted animals compared to *ad libitum* during the most intensely restricted period (Hocking et al., 2001) and also lower stress levels in animals experiencing more gradual changes in feeding level even when different restriction regimens led to equivalent body masses (Hocking et al., 1996). The welfare indices used in these studies included lower water intake, lower HLRs, lower circulating CORT levels and lower basophil counts (Hocking et al., 1996).

Paper summaries

Theme 1: Early growth in feed-restricted broiler breeders

I. Slow and steady wins the race?

In this paper we started out by exploring the idea that small and large individuals within a broiler breeder flock grow at different rates as a result of different coping strategies. Data collected on growth, behavior and body composition in farm-raised broiler breeder hens at 4 weeks of age did not support our initial hypothesis, which was thus quickly abandoned. We did, however, find consistent differences suggesting that small birds were less fearful, less stressed and had relatively enlarged gastrointestinal organs compared to larger individuals.

II. Growth heterogeneity is settled before feed restriction

Following up on the results from paper I, we followed a cohort of almost 500 farm-raised broiler breeder chicks from just after hatching until 4 weeks old. Data collected from the farm was complemented by raising two cohorts of 120 breeder chicks each under farm-like conditions in the lab and one cohort of 40 broiler chicks under simplified feed-restricted conditions. While it has been generally accepted that growth heterogeneity is a result of restrictive feeding, we found that the different growth patterns were already largely settled within 24 h of first access to feed and appeared to be the result of differences in voluntary food and water intake.

Theme 2: Intermittent fasting in Red Junglefowl

III. Physiological and neuroendocrine correlates of hunger

In an attempt to understand the effects of intermittent fasting and its effects on chicken hunger we raised Red Junglefowl, the closest wild ancestor of domestic chickens which is not prone to over-eating, under three different feeding regimens: *ad libitum* food access, daily restriction (at 70% of *ad libitum* intake) and a 2:1 intermittent fasting schedule. The different feeding regimens elicited large differences in hepatic energy stores (especially glycogen) and appetite-related gene expression in the arcuate nucleus, and induced binge-eating in both restricted groups. Animals subjected to a daily restricted diet displayed elevated expression of AgRP especially, and this hunger signal was significantly associated with increased gizzard content and decreases in liver mass as well as in fat around both the gizzard and clavicles.

IV. On-site measurements of whole-blood ketones

In this methodological paper we used Red Junglefowl fed *ad libitum* or according to an intermittent fasting schedule to assess the validity and usefulness of measuring circulating levels of the ketone body β -hydroxybutyrate with a handheld point-of-care device producing results within seconds. While the handheld device was prone to a

slight over-estimation of ketone levels in the chickens, we found good correlation with a common laboratory-based method and a low random error rate. Circulating ketone levels clearly distinguished between birds expected to be experiencing different levels of hunger, ranging from around 1 mM in *ad libitum*-fed birds to an average of 6.3 mM in birds fasted for 40 h.

V. Intermittent fasting chronically changes gene expression

Using the same experimental design as in papers III and IV, we collected liver samples from 5-week-old Red Junglefowl fed *ad libitum*, restricted to 70% or fed according to a 2:1 intermittent fasting schedule. The hepatic transcriptomes of these birds were investigated using a microarray which showed large effects on gene expression across most metabolic pathways. We also found signs of increased cell proliferation and/or improved proliferative control in these birds and these effects were chronic under intermittent fasting (i.e. not only occurring on feeding days), suggesting that birds may experience similar health benefits from these diets as mammals.

Theme 3: Intermittent fasting in broiler breeders

VI. Effects of daily vs 5:2 feed restriction in broiler breeders

In this last theme of the thesis, we finally move on to the applied situation of intermittent fasting in broiler breeders. For this paper, farm-raised animals were fed according to either a daily restriction schedule or a 5:2 intermittent schedule introduced gradually from 6 weeks of age. At 12 weeks of age we compared body composition, behavior, bone strength, activity and white blood cell counts. As expected, the results suggest a compromised welfare situation in both groups. The 5:2-fed group showed body composition changes suggestive of increased stress, possibly linked to perceived unpredictable feeding, but appeared less anxious around feeding time. We also found signs of reduced fear and/or increased risk-taking in the 5:2-fed group, but no effects on bone strength or vaccination-induced immunity.

VII. Disentangling effects of intermittent fasting and caloric restriction

To better understand the interaction between intermittent fasting and quantitative restriction, we also raised broiler chickens on either daily feeding or alternate-day IF – a seemingly more predictable schedule than 5:2 – at two different restriction levels roughly corresponding to commercial restriction levels (30%) and the expected welfare optimum (60%). Their growth, activity levels, perching and responses to an insulin challenge were tracked, and show that the physiological effects of intermittent fasting (insulin response, liver mass) are robust across the different restriction levels, while the behavioral responses (activity, perching) were highly affected by restriction level. As expected, birds restricted to 30% of *ad libitum* appear more affected by current feeding status and also exhibit a clear pattern of food anticipatory activity, which was not apparent at 60% feed allowance.

Discussion

Theme 1: Early growth in feed-restricted broiler breeders

Early work for this thesis started out exploring growth heterogeneity and its potential links to individual differences in animal welfare in young broiler breeders (**Papers I-II**). Heterogeneous growth, and the poor flock uniformity that comes with it, is a major management problem in broiler breeders, but has also been pointed out as a possible sign of poor welfare (Dawkins et al., 2013; Zuidhof et al., 1995). In these papers, we focused on the first four weeks of life when feed restriction is introduced but farmers were not yet implementing efforts such as grading or intermittent fasting to reduce heterogeneous growth. Although it has often been argued that poor uniformity in growth is the effect of a competitive feeding environment, our results indicate that growth variation appears before the onset of feed restriction and feed competition, and thus rather stems from individual differences that already appear to be present in newly hatched chicks (Paper II). We argued that growth heterogeneity is maintained by feed restriction rather than caused by it. While the recent studies employing precision feeding systems have achieved extremely uniform flocks, they also highlight an enormous variation in attempts to access feed in these experiments which points towards large individual differences in feeding motivation (Zuidhof, 2018).

Although early feed intake and weight gain appear to have long-lasting implications both on intestinal development (Lilburn and Loeffler, 2015) and growth performance (Paper II), our attempts to pinpoint mechanisms tied to these differences have been mostly unsuccessful. Our initial attempt to tie growth differences to behavioral coping strategies showed that if anything small birds, which were hypothesized to be unsuccessful feeders due to a reactive coping style, were behaving more proactively in unknown situations and were more active in the home pen (Paper I). Although we found links between behavior and 4-week body mass, with smaller birds vocalizing more in an open field arena and being quicker to leave tonic immobility (Paper I, Paper II), this did not fit well with a coping style explanation and may rather have been an example of increased risk-taking in individuals experiencing a more restrictive feeding situation (Caraco, 1981; Verma et al., 2016). An earlier hypothesis that smaller birds were more socially motivated and that this could help them cope with the stress of feed restriction suggested in Paper I did not hold up to closer scrutiny (Paper II). We also explored the idea that slow growth in young broiler breeders is an effect of a slower thermocompetence development affecting basal metabolism but could not pick up any such differences (Paper II). To date, the only slightly predictive difference we have seen is that higher levels of circulating β HB before first access to feed significantly reduces later growth performance (Paper VII).

The effects of different early growth patterns have been less elusive, and small birds have consistently exhibited relatively larger hearts and gastrointestinal organs, and occasionally also larger livers and lungs (Paper I, Paper II). While we expect that

different growth trajectories must be formed by individual differences in feed intake, weighing the crop contents at the time of dissection did not reveal any such differences (Paper I) and it is possible that the growth heterogeneity present at 4 weeks of age is the result of previous rather than contemporary differences in feed intake. In Paper II, where we found increased relative lung sizes in small birds at 4 weeks of age this difference was not only retained two months later but the difference in relative heart and lung mass at 12 weeks of age had actually increased. These findings suggest that slow early growth should not necessarily be considered poor growth as it may come with long-term benefits. This picture is further supported by the significant (albeit weak) positive correlation found between heterophil to lymphocyte ratios (HLR), an expected stress marker, and body weight at 33 days of age (Paper I).

Overall, the results from this theme support the common notion that feed intake and growth during the first days to the first week of life is crucial for chick development. A better understanding of how individual differences affect growth is important not only to the rearing of broiler breeders, who have limited capacity for catch-up growth due to restricted feeding, but also to the emerging practice of feeding broiler chicks immediately after hatch (van de Ven et al., 2009). This practice has gained much interest in recent years and is expected to promote both growth and welfare but may sometimes lead to increased heterogeneity in growth (unpublished data).

Theme 2: Intermittent fasting in Red Junglefowl

One of the major challenges for any research trying to address the welfare of broiler breeders is the lack of a proper control group. The extreme selection that these animals are the result of have left them “in (a) freely expressed adult state (...), completely unfit for life” (Renema and Robinson, 2004), and *ad libitum*-fed broilers display physiological peculiarities such as a lack of diurnal variations in e.g. body temperature, heart rate and blood glucose that render them unsuitable as a control group (de Jong et al., 2002). Other options for control groups would be laying hens or slower-growing meat-type chickens such as those used in organic production, but while these birds are different from conventional broiler breeds, they are also the result of intense selection pressures. Therefore, we chose a captive population of the wild-type Red Junglefowl as control group in an attempt to better understand the normal physiological response to IF in chickens (**Papers III-V**).

Our studies of IF in Red Junglefowl have included three different feeding treatments: an *ad libitum*-fed control group (AL), a group experiencing daily restriction to around 70% of *ad libitum* intake (CR) and an IF group offered 150% of the *ad libitum* intake daily on two consecutive feeding days and then fasted on the third day. Unlike intermittently fasted broiler breeders, which are simultaneously experiencing quantitative feed restriction, these birds were offered the full amount of feed that they were expected to voluntarily ingest. In reality however, they were not able to match the feed intake of the AL birds, probably as they would have needed more time to finish their full rations, and grew at rates similar to the CR treatment (Paper III).

Binge eating with enlarged crops was seen in both the CR and IF treatments (Paper III), and should probably be considered similar to the food hoarding seen in some mammals (Thomas and Xue, 2018). The CR treatment led to the lowest relative liver masses, but with no effect on the concentration of glycogen or lipids. Intermittently fasted birds instead showed similar liver masses as the AL group on fasting days, with increased liver masses on feeding days and an increased concentration of lipids on the second day of feeding (Paper III). Glycogen concentration in the livers of IF animals also fluctuated between days, peaking significantly above both AL and CR levels on the first day of re-feeding and falling significantly below AL levels during fasting (Paper III). These patterns are similar to what we have seen in IF-fed and daily-fed broiler breeders (unpublished data), suggesting the hepatic response to fasting and re-feeding has not been majorly affected by selection.

Circulating levels of β HB in these birds increase above AL levels from the time of missed feeding on the fasting day and then keep increasing until the birds are re-fed. When birds are re-fed, they first fall below AL levels, then become elevated again before stabilizing after feeding on the second fed day (Paper IV). This suggests to us that intermittently fasted Red Junglefowl need the full two days of feeding to make up for the energy deficit they sustained during fasting, but that they are indeed able to prepare for fasting by building energy reserves (Paper IV). This is in contrast with the CR group who followed a similar growth curve but exhibited no signs of building energy reserves, not only in the liver but also in terms of smaller extra-hepatic fat stores surrounding the gizzard and clavicles (Paper III).

A principal component analysis revealed that physiological parameters mostly related to recent food ingestion (crop fill, gizzard fill, liver glycogen) were not related with ARC expression, while indicators of increased energy storage (clavicular and gizzard fat) were negatively related with expression of AgRP and positively correlated with POMC (Paper III), which agrees with the previous finding that a more dilute diet did not change ARC expression (Boswell and Dunn, 2017). In general, AgRP expression was elevated in the CR treatment but not significantly affected by IF, which supports the idea that AgRP offers a long-term representation of feed intake (Paper III). As expected, POMC showed a mostly opposite pattern to that of AgRP with reduced expression in CR compared to AL. Although the POMC expression of IF-fed birds was not significantly different from AL, there were significant differences between the different days of the feeding schedule. Overall, POMC also appeared to respond more to a long-term energy deficit than an acute one (Paper III). Expression of NPY was elevated above AL levels in fasting, suggesting it more closely reflects an acute lack of food in chickens. Although NPY expression was not associated with any carcass trait measured in that study (Paper III), it did show significant correlation with the expression of two hepatic gene clusters in a follow-up microarray study (Paper V). These clusters were heavily loaded with genes involved in lipid and carbohydrate metabolism and also correlated with the concentration of hepatic glycogen (Paper V).

The two other clusters in the microarray instead correlated with AgRP, POMC and

relative liver mass and were heavily loaded with genes associated with amino acid metabolism and cell cycle progression (Paper V). The expression patterns of the latter cluster appear to suggest a potential for increased hepatocyte proliferation in IF-fed birds but show the opposite pattern in CR (Paper V). Similar effects have been previously reported on the transcriptomic level in acutely fasted chickens (Désert et al., 2008), and matches reports of increased cell proliferation in the liver of fasted and re-fed rats (Kouda et al., 2004). In Paper V, a total of ten genes received special attention for showing a chronic (i.e. present in both fed and fasted states) over-expression in IF compared to the livers of both AL- and CR-fed birds. These ten genes also seem to be important for tissue plasticity in response to nutrient availability (Paper V) and are reminiscent of the beneficial effects of IF reported in mammals.

Also of interest is the effect on hepatic expression of genes involved in the regulation of circadian rhythm. Of special note is PER3, closely related to PER2 which has been associated with the peripheral clock involved in food anticipation behaviors in rodents (Thomas and Xue, 2018), and NR1D2 which is crucial for keeping a circadian rhythm in hepatic lipid metabolism (Bugge et al., 2012). Both of these genes are chronically over-expressed in the livers of IF birds compared to both the AL and CR treatments (Paper V). Although Red Junglefowl are not used in commercial chicken rearing of any kind, a better understanding of how feeding strategies and expected hunger affect the circadian rhythm of chickens would be important in the development of better behavioral welfare assessments for broiler breeders.

Theme 3: Intermittent fasting in broiler breeders

The final two papers of this thesis look at IF in broiler breeders (**Papers VI-VII**). While in Paper VI we tried to assess the on-farm welfare effects of implementing a 5:2 IF schedule, suggested in the literature to be a gentler form of IF than the traditional alternate-day fasting (Renema and Robinson, 2004), Paper VII is an attempt to disentangle the effects of the IF treatment from the level of feed restriction in lab-raised chickens.

Similar to the smaller birds in Papers I-II, birds fed an IF diet grew less muscle mass but instead displayed larger intestines, proventriculi, pancreases, livers and hearts at 12 weeks of age (Paper VI). This is surprising from an IF perspective, as mammalian studies typically suggest increased retention of lean mass in IF-fed animals compared to daily CR regimens (Anton et al., 2018) but may be the result of IF having a more growth-retarding effect as these animals are undergoing rapid juvenile growth, or simply an effect of poor protein-sparing capability in chickens (Belo et al., 1976). The growth-retarding interpretation is supported by the length of the tarsometatarsal bone, a commonly used indicator of frame size in chickens, also being shorter in IF birds (Paper VI). These birds also showed a higher degree of abdominal adiposity which is counter to the IF effects usually reported in mammals but may simply be the result of increased propensity for energy storage on feeding days in IF regimens similar to what we previously reported in Red Junglefowl (Paper III). Also in contrast

with most mammalian reports, we found a generally reduced insulin sensitivity in IF-fed birds that was independent of the level of feed restriction (Paper VII). This may have simply been an effect of higher endogenous insulin levels in IF birds under fed conditions effectively diluting the effect of the insulin challenge, or it could be a peculiarity of broiler chickens. A similar effect has been reported in hypercholesterolemic rats who, unlike wild-type rats, develop increased adiposity and reduced insulin sensitivity under IF conditions (Dorighello et al., 2014). It is however worth noting that the lower insulin sensitivity was not a chronic condition in our IF-fed chickens, as fasting IF birds responded to insulin injection with similar reductions in blood glucose as the daily-fed birds (Paper VII).

From the increased expression of circadian genes in the hepatic transcriptome of IF-fed Red Junglefowl (Paper III), it could be expected that IF birds would show increased signs of food anticipation as these behaviors have been linked to liver-based peripheral clocks (Thomas and Xue, 2018). Our farm-reared birds did, however, exhibit more anticipatory behavior before feeding time when fed on a daily restriction schedule. Daily-fed birds also displayed increased activity in the post-feeding period which may have been a sign of frustration as these birds received very limited amounts of feed and may not have been able to fulfill their need for foraging behaviors. Intermittently fasted birds displayed a similar activity increase around the expected feeding time on fasting days (Paper VI). Food anticipatory behaviors are a well-known response to feed restriction in both rodents (Thomas and Xue, 2018) and chickens (Kostal et al., 1992), and it is conceivable that the slightly relaxed restriction level implemented in the IF treatment to achieve matching growth curves for the two groups (Paper VI) was enough to reduce behavioral signs of food anticipation. This seems unlikely however, as birds fed to the 30% level in Paper VII, a restriction expected to be slightly less harsh than that applied in commercial broiler breeder rearing, showed daily activity patterns with clear peaks around feeding time. In contrast, birds fed to the 60% feeding level exhibited activity patterns corresponding to the light/dark cycle and did not show signs of food anticipation (Paper VII).

Although the time around feeding itself appears to be more stressful for daily-fed birds, many of the physiological effects of IF mimic the expected effects of chronically elevated CORT levels, which would suggest a more stressful situation overall for IF birds (Paper VI). These effects include the higher adiposity already mentioned as well as elevated HLRs, but the elevated eosinophil counts seen in IF does not fit the picture and it is possible that birds were either habituated to the IF regimen at 12 weeks of age, or that CORT-related effects in IF birds are part of a functional metabolic response rather than a sign of psychological stress (Paper VI). Another possibility is that the 5:2 fasting schedule, which imparts variation in the time between fasting days, is too complicated and causes psychological stress because of its unpredictability (Paper VI). Other behavioral tests did not reveal higher levels of perceived stress in IF, with no difference in TI duration (Paper VI) and no difference in perching frequency in fasting IF birds compared to daily-fed birds at restriction to 30% (Paper VII). Perching is expected to be a sign of reduced stress, as stress from

feeding frustration typically increases foraging efforts which cannot be carried out from a perch (Jones et al., 2004). At the commercial-like restriction level, only the fed IF condition stood out in terms of perching with these animals perching significantly more than others (Paper VII). In novel object tests carried out on the farm, fasting IF birds were significantly more explorative, as were birds tested in the afternoon compared to morning birds. While the test is validated for welfare assessment in laying hens with increased exploration of the object indicating reduced fear and improved welfare, our results may rather be the effect of increased risk-taking behaviors in energy-deficient animals and novel object exploration may represent a form of foraging effort in broiler breeders (Paper VI). Interestingly, IF birds were more prone to explore the object on fed days than were daily-fed birds even though they had received more feed, but we cannot rule out the possibility that this is a form of increased risk-taking in anticipation of future energy deficits (Paper VI). This may, however, still come with positive welfare effects for IF-fed birds, as rodents show reduced fear-learning when fasting (Verma et al., 2016) and thus stressful handling procedures may impose less stress when carried out on fasting days (Paper VI).

Conclusions

In conclusion, we still find it difficult to compare the welfare situation experienced by chickens under IF regimens compared to daily feeding and this is largely the effect of a lack of well-validated welfare indicators for feed-restricted broiler breeders (D'Eath et al., 2009). The comparison is made even more complicated by the fact that animal welfare in IF is expected to vary between fed and fasted days, which poses the question of to what extent positive experiences can be expected to counter-act negative ones (Duncan, 2002). Judging from the available data, however, we cannot see that IF regimens are obviously worse for animal welfare despite being illegal for this reason in several European countries. This conclusion comes from the fact that several of the expected welfare indicators used as part of this thesis, e.g. perching (Paper VII), overall activity (Paper VI) and hepatic energy reserves (Paper III), appear to indicate similar welfare in fasting IF conditions as in daily feeding with welfare improvements on fed IF days. At the same time, caution must clearly be exercised as we have also seen that physiological measurements tend to respond similarly across restriction levels even when behavioral indicators suggest very different welfare situations for the birds (Paper VII). This highlights the need for including behavioral measurements whenever attempts are made to extrapolate any information concerning animal welfare from physiological measurements. To this end, more information about how the behavioral differences between feeding and fasting days relate to welfare differences is sorely needed.

Finally, I would argue that it is time to start talking about how we can relax restriction for broiler breeders without severely impacting their health and fertility. The reduced growth in IF regimens has often been mentioned as a productivity drawback, but I would argue that it should be seen as a welfare-promoting opportunity. As an example, we were able to increase feed amounts by around 5% using a 5:2 IF schedule (Paper VI) while achieving similar growth rates, and measured bone lengths still suggested that the IF birds grew to a slightly smaller frame size. Although profit margins are allegedly small in the chicken industry, it does not make much sense to simultaneously say that broiler breeders are not economically important enough to majorly impact broiler breeding goals, while also trying to maximize their feed conversion ratios for increased profits. Although restriction to 30% of *ad libitum* may seem so extreme that slight relaxations of the restriction level wouldn't make much difference for animal welfare, it is important to remember that both we (Paper VII) and others (de Jong et al., 2003; Hocking et al., 1996) have demonstrated that the welfare optimum for broiler breeders is reached around 50% of *ad libitum* intake, and any increase in feeding bringing feed amounts closer to that 50% should be beneficial from an animal welfare standpoint.

Acknowledgments

The cover of this thesis bears only one name, but as always it has relied on the contributions of a vast number of people. I was extremely lucky to start this project with some truly amazing master students by my side, Andreas Calais and Johan Jönsson, and I can honestly say that without your hard work this little book would be much thinner. Since then Anouschka Middelkoop, Alexandra Johansson and Marloes Busscher have also made important contributions as part of their MSc projects for which I am very grateful.

To members of the CADE/Altimiras lab past and present, a big thank you for everything. Special thanks to Magnus for taking the time to teach me “chicken juggling” (not as bad as it sounds, I promise!), to Hanna for the grad school survival training, to “princess” Petros for your hard work and humor (you do know I never really tried to kill you, right?) and to John for more help and input than can fit on this page. And finally, of course, to my supervisor Jordi Altimiras. I guess most people pick a project for their PhD and end up with a supervisor, but I picked a supervisor instead and have not regretted it for a second. Thanks for all your work, for letting me (and sometimes forcing me to) be independent and for always supporting me when I needed it.

The biology department has been a great workplace over these last five(-ish) years, and there is no way I can name and thank all of you for the lunchroom laughs and good times. That said, a few people do deserve a special mention. Ann-Charlotte and Tove for noticing the little things and making sure that we all have felt seen and welcome. Jesper for never hesitating to step in when teaching got out of hand. Lejla for coming on a long road trip with us to do extremely tedious chicken work in near-tropical conditions on farm. And finally, Rebecca, Enya, Laura, Louise and Robin for helping out when my final experiment proved way too much for just my own set of hands.

Writing up a thesis is crazy making at times. Huge thanks to Robin for bringing chocolate, friendship and for proof-reading this thesis, to Gen for the whine-and-cinnamon-bun breaks and to Mattias for proof-reading from an outsider’s perspective and for pointing out that “hardly anyone will ever read the thesis anyway” when that was exactly what I needed to hear.

Last but certainly not least, thanks to Mattias, Lukas and the baby to come. You’ve given me more important things than a thesis to worry about, and what more can anyone really ask for? ♥

References

- Anton, S.D., Moehl, K., Donahoo, W.T., Marosi, K., Lee, S.A., Mainous, A.G., Leeuwenburgh, C., Mattson, M.P., 2018. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting: Flipping the Metabolic Switch. *Obesity* 26, 254–268. <https://doi.org/10.1002/oby.22065>
- Arrazola, A., Mosco, E., Widowski, T.M., Guerin, M.T., Kiarie, E.G., Torrey, S., 2019a. The effect of alternative feeding strategies for broiler breeder pullets: 1. Welfare and performance during rearing. *Poult. Sci.* 98, 3377–3390. <https://doi.org/10.3382/ps/pez170>
- Arrazola, A., Torrey, S., 2019. Conditioned place avoidance using encapsulated calcium propionate as an appetite suppressant for broiler breeders. *PLOS ONE* 14, e0206271. <https://doi.org/10.1371/journal.pone.0206271>
- Arrazola, A., Widowski, T.M., Guerin, M.T., Kiarie, E.G., Torrey, S., 2019b. The effect of alternative feeding strategies for broiler breeder pullets: 2. Welfare and performance during lay. *Poult. Sci.* pez447. <https://doi.org/10.3382/ps/pez447>
- Barbato, G.F., Siegel, P.B., Cherry, J.A., Nir, I., 1984. Selection for Body Weight at Eight Weeks of Age. 17. Overfeeding. *Poult. Sci.* 63, 11–18. <https://doi.org/10.3382/ps.0630011>
- Bartov, I., Bornstein, S., Lev, Y., Pines, M., Rosenberg, J., 1988. Feed Restriction in Broiler Breeder Pullets: Skip-a-Day Versus Skip-Two-Days. *Poult. Sci.* 67, 809–813. <https://doi.org/10.3382/ps.0670809>
- Belo, P.S., Romsos, D.R., Leveille, G.A., 1976. Blood Metabolites and Glucose Metabolism in the Fed and Fasted Chicken. *J. Nutr.* 106, 1135–1143. <https://doi.org/10.1093/jn/106.8.1135>
- Bennett, C.D., Leeson, S., 1989. Research Note: Growth of Broiler Breeder Pullets with Skip-a-Day Versus Daily Feeding. *Poult. Sci.* 68, 836–838. <https://doi.org/10.3382/ps.0680836>
- Berghman, L.R., 2016. Immune responses to improving welfare. *Poult. Sci.* 95, 2216–2218. <https://doi.org/10.3382/ps/pew159>
- Bessei, W., 2006. Welfare of broilers: a review. *Worlds Poult. Sci. J.* 62, 455–466. <https://doi.org/10.1079/WPS2005108>
- Bokkers, E.A.M., Koene, P., 2003. Eating behaviour, and preprandial and postprandial correlations in male broiler and layer chickens. *Br. Poult. Sci.* 44, 538–544. <https://doi.org/10.1080/00071660310001616165>
- Boswell, T., Dunn, I.C., 2017. Regulation of Agouti-Related Protein and Pro-Opiomelanocortin Gene Expression in the Avian Arcuate Nucleus. *Front. Endocrinol.* 8. <https://doi.org/10.3389/fendo.2017.00075>
- Bruggeman, V., Onagbesan, O., D'Hondt, E., Buys, N., Safi, M., Vanmontfort, D., Berghman, L., Vandesande, F., Decuypere, E., 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. *Poult. Sci.* 78, 1424–1434. <https://doi.org/10.1093/ps/78.10.1424>
- Bugge, A., Feng, D., Everett, L.J., Briggs, E.R., Mullican, S.E., Wang, F., Jager, J., Lazar, M.A., 2012. Rev-erb and Rev-erb coordinately protect the circadian clock and normal metabolic function. *Genes Dev.* 26, 657–667. <https://doi.org/10.1101/gad.186858.112>
- Butterworth, A., Arnould, C., Niekerk, T.F. (Eds.), 2009. Assessment protocol for poultry. Welfare Quality Consortium, Lelystad.
- Caraco, T., 1981. Energy budgets, risk and foraging preferences in dark-eyed juncos (*Junco hyemalis*). *Behav. Ecol. Sociobiol.* 8, 213–217. <https://doi.org/10.1007/BF00299833>
- Carpenter, R.G., Grossman, S.P., 1983. Plasma fat metabolites and hunger. *Physiol. Behav.* 30, 57–63. [https://doi.org/10.1016/0031-9384\(83\)90038-0](https://doi.org/10.1016/0031-9384(83)90038-0)
- Chavan, R., Feillet, C., Costa, S.S.F., Delorme, J.E., Okabe, T., Ripperger, J.A., Albrecht, U., 2016. Liver-derived ketone bodies are necessary for food anticipation. *Nat. Commun.* 7, 10580. <https://doi.org/10.1038/ncomms10580>
- Cheema, M., Qureshi, M., Havenstein, G., 2003. A comparison of the immune response of a

- 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. *Poult. Sci.* 82, 1519–1529. <https://doi.org/10.1093/ps/82.10.1519>
- Chen, S.E., McMurtry, J.P., Walzem, R.L., 2006. Overfeeding-Induced Ovarian Dysfunction in Broiler Breeder Hens Is Associated with Lipotoxicity. *Poult. Sci.* 85, 70–81. <https://doi.org/10.1093/ps/85.1.70>
- Cherel, Y., Le Maho, Y., 1985. Five months of fasting in king penguin chicks: body mass loss and fuel metabolism. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 249, R387–R392. <https://doi.org/10.1152/ajpregu.1985.249.4.R387>
- Cherel, Y., Stahl, J.-C., Maho, Y.L., 1987. Ecology and Physiology of Fasting in King Penguin Chicks. *The Auk* 104, 254–262. <https://doi.org/10.1093/auk/104.2.254>
- CIWF, 2019. Statistics: Broiler Chickens. <https://www.ciwf.org.uk/media/5235303/Statistics-Broiler-chickens.pdf>
- Council Directive, 2007. Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production.
- Dawkins, M., Layton, R., 2012. Breeding for better welfare: genetic goals for broiler chickens and their parents. *Anim. Welf.* 21, 147–155. <https://doi.org/10.7120/09627286.21.2.147>
- Dawkins, M.S., Cain, R., Merelie, K., Roberts, S.J., 2013. In search of the behavioural correlates of optical flow patterns in the automated assessment of broiler chicken welfare. *Appl. Anim. Behav. Sci.* 145, 44–50. <https://doi.org/10.1016/j.applanim.2013.02.001>
- de Beer, M., Coon, C.N., 2007. The Effect of Different Feed Restriction Programs on Reproductive Performance, Efficiency, Frame Size, and Uniformity in Broiler Breeder Hens. *Poult. Sci.* 86, 1927–1939. <https://doi.org/10.1093/ps/86.9.1927>
- de Beer, M., McMurtry, J.P., Brocht, D.M., Coon, C.N., 2008. An Examination of the Role of Feeding Regimens in Regulating Metabolism During the Broiler Breeder Grower Period. 2. Plasma Hormones and Metabolites. *Poult. Sci.* 87, 264–275. <https://doi.org/10.3382/ps.2007-00196>
- de Beer, M., Rosebrough, R.W., Russell, B.A., Poch, S.M., Richards, M.P., Coon, C.N., 2007. An Examination of the Role of Feeding Regimens in Regulating Metabolism During the Broiler Breeder Grower Period. 1. Hepatic Lipid Metabolism. *Poult. Sci.* 86, 1726–1738. <https://doi.org/10.1093/ps/86.8.1726>
- de Jong, I.C., Enting, H., van Voorst, A., Blokhuis, H.J., 2005. Do low-density diets improve broiler breeder welfare during rearing and laying? *Poult. Sci.* 84, 194–203. <https://doi.org/10.1093/ps/84.2.194>
- de Jong, I.C., van Voorst, A.S., Blokhuis, H.J., 2003. Parameters for quantification of hunger in broiler breeders. *Physiol. Behav.* 78, 773–783. [https://doi.org/10.1016/S0031-9384\(03\)00058-1](https://doi.org/10.1016/S0031-9384(03)00058-1)
- de Jong, I.C., Voorst, S.V., Ehlhardt, D.A., Blokhuis, H.J., 2002. Effects of restricted feeding on physiological stress parameters in growing broiler breeders. *Br. Poult. Sci.* 43, 157–168. <https://doi.org/10.1080/00071660120121355>
- D'Eath, R.B., Tolkamp, B.J., Kyriazakis, I., Lawrence, A.B., 2009. 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. *Anim. Behav.* 77, 275–288. <https://doi.org/10.1016/j.anbehav.2008.10.028>
- Decuyper, E., Bruggeman, V., Everaert, N., Li, Y., Boonen, R., De Tavernier, J., Janssens, S., Buys, N., 2010. The Broiler Breeder Paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. *Br. Poult. Sci.* 51, 569–579. <https://doi.org/10.1080/00071668.2010.519121>
- Désert, C., Duclos, M.J., Blavy, P., Lecerf, F., Moreews, F., Klopp, C., Aubry, M., Herault, F., Le Roy, P., Berri, C., Douaire, M., Diot, C., Lagarrigue, S., 2008. Transcriptome profiling of the feeding-to-fasting transition in chicken liver. *BMC Genomics* 9, 611. <https://doi.org/10.1186/1471-2164-9-611>
- Dorighello, G.G., Rovani, J.C., Luhman, C.J.F., Paim, B.A., Raposo, H.F., Vercesi, A.E., Oliveira, H.C.F., 2014. Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in

- hypercholesterolaemic mice. *Br. J. Nutr.* 111, 979–986.
<https://doi.org/10.1017/S0007114513003383>
- Duchamp, C., Barre, H., Delage, D., Rouanet, J.L., Cohen-Adad, F., Minaire, Y., 1989. Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 257, R744–R751.
<https://doi.org/10.1152/ajpregu.1989.257.4.R744>
- Duncan, I.J.H., 2002. Poultry welfare: Science or subjectivity? *Br. Poult. Sci.* 43, 643–652.
<https://doi.org/10.1080/0007166021000025109>
- Dunn, I.C., Wilson, P.W., Smulders, T.V., Sandilands, V., D'Eath, R.B., Boswell, T., 2013. Hypothalamic Agouti-Related Protein Expression Is Affected by Both Acute and Chronic Experience of Food Restriction and Re-Feeding in Chickens. *J. Neuroendocrinol.* 25, 920–928. <https://doi.org/10.1111/jne.12088>
- EFSA Panel on Animal Health and Welfare, 2010. Scientific Opinion on welfare aspects of the management and housing of the grand-parent and parent stocks raised and kept for breeding purposes. *EFSA J.* 8, 1667. <https://doi.org/10.2903/j.efsa.2010.1667>
- Gibson, A.A., Seimon, R.V., Lee, C.M.Y., Ayre, J., Franklin, J., Markovic, T.P., Caterson, I.D., Sainsbury, A., 2015. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis: Do ketogenic diets really suppress appetite? *Obes. Rev.* 16, 64–76.
<https://doi.org/10.1111/obr.12230>
- Girard, T.E., Zuidhof, M.J., Bench, C.J., 2017a. Aggression and social rank fluctuations in precision-fed and skip-a-day-fed broiler breeder pullets. *Appl. Anim. Behav. Sci.* 187, 38–44. <https://doi.org/10.1016/j.applanim.2016.12.005>
- Girard, T.E., Zuidhof, M.J., Bench, C.J., 2017b. Feeding, foraging, and feather pecking behaviours in precision-fed and skip-a-day-fed broiler breeder pullets. *Appl. Anim. Behav. Sci.* 188, 42–49. <https://doi.org/10.1016/j.applanim.2016.12.011>
- Griffin, A.M., Renema, R.A., Robinson, F.E., Zuidhof, M.J., 2005. The Influence of Rearing Light Period and the Use of Broiler or Broiler Breeder Diets on Forty-Two-Day Body Weight, Fleshing, and Flock Uniformity in Broiler Stocks. *J. Appl. Poult. Res.* 14, 204–216. <https://doi.org/10.1093/japr/14.2.204>
- Griffin, H.D., Goddard, C., 1994. Rapidly growing broiler (meat-type) chickens. Their origin and use for comparative studies of the regulation of growth. *Int. J. Biochem.* 26, 19–28. [https://doi.org/10.1016/0020-711X\(94\)90190-2](https://doi.org/10.1016/0020-711X(94)90190-2)
- Groscolas, R., 1986. Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. *J. Comp. Physiol. B* 156, 521–527. <https://doi.org/10.1007/BF00691038>
- Groscolas, R., Robin, J.-P., 2001. Long-term fasting and re-feeding in penguins. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 128, 643–653.
[https://doi.org/10.1016/S1095-6433\(00\)00341-X](https://doi.org/10.1016/S1095-6433(00)00341-X)
- Hadinia, S.H., Carneiro, P.R.O., Ouellette, C.A., Zuidhof, M.J., 2018. Energy partitioning by broiler breeder pullets in skip-a-day and precision feeding systems. *Poult. Sci.* 97, 4279–4289. <https://doi.org/10.3382/ps/pey283>
- Han, P.F.-S., Smyth, J.R., 1972. The Influence of Growth Rate on the Development of Marek's Disease in Chickens. *Poult. Sci.* 51, 975–985. <https://doi.org/10.3382/ps.0510975>
- Havenstein, G., Ferket, P., Qureshi, M., 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poult. Sci.* 82, 1509–1518. <https://doi.org/10.1093/ps/82.10.1509>
- Hocking, P.M., 2006. High-fibre pelleted rations decrease water intake but do not improve physiological indexes of welfare in food-restricted female broiler breeders. *Br. Poult. Sci.* 47, 19–23. <https://doi.org/10.1080/00071660500468041>
- Hocking, P.M., 1993. Welfare of broiler breeder and layer females subjected to food and water control during rearing: Quantifying the degree of restriction. *Br. Poult. Sci.* 34, 53–64. <https://doi.org/10.1080/00071669308417562>
- Hocking, P.M., Maxwell, M.H., Mitchell, M.A., 1996. Relationships between the degree of food restriction and welfare indices in broiler breeder females. *Br. Poult. Sci.* 37, 263–278. <https://doi.org/10.1080/00071669608417858>
- Hocking, P.M., Maxwell, M.H., Robertson, G.W., Mitchell, M.A., 2001. Welfare assessment of

- modified rearing programmes for broiler breeders. *Br. Poult. Sci.* 42, 424–432. <https://doi.org/10.1080/00071660120070677>
- Hocking, P.M., Waddington, D., Walker, M.A., Gilbert, A.B., 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. *Br. Poult. Sci.* 30, 161–173. <https://doi.org/10.1080/00071668908417134>
- Jones, E.K.M., Zaczek, V., MacLeod, M., Hocking, P.M., 2004. Genotype, dietary manipulation and food allocation affect indices of welfare in broiler breeders. *Br. Poult. Sci.* 45, 725–737. <https://doi.org/10.1080/00071660400014226>
- Katanbaf, M.N., Dunnington, E.A., Siegel, P.B., 1989. Restricted feeding in early and late-feathering chickens. 1. Growth and physiological responses. *Poult. Sci.* 68, 344–351. <https://doi.org/10.3382/ps.0680344>
- Kiarie, E., Romero, L.F., Ravindran, V., 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase1. *Poult. Sci.* 93, 1186–1196. <https://doi.org/10.3382/ps.2013-03715>
- Kiarie, E., Walsh, M.C., Romero, L.F., Arent, S., Ravindran, V., 2017. Nutrient and fiber utilization responses of supplemental xylanase in broiler chickens fed wheat based diets are independent of the adaptation period to test diets2. *Poult. Sci.* 96, 3239–3245. <https://doi.org/10.3382/ps/pex100>
- Knowles, T.G., Kestin, S.C., Haslam, S.M., Brown, S.N., Green, L.E., Butterworth, A., Pope, S.J., Pfeiffer, D., Nicol, C.J., 2008. Leg Disorders in Broiler Chickens: Prevalence, Risk Factors and Prevention. *PLoS ONE* 3, e1545. <https://doi.org/10.1371/journal.pone.0001545>
- Korte, S.M., Olivier, B., Koolhaas, J.M., 2007. A new animal welfare concept based on allostasis. *Physiol. Behav.* 92, 422–428. <https://doi.org/10.1016/j.physbeh.2006.10.018>
- Kostal, L., Savory, C.J., Hughes, B.O., 1992. Diurnal and individual variation in behaviour of restricted-fed broiler breeders. *Appl. Anim. Behav. Sci.* 32, 361–374. [https://doi.org/10.1016/S0168-1591\(05\)80028-0](https://doi.org/10.1016/S0168-1591(05)80028-0)
- Kouda, K., Nakamura, H., Kohno, H., Ha-Kawa, S.K., Tokunaga, R., Sawada, S., 2004. Dietary restriction: effects of short-term fasting on protein uptake and cell death/proliferation in the rat liver. *Mech. Ageing Dev.* 125, 375–380. <https://doi.org/10.1016/j.mad.2004.02.006>
- Le Ninan, F., Cherel, Y., Robin, J.-P., Leloup, J., Le Maho, Y., 1988a. Early changes in plasma hormones and metabolites during fasting in king penguin chicks. *J. Comp. Physiol. B* 158, 395–401. <https://doi.org/10.1007/BF00691136>
- Le Ninan, F., Cherel, Y., Sardet, C., Le Maho, Y., 1988b. Plasma hormone levels in relation to lipid and protein metabolism during prolonged fasting in king penguin chicks. *Gen. Comp. Endocrinol.* 71, 331–337. [https://doi.org/10.1016/0016-6480\(88\)90261-4](https://doi.org/10.1016/0016-6480(88)90261-4)
- Lees, J.J., Lindholm, C., Batakis, P., Busscher, M., Altimiras, J., 2017. The physiological and neuroendocrine correlates of hunger in the Red Junglefowl (*Gallus gallus*). *Sci. Rep.* 7, 17984. <https://doi.org/10.1038/s41598-017-17922-w>
- Leeson, S., Summers, J.D., 1985. Effect of Cage Versus Floor Rearing and Skip-a-Day Versus Every-Day Feed Restriction on Performance of Dwarf Broiler Breeders and Their Offspring. *Poult. Sci.* 64, 1742–1749. <https://doi.org/10.3382/ps.0641742>
- Leshchinsky, T.V., Klasing, K.C., 2001. Divergence of the inflammatory response in two types of chickens. *Dev. Comp. Immunol.* 25, 629–638. [https://doi.org/10.1016/S0145-305X\(01\)00023-4](https://doi.org/10.1016/S0145-305X(01)00023-4)
- Leung, H., Arrazola, A., Torrey, S., Kiarie, E., 2018. Utilization of soy hulls, oat hulls, and flax meal fiber in adult broiler breeder hens. *Poult. Sci.* 97, 1368–1372. <https://doi.org/10.3382/ps/pex434>
- Li, C., Hu, Q., Lesuisse, J., Schallier, S., Bautil, A., Lamberigts, C., Driessen, B., Everaert, N., Lin, H., Buyse, J., 2019. The effect of reduced balanced protein diet on the behavior of female broiler breeders in 2 generations. *Poult. Sci.* 98, 4301–4312. <https://doi.org/10.3382/ps/pez347>

- Lilburn, M.S., Loeffler, S., 2015. Early intestinal growth and development in poultry. *Poult. Sci.* 94, 1569–1576. <https://doi.org/10.3382/ps/pev104>
- Lindholm, C., Altimiras, J., Lees, J., 2019. Measuring ketones in the field: rapid and reliable measures of β -hydroxybutyrate in birds. *Ibis* 161, 205–210. <https://doi.org/10.1111/ibi.12643>
- Lindholm, C., Calais, A., Jönsson, J., Yngwe, N., Berndtson, E., Hult, E., Altimiras, J., 2015. Slow and steady wins the race? No signs of reduced welfare in smaller broiler breeder hens at four weeks of age. *Anim. Welf.* 24, 447–454. <https://doi.org/10.7120/09627286.24.4.447>
- Lindholm, C., Johansson, A., Middelkoop, A., Lees, J.J., Yngwe, N., Berndtson, E., Cooper, G., Altimiras, J., 2018. The Quest for Welfare-Friendly Feeding of Broiler Breeders: Effects of Daily vs. 5:2 Feed Restriction Schedules. *Poult. Sci.* 97, 368–377. <https://doi.org/10.3382/ps/pex326>
- Lindholm, C., Jönsson, J., Calais, A., Middelkoop, A., Yngwe, N., Berndtson, E., Lees, J.J., Hult, E., Altimiras, J., 2017. Growth heterogeneity in broiler breeder pullets is settled before the onset of feed restriction but is not predicted by size at hatch. *J. Anim. Sci.* 95, 182–193. <https://doi.org/10.2527/jas.2016.0929>
- Mattson, M.P., Moehl, K., Ghena, N., Schmaedick, M., Cheng, A., 2018. Intermittent metabolic switching, neuroplasticity and brain health. *Nat. Rev. Neurosci.* 19, 80–80. <https://doi.org/10.1038/nrn.2017.156>
- Mench, J.A., 2002. Broiler breeders: feed restriction and welfare. *Worlds Poult. Sci. J.* 58, 23–29. <https://doi.org/10.1079/WPS20020004>
- Morrissey, K.L.H., Widowski, T., Leeson, S., Sandilands, V., Arnone, A., Torrey, S., 2014. The effect of dietary alterations during rearing on growth, productivity, and behavior in broiler breeder females. *Poult. Sci.* 93, 285–295. <https://doi.org/10.3382/ps.2013-03265>
- Nielsen, B.L., Thodberg, K., Malmkvist, J., Steinfeldt, S., 2011. Proportion of insoluble fibre in the diet affects behaviour and hunger in broiler breeders growing at similar rates. *Animal* 5, 1247–1258. <https://doi.org/10.1017/S1751731111000218>
- Noy, Y., Sklan, D., 1999. Energy utilization in newly hatched chicks. *Poult. Sci.* 78, 1750–1756. <https://doi.org/10.1093/ps/78.12.1750>
- Olkowski, A.A., Wojnarowicz, C., Nain, S., Ling, B., Alcorn, J.M., Laarveld, B., 2008. A study on pathogenesis of sudden death syndrome in broiler chickens. *Res. Vet. Sci.* 85, 131–140. <https://doi.org/10.1016/j.rvsc.2007.08.006>
- Pedersen, I., Forkman, B., 2019. Improving Leg Health in Broiler Chickens: A Systematic Review of the Effect of Environmental Enrichment. *Anim. Welf.* 28, 215–230. <https://doi.org/10.7120/09627286.28.2.215>
- Pollock, D., 1999. A geneticist's perspective from within a broiler primary breeder company. *Poult. Sci.* 78, 414–418. <https://doi.org/10.1093/ps/78.3.414>
- Powell, T.S., Gehle, M.H., 1976. Effect of Various Pullet Restriction Methods on Performance of Broiler Breeders. *Poult. Sci.* 55, 502–509. <https://doi.org/10.3382/ps.0550502>
- Puchalska, P., Crawford, P.A., 2017. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metab.* 25, 262–284. <https://doi.org/10.1016/j.cmet.2016.12.022>
- Rees, A., Harvey, S., Phillips, J.G., 1985. Adrenocortical responses to novel stressors in acutely or repeatedly starved chickens. *Gen. Comp. Endocrinol.* 59, 105–109. [https://doi.org/10.1016/0016-6480\(85\)90424-1](https://doi.org/10.1016/0016-6480(85)90424-1)
- Renema, R.A., Robinson, F.E., 2004. Defining normal: comparison of feed restriction and full feeding of female broiler breeders. *Worlds Poult. Sci. J.* 60, 508–522. <https://doi.org/10.1079/WPS200434>
- Renema, R.A., Rustad, M.E., Robinson, F.E., 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. *Worlds Poult. Sci. J.* 63, 457–472. <https://doi.org/10.1017/S0043933907001572>
- Robin, J.P., Frain, M., Sardet, C., Groscolas, R., Le Maho, Y., 1988. Protein and lipid utilization during long-term fasting in emperor penguins. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 254, R61–R68.

- <https://doi.org/10.1152/ajpregu.1988.254.1.R61>
- Robinson, F.E., Yu, M.W., Lupicki, M.E., Hardin, R.T., 1993. Short-term consequences of a sudden increase in feed allowance in broiler breeder hens. *Can. J. Anim. Sci.* 73, 159–167. <https://doi.org/10.4141/cjas93-015>
- Rushen, J., 2003. Changing concepts of farm animal welfare: bridging the gap between applied and basic research. *Appl. Anim. Behav. Sci.* 81, 199–214. [https://doi.org/10.1016/S0168-1591\(02\)00281-2](https://doi.org/10.1016/S0168-1591(02)00281-2)
- Sandilands, V., Tolkamp, B., Kyriazakis, I., 2005. Behaviour of food restricted broilers during rearing and lay—effects of an alternative feeding method. *Physiol. Behav.* 85, 115–123. <https://doi.org/10.1016/j.physbeh.2005.03.001>
- Sandilands, V., Tolkamp, B.J., Savory, C.J., Kyriazakis, I., 2006. Behaviour and welfare of broiler breeders fed qualitatively restricted diets during rearing: Are there viable alternatives to quantitative restriction? *Appl. Anim. Behav. Sci.* 96, 53–67. <https://doi.org/10.1016/j.applanim.2005.04.017>
- Savory, C.J., Hocking, P.M., Mann, J.S., Maxwell, M.H., 1996. Is Broiler Breeder Welfare Improved by Using Qualitative Rather Than Quantitative Food Restriction to Limit Growth Rate? *Anim. Welf.* 5, 105–127.
- Savory, C.J., Lariviere, J., 2000. Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. *Appl. Anim. Behav. Sci.* 69, 135–147.
- Seroussi, E., Cinnamon, Y., Yosefi, S., Genin, O., Smith, J.G., Rafati, N., Bornelöv, S., Andersson, L., Friedman-Einat, M., 2016. Identification of the Long-Sought Leptin in Chicken and Duck: Expression Pattern of the Highly GC-Rich Avian *leptin* Fits an Autocrine/Paracrine Rather Than Endocrine Function. *Endocrinology* 157, 737–751. <https://doi.org/10.1210/en.2015-1634>
- Skinner-Noble, D.O., Teeter, R.G., 2009. An examination of anatomic, physiologic, and metabolic factors associated with well-being of broilers differing in field gait score. *Poult. Sci.* 88, 2–9. <https://doi.org/10.3382/ps.2006-00450>
- Statens jordbruksverk, 2010. Statens jordbruksverks föreskrifter och allmänna råd om djurhållning inom lantbruket m.m. SJVFS 2010:15, saknr L 100.
- Thomas, M.A., Xue, B., 2018. Mechanisms for AgRP neuron-mediated regulation of appetitive behaviors in rodents. *Physiol. Behav.* 190, 34–42. <https://doi.org/10.1016/j.physbeh.2017.10.006>
- van de Ven, L.J.F., van Wagenberg, A.V., Groot Koerkamp, P.W.G., Kemp, B., van den Brand, H., 2009. Effects of a combined hatching and brooding system on hatchability, chick weight, and mortality in broilers. *Poult. Sci.* 88, 2273–2279. <https://doi.org/10.3382/ps.2009-00112>
- Van Krimpen, M.M., De Jong, I.C., 2014. Impact of nutrition on welfare aspects of broiler breeder flocks. *Worlds Poult. Sci. J.* 70, 139–150. <https://doi.org/10.1017/S0043933914000129>
- Veissier, I., Boissy, A., 2007. Stress and welfare: Two complementary concepts that are intrinsically related to the animal's point of view. *Physiol. Behav.* 92, 429–433. <https://doi.org/10.1016/j.physbeh.2006.11.008>
- Verma, D., Wood, J., Lach, G., Herzog, H., Sperk, G., Tasan, R., 2016. Hunger Promotes Fear Extinction by Activation of an Amygdala Microcircuit. *Neuropsychopharmacology* 41, 431–439. <https://doi.org/10.1038/npp.2015.163>
- Warriss, P.D., Kestin, S.C., Brown, S.N., Bevis, E.A., 1988. Depletion of glycogen reserves in fasting broiler chickens. *Br. Poult. Sci.* 29, 149–154. <https://doi.org/10.1080/00071668808417036>
- Weeks, C.A., Danbury, T.D., Davies, H.C., Hunt, P., Kestin, S.C., 2000. The behaviour of broiler chickens and its modification by lameness. *Appl. Anim. Behav. Sci.* 67, 111–125. [https://doi.org/10.1016/S0168-1591\(99\)00102-1](https://doi.org/10.1016/S0168-1591(99)00102-1)
- Wideman, R.F., Rhoads, D.D., Erf, G.F., Anthony, N.B., 2013. Pulmonary arterial hypertension (ascites syndrome) in broilers: A review. *Poult. Sci.* 92, 64–83. <https://doi.org/10.3382/ps.2012-02745>
- Yi, J., Yuan, J., Gilbert, E.R., Siegel, P.B., Cline, M.A., 2017. Differential expression of

- appetite-regulating genes in avian models of anorexia and obesity. *J. Neuroendocrinol.* 29, e12510. <https://doi.org/10.1111/jne.12510>
- Yunis, R., Ben-David, A., Heller, E.D., Cahaner, A., 2000. Immunocompetence and Viability Under Commercial Conditions of Broiler Groups Differing in Growth Rate and In Antibody Response to *Escherichia coli* Vaccine. *Poult. Sci.* 79, 810–816. <https://doi.org/10.1093/ps/79.6.810>
- Zuidhof, M.J., 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. *Poult. Sci.* 97, 3921–3937. <https://doi.org/10.3382/ps/pey252>
- Zuidhof, M.J., Fedorak, M.V., Ouellette, C.A., Wenger, I.I., 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. *Poult. Sci.* 96, 2254–2263. <https://doi.org/10.3382/ps/pex013>
- Zuidhof, M.J., Robinson, F.E., Feddes, J.J.R., Hardin, R.T., Wilson, J.L., McKay, R.I., Newcombe, M., 1995. The Effects of Nutrient Dilution on the Well-Being and Performance of Female Broiler Breeders. *Poult. Sci.* 74, 441–456. <https://doi.org/10.3382/ps.0740441>
- Zuidhof, M.J., Schneider, B.L., Carney, V.L., Korver, D.R., Robinson, F.E., 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poult. Sci.* 93, 2970–2982. <https://doi.org/10.3382/ps.2014-04291>

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-160814>

FACULTY OF SCIENCE AND ENGINEERING

Linköping Studies in Science and Technology, Dissertation No. 2005, 2019
Department of Physics, Chemistry and Biology (IFM)

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se