**Implications of altering maternal feed availability and feeding system on offspring performance**

by

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**Abstract**

In the pursuit of increasing the efficiency of hatching egg production the poultry industry may have inadvertently decreased the growth potential of the broiler. Research has found that matching the offspring feeding environment to the maternal feeding environment has beneficial effects on the final offspring BW. This project consisted of 2 experiments, each used offspring from 2 maternal treatments (**MT**), 2 sexes and 3 feeding treatments. In experiment 1, the MT were Ross 708 broiler breeder hens raised on standard breeder-recommended target BW (**SBW**), or 121% of SBW (**HBW**). In experiment 2, the MT was Cobb grandparent breeder hens fed using a conventional (**CON**) or a precision feeding system (**PF**). Both broiler trials were organized in a 2 x 2 x 3 factorial arrangement of treatments with 2 MT treatments, 2 sexes, and 3 feeding treatments. Broilers from experiment 1 were housed in cages and fed using conventional feeding methods. Broiler from experiment 2 were housed in floor pens and fed using precision feeding techniques. Broilers from both experiments were fed ad libitum until d 28. From 29 to 42 d of age, they were provided feed ad libitum (**AL**), or at 80 or 60% of AL. At 28, 35 and 42 d of age, carcass yields were determined after dissection. Blood was also collected for hormone analysis. The circulating levels of $T_4$ were 11.7% higher in HBW offspring than in SBW offspring, suggesting that the metabolic system of the bird was trying to stimulate growth in an attempt to reach the birds growth potential because $T_4$ is known to be associated with increased growth. Offspring BW for trial 1 was 4.0% higher in the HBW offspring compared to the SBW offspring. There were no MT effects on BW in experiment 2. These led us to conclude that raising broiler breeder target BW could have a positive effect on the performance of their offspring by increasing final BW.

**Preface**

This thesis is an original work by Katelyn Humphreys. Funding for this project was provided by Alberta Agriculture and Forestry and Cargill, Inc. Publication is intended for chapters 3 and 4 with co-authors S.A.S. van der Klein, L.E. Ellestad, J.L. Aalhus, and M.J. Zuidhof. The research projects included as part of this thesis were approved by the University of Alberta Research Ethics Board, AUP00000121.

**Dedication**

Before I started this project, I was under the impression that the most significant information that I would learn would be related to maternal nutrition and the effect that it has on offspring performance. I did learn a great deal about chickens and I can confidently say that I know more than I did before I started but it was not the most significant thing that I discovered. The most valuable information that I took away from this program was about myself, what I can accomplish and how valuable it is to have good people around you. I would not have been able to be as successful as I was without all the wonderful people that helped me along the way. I do not think that I could ever do enough to thank them for everything that they have done for me.

To anyone that is doing a Masters degree: It may seem frivolous in the moment but the true value of the process cannot be seen until it is complete.

**Acknowledgments**

Of the many excellent and enthusiastic educators I had the pleasure of learning from in the 7 years that I was in university, Dr. Martin Zuidhof has had the most significant impact on my university career. His enthusiasm and love for teaching and agriculture is infectious and unavoidable. I would like to thank Dr. Martin Zuidhof for his unconditional support through out this whole process. I appreciate beyond words the patience and constant help he was able to provide for me. He has taught me many things that go far beyond poultry production. I would also like to thank Dr. Zuidhof for giving me the opportunity to travel; I have seen many places and met many wonderful people and none of that would have been possible without you.

To my parents, I would not have had all the opportunities that led me to where I am now if it wasn't for everything that both of you have done for me. I will never be able to put into words how much I appreciate the unconditional love and support that you have given me.

To Sasha, thank you for putting up with all my questions and problems at all hours of the day. Your guidance and mentorship made this process much more bearable.

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**List of Abbreviations**

| | |
|---|---|
| ACTH | Adrenocorticotropic Hormone |
| ADFI | Average Daily Feed Intake |
| ADG | Average Daily Gain |
| AL | Ad Libitum |
| BW | Body Weight |
| ℃ | Degrees Celsius |
| CON | Conventionally Fed |
| CRH | Corticotropin Releasing Hormone |
| CORT | Corticosterone |
| d | Day |
| dL | Deciliter |
| DOA | Days of Age |
| D1 | Type 1 Deiodinase |
| D2 | Type 2 Deiodinase |
| D3 | Type 3 Deiodinase |
| FCR | Feed Conversion Ratio |
| FI | Feed Intake |
| *g* | gram |
| G6P | Glucose-6-Phosphate |
| GAS | General Adaptation Syndrome |
| GH | Growth Hormone |
| GHRH | Growth Hormone-releasing Hormone |
| G:I | Glucagon to insulin ratio |
| GnRH | Gonadotropin-releasing Hormone |
| GP | Glucidic Potential |
| HBW | High Body Weight |
| HRP | Horseradish Peroxidase |
| IGF-1 | Insulin like Growth Factor |
| mL | Millilitre |
| mM | Millimol |
| MT | Maternal Treatment |
| *ng* | Nanogram |
| PF | Precision Fed |
| *pg* | Picogram |
| PRS | Precision Feeding Station |
| rpm | Rotations per Minute |
| $rT_3$ | 3,3',5' - triiodothyronine |
| SBW | Standard Body Weight |
| $T_3$ | Triiodothyronine |
| $T_4$ | Thyroxine |
| TMB | 3,3',5,5'-Tetramethylbenzidine |
| TRH | Thyroid Releasing Hormone |
| TSH | Thyroid Stimulating Hormone |

| | |
|---|---|
| uIU | micro-International Units |
| µL | Microliter |
| µmol.$g^{-1}$ | micro-Mol per Gram |

## 1.0 Introduction

Modern broiler breeders are subjected to feed restriction in an attempt to reduce their growth rate and increase their reproductive efficiency, while their broiler offspring are given full access to feed. In the chicken, it has been found that increasing the BW of the mother can significantly increase the final BW of offspring (van Emous et al., 2015). Similar patterns have been observed in humans, obese mothers tend to have obese children (Herrera et al., 2011). It has been discovered that mismatching the maternal and offspring nutritional environment of broiler breeders and broilers (restricted and non-restricted feeding treatments) can reduce the growth observed in broilers by as much as 1.3%, as well as increasing the proportion of abdominal fat as a proportion of the carcass by at least 50% (van der Waaij et al., 2011). It is common practice in the broiler industry to feed broilers at their ad libitum level of intake. However, broiler breeders are often restricted to 25 – 35% of their ad libitum intake (de Jong et al., 2002). If what van der Waaij et al. (2011) discovered is true, this may be leading to reduced productivity in broilers.

If the poultry industry was able to more fully understand how maternal feeding affects the growth and development of the offspring, it might be used to increase the efficiency of broilers. The primary objectives of this thesis were:

1) To review how hormones associated with growth and metabolism affect broiler growth and how these hormones can be measured, as well as, a review of how the maternal environment affects offspring performance (Chapter 2).
2) To determine the effect of maternal body weight on offspring performance (Experiment 1).

3) To determine the effect of maternal feeding system on offspring performance (Experiment 2).
4) To summarize the finding of this thesis as a whole (Chapter 5).

A literature review of the hormonal systems associated with growth and metabolism in the chicken, as well as the effect of maternal environment on offspring performance is given in chapter 2 of this thesis. Chapter 3 describes the effect of both maternal studies (maternal body weight and maternal feeding system) on offspring performance. Chapter 4 describes the effect of maternal body weight and maternal feeding system on the hormones affecting growth and metabolism of the chicken. Chapter 5 is a synthesis of all aspects of this thesis and recommendations for changes in broiler breeder management.

## 1.1 Experimental Objectives

The main objective of this thesis was to determine the effect of maternal environment of offspring performance in broiler chickens. Within the main objective, each experiment that was done had its own set of objectives, which included the comparison of the effects of standard and high maternal body weight on offspring performance, as well as, a comparison of a conventional and precision fed maternal feeding methods on offspring performance. The specific experimental objectives were:

1) To determine the effect of increasing the maternal body weight on offspring performance (Experiment 1).
2) To determine the effect of raising the maternal body weight on the circulating level of growth hormone, insulin-like growth factor 1, corticosterone, glucagon, triiodothyronine, thyroxine and insulin in offspring (Experiment 1).
3) To determine the effect of maternal feeding system on the growth performance of offspring (Experiment 2).
4) To determine the effect of maternal feeding system on the circulating levels of hormones growth hormone, insulin-like growth factor 1, corticosterone, glucagon, triiodothyronine, thyroxine and insulin in offspring (Experiment 2).

## 1.2 Works Cited

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# 2.0 Literature Review

## 2.1 Introduction to Endocrine Control of Growth

The endocrine system is comprised of hormones and glands that are used to communicate throughout the body and is the most complicated body system that the avian possesses (Scanes, 2015a). Research is constantly being done to increase our understanding of how the endocrine system functions, we are constantly discovering previously unknown functions for hormones. It has become obvious that there are many factors that influence the workings of the endocrine system, they are often a combination of individual and environmental factors. Environmental factors that influence the endocrine system include: temperature, light, humidity, social interaction, stressors, feed quality and quantity, water quality and quantity, among many others (Scanes, 2015a). Individual factors that influence the endocrine system include: epigenetic factors passed down from the parents, genetic abnormalities, disease and individual behaviors (Dupont et al., 2009; Scanes, 2009; Scanes, 2015a).

Hormones are signaling molecules produced by various glands in the body that are transported through the circulatory system. They are used to regulate metabolism, digestion, growth, reproduction, mood, sleeping habits and eating habits (Scanes, 2015a). Hormones in the body tend to have multiple functions and work in close relation with other hormones. The most well-known example of hormones working together to maintain homeostasis is insulin and glucagon, which will be examined in the next paragraph. The main endocrine organ that controls hormone secretion is the hypothalamus. The hypothalamus secretes thyrotropin-releasing hormone (**TRH**), gonadotropin-releasing hormone (**GnRH**), growth hormone-releasing hormone (**GHRH**) and corticotropin-releasing hormone (**CRH**), among many others (Scanes, 2015a). The anterior pituitary gland is acted on by the hormones of the hypothalamus to secrete growth hormone (**GH**), thyroid-stimulating hormone, adrenocorticotropic hormone (**ACTH**) and many

others. A delicate mixture of GH, insulin-like growth factor-1 (**IGF-1**) and the thyroid hormones are thought to be required for successful growth (Scanes et al., 1986; Goddard et al., 1988). This review will focus on the hormones that are responsible for growth and metabolism in the chicken.

**2.2 Blood Glucose, Insulin and Glucagon**

Glucose is an important component of muscle and brain function and can cause severe cellular damage when levels are out of the optimal range so therefore is highly regulated within the body. The normal level of plasma glucose in the chicken is between 200 and 250 mg/dL (Scanes, 2015b). After a chicken consumes a meal, the glucose concentration in the bloodstream increases. Glucose is used as an energy source by the chicken. After a meal, plasma glucose levels return to basal levels within an hour (Scanes, 2015b). Insulin is a hormone produced by the islet cells of the pancreas and is secreted when circulating blood glucose levels are high and functions to lower the level of blood glucose by allowing cells in the body to store glucose for energy (Scanes, 2015b). Insulin also directs the body to store glucose in the form of glycogen for later use. When glucose is absorbed out of the bloodstream it is usually converted into glycogen through the process of glycogenesis or it is turned into fats through the process of lipogenesis. In birds and mammals, insulin has been found to serve the same function. Extensive research on the effect of feed restriction on the function of insulin has been reported. Feed restriction has no consistent effect on insulin levels in blood plasma (Krestel-Rickert et al., 1986). Goddard et al. (1988) reported higher levels of plasma insulin level in chickens that are selected for high growth rate when compared to low growth rate chickens. It is hypothesized that because the body has tight control on blood glucose levels, there is little variation in circulating insulin levels, regardless of changes in feed availability. If glucose levels are out of their normal range, either

too high or too low, it can cause serious health problems for the individual and for this reason, the body keeps glucose under tight controls using insulin and its antagonist, glucagon.

Glucagon is a hormone that works in opposition to insulin and is produced by the alpha cells of the pancreas (Scanes, 2015a). Glucagon is the most aggressive stimulator of lipolysis in the chicken. When circulating blood glucose levels are low, glucagon acts on liver cells to cause glycogenolysis and release it into the bloodstream (Scanes, 2015a). Research has found that 6 hours after feed restriction was implemented, plasma glucagon levels increased and stayed elevated for 12, 18, and 24 hours after fasting began (Christensen et al., 2013). Christensen et al. (2013) also found that plasma glucagon levels rose 3.5 to 3.7-fold 6 h after feed restriction began. When talking about glucagon and insulin some researchers like to report their results as the glucagon to insulin (**G:I**) ratio. At a high G:I, ratio glucagon is being used to mobilize glycogen and increase the concentration of glucose in the bloodstream. The rate of gluconeogenesis, glycogenolysis and fat breakdown also increase with a high G:I ratio. A low G:I ratio will result in the blood concentration of glucose decreasing and the amount of glucose being stored as glycogen to increase.

### 2.3 Thyroid Hormones

Both triiodothyronine (**$T_3$**) and thyroxine (**$T_4$**) are hormones released by the thyroid gland. For thyroid hormone (**TH**) release to occur, the hypothalamus releases thyrotropin releasing hormone (**TRH**), which stimulates the anterior pituitary gland to release thyroid stimulating hormone (**TSH**). TSH stimulates the thyroid gland to release TH. A chicken's thyroid gland predominantly releases $T_4$. $T_4$ is then de-iodinated by type 1 deiodinase (**D1**) or type 2 deiodinase (**D2**) to form $T_3$, a more active substance and responsible for most of the changes in the chicken than $T_4$ (Klandorf et al., 1981). The conversion of $T_4$ into $T_3$ happens in the thyroid gland, liver,

kidney, and target tissues. When the conversion occurs in the target tissue it would not be detectable in the plasma. $T_4$ can also be de-iodinated by D1 or type 3 deiodinase (**D3**) to form reverse $T_3$ (**r$T_3$**). $T_3$ when compared with $T_4$ plays a larger role in $O_2$ consumption (Bobek et al., 1977). $T_3$ can be degraded into 3,5-diiodo-L-thyronine (**$T_2$**), which is a less active substance than $T_3$ and $T_4$ (Darras et al., 2000). The TH are involved with metabolic regulation and their functions are especially important during times of negative energy balance (Gyorffy et al., 2009; Rimbach et al., 2016). Recently, $T_3$ has been found to be in higher concentrations in the plasma of high growth rate birds when compared to the plasma of low growth rate birds (Xiao et al., 2017). Thyroid hormones involvement in growth has also been demonstrated by a reduction in the growth of thyroidectomized birds (Scanes, 2015a). However, when chickens with a normal thyroid gland were given exogenous $T_4$ or $T_3$, a negative effect on growth was observed (Decuypere et al., 1987). Therefore, we can hypothesize that the thyroid hormones are necessary for growth, but not sufficient in and of themselves to promote growth. If $T_4$ and $T_3$ were wholly involved with increasing growth, exogenous administration of either hormone would result in an increase in growth. Feed restriction significantly lowers the circulating $T_3$ and $T_4$ levels when compared with unrestricted birds (Zhan et al., 2007; Gyorffy et al., 2009). $T_3$ and $T_4$ are known to be involved in the regulation of metabolism so the decrease in circulating concentration of those hormones may cause a reduction in metabolism that is the result of feed restriction and the need to conserve energy.

### 2.4 Growth Hormone

Growth hormone (**GH**) is a peptide hormone produced by the anterior pituitary in response to the release of Growth hormone releasing hormone (**GHRH**) and TRH by the hypothalamus. Receptors for GH are located on tissues throughout the body, including the liver, muscle and

adipose tissue. The release of GH is controlled through a negative feedback system. Elevated GH levels will reduce the release of GHRH and increase the release of somatostatin. The increase in somatostatin level will further reduce the level of GHRH, this will cause the level of GH to decrease eventually relieving the negative feedback system. GH is thought to be involved in cell growth, reproduction and regeneration. An early study that examined GH found that in the early growth period of the chicken, plasma concentration of GH increased and as the birds became older, the concentration decreased (Scanes and Harvey, 1981). Scanes et al. (1986) found that growth could be restored in hypophysectomized chickens (removal of the pituitary gland) by administering $T_3$ but not by administering GH. Despite the name, it is unlikely that GH is the sole hormone responsible for growth in avian species (Cabello and Wrutniak, 1989; Harvey, 2013). Broiler chickens given GH in a pulsatile manner had decreased growth rates and feed intake (Vasilatos-Youken et al., 2000). Rosebrough et al. (1991) found that GH administration had no effect on growth rate. Bowen et al. (1987) also found that sex-linked dwarf chickens had higher levels of circulating GH than White Leghorns. This would further lead us to believe that GH is not the sole hormone responsible for growth in the chicken. Studies on growth have posed that insulin, IGF and the thyroid hormones may work in concert with GH to promote growth (McMurtry et al., 1988; Harvey, 2013). A direct relationship has been found between GH and $T_3$; GH reduces the activity of enzymes that degrade $T_3$ into $T_2$, which could promote growth (Darras et al., 1992). The level of circulating GH can be affected by feed restriction. McMurtry et al. (1988) found that feed restricted birds had significantly lower levels of GH on d 12 and significantly higher levels on d 42, than non-restricted birds. However, Ghazanfari et al. (2010) concluded that feed restriction did not significantly affect the plasma GH level. More research on

GH and what affects the plasma concentration of GH needs to be completed to fully understand how GH fits into the metabolic regulation of growth in the broiler chicken.

### 2.5 Insulin-Like Growth Factor 1

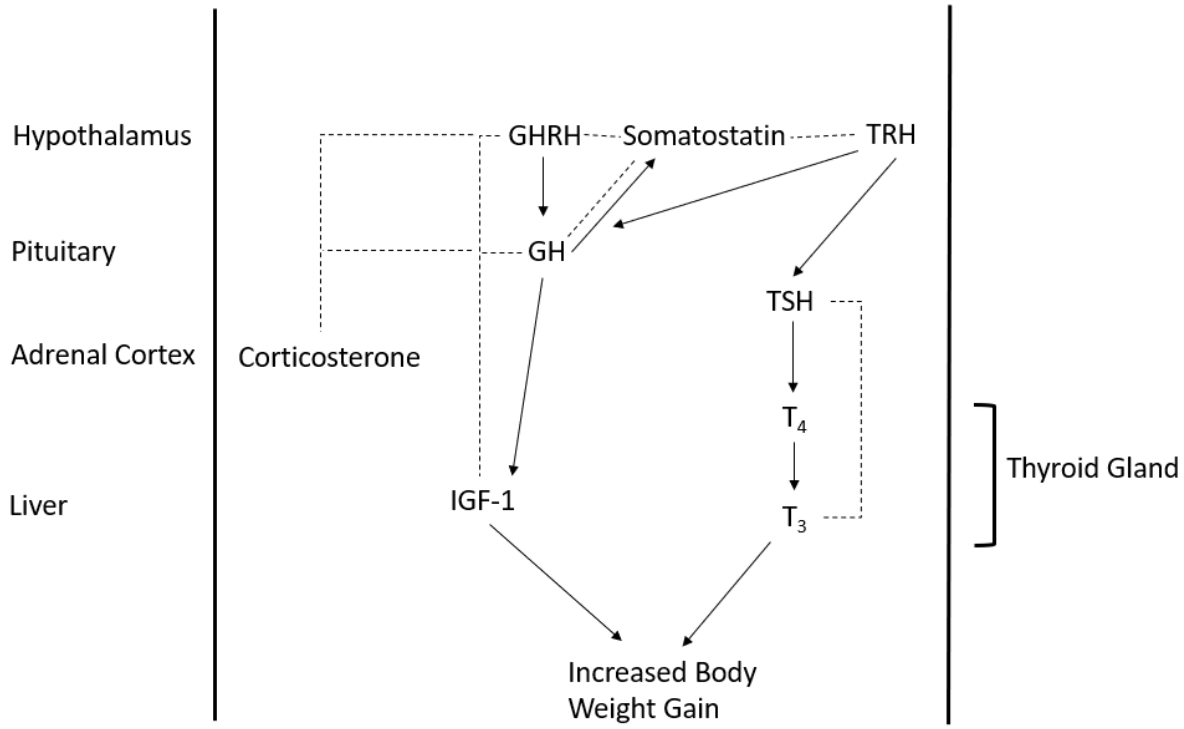
Insulin-like Growth Factor 1 (**IGF-1**) is a hormone that is produced by the liver and as the name suggests, is similar in structure to insulin. The role of IGF-1 in the chicken is not well understood. IGF-1 is related to insulin with metabolic and anabolic properties (McMurtry et al., 1997). It is hypothesized that increasing levels of IGF-1 will lead to an increase in growth and metabolic rate (Goddard et al., 1988; Duclos, 2005; Jia et al., 2018). McMurtry et al. (1997) proposed that IGF was responsible for many processes associated with metabolism and growth, such as stimulating the proliferation of muscle and bone cells, increasing the uptake of glucose and amino acids and increasing DNA and protein synthesis. In the chicken, the amount of IGF-1 in circulation increases with advancing age (Goddard et al., 1988; McMurtry, 1998). Increased weight gain is associated with advancing age but research has not yet associated the two with increased levels of IGF-1 (McMurtry, 1998). However, Goddard et al. (1988) found that increased IGF-1 levels were significantly correlated with body weight gain in chickens up to 10 weeks of age. Xiao et al. (2017) found that high growth rate broiler have higher levels of IGF-1 present in their plasma than low growth rate broilers. Yu et al. (2015) found that administration of IGF-1 to chicken embryos was associated with the upregulation of genes associated with muscle growth and development. More recently, Lertpimonoan et al. (2019) found that broilers fed fermented potato protein had improved growth performance compared to broiler not fed fermented potato protein, which they attributed to the increased stimulation of IGF-1 gene expression. It has been concluded that IGF-1 levels are increased in high growth rate broilers (Jawasreh et al., 2019).

Like other hormones reviewed above, the circulating concentration of IGF-1 is also affected by feed restriction (McMurtry et al., 1998). Kim et al. (1991), Morishita et al. (1993), and Li et al. (2007) found that serum IGF-1levels of feed restricted birds were significantly lower than ad libitum fed controls. Feed restriction is not the only factor that is thought to affect the circulating concentration of IGF-1. The circulating concentration of TH and GH may have an effect on the level of IGF-1 present in the bloodstream and will be discussed in the following paragraph.

**2.6 Interactions between TH, GH and IGF-1**

Hypophysectomized chickens have decreased GH, IGF-1 and TH levels with a corresponding decrease in growth rate (Scanes, 2015a). The main evidence for a working relationship between TH and GH, is the inhibitory effect that $T_3$ has on GH. When $T_3$ was administered to growing chickens, the circulating concentration of GH decreased (Scanes et al., 1986; Bowen et al., 1987; O'Neill et al., 1990). Krestel-Rickert et al. (1986) found that as insulin and glucose levels in plasma increased, GH levels decreased, when chickens were exposed to a 4-hour fasting period. Growth can be increased in sex-linked dwarf chicken by administration of $T_3$ (Bowen et al., 1987). GH has a positive effect on the plasma concentration of IGF-1, as the level of GH increased, the concentration of IGF-1 also increased. McMurtry et al. (1987) found that pulsatile administration of GH increased the circulating concentration of IGF-1 but continuous GH administration had no effect on IGF-1 concentration. Increased levels of insulin or glucagon decreased plasma IGF-1 levels (Lazarus and Scanes, 1987). Figure 1 shows a graphical representation of the effect of $T_3$, IGF-1, and GH on growth.

**Figure 1**. The hormonal control of growth and metabolism requires a complicated interplay between growth hormone releasing hormone (GHRH), thyrotropin releasing hormone (TRH), triiodothyronine ($T_3$), insulin-like growth factor 1 (IGF-1), somatostatin, corticosterone and growth hormone (GH). Solid lines represent positive feedback systems, and broken lines represent negative feedback systems.

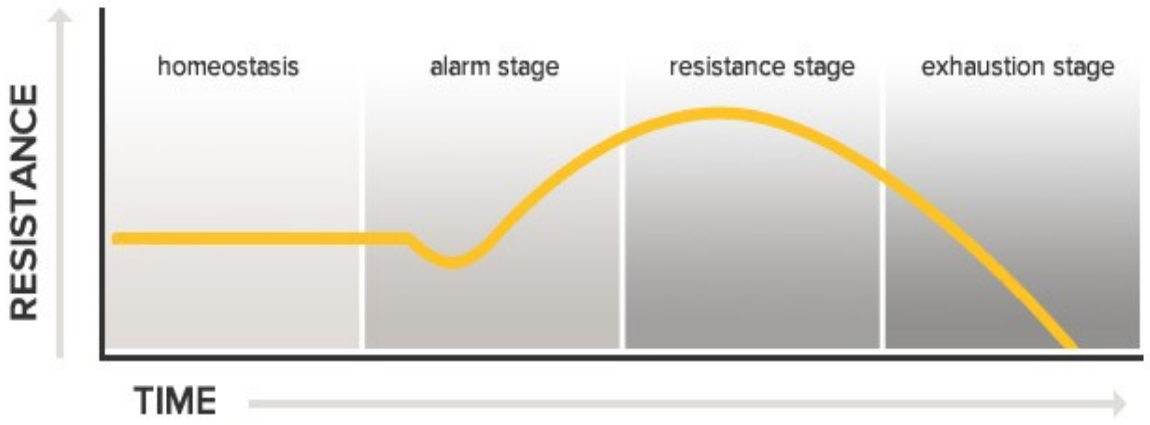



## 2.7 Corticosterone

Corticosterone is the main steroid hormone secreted by the adrenal glands in avian species and is most widely known for its role in the stress response. The function of corticosterone was discovered by Hans Selye when he created the idea of General Adaptation Syndrome (**GAS**) (Selye, 1950). GAS is an axis divided in stages that describes the bodily response to prolonged stress (Figure 2). In the "Alarm" stage, corticosterone is released as a result of the sympathetic nervous system in response to an initial stress in order to increase heart rate, breathing rate and mobilization of sugar from the liver. In the "Resistance" stage the parasympathetic nervous system compensates for prolonged stress and brings the body back to homeostasis. In the "Exhaustion" stage, the body can no longer compensate for prolonged stress and becomes

increasingly susceptible to disease and death (Selye, 1950). Corticosterone is one of the main glucocorticoids, which are steroid hormones named for their role in regulating glucose metabolism. In birds, the main function of glucocorticoids is to mediate the stress response and one way that this occurs is through the maintenance of glucose homeostasis. There is a positive relationship between the level of blood glucose and the circulating level of corticosterone (Kafri et al., 1988). Administration of corticosterone is also associated with increased fat pad weight (Bartov, 1985; Kafri et al., 1988; Jiang et al., 2008; Hu et al., 2018). Along with increased fat pad weight, administration of corticosterone increased lipogenesis in the liver and circulating concentrations of free-fatty acids (Kafri et al., 1988; Jiang et al., 2008). The relationship between corticosterone and feed intake, as well as growth is inversely related (Bartov, 1985). Feed restriction has been found to significantly increase the plasma concentration of corticosterone in both the short term and long term when compared to ad libitum fed chickens (de Jong et al., 2002; Rajman et al., 2006).

**Figure 2**. Hans Selye's General Adaptation Syndrome (Lucille, 2016). The General Adaptation Syndrome describes how a living being responds to long-term and short-term stress. The y-axis describes the level of resistance to the stressor and the x-axis is the time that the stressor has been present. The yellow line represents the bodies level of adaptation to the perceived stressor.



## 2.8 Glucidic Potential

Glucidic Potential (**GP**) is an index calculated as the sum of glycogen, ½ lactate and the intermediate metabolites, glucose and glucose-6-phosphate (**G6P**) in tissue (Yambayamba et al., 1996).

$$GP = glycogen + \frac{1}{2}(lactate) + glucose + G6P$$

GP allows estimation of the amount of glucose substrates available at the point of death to fuel post-mortem metabolism. By analyzing glycogen, the amount of residual glycogen remaining in the tissue can be determined. The amount of lactate present in the muscle and liver tissue tells the extent of glycogen breakdown that has occurred. Muscle and liver tissue are measured because they are the main storage sites for glucose in the body. Glucose-6-phosphate is most commonly not measured as it is minor component and it requires a separate sample to be analyzed.

## 2.9 Plasma Analysis

### *2.9.1 Enzyme Linked Immunosorbent Assay (ELISA)*

There are four types of ELISA that can be used to determine the concentration of a hormone in a substrate. For the purpose of this explanation antigen will refer to the substance that is being measured within the sample. A ELISA allows for the detection of the antigen of interest in a sample by employing antibody-antigen interactions (Clarke, 2004). The sample containing the antigen of interest and an antibody with affinity for the antigen are incubated together. A signal is produced by the binding of the antigen and antibody (usually a color change); the intensity of

the color change can be used to determine the amount of antigen in the sample. The first of which is known as a "direct" ELISA. To perform a direct ELISA, the sample to be tested is pipetted into the wells of the ELISA plate and the antigen of interest is given time to attach to the well surface. Allowing the antigen to bind to the well surface will allow the user to determine how much antigen is in the sample. A primary antibody that is labeled with horseradish peroxidase (**HRP**) is then added to each well, which will bind specifically to the antigen being tested. HRP is used to cause intense color reactions when mixed with the substrate (e.g. plasma sample) in the next step (Aydin, 2015). After the primary antibody is given time to attach, a HRP is added that produces a color change when it comes into contact with the primary antibody attached to the antigen. A strong color change indicates that there is a large amount of the primary antibody bound to the antigen. Using a spectrophotometer, the degree of color change can be measured and used to quantify the antigen concentration in the sample. Direct ELISA are the simplest form of ELISA and there is little cross-reactivity due to there only being one antibody/antigen interaction (Aydin, 2015). The downfall of "direct" ELISA is the lack of sensitivity compared to other ELISA types. The "direct" ELISA lacks a secondary antibody which does not allow for signal amplification.

The second type is known as an "indirect" ELISA. Similar to the direct ELISA explained above but "indirect" ELISA requires addition of an enzyme-labeled secondary antibody, which specifically binds to the primary antibody that is bound to the antigen of interest. After the enzyme-labeled secondary antibody is given time to bind, a substrate is added to produce a color change and the sample can then be analyzed using a spectrophotometer. The enzyme-labeled secondary antibody enhances the signal of the primary antibody, allowing the "indirect" ELISA to be more sensitive than the "direct" ELISA, which allows this assay to detect lower levels of

antibody than the “direct” ELISA (Hnasko, 2015). However, the enzyme-labeled secondary antibody can produce a high background signal which reduces the accuracy of the ELISA. Background signals can be cause by non-specific binding, however if the assay is conducted properly this can be controlled.

The third type is known as a sandwich ELISA. The wells of the plate are pre-coated with a target specific capture antibody, which has an affinity for the antigen being measured. When the sample is added to the wells, the antigen will bind to the antigen specific capture antibodies. After this step, a second antibody, known as the detector antibody is added that also has affinity for the antigen in question. Horseradish Peroxidase conjugate is added which binds to the detector antibody and increases the strength of the color change. A 3,3’,5,5’-Tetramethylbenzidine (**TMB**) substrate is then added that reacts with the HRP to produce a color change. When using a sandwich ELISA, the antigen of interest must be large enough to allow binding of two antibodies which reduces the number of hormones that can be tested using this method. However, due to the use of the capture antibody, as well as, the detector antibody the sandwich ELISA is more specific than the direct and indirect ELISA (Aydin, 2015).

The last type is known as a competitive ELISA. Similar to the sandwich ELISA, the well is pre-coated with a specific antibody. The sample of interest is then mixed with a solution that contains tracer, or an enzyme-conjugated version of the hormone being measured. This mixture is added to the wells and the antigen in the sample will compete for the binding sites with the enzyme conjugated version of the sample (Aydin, 2015). A high sample antigen concentration will result in less enzyme-conjugated antigen binding and vice versa. This is the most complex ELISA to perform but it is very specific because two antibodies are being used to bind the antigen.

### *2.9.2 Radioimmunoassay*

Radioimmunoassay (**RIA**), another competitive binding assay, takes advantage of competition for specific antibody sites between labeled and unlabeled antigen (Goldsmith, 1975). Radioimmunoassay is similar to the competitive binding ELISA but it uses a radioactive isotope to label the tracer instead of an enzyme. A known amount of the antigen in question is labeled with a radioactive isotope to produce the tracer. This radiolabeled tracer is then mixed with a limiting quantity of antibody that will bind to that antigen. Serum or extracted plasma from the bird that is being measured is then added to the previous mixture. The antigen in the serum and the radioactive antigen compete for the antibody binding sites. The level of radioactivity measured is inversely related to the concentration of antigen in the sample.

## 2.10 Epigenetics

Epigenetics can be defined as a heritable change in gene expression that is not the result of a change in the DNA sequence. These completely random changes in gene expression can be caused by nutritional availability, feeding system, temperature, and stress level. If these changes in gene expression cause a change in fitness that increases the individuals ability to pass on genes, these changes may be passed on to the next generation. Gene expression is modified through histone modification or DNA methylation. DNA methylation occurs when methyl groups are added to a DNA molecule, which can occur as a result of an environmental change such as feed availability and can be used to differentiate the expression of genes in a parent-of-origin-specific manner (Li et al., 1993). Histone modifications are a post-translational modification to the histone proteins, these modifications can include methylation, phosphorylation, acetylation (addition of an acetyl group), ubiquitination (addition of one or more ubiquitin molecules), and sumoylation (addition of small ubiquitin-like modifiers; Shiio

and Eisenman, 2003). Histone modifications can have an effect on gene expression by altering the chromatin structure, which may repress transcription (Dong and Weng, 2013; Stoll et al., 2018). These changes do not only affect an individual but are heritable (Ferguson-Smith., 2011). Epigenetic changes can be both advantageous and disadvantageous; the more successful they make an individual the more likely they are to be retained in a population.

Epigenetic mechanisms have been used to explain increased chronic disease risk in humans from mothers that were nutritionally deprived early in gestation. Genetic alterations caused by nutrient restriction are passed on to offspring that increase risk for chronic disease (Roseboom et al., 2006). The Dutch famine produced a situation to study the effect of nutrient deprivation during pregnancy. A blockade cutting off food deliveries to large portions of the Netherlands by Nazi forces in 1944-45 caused a severe food shortage (Roseboom et al., 2006). Before the onset of the famine, the Dutch people were not exposed to nutrient restriction and the end of the famine was sudden. Children that were born to mothers that experienced the famine during early or mid-gestation were more likely to have a lower birth weight, increased risk of obesity later in life, develop airway disease and have increased risk of insulin related disorders (Lumey and Stein, 1997; Rosebloom et al., 2006; Heijmnas et al., 2008). However, due to a lack of research it is not yet known the full effect of early gestational undernutrition on 2$^{nd}$ generation birth weight.

Recently, there has been more research done on epigenetic mechanisms in poultry. Researchers have found that immune function, behavior, temperature regulation, response to stress and growth efficiency in chickens can be altered by epigenetic mechanisms, and all of these factors can change the rate of DNA methylation and histone modification of certain sections of DNA (Li et al., 1993; Bélteky et al., 2018; Kisliouk et al., 2017). Broiler breeders are of interest to researchers studying epigenetics because breeders are feed restricted from what they would

consume ad libitum (Renema and Robinson, 2004). The reproductive issues previously seen in broiler breeders have most likely been reduced by selection by the primary producers. If this is in fact true, the level of restriction imposed on broiler breeders could be relaxed. Van Emous et al. (2015) determined that offspring from broiler breeders that had an increase BW target during the rearing period were 1.4% heavier than offspring from broiler breeders on a standard BW target during rearing. As part of the same study, they found offspring feed intake increased when broiler breeder crude protein intake was lowered. Van Emous et al. (2015) did not directly determine if their results were due to epigenetic changes; however, epigenetic changes could offer a reasonable explanation for the observed results. There may also be an argument for the differences observed by Van Emous et al. (2015) to be the result of differences in egg nutrient composition. Van der Waaij et al. (2011) determined that matching offspring feeding environment to the maternal feeding environment increased the efficiency of offspring growth. Ad libitum-fed offspring from restricted mothers grew to be 18% heavier and had 51.9% more abdominal fat than restricted offspring from restricted mothers. Van der Waaij et al. (2011) concluded that mothers pass on genetic information to their offspring that prepares them for similar nutritional environments to their mother and restricting mothers while full feeding offspring may be causing offspring to be less efficient. Broilers hatched from feed restricted mothers had 2.5% lower hatch weights when compared to broilers hatched from ad libitum mothers, however this result was non-significant (van der Waaij et al., 2011). Bowling et al. (2018) found that male offspring from restricted hens were on average 220 g lower in weight at wk 6 than male offspring from non-restricted hens. Bowling et al. (2018) also measured the amount of yolk corticosterone in eggs from restricted and non-restricted hens and found that levels were increased in eggs from restricted hens. This suggests that the level of stress that the

hen is exposed to due to the level of feed restriction may affect the hormone levels in the egg and therefore affect the offspring. To determine if this is the result of maternal nutritions effect on the embryo/offspring or if this is in fact the result of an epigenetic change more research will need to be completed.

Even though the physiological and hormonal systems that control growth and development in the chicken have been studied for years, much remains unknown. Much of the work that has been done on endocrine regulation of growth and metabolism was done over 20 years ago. Due to the rapid advances in genetic selection, birds are genetically very different than they were 20 years ago. This review identified gaps in our knowledge that relate to how these hormonal systems are affect in offspring by maternal nutrition. This topic is of importance due to the severe nature of feed restriction that broiler breeders are exposed to. If maternal nutrition does in fact alter offspring performance understanding what occurs physiologically could allow researchers and industry to take advantage of these epigenetic effects. The main objectives of this project were to:

1) To determine the effect of increasing maternal body weight on offspring growth performance (Experiment 1).
2) To determine the effect of increasing maternal body weight on the circulating level of hormones associated with growth and metabolism in offspring (Experiment 1).
3) To determine the effect of maternal feeding system on the growth performance of offspring (Experiment 2).
4) To determine the effect of maternal feeding system on the circulating levels of hormones associated with growth and metabolism in offspring (Experiment 2).

With what has been discovered by previous research it is believed that more closely matching the offspring and maternal environments will result in broilers being more efficient by growing to heavier weights with the same feed intake as their mismatched counterparts. Experiments that examine the above objectives can be found in this thesis.

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## 3.0 The effect of maternal body weight and feeding system on the growth performance of broiler offspring

### 3.1 ABSTRACT

A significant body of evidence exists that suggests maternal nutrition could influence progeny performance in chickens. Maternal feeding system, one feeding per day vs. multiple feedings per day, may also significantly alter body composition in progeny. It is believed that feed restriction and feeding system may alter gene expression in parents and those changes in gene expression could be passed onto offspring, altering their metabolic and growth patterns. The objective of this study was to determine if maternal feed availability and maternal feeding system influenced offspring performance. We hypothesized that 1) relaxing maternal feed restriction would increase growth efficiency in broilers fed ad libitum (Experiment 1) and, 2) feeding broiler breeders with the precision feeding system will result in leaner offspring (Experiment 2). This project consisted of 2 experiments, each using offspring from 2 maternal treatments (**MT**), 2 sexes and 3 feeding treatments (**FT)**. The MT for experiment 1 consisted of Ross 708 (n= 264) broilers from mothers raised on standard breeder-recommended target BW (**SBW**), or 121% of SBW (**HBW**). MT for experiment 2 consisted of Cobb broilers (n = 268) from mothers fed using a conventional feeding system (**CON**) and broilers from mothers fed using precision feeding techniques (**PF**). Broilers from both experiments were fed ad libitum until d 28. From 29 to 42 d of age, birds were provided feed ad libitum (AL), or at 80 or 60% of AL. Average broiler BW for experiment 1 was 4% higher in HBW offspring when compared to SBW offspring. There was no significant difference in BW between MT for experiment 2. Increasing feed intake of broiler breeder hens increased growth performance of offspring. In agreement with the first hypothesis, HBW offspring were heavier than SBW offspring throughout experiment 1. No MT difference were found in experiment 2, therefore the 2$^{nd}$ hypothesis that feeding broiler breeders using the

precision feeding system will result in leaner offspring was rejected. In conclusion, increasing the BW of broiler breeders in a precision feeding system resulted in heavier broilers.

**Key words:** broiler chicken, epigenetic, maternal body weight, feeding system, precision feeding

## 3.2 INTRODUCTION

In chickens, nutritional availability and plane of nutrition during lay affects hatch weight, growth potential and susceptibility to disease later in life (van der Waaij et al., 2011; Moraes et al., 2014; Moraes et al., 2019). In chickens and turkeys, maternal nutrition can affect egg composition, egg and hatch weight, growth potential and fat deposition in offspring (Wilson, 1997; Sun and Coon, 2005; Bhattacharyya et al., 2018). Van der Waaij et al. (2011) discovered that when the offspring's nutritional environment was more abundant than that of the hen, the proportion of body fat increases and the proportion of muscle decreases in broilers. Van Emous et al. (2015) also found that offspring from heavier broiler breeders had higher body weights at the end of life compared to offspring from lower body weight broiler breeders. Presently in industry, the broiler nutritional environment does not match that of their mother's, and this may be reducing broiler growth rate and feed efficiency and affecting development. Increasing overall BW while also reducing the proportion of broiler body weight that is comprised of fat and increasing the proportion of muscle will lead to increased profits for producers as fat is a less desirable product than muscle. It also takes less energy to build muscle than to build fat so broilers with increased proportions of muscle compared to fat in theory should be more feed efficient. However, feed efficiency might be reduced as higher amino acid requirements and energy for lean tissue deposit will increase feed intake. Mechanisms exist which allow mothers to pass genetic information pertaining to their environment to offspring without altering the DNA sequence. Genetic information may be able to be passed onto offspring that would make them better prepared to

efficiently survive in an environment similar to their mothers. Information about environmental stressors, such as, light, temperature and nutritional availability can be passed to offspring through epigenetic mechanisms (Ferguson-Smith., 2011).

The objective of the current project was to determine how maternal feed availability and feeding system affects the growth of offspring. We hypothesized that 1) relaxing maternal feed restriction would increase growth efficiency in broilers and, 2) increased feed efficiency would be seen in feed restricted broilers from feed restricted hens, 3) feeding broiler breeder hens using the precision feeding system would result in leaner offspring.

## 3.3 MATERIALS AND METHODS

This research was conducted at the Poultry Research Centre at the University of Alberta (Edmonton, AB, Canada). Animal use in this study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed animal care principles established by the Canadian Council on Animal Care (Canadian Council on Animal Care, 2009).

### *3.3.1 Maternal Body Weight – Experiment 1*

#### *3.3.1.1 Treatments and experimental design*

A 2 x 2 x 3 factorial arrangement of treatments was used in this study with offspring being hatched from broiler breeders fed on a HBW curve or a SBW curve (van der Klein et al., 2018)., both male and female offspring were used and offspring were assigned to an ad libitum, 80% of ad libitum, or 60% of ad libitum feed treatment. Eggs were collected from 40 to 41 wk old hens. Birds in this study were the offspring of precision fed Ross 708 Broiler Breeders on a breeder recommended standard BW curve (SBW) beginning at photostimulation or breeders raised on a high BW, which was 21% above the SBW curve (van der Klein et al., 2018). Male breeders were

fed to the recommended target BW curve (Aviagen, 2016). This experiment started on d 0 and was completed on d 42. Offspring were feather sexed at hatch, fitted with a unique necktag for identification, separated into male and female groups at hatch, and assigned to one of three feeding treatments: ad libitum, 60% of ad libitum and 80% of ad libitum. The 60% of ad libitum broiler feeding treatment was calculated as 60% of the Ross 708 broiler performance objectives, the 80% of ad libitum broiler feeding treatment was calculated as 80% of the Ross 708 broiler performance objectives (Aviagen, 2014). After feeding treatments were assigned birds were assigned to 1 of 72 sex separate cages based on feeding treatment. The sex of each bird was confirmed upon dissection at 28, 25, and 42 d of age. The cage was used as the experimental unit.

#### *3.3.1.2 Animals and housing*

Two hundred sixty-four Ross 708 broilers were raised sex-separately from 0 to 42 d in above floor pullet rearing cages. The cage dimensions were 1.19 m x 0.53 m and were stocked with 2 to 5 birds depending on treatment combinations. Dissections were completed on d 28, 35, and 42 so not all birds were raised to d 42. On d 0 all birds were fitted with a neck tag that provided a unique code specific to each bird. All birds were exposed to 24L:0D (0 to 3d), followed by 18L:6D (4 to 42d). A Ross 708 temperature schedule was used that began at 34°C on d 0 and decreased by 1°C daily until 23°C was reached on d 11, which was maintained for the remainder of the study. The number of birds allocated to each cage could not be kept equal due to a shortage of male offspring from the SBW mothers, and 2 to 5 birds were in each cage. At the beginning of the study there were 27 male birds in both the ad libitum and 80% of ad libitum treatments from high BW hens. Twenty-seven, twenty-seven, and twenty-eight male birds were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively.

Eighteen, eighteen, and nineteen male birds were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively. Twenty-two, twenty-three, and twenty female birds from high BW hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatment, respectively. Seventeen, fourteen, and seventeen female birds from standard BW hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively. Each cage contained three drinking nipples. Litter consisted of pine shavings at a depth of 5 cm throughout each cage. Individual BW was recorded weekly.

#### *3.3.1.3 Feeding and diets*

On d 28 birds were assigned to either ad libitum, 60% of ad libitum, or 80% of ad libitum feeding treatment. Prior to feed restriction birds were fed ad libitum. Feed restriction began on d 28 because maternal treatment effects wanted to be examined under both ad libitum and restricted feed intake conditions, the latter of which more closely matched maternal feed intake. Feed was added daily and was weighed back once per week to determine feed intake. When feed restriction began, the birds exposed to feed restriction treatments were fed at 1:00 PM daily. Birds on the ad libitum treatment had continued free access to feed. All birds were fed a starter pellet diet (23% CP, 3067.51 kcal/kg) from 0 to 2 wk of age, a grower pellet diet (20.20% CP, 3152.00 kcal/kg) from 2 to 4 wk of age and a finisher pellet diet (19% CP, 3196.00 kcal/kg) from 4 to 6 wk of age (Table 3.1).

### *3.3.2 Maternal Feeding System Trial – Experiment 2*

#### *3.3.2.1 Maternal treatments and experimental design*

A 2 x 2 x 3 factorial arrangement of treatments was used in this study with offspring being hatched from broiler breeders fed using either conventional methods (CON) or by precision feeding (PF) (Zuidhof, 2018), both male and female offspring were used and offspring were

assigned to an ad libitum, 80% of ad libitum, or 60% of ad libitum feed treatment. Eggs were collected from 51 to 52 wk of age. Offspring were placed into one of three mixed sex pens. Each bird was an experimental unit because in Experiment 2, feed was provided to each bird on an individual basis with the precision feeding system from 14 d of age onward.

#### *3.3.2.2 Animals and housing*

Using a precision feeding system (Zuidhof et al., 2017; Zuidhof, 2018), 300 Cobb broilers were raised from 0 to 42 d. Mixed sex birds were divided into 3 pens that were 5.40 m x 4.50 m. Fifty, forty-nine, and fifty-one birds hatched from conventionally fed hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum. Fifty, fifty-one, and forty-nine birds from precision fed hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum. Dissections occurred on d 28, 35, and 42 so not all birds were raised until d 42. On d 0, chicks were allocated to one of three pens, each containing three precision feeding (PF) stations. One hundred chicks were allocated to each pen. PF stations controlled feed intake for each individual bird. In this experiment, all birds were divided into groups of 3 based on BW on d 28. The heaviest bird of the group was fed ad libitum, and using real-time pair feeding with the PF system, the middle BW bird was fed 80% of what the ad libitum bird consumed and the lightest BW bird was fed 60% of what that ad libitum bird consumed. All birds were exposed to 24L:0D (0 to 3 d) and 18L:6D (4 to 42 d). A standard temperature schedule was used that began at 33°C on d 0 and decreased linearly by 0.5°C to 23°C on d 28, which was maintained for the remainder of the study. Two hanging nipple drinkers were placed in each pen, each drinker consisted of 8 nipples. Litter consisted of pine shavings at a depth of 5 cm throughout each pen. Starting at d 0, until individual PF was implemented at d 14, birds were manually weighed once per day to ensure that they were eating and gaining weight.

#### *3.3.2.3 Precision Feeding System*

The PF system was used to identify individual birds through the use of a radio frequency identification tag and feed them according to how their BW compared to other birds using pair feeding. Pair feeding is described in the section below. The ad libitum fed birds were allowed access to as much feed as desired. From 4 to 6 wk of age, the 80% of ad libitum and 60% of ad libitum fed birds were fed 80% and 60%, respectively, of what the ad libitum bird consumed. BW of individual birds were measured using a scale in the PF station. If the bird's intake was below the desired intake compared to the ad libitum bird, the PF system allowed them access to 30 g of feed for 60 sec in the main stage of the PF station. If the bird's intake was at the desired intake level the bird was sent out of the station and was not fed. Individual feed intake and BW were measured by the PF station so the bird was used as the experimental unit.

#### *3.3.2.4 Feeding and diets*

All birds were fed the same pelleted rations: starter from 0 to 2 wk of age, grower from 2 to 4 wk of age, and finisher from 4 to 6 wk of age (Table 3.1). Three PF stations were in each pen. To train the birds to eat, three paper plates, each with 200 g of feed, were placed around the ramp of the station and 200 g of feed was placed on the ramp. One paper plate with 200 g of feed was placed in both the scale stage and feeding stage. Feed was replaced on the plates as it was consumed, and plates stopped being filled once the majority of birds were searching for feed inside the stations. From d 0 to d 13 (training period) every morning birds were weighed manually to ensure that each bird was gaining weight. Daily feeding reports that showed the level of feed consumption for every bird that entered the PF stations were used to determine which birds were consuming feed. If a bird was heavier than the previous day it was assumed to be eating and was returned to the pen. If a bird was lower in weight than the previous day, it was

shown where the feed was in the station. If the same bird did not gain the desired amount of weight for 2 days in a row, it was put into a training pen. From d 0 to d 13, 25 birds were assigned to the training pen. The training pen contained one station and one drinker with eight nipples. In the training pen, birds were manually put through the stations until they were observed consuming feed. Feed left in the station from the previous day was weighed and recorded. All feed added to stations was weighed and recorded. During training, feed intake (**FI**) was measured at the pen level; from 0 to 13 d of age, FI was measured by subtracting the amount of feed left in the pen from the previous day from the total feed added to the pen, this number was then divided by the number of birds in the pen to determine average intake per bird.

At d 13 each bird was fitted with a radio frequency identification tag on its left wing, so individual birds could be identified by the PF stations. On d 14, individual feeding mode was started, and all birds were allowed ad libitum access to feed until d 28. If a bird was found not to be eating it was trained by putting the bird in the station, showing the bird where the feed was and ensuring that it consumed feed. Prior to 28 d of age, each time a bird entered the station it was allowed access to feed for 45 sec. Feed restriction treatments were implemented from 28 to 42 d of age.

#### *3.3.2.5 Pair Feeding*

When feed restriction began, birds were ranked from highest to lowest BW and were placed into groups of 3, with the birds closest in BW being grouped together. The heaviest bird in each group was assigned to be the master and was fed ad libitum for the remainder of the experiment, this was not a random selection. The middle BW bird was assigned to the 80% of the intake of the master bird. The lowest BW bird was assigned to 60% of the intake of the master bird. Feed

intake of the master bird was cumulative from the time the pairings occurred to the end of the experiment, which determined what the restricted birds were fed.

### *3.3.3 Sample Collection – Experiment 1 and Experiment 2*

#### *3.3.3.1 Dissection*

Dissections were carried out on d 28, 35 and 42 and all birds randomly selected for dissection were euthanized via cervical dislocation. On d 28 (start of feed restriction treatment implementation period), 22 birds were selected for dissection and on d 35 and 42, 96 birds were selected for dissection. Pectoralis major and Pectoralis minor muscles were weighed individually and summed to determine breast weight. Heart, fat pad, liver, gut (2.5 cm below the crop to 0.5 cm above the cloaca), crop, and gonads were all weighed individually. Feed was not withheld from the ad libitum fed birds before dissection. Fat adhering to the gizzard but not to the proventriculus was included as part of abdominal fat pad weight. Guts were not emptied at the time of weighing. Sex was confirmed during dissection. In experiment 2, the birds were not feather sexable so sex was determined during dissection.

#### *3.3.3.2 Egg Composition*

Eggs were collected from every hen that produced a chick and were used for egg proportion analysis. Eggs were separated into yolk, albumen and shell. Wet weights were taken, then eggs were placed into drying ovens at 60°C for 4 days. After drying was complete, eggs were removed from the dryer and dry weights of yolk, albumen and shell were recorded.

### *3.3.4 Statistical Analysis*

#### *3.3.4.1 Statistical Analysis – Experiment 1*

All ANOVA were conducted using the HP MIXED and MIXED procedures of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Individual bird was used as the experimental unit for dissection data, blood plasma data and glucidic analysis. The pen was used as the experimental unit for feed intake analysis. Egg weight was used as a covariate in the model to account for differences in BW caused by differences in egg weight. Due to model convergence issues, age was removed from the random statement in the analysis of ADFI; MT, FT, and sex were included in the random statement. MT, FT, and sex were used as sources of variation. Means were reported as different where $P \leq 0.05$. The model for dissection, feed intake and efficiency analysis included MT, FT, age, sex and all 2, 3, and 4-way interactions. Tukey's range test was used for multiple mean comparisons to reduce type II errors (false positives).

#### *3.3.4.2 Statistical Analysis – Experiment 2*

All ANOVA were conducted using the HP MIXED and MIXED procedures of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Individual bird was used as the experimental unit. Due to model convergence issues, age was removed from the random statement in the analysis of ADFI; MT, FT, and sex were included in the random statement. MT, FT, and sex were used as sources of variation. Means were reported as different where $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$. The model for dissection, feed intake and efficiency analysis included MT, FT, age, sex and all 2, 3, and 4-way interactions. Tukey's range test was used for multiple mean comparisons to reduce type II errors (false positives).

## 3.5 RESULTS AND DISCUSSION

### *3.5.1 Maternal Body Weight Treatment (Experiment 1)*

At d 35, HBW broilers were 3% heavier and 4% heavier at d 42 than SBW broilers ($P = 0.012$; Table 3.2). Hatch weight was not significantly different in offspring of HBW hens than in

offspring of SBW hens (Table 3.2). Eggs from HBW hens were 3.8% larger than eggs from SBW hens (Table 3.3). The difference in BW through out the experiment may be due to the difference in egg and hatch weight, however it is unlikely. Egg weight was included as a covariate in the statistical model and it was determined that for every 1 g increase in egg weight there would be a 0.7 g increase in final BW. This agrees with the conclusions of Tahir et al. (2011) that the relationship between egg weight, chick weight, and final BW has decreased with genetic selection for growth and may not be a good predictor of final BW. Ulmer-Franco et al. (2010) found that broilers from eggs that were 3 to 6 g heavier than average were 18 g lighter at d 41 than broilers from eggs that were average weight. Pinchasov (1991) and Iqbal et al. (2017) concluded that the correlation between egg weight and BW significantly diminishes after d 5. There was a trend towards eggs from HBW hens having larger dry albumen weights than SBW hens (Table 3.3), however these differences were not large enough to cause a change in chick performance. Dry albumen from HBW hens was 20% of the dry egg weight compared to 19% from eggs of SBW hens. Bowling et al. (2018) found that male broiler BW was 8.5% higher when their mother's BW was maintained at a level 15% higher than a standard BW target. van der Waaij et al. (2011) reported that they also observed offspring of ad libitum fed hens produced offspring that grew to be heavier than offspring of restricted mothers. Bowling et al. (2018) and van der Waaij et al. (2011) did not report egg weight data so it could be conceivable that their results could be attributed to differences in egg weight. Broiler breeders have been heavily feed restricted to prevent reproductive disorders associated with a high feed intake. However, selection by the primary breeders for reproductive efficiency may have diminished the likelihood that breeders will experience reproductive dysfunction if their feed intake is increased. There has been no research published that can support this statement; future research should focus on

examining difference in feeding level on modern broiler breeder reproductive performance. Therefore, the target BW of breeders could be raised without any detrimental effects on their reproductive performance.

BW was also significantly affected by MT, however the results were dependant on FT and sex (P = 0.004). At 6 wk of age ad libitum fed male broilers from HBW broiler breeders were 9.0% heavier than ad libitum fed males from SBW broiler breeders. Female broilers fed 60% of AL from HBW broiler breeders were 21% heavier than females fed 60% of AL from SBW broiler breeders. In the current experiment the offspring were from precision fed broiler breeders, however, the similar results have been seen in the offspring of conventionally fed broiler breeders. Bowling et al. (2018) found that at 6 wk of age male broilers from high BW hens were 8.5% heavier than males from low BW hens.

MT significantly affected ADG; ADG of broilers from HBW hens was 57.6 g/d, which was 1.6 g/d higher than that of broilers from SBW hens (Table 3.4). Significant differences in final BW caused by MT were not found due to the variability of the BW values. However, there was less variation in the values for ADG and a difference due to MT was detected. ADFI and FCR were not significantly affected by MT in experiment 1. HBW broilers had guts than were 12.7% heavier than SBW broilers (Table 3.5); a larger gut may have allowed the HBW broilers to make more efficient use of their feed due to the larger surface area of the gut when compared to SBW broilers, therefore increasing their ADG. Increased gut size has been linked with increased digestive and absorptive capacity in broilers (Jackson and Diamond, 1996). However, guts were not flushed of their contents so this likely contributed to the observed differences. The difference in egg weight reported above could also offer a logical explanation for the differences observed (Pinchasov, 1991; Iqbal et al., 2017). HBW hens laid larger eggs than the SBW hens, therefore it

would be conseivable that larger eggs would result in larger broilers. However, there is a significant body of research that suggests egg weight is no longer a good predictor of final BW.

### *3.5.2 Maternal Feeding System Treatment (Experiment 2)*

Zuidhof (2018) found that PF hens laid eggs that were 3.1 g larger than the eggs of CON fed hens and that there was no interaction between age and the maternal feeding treatment (P =0.14). Eggs for the current study were collected from wk 51 to 52 and were not found to be significantly different in weight, the short time span of egg collection and the smaller sample size may have caused the discrepancy between the results of this study and Zuidhof (2018). On average PF breeders had yolks that were 0.80 g heavier than CON broilers (Table 3.6). Maternal treatment did not influence egg weight or hatch weight (Table 3.6; Table 3.7). Researchers that have reported differences in yolk weight attribute it to a difference in egg size (Iqbal et al., 2014; Iqbal et al., 2017), which was not seen in the current experiment. FCR and BW were not significantly affected by MT (Table 3.7; Table 3.8). CON offspring had an ADG of 55.5 g/d, which was 1.4 g/d higher than the PF offspring group (Table 3.8). Conventionally fed mothers consumed feed once per day, whereas precision fed mothers fed more than once per day if they did not surpass their BW target. This may have resulted in changes to the hen's metabolism similar to the changes seen when feed restriction is relaxed; however, more research needs to be conducted to fully understand these changes. We observed a significant difference in ADFI between MT between 5 and 6 wk of age; CON offspring consumed 123.7 g/d of feed, which was 8.8 g/d more than the PF offspring group (Table 3.9). It is unknown why this occurred, FCR and ADG were not affected by the interaction between MT and age.

For all dissections, CON offspring had livers that were 8% larger than the livers of PF offspring (Table 3.10). The ADG of CON offspring was 2.6% higher than the ADG of PF offspring (Table

3.8). Zaefarian et al. (2019) concluded that larger livers in broilers may allow them to process nutrients more efficiently than broilers with smaller livers. The larger livers of the CON offspring may be related to more efficiently metabolize nutrients which may have resulted in the increase in ADG seen in the CON offspring. Offspring of CON fed hens had guts that were 8.7% heavier than those of offspring from PF fed hens ($P < 0.001$). Guts were not emptied before weighing which could contribute to the variation. There were observed differences in gut and liver weight, however there was no significant difference in fat pad weight within MT. If epigenetic mechanisms passed information about the maternal nutritional environment onto the offspring it would be possible that offspring from CON fed hens would have heavier fatpads than the offspring of PF hens. CON fed hens consume feed once per day and would have to store the extra energy that they are consuming as fat for later use. Increased fatpad size would give the CON offspring access to more energy reserves than the smaller fatpads of the PF offspring, which would make the CON offspring more successful in an environment where feed was not always in abundance. Spratt and Leeson, (1987) found that broilers from hens that were calorically restricted deposited more carcass fat than broilers from hens that were not calorically restricted. It could also be conceivable that the CON hens appeared to their metabolism that they were in a nutrient abundant environment. CON hens carried more weight as fat because once a day they consumed a large meal. The fatpad size would signal to the metabolism that nutrients were abundant in the environment. In this case it would be less of a priority for offspring of the CON hens to store fat and that is why there was no significant difference found between fatpad size of PF and CON offspring. To determine whether epigenetic mechanisms are responsible for the observed differences, genes associated with growth and development with special focus on DNA methylation and histone modification should be examined.

### 3.6 Conclusions

Based on the current results, the data is consistent with our hypothesis that maternal plane of nutrition in a precision feeding system and feeding system has an influence on final offspring BW. The first obvious reason for the observed differences is differences in egg weight between the two maternal treatments. However, previous research done by Pinchasov et al, (1991), Ulmer-Franco et al. (2010), Tahir et al. (2011), and Iqbal et al, (2017) suggests that egg and chick weight are not as closely related to final BW; the $R^2$ values for the relationship between egg weight and broiler weight have been reported in the range of 0.142 and 0.544. The magnitude of the effect of maternal BW on the final offspring BW is large enough that it would be unlikely to be caused by egg weight, chick weight or composition alone. An epigenetic mechanism would offer another plausible explanation for these results. However, to determine if an epigenetic mechanism was responsible, a thorough genetic examination would have to be performed to determine changes such as DNA methylation or histone modification. Further research should focus on characterising how epigenetic changes occur in the chicken. Based on the results of this study as well as Bowling et al. (2018), van Emous et al. (2015) and van der Waaij et al. (2011), increasing the BW target and the amount of feed available to broiler breeders in a precision feeding system may increase final broiler BW. More research needs to be completed to determine the affect that these changes will have on producer profits.

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## 3.8 TABLES

**Table 3.1.** Composition and calculated analysis of broiler diets.

| Ingredient | Starter | Grower | Finisher |
|---|---|---|---|
| Ingredient composition, g/kg | | | |
| Yellow Corn | 180.00 | 180.00 | 150.00 |
| Canola Oil | 37.73 | 33.60 | 41.30 |
| Fish Meal | 30.00 | 50.00 | 35.10 |
| Soybean Meal | 268.66 | 162.30 | 151.00 |
| Wheat | 429.31 | 532.20 | 580.30 |
| Calcium Carbonate | 14.99 | 10.50 | 10.70 |
| Dicalcium Phosphate | 15.45 | 10.00 | 10.80 |
| Salt | 4.25 | 3.40 | 3.60 |
| L-Lysine | 2.32 | 1.50 | 1.54 |
| DL-Methionine | 2.29 | 1.00 | 0.90 |
| L-Threonine | 0.48 | 1.00 | 0.26 |
| Broiler Vitamin Premix (0.5% inclusion) | 5.00 | 5.00 | 5.00 |
| Choline Premix (0.5% inclusion) | 5.00 | 5.00 | 5.00 |
| Vitamin E 5000 IU/kg | 3.00 | 3.00 | 3.00 |
| Avizyme | 0.50 | 0.50 | 0.50 |
| Coban | 0.50 | 0.50 | 0.50 |
| | | | |
| Calculated nutrient analysis, as-fed basis | | | |
| CP (%) | 23.00 | 20.20 | 19.00 |
| ME (kcal/kg) | 3,067.51 | 3,152.00 | 3,196.00 |
| Calcium (%) | 1.10 | 0.9 | 0.85 |
| Nonphytate phosphorous (%) | 0.50 | 0.45 | 0.42 |
| Lysine (%) | 1.35 | 1.10 | 1.01 |
| Methionine (%) | 0.60 | 0.46 | 0.42 |

[1]Broiler Vitamin Premix: 24,000 mg/kg of Manganese, 20,000 mg/kg of Zinc, 16,000 mg/kg of Iron, 4,000 mg/kg of Copper, 330 mg/kg of Iodine, 2,000,000 IU/kg of Vitamin A, 800,000 IU/kg of Vitamin D, 10,000 IU/kg of Vitamin E, 800 mg/kg of Vitamin K, 800 mg/kg of Thiamin, 2,000 mg/kg of Riboflavin, 13,000 mg/kg of Niacin, 1000 mg/kg of Pyridoxine, 3,000 mg/kg of d-Pantothenic Acid, 4,000 mg/kg of Vitamin B12, 400 mg/kg of Folic Acid, 40,000 mcg/kg of Biotin.
[2]Choline Premix: 80,000 mg/kg of Choline.

**Table 3.2.** BW of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]), on 3 feeding treatments (FT: ad libitum (100%), 80 or 60% of ad libitum), from 0 to 6 wk of age (Experiment 1).

| | | | Age (wk) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | FT | MT | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| | | | g | | | | | | |
| F | | | 43.8$^{a}$ | 176$^{a}$ | 456$^{a}$ | 874$^{a}$ | 1,360$^{b}$ | 1,840$^{b}$ | 2,309$^{b}$ |
| M | | | 44.1$^{a}$ | 183$^{a}$ | 472$^{a}$ | 898$^{a}$ | 1,428$^{a}$ | 1,985$^{a}$ | 2,566$^{a}$ |
| SEM | | | 0.15 | 3.2 | 26.6 | 11.1 | 17.0 | 22.0 | 26.3 |
| | 100 | | 43.8$^{b}$ | 179.5$^{a}$ | 464$^{a}$ | 878$^{a}$ | 1,397$^{a}$ | 1,996$^{a}$ | 2,603$^{a}$ |
| | 80 | | 44.1$^{a}$ | 178.2$^{a}$ | 463$^{a}$ | 881$^{a}$ | 1,375$^{a}$ | 1,921$^{a}$ | 2,492$^{b}$ |
| | 60 | | 44.0$^{b}$ | 179.8$^{a}$ | 465$^{a}$ | 898$^{a}$ | 1,410$^{a}$ | 1,821$^{b}$ | 2,218$^{c}$ |
| SEM | | | 0.17 | 3.8 | 25.0 | 13.6 | 20.8 | 26.9 | 32.2 |
| | | SBW | 43.8$^{a}$ | 178$^{a}$ | 463$^{a}$ | 875$^{a}$ | 1,376$^{a}$ | 1,876$^{b}$ | 2,389$^{b}$ |
| | | HBW | 44.1$^{a}$ | 181$^{a}$ | 465$^{a}$ | 897$^{a}$ | 1,412$^{a}$ | 1,949$^{a}$ | 2,486$^{a}$ |
| SEM | | | 0.14 | 3.3 | 19.7 | 11.2 | 17.0 | 22.0 | 26.3 |
| F | 100 | SBW | 43.7$^{abc}$ | 169$^{c}$ | 442$^{ab}$ | 859$^{ab}$ | 1,345$^{bc}$ | 1,922$^{abc}$ | 2,438$^{cde}$ |
| | | HBW | 44.0$^{abc}$ | 191$^{ab}$ | 483$^{ab}$ | 912$^{a}$ | 1,410$^{ab}$ | 2,030$^{ab}$ | 2,619$^{abc}$ |
| | 80 | SBW | 43.8$^{abc}$ | 169$^{bc}$ | 450$^{b}$ | 857$^{ab}$ | 1,332$^{bc}$ | 1,825$^{cd}$ | 2,323$^{e}$ |
| | | HBW | 44.1$^{ab}$ | 180$^{abc}$ | 458$^{ab}$ | 861$^{ab}$ | 1,377$^{bc}$ | 1,897$^{bc}$ | 2,376$^{de}$ |
| | 60 | SBW | 43.0$^{c}$ | 168$^{bc}$ | 428$^{ab}$ | 827$^{b}$ | 1,285$^{c}$ | 1,621$^{e}$ | 2,019$^{f}$ |
| | | HBW | 43.9$^{abc}$ | 168$^{c}$ | 442$^{ab}$ | 859$^{ab}$ | 1,345$^{bc}$ | 1,922$^{abc}$ | 2,438$^{cde}$ |
| M | 100 | SBW | 43.8$^{bc}$ | 190$^{a}$ | 486$^{a}$ | 889$^{ab}$ | 1,436$^{ab}$ | 1,999$^{b}$ | 2,562$^{bc}$ |
| | | HBW | 43.5$^{bc}$ | 170$^{bc}$ | 448$^{ab}$ | 854$^{ab}$ | 1,397$^{abc}$ | 2,032$^{ab}$ | 2,791$^{a}$ |
| | 80 | SBW | 43.9$^{abc}$ | 179$^{abc}$ | 476$^{ab}$ | 883$^{ab}$ | 1,343$^{bc}$ | 1,903$^{bc}$ | 2,560$^{bcd}$ |
| | | HBW | 44.5$^{a}$ | 185$^{a}$ | 469$^{ab}$ | 922$^{a}$ | 1,447$^{ab}$ | 2,060$^{a}$ | 2,709$^{ab}$ |
| | 60 | SBW | 44.3$^{ab}$ | 191$^{a}$ | 498$^{ab}$ | 933$^{a}$ | 1,516$^{a}$ | 1,987$^{b}$ | 2,434$^{cde}$ |
| | | HBW | 44.3$^{ab}$ | 179$^{ab}$ | 458$^{ab}$ | 906$^{a}$ | 1,426$^{ab}$ | 1,929$^{abc}$ | 2,339$^{e}$ |
| SEM | | | 0.35 | 7.1 | 38.2 | 26.8 | 41.3 | 53.6 | 64.0 |

| Source of Variation | Probability |
|---|---|
| MT | < 0.001 |
| FT | < 0.001 |
| Age | < 0.001 |
| Sex | < 0.001 |
| MT x FT | 0.22 |
| MT x Age | 0.012 |
| MT x Sex | 0.022 |
| FT x Age | < 0.001 |
| FT x Sex | < 0.001 |
| Age x Sex | < 0.001 |
| MT x FT x Age | 0.63 |
| MT x FT x Sex | 0.004 |
| MT x Age x Sex | 0.024 |
| FT x Age x Sex | 0.040 |
| MT x FT x Age x Sex | 0.087 |

[1] Maternal treatments consisted of standard maternal BW (SBW); 121% of SBW at 18 wk (HBW)
[a-e] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.3.** Dry shell, albumen, yolk and total wet egg weight of eggs from high and low BW maternal treatments (MT[1]), collected from 40 to 41 wk of age (Experiment 1).

| MT | Shell | Albumen | Yolk | Egg Weight |
|---|---|---|---|---|
| | g | | | |
| SBW | 7.6 | 5.1 | 13.6 | 55.2[b] |
| HBW | 7.5 | 5.5 | 13.5 | 57.3[a] |
| SEM | 0.20 | 0.15 | 0.35 | 0.35 |
| Source of Variation | Probability | | | |
| MT | 0.80 | 0.085 | 0.60 | < 0.001 |

[1] Maternal treatments consisted of standard maternal BW (SBW); 121% of SBW at 18 wk (HBW)
[a-b] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.4.** ADFI, ADG, and FCR of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]), on 3 feeding treatments (FT: ad libitum (100%), 80 or 60% of ad libitum), from 0 to 6 wk of age (Experiment 1).

| MT | FT | Age | Sex | ADFI | SEM | ADG | SEM | FCR | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | | | | g/d | | | | | |
| SBW | | | | 95.2 | 2.4 | 56.0[b] | 0.5 | 1.635 | 0.034 |
| HBW | | | | 95.3 | 1.8 | 57.6[a] | 0.5 | 1.644 | 0.033 |
| | 100 | | | 96.3[ab] | 4.0 | 61.1[a] | 0.8 | 1.566 | 0.059 |
| | 80 | | | 102.0[a] | 1.5 | 57.8[b] | 0.6 | 1.696 | 0.028 |
| | 60 | | | 87.6[b] | 1.4 | 51.4[c] | 0.5 | 1.655 | 0.025 |
| | | 0 to 1 | | 23.0[e] | 3.7 | 19.2[d] | 0.3 | 1.197[c] | 0.058 |
| | | 1 to 2 | | 62.0[d] | 3.7 | 40.7[c] | 0.6 | 1.544[b] | 0.058 |
| | | 2 to 3 | | 104.0[c] | 3.7 | 60.2[b] | 1.0 | 1.778[b] | 0.058 |
| | | 3 to 4 | | 116.0[bc] | 3.7 | 73.3[a] | 0.9 | 1.592[b] | 0.058 |
| | | 4 to 5 | | 121.0[b] | 3.7 | 74.6[a] | 1.0 | 1.665[b] | 0.058 |
| | | 5 to 6 | | 145.0[a] | 3.7 | 72.7[a] | 1.3 | 2.060[a] | 0.058 |
| | | | F | 86.5[b] | 1.5 | 54.3[b] | 0.4 | 1.565[b] | 0.025 |
| | | | M | 104.0[a] | 2.6 | 59.2[a] | 0.6 | 1.714[a] | 0.040 |

| Sources of Variation | Probability | | |
|---|---|---|---|
| MT | 0.97 | 0.035 | 0.84 |
| FT | < 0.001 | < 0.001 | 0.13 |
| Age | < 0.001 | < 0.001 | < 0.001 |
| Sex | < 0.001 | < 0.001 | 0.002 |
| MT x FT | 0.44 | 0.060 | 0.89 |
| MT x Age | 0.26 | 0.062 | 0.50 |
| MT x Sex | 0.89 | 0.42 | 0.69 |
| FT x Age | < 0.001 | < 0.001 | 0.023 |
| FT x Sex | 0.54 | 0.050 | 0.15 |
| Age x Sex | 0.002 | < 0.001 | 0.023 |
| MT x FT x Age | 0.69 | 0.24 | 0.55 |
| MT x FT x Sex | 0.41 | 0.060 | 0.02 |
| MT x Age x Sex | 0.44 | 0.34 | 0.36 |
| FT x Age x Sex | 0.98 | 0.077 | 0.71 |
| MT x FT x Age x Sex | 0.97 | 0.59 | 0.62 |

[1]Standard maternal BW (SBW); 121% of S at 18 wk (HBW)

[a-e] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.5.** Breast, fat pad, gut, heart and liver weights of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]), on 3 feeding treatments (FT: ad libitum (100%), 80 or 60% of ad libitum), from 0 to 6 wk of age (Experiment 1).

| MT | FT | Age (wk) | Sex | Breast | SEM | Fat pad | SEM | Gut | SEM | Heart | SEM | Liver | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | g | | | | | | | | | |
| SBW | | | | 308.8 | 8.6 | 23.8[b] | 1.1 | 104.6[b] | 2.4 | 7.0 | 0.2 | 28.2 | 0.8 |
| HBW | | | | 323.2 | 5.8 | 27.6[a] | 0.8 | 111.3[a] | 1.6 | 7.2 | 0.2 | 29.4 | 0.5 |
| | 100 | | | 332.3[a] | 8.7 | 28.0[a] | 1.1 | 115.7[a] | 2.4 | 7.3 | 0.2 | 30.5[a] | 0.8 |
| | 80 | | | 316.4[ab] | 9.5 | 26.0[ab] | 1.3 | 111.2[a] | 2.6 | 7.4 | 0.3 | 29.0[ab] | 0.9 |
| | 60 | | | 299.3[b] | 8.7 | 23.1[b] | 1.1 | 97.0[b] | 2.4 | 6.7 | 0.2 | 26.9[b] | 0.8 |
| | | 0 | | 0.6[d] | 13.3 | - | - | 5.3[c] | 3.6 | 0.3[c] | 0.4 | 1.1[d] | 1.2 |
| | | 4 | | 281.7[c] | 10.8 | 17.3[c] | 1.4 | 135.1[b] | 3.0 | 8.1[b] | 0.3 | 33.8[c] | 1.0 |
| | | 5 | | 412.6[b] | 8.6 | 24.0[b] | 1.1 | 138.9[b] | 2.4 | 9.0[b] | 0.2 | 38.1[b] | 0.8 |
| | | 6 | | 569.2[a] | 7.7 | 35.8[a] | 1.0 | 152.7[a] | 2.1 | 11.2[a] | 0.2 | 42.2[a] | 0.7 |
| | | | F | 316.9 | 8.4 | 28.3[a] | 1.0 | 101.2[b] | 2.3 | 6.8[b] | 0.2 | 28.9 | 0.8 |
| | | | M | 315.1 | 6.1 | 23.2[b] | 0.9 | 114.8[a] | 1.7 | 7.5[a] | 0.2 | 28.7 | 0.6 |
| Sources of Variation | | | | Probability | | | | | | | | | |
| MT[1] | | | | 0.167 | | < 0.001 | | 0.020 | | 0.41 | | 0.24 | |
| FT | | | | 0.028 | | 0.001 | | < 0.001 | | 0.100 | | 0.008 | |
| Age | | | | < 0.001 | | < 0.001 | | < 0.001 | | < 0.001 | | < 0.001 | |
| Sex | | | | 0.86 | | < 0.001 | | < 0.001 | | 0.024 | | 0.79 | |
| MT x FT | | | | 0.83 | | 0.67 | | 0.81 | | 0.75 | | 0.75 | |
| MT x Age | | | | 0.60 | | 0.73 | | 0.25 | | 0.21 | | 0.75 | |
| MT x Sex | | | | 0.41 | | 0.27 | | 0.37 | | 0.51 | | 0.97 | |
| FT x Age | | | | 0.020 | | 0.31 | | < 0.001 | | 0.13 | | < 0.001 | |
| FT x Sex | | | | 0.35 | | 0.60 | | 0.57 | | 0.36 | | 0.70 | |
| Age x Sex | | | | 0.80 | | 0.71 | | 0.010 | | 0.004 | | 0.47 | |
| MT x FT x Age | | | | 0.36 | | 0.80 | | 0.88 | | 0.86 | | 0.99 | |
| MT x FT x Sex | | | | 0.75 | | 0.71 | | 0.37 | | 0.58 | | 0.87 | |
| MT x Age x Sex | | | | 0.85 | | 0.81 | | 0.67 | | 0.80 | | 0.79 | |
| FT x Age x Sex | | | | 0.37 | | 0.36 | | 0.61 | | 0.73 | | 0.65 | |
| MT x FT x Age x Sex | | | | 0.85 | | 0.72 | | 0.85 | | 0.99 | | 0.87 | |

[1] Standard maternal BW (SBW); 121% of S at 18 wk (HBW)

[a-d] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.6.** Dry shell, albumin, yolk and total wet egg weight of eggs from hens fed using conventional or precision fed feeding methods (MT[1]). Eggs were dried at 60°C for 4 days (Experiment 2).

| MT | Shell | Albumin | Yolk | Egg Weight |
|---|---|---|---|---|
| | g | | | |
| CON | 5.78 | 3.72 | 13.1[b] | 66.1 |
| PF | 5.79 | 3.84 | 13.9[a] | 68.6 |
| SEM | 0.10 | 0.20 | 0.30 | 1.0 |
| Source of Variation | Probability | | | |
| | 0.94 | 0.61 | 0.037 | 0.071 |

[1] Conventional feeding (CON); Precision feeding (PF)

[a-b] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.7.** BW of female and male broilers from conventionally (CON) and precision fed (PF) hens (MT[1]), raised from 0 to 6 wk of age (Experiment 2).

| | | | Age (wk) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sex | FT | MT | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| | | | g | | | | | | |
| F | | | 46.3 | 184.2 | 510[b] | 849[b] | 1,368[b] | 1,839[b] | 2,281[b] |
| M | | | 46.1 | 184.1 | 541[a] | 936[a] | 1,524[a] | 1,994[a] | 2,471[a] |
| SEM | | | 1.30 | 3.30 | 7.5 | 11.3 | 13.1 | 12.0 | 17.6 |
| | 100 | | 46.9 | 183.4 | 527 | 892 | 1,453 | 2,099[a] | 2,673[a] |
| | 80 | | 46.0 | 183.4 | 524 | 887 | 1,437 | 1,913[b] | 2,378[b] |
| | 60 | | 45.7 | 185.7 | 524 | 898 | 1,449 | 1,738[c] | 2,078[c] |
| SEM | | | 1.47 | 3.97 | 9.5 | 13.7 | 16.0 | 14.6 | 21.7 |
| | | CON | 45.7 | 186.6 | 534 | 895 | 1,449 | 1,921 | 2,376 |
| | | PF | 46.7 | 181.7 | 517 | 891 | 1,443 | 1,912 | 2,376 |
| SEM | | | 1.30 | 3.30 | 7.8 | 11.2 | 13.1 | 11.95 | 17.8 |
| F | 100 | CON | 46.8 | 190 | 530[abc] | 862[cdef] | 1,397[b] | 2,033[b] | 2,530[c] |
| | | PF | 47.4 | 177 | 497[cd] | 833[f] | 1,351[b] | 2,010[b] | 2,608[bc] |
| | 80 | CON | 43.6 | 182 | 504[bcd] | 835[ef] | 1,349[b] | 1,832[c] | 2,286[de] |
| | | PF | 47.1 | 186 | 509[bcd] | 854[def] | 1,371[b] | 1,828[c] | 2,275[d] |
| | 60 | CON | 46.0 | 195[a] | 530[abc] | 870[bcdef] | 1,395[b] | 1,695[d] | 2,008[f] |
| | | PF | 47.0 | 176[b] | 487[d] | 841[ef] | 1,347[b] | 1,636[d] | 1,981[f] |
| M | 100 | CON | 47.1 | 181 | 540[abcd] | 910[abcde] | 1,522[a] | 2,168[a] | 2,795[a] |
| | | PF | 46.3 | 186 | 542[ab] | 965[a] | 1,542[a] | 2,184[a] | 2,757[ab] |
| | 80 | CON | 46.2 | 182 | 546[ab] | 947[a] | 1,523[a] | 1,990[b] | 2,480[c] |
| | | PF | 47.2 | 183 | 535[abcd] | 914[abcd] | 1,504[a] | 2,002[b] | 2,471[c] |
| | 60 | CON | 44.7 | 189 | 551[a] | 944[ab] | 1,508[a] | 1,810[c] | 2,158[e] |
| | | PF | 45.0 | 183 | 529[abcd] | 937[abc] | 1,545[a] | 1,809[c] | 2,165[de] |
| SEM | | | 2.42 | 6.92 | 16.1 | 24.1 | 27.7 | 25.6 | 37.0 |

| Source of Variation | Probability |
|---|---|
| MT | 0.33 |
| FT | < 0.001 |
| Age | < 0.001 |
| Sex | < 0.001 |
| MT x FT | 0.48 |
| MT x Age | 0.63 |
| MT x Sex | 0.29 |
| FT x Age | < 0.001 |
| FT x Sex | 0.80 |
| Age x Sex | < 0.001 |
| MT x FT x Age | 0.98 |
| MT x FT x Sex | 0.22 |
| MT x Age x Sex | 0.63 |
| FT x Age x Sex | 1.00 |
| MT x FT x Age x Sex | 0.76 |

[1] Maternal treatments consisted of conventionally fed hens (CON); Precision fed hens (PF)

[a-d] Means with no common superscript differ ($P < 0.05$)

**Table 3.8.** ADFI, ADG, and FCR of female and male broilers from conventionally (CON) and precision fed (PF) hens (MT[1]), raised from 0 to 6 wk of age (Experiment 2).

| MT | FT | Age | Sex | ADFI | SEM | ADG | SEM | FCR | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | | | | g/d | | | | | |
| CON | | | | 86.9 | 0.5 | 55.5[a] | 0.5 | 1.441 | 0.017 |
| PF | | | | 85.5 | 0.6 | 54.1[b] | 0.4 | 1.477 | 0.016 |
| | 100 | | | 97.0[a] | 0.8 | 62.1[a] | 0.5 | 1.413[b] | 0.020 |
| | 80 | | | 85.8[b] | 0.7 | 54.6[b] | 0.5 | 1.437[b] | 0.018 |
| | 60 | | | 75.8[c] | 0.7 | 47.8[c] | 0.5 | 1.528[a] | 0.022 |
| | | 0 to 1 | | 25.2[e] | 0.8 | 19.7[f] | 0.3 | 1.369[b] | 0.024 |
| | | 1 to 2 | | 45.8[d] | 0.8 | 48.7[e] | 0.4 | 0.953[c] | 0.024 |
| | | 2 to 3 | | 90.7[c] | 0.8 | 52.5[d] | 0.8 | 1.334[b] | 0.024 |
| | | 3 to 4 | | 113.4[b] | 0.8 | 79.2[a] | 0.7 | 1.423[b] | 0.024 |
| | | 4 to 5 | | 122.5[a] | 0.8 | 66.4[b] | 0.9 | 1.889[a] | 0.025 |
| | | 5 to 6 | | 119.3[a] | 1.1 | 62.5[c] | 1.0 | 1.789[a] | 0.036 |
| | | | F | 84.9[b] | 0.6 | 53.1[b] | 0.4 | 1.469 | 0.016 |
| | | | M | 87.4[a] | 0.6 | 56.5[a] | 0.4 | 1.450 | 0.017 |

| Sources of Variation | Probability | | |
|---|---|---|---|
| MT | 0.087 | 0.020 | 0.12 |
| FT | < 0.001 | < 0.001 | < 0.001 |
| Age | < 0.001 | < 0.001 | < 0.001 |
| Sex | 0.003 | < 0.001 | 0.42 |
| MT x FT | 0.73 | 0.98 | 0.95 |
| MT x Age | 0.004 | 0.12 | 0.29 |
| MT x Sex | 0.75 | 0.95 | 0.72 |
| FT x Age | < 0.001 | < 0.001 | < 0.001 |
| FT x Sex | 0.62 | 0.72 | 0.81 |
| Age x Sex | < 0.001 | < 0.001 | < 0.001 |
| MT x FT x Age | 0.68 | 0.79 | 0.71 |
| MT x FT x Sex | 0.72 | 0.81 | 0.30 |
| MT x Age x Sex | 0.73 | 0.75 | 0.20 |
| FT x Age x Sex | 0.95 | 0.94 | 0.25 |
| MT x FT x Age x Sex | 0.21 | 0.59 | 0.51 |

[1] Conventional feeding (CON); Precision feeding (PF)

[a-e] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 3.9.** ADFI of Cobb broilers from conventionally (CON) and precision fed (PF) hens (MT[1]), raised from 0 to 6 wk of age (Experiment 2).

| MT | CON | SEM | PF | SEM |
|---|---|---|---|---|
| Age | g/d | | | |
| 0 to 1 | 25.2[e] | 1.1 | 25.3[e] | 1.1 |
| 1 to 2 | 45.7[d] | 1.1 | 45.8[d] | 1.1 |
| 2 to 3 | 89.7[c] | 1.1 | 91.7[c] | 1.1 |
| 3 to 4 | 113.6[b] | 1.1 | 113.3[b] | 1.1 |
| 4 to 5 | 123.2[a] | 1.1 | 121.9[a] | 1.1 |
| 5 to 6 | 123.7[a] | 1.6 | 114.9[b] | 1.6 |

[1] Conventionally fed hens (CON); Precision fed hens (PF)

[a-e] Means within column and within row with no common superscript differ ($P < 0.05$)

**Table 3.10.** Breast, fatpad, gut, heart and liver weights of female and male Cobb broilers from conventionally and precision fed broiler breeders (MT[1]), fed on 3 treatments (FT: ad libitum (100), 80 or 60% of ad libitum), raised from 0 to 6 wk of age (Experiment 2).

| MT | FT | Age | Sex | Breast | SEM | Fat pad | SEM | Gut | SEM | Heart | SEM | Liver | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | g | | | | | | | | | |
| CON | | | | 291.1[a] | 4.5 | 25.1 | 1.3 | 135.3[a] | 2.4 | 7.8 | 0.2 | 31.4[a] | 0.7 |
| PF | | | | 273.2[b] | 4.4 | 24.1 | 1.3 | 123.5[b] | 2.4 | 7.6 | 0.2 | 28.9[b] | 0.7 |
| | 100 | | | 303.0[a] | 5.5 | 27.6[a] | 1.6 | 136.2[a] | 3.0 | 9.0[a] | 0.2 | 35.2[a] | 0.8 |
| | 80 | | | 287.5[a] | 5.5 | 24.7[ab] | 1.6 | 128.2[ab] | 3.0 | 7.5[b] | 0.2 | 29.5[b] | 0.8 |
| | 60 | | | 256.0[b] | 5.3 | 21.5[b] | 1.5 | 123.8[b] | 2.9 | 6.7[c] | 0.2 | 25.7[c] | 0.8 |
| | | 0 | | 0.6[d] | 6.2 | 0.0[d] | 1.8 | 5.3[d] | 3.4 | 0.3[d] | 0.2 | 1.2[d] | 0.9 |
| | | 4 | | 245.9[c] | 9.1 | 23.1[c] | 2.6 | 169.2[b] | 4.9 | 8.4[c] | 0.3 | 33.1[c] | 1.4 |
| | | 5 | | 379.7[b] | 4.2 | 32.5[b] | 1.2 | 154.7[c] | 2.3 | 10.2[b] | 0.1 | 37.2[b] | 0.6 |
| | | 6 | | 502.4[a] | 4.3 | 42.8[a] | 1.3 | 188.4[a] | 2.3 | 11.8[a] | 0.1 | 49.0[a] | 0.7 |
| | | | F | 282.3 | 4.0 | 25.5 | 1.2 | 120.0[b] | 2.2 | 7.4[b] | 0.1 | 30.1 | 0.6 |
| | | | M | 282.1 | 4.8 | 23.6 | 1.4 | 138.8[a] | 2.6 | 8.0[a] | 0.2 | 30.2 | 0.7 |

| Sources of Variation | Probability | | | | |
|---|---|---|---|---|---|
| MT[1] | 0.005 | 0.57 | < 0.001 | 0.46 | 0.012 |
| FT | < 0.001 | 0.024 | 0.012 | < 0.001 | < 0.001 |
| Age | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Sex | 0.98 | 0.30 | < 0.001 | 0.015 | 0.89 |
| MT x FT | 0.90 | 0.79 | 0.27 | 0.71 | 0.73 |
| MT x Age | 0.13 | 0.86 | 0.003 | 0.21 | 0.25 |
| MT x Sex | 0.59 | 0.47 | 0.015 | 0.73 | 0.82 |
| FT x Age | < 0.001 | 0.36 | 0.006 | < 0.001 | < 0.001 |
| FT x Sex | 0.67 | 0.74 | 0.23 | 0.073 | 0.55 |
| Age x Sex | 0.95 | 0.79 | < 0.001 | 0.15 | 0.081 |
| MT x FT x Age | 0.99 | 0.99 | 0.33 | 0.44 | 0.92 |
| MT x FT x Sex | 0.81 | 0.17 | 0.94 | 0.85 | 0.62 |
| MT x Age x Sex | 0.23 | 0.23 | < 0.001 | 0.96 | 0.92 |
| FT x Age x Sex | 0.97 | 0.96 | 0.93 | 0.61 | 0.74 |
| MT x FT x Age x Sex | 0.31 | 0.60 | 0.44 | 0.88 | 0.80 |

[1] Conventionally fed hens (CON); Precision fed hens (PF)

[a-d] Means within column within effect and within row with no common superscript differ ($P < 0.05$)

## 4.0 The effect of maternal body weight and feeding system on the hormones that influence metabolism and growth in offspring

### 4.1 ABSTRACT

It has been proposed that significant nutrient restriction alters expression of genes associated with growth and metabolism and these changes in gene expression can be passed to offspring providing them with an advantage in similar nutritional environments. The objective of this experiment was to determine how maternal feeding strategies affect growth and metabolic processes of offspring. It was hypothesized that 1) relaxing maternal feed restriction would increase circulating concentrations of GH, IGF-1, $T_3$ and $T_4$ which will increase final BW in offspring and 2) offspring of birds fed using a precision feeding system will store less glycogen in the liver because they consume feed more than once per day. This research consisted of 2 experiments, each using offspring from 2 maternal treatments (**MT**), 2 sexes and 3 feeding treatments (**FT)**. The MT for Experiment 1 consisted of Ross 708 (n = 264) broilers from mothers raised on standard breeder-recommended target BW (**SBW**), or 121% of SBW (**HBW**). The MT for Experiment 2 consisted of Cobb broilers (n = 268) from mothers fed using a conventional feeding system (**CON**) and broilers from mothers fed using a precision feeding (**PF**) system. Broilers from both trials were fed ad libitum until d 28. From 29 to 42 d of age, they were provided feed ad libitum (AL), or at 80% or 60% of AL. The circulating levels of $T_4$ were 11.7% higher in HBW offspring than in SBW offspring, suggesting there may be an increased potential for growth in the HBW offspring. Significant results were not seen in other hormones analyzed, however receptor quantities and quantities in target tissues were not examined. Maternal feeding system was not found to increase glycogen stored in liver and muscle tissue, therefore the 2$^{nd}$ hypothesis was rejected.

**Key words:** broiler chicken, epigenetic, maternal body weight, feeding system, precision feeding

## 4.2 INTRODUCTION

Broiler breeders are restricted to 25 to 35% of what would be their ad libitum feed intake to prevent them from growing to their full potential and to improve their reproductive efficiency (de Jong et al., 2002). The severity of feed restriction reported by de Jong and colleagues in 2002 may be even lower due to continued selection for growth rate, efficiency and yield since that time, and little change in breeder target BW. In North America, feed restriction in broiler breeders is most commonly implemented by feeding an appropriate amount of feed with 24 hours of time between feeding times. Zuidhof (2018) found that broiler breeders fed once per day proportion more of their nutrients towards adipose tissue development, while precision fed birds, which consume feed multiple times per day, direct more of their nutrients towards muscle tissue development. This change in how nutrients are distributed suggests that altering the birds' feeding system also changes how the birds' metabolic system functions.

Overall metabolism and growth is regulated by hormones such as $T_3$, $T_4$, insulin, glucagon, growth hormone (GH) and insulin-like growth factor-I (IGF-1). $T_3$, $T_4$, GH, and IGF-1 are all known to be involved in increased final BW and muscle development (Xiao et al., 2017). The activity of all could be affected by altering the amount of feed given and the pattern in which the feed is consumed. Metabolic changes induced by feed availability or changes in the feeding system in broiler breeders may be passed onto their offspring through epigenetic mechanisms (van der Waaij et al., 2011). Epigenetics has been defined as a heritable change in gene expression with no alteration in the DNA sequence; changes in gene expression often involve DNA methylation or histone modification (Marchlewicz et al., 2016). Information about feed availability could be passed on to the offspring through heritable changes affecting gene

expression, giving them a better chance of survival in a similar environment. The changes that are passed on to the offspring give them a genetic advantage that allows them to thrive in environments where offspring that did not have the same genetic changes passed to them would not. An estimate of energy stored in these tissues can be measured as glucidic potential (GP), which is the sum of glycogen, ½ lactate and its intermediate metabolites (glucose and glucose-6-phosphate; Dalrymple and Hamm, 1973; Yambayamba et al., 1996). GP is a measure of the available glucose substrate that can be utilized for energy (Yambayamba et al., 1996). In an environment where feed is not readily available, the birds may benefit from storing more of the glucose they consume as glycogen in their liver or muscle tissue (Bennett et al., 2007). If the maternal nutritional environment causes changes in how the offspring develop and proportion nutrients for growth, it may become apparent in how the offspring store energy. Altering the way offspring store energy to better match the nutritional availability in the environment would make them more successful in their environment and therefore the genetic changes would be conserved. The objective of the study was to determine how maternal feed availability and feeding system affects the growth and metabolic processes of offspring. We hypothesized that 1) relaxing maternal feed restriction would increase circulating concentrations of GH, IGF-1, $T_3$ and $T_4$ which would increase final BW in offspring and 2) birds fed using a precision feeding system would store less glycogen in the liver because they are consuming feed more than once per day.

## 4.3 MATERIALS AND METHODS

This research was conducted at the Poultry Research Centre at the University of Alberta (Edmonton, AB, Canada). Animal use in this study was approved by the University of Alberta

Animal Care and Use Committee for Livestock and followed animal care principles established by the Canadian Council on Animal Care (Canadian Council on Animal Care, 2009).

### *4.3.1 Experiment 1: Maternal Body Weight*

#### *4.3.1.1 Treatments and Experimental Design.*

A 2 x 2 x 3 factorial arrangement of treatments was used in this study with offspring being hatched from broiler breeders fed on a HBW curve or a SBW curve (van der Klein et al., 2018)., both male and female offspring were used and offspring were assigned to an ad libitum, 80% of ad libitum, or 60% of ad libitum feed treatment. Eggs were collected from 40 to 41 wk old hens. Birds in this study were the offspring of precision fed Ross 708 Broiler Breeders on a breeder recommended standard BW curve (SBW) beginning at photostimulation or breeders raised on a high BW, which was 21% above the SBW curve (van der Klein et al., 2018). Male breeders were fed to the recommended target BW curve (Aviagen, 2016). This experiment started on d 0 and was completed on d 42. Offspring were feather sexed at hatch and separated into male and female groups at hatch then assigned to one of three feeding treatments: ad libitum, 60% of ad libitum and 80% of ad libitum. The 60% of ad libitum broiler feeding treatment was calculated as 60% of the Ross 708 broiler performance objectives, the 80% of ad libitum broiler feeding treatment was calculated as 80% of the Ross 708 broiler performance objectives (Aviagen, 2014). After feeding treatments were assigned birds were assigned to 1 of 72 sex separate cages based on feeding treatment. The sex of each bird was confirmed upon dissection at 28, 25, and 42 d of age. The cage was used as the experimental unit.

#### *4.3.1.2 Animals and Housing.*

Two hundred sixty-four Ross 708 broilers were raised sex-separately from 0 to 42 d in above floor cages. The cage dimensions were 1.19 m x 0.53 m and were stocked with 2 to 5 birds depending on treatment combinations. Dissections were completed on d 28, 35, and 42 so not all birds were raised to d 42. On d 0 all birds were fitted with a neck tag that provided a unique code specific to each bird. All birds were exposed to 24L:0D (0 to 3d), followed by 18L:6D (4 to 42d). A Ross 708 temperature schedule was used that began at 34°C on d 0 and decreased by 1°C daily until 23°C was reached on d 11, which was maintained for the remainder of the study. The number of birds allocated to each cage could not be kept equal due to a shortage of male offspring from the SBW mothers, and 2 to 5 birds were in each pen. At the beginning of the study there were 27 male birds in both the ad libitum and 80% of ad libitum treatments from high BW hens. Twenty-seven, twenty-seven, and twenty-eight male birds were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively. Eighteen, eighteen, and nineteen male birds were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively. Twenty-two, twenty-three, and twenty female birds from high BW hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatment, respectively. Seventeen, fourteen, and seventeen female birds from standard BW hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum treatments, respectively. Each pen contained three drinking nipples. Litter consisted of pine shavings at a depth of 5 cm throughout each cage. Individual BW was recorded weekly.

#### *4.3.1.3 Feeding and Diets.*

On d 28 birds were assigned to either ad libitum, 60% of ad libitum, or 80% of ad libitum feeding treatment. Prior to feed restriction birds were fed ad libitum. Feed restriction began on d 28 for maternal treatment effects to be examined under both ad libitum fed and restrictive

conditions. Feed was added daily and was weighed back once per week to determine feed intake. When feed restriction began, the birds exposed to feed restriction treatments were fed at 1:00 PM daily. Birds on the ad libitum treatment had continued free access to feed. All birds were fed a starter pellet diet (23% CP, 3067.51 kcal/kg) from 0 to 2 wk of age, a grower pellet diet (20.20% CP, 3152.00 kcal/kg) from 2 to 4 wk of age and a finisher pellet diet (19% CP, 3196.00 kcal/kg) from 4 to 6 wk of age (Table 3.1).

### *4.3.2 Experiment 2: Maternal Feeding System*

#### *4.3.2.1 Treatments and Experimental Design.*

A 2 x 2 x 3 factorial arrangement of treatments was used in this study. The maternal treatment (MT) consisted of offspring being hatched from broiler breeders fed using either conventional methods (CON) or by precision feeding (PF) (Zuidhof, 2018), both male and female offspring (sex confirmed at dissection) were used and offspring were assigned to an ad libitum, 80% of ad libitum, or 60% of ad libitum feed treatment. Eggs were collected from MT hens from 51 to 52 wk of age. Offspring were placed into one of three mixed sex pens. The bird was used as the experimental unit.

#### *4.3.2.2 Animals and Housing.*

Three hundred Cobb broilers were raised from 0 to 42 d. Fifty, forty-nine, and fifty-one birds hatched from conventionally fed hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum. Fifty, fifty-one, and forty-nine birds from precision fed hens were allocated to the ad libitum, 80% of ad libitum, and 60% of ad libitum. Dissections occurred on d 28, 35, and 42 so not all birds were raised until d 42. On d 0, chicks were allocated to one of three pens, each containing three precision feeding (PF) stations (Zuidhof et al., 2017; Zuidhof, 2018). One

hundred chicks were allocated to each pen. PF stations control feed intake for each individual bird; in this experiment all birds were divided into groups of 3 based on BW. Birds were divided into groups on d 28. The heaviest bird was fed ad libitum, the middle BW bird was fed 80% of what the ad libitum bird consumed and the lightest BW bird was fed 60% of what that ad libitum bird consumed. All birds were exposed to 24L:0D (0 to 3 d) and 18L:6D (4 to 42 d). A standard temperature schedule was used that began at 33°C on d 0 and decreased by 1°C to 23°C on d 28, which was maintained for the remainder of the study. Mixed sex birds were divided into 3 pens that measured 5.40 m x 4.50 m. Two hanging nipple drinkers, with 8 nipples per drinker were placed in each pen. Litter consisted of pine shavings at a depth of 5 cm throughout each pen. Starting at d 0 birds were weighed once per day to determine their success in using the PF stations and to monitor their weight gains prior to the point at which individual feeding was implemented.

#### *4.3.2.3 Precision Feeding System.*

The PF system was used to identify individual birds through the use of a radio frequency identification tag and feed them according to how their BW compared to other birds using pair feeding. Pair feeding is described in the section below. The ad libitum fed birds were allowed access to as much feed as desired. From 4 to 6 wk of age, the 80% of ad libitum and 60% of ad libitum fed birds were allowed access to 80% and 60% of what the ad libitum bird consumed, respectively. BW of individual birds were measured using a scale in the PF station. If the bird's intake was below the desired intake compared to the ad libitum bird, the PF system allows them access to feed in the main stage of the PF station. If the bird's intake was at the desired intake level the bird was sent out of the station and was not fed. Individual feed intake and BW was measured by the PF station so the bird was used as the experimental unit.

#### *4.3.2.4 Feeding and Diets.*

All birds were fed a starter pellet ration from 0 to 2 wk of age, a grower pellet ration from 2 to 4 wk of age and a finisher pellet ration from 4 to 6 wk of age (Table 3.1). Three PF stations were in each pen. To train the birds to eat, three paper plates with 200 g of feed were placed around the ramp of the station and 200 g of feed was placed on the ramp. One paper plate with 200 g of feed was placed in both the scale stage and feeding stage. Feed was replaced on the plates as it was consumed, and plates stopped being filled once the majority of birds were searching for feed inside the stations. From d 0 to d 13 (training period) every morning birds were weighed to determine whether every bird was gaining weight. If a bird was heavier than the previous day it was assumed to be eating and was returned to the pen. If a bird was lower in weight than the previous day, that bird was shown where the feed was in the station. If the same bird did not gain the desired amount of weight for 2 days in a row, it was put into a training pen. The training pen contained one station and one drinker with eight nipples. Feed left in the station from the previous day was weighed and recorded. All feed added to stations was weighed and recorded. During training feed intake (**FI**) was measured at the pen level. FI was measured by subtracting the amount off feed left in the pen from the previous day from the total feed added to the pen, this number was then divided by the number of birds in the pen to determine average intake per bird.

At d 13 each bird was fitted with a radio frequency identification tag on its left wing, so individual birds could be identified by the PF stations. On d 14, individual feeding mode was started, during this time all birds were allowed ad libitum access to feed until d 28. Daily feeding reports that showed the level of feed consumption for every bird that entered the PF stations were used to determine which birds were consuming feed. If a bird was found not to be eating it was

trained by putting the bird in the station, showing the bird where the feed was and ensuring that it consumed feed. Prior to 28 d of age, each time a bird entered the station it was allowed access to feed for 45 sec. Feed restriction began at 4 wk of age for the birds chosen to be part of the feed restriction treatments.

#### *4.3.2.5 Pair Feeding*

When feed restriction began, birds were ranked from highest to lowest BW and were placed into groups of 3, with the birds closest in BW being grouped together. The heaviest bird in each group was assigned to be the master and was fed ad libitum for the remainder of the experiment, this was not a random selection. The middle BW bird was assigned to the 80% of the intake of the master bird. The lowest BW bird was assigned to 60% of the intake of the master bird. Feed intake of the master bird was cumulative from the time the pairings occurred to the end of the experiment, which determined what the restricted birds were fed.

### *4.3.3 Sample Collection – Experiment 1 and Experiment 2*

#### *4.3.3.1 Blood Collection and Analysis*

Blood samples were taken at 27, 34, and 41 d of age from four randomly selected birds per MT x FT x sex interaction. Blood was collected in 4 mL sodium heparin vacutainers by venipuncture of the left brachial vein 1 to 3 h after the lights were turned on. Blood collection was repeated weekly on the same birds. Immediately after collection, blood was centrifuged (3000 rpm) for 15 minutes at 4°C. Plasma was allocated into 2 mL centrifuge tubes and frozen at -25°C until analysis. For all hormones analyzed, standard curves were used to ensure that all concentrations were accounted for. All samples were analyzed in a single assay run, so there is no inter-assay CV. CV was also calculated from the duplicated reads of the samples, which is why some are high.

Plasma levels of $T_3$ and $T_4$ were measured using coated tube radioimmunoassay kits (MP Biomedicals, Solon, OH) with the following modifications (Appendix A). Sensitivities of both standard curves were extended to 0.03 ng/mL ($T_3$) and 1.5 ng/mL ($T_4$) by performing a series of 2-fold dilutions with steroid-free serum (MP Biomedicals, Solon, OH). Samples were diluted 1:5 (T3 only) using steroid-free serum (MP Biomedicals, Solon, OH), and tubes were incubated for 16 h at 4°C following addition of tracer. Radioactivity retained in each tube was counted for 1 min with a gamma counter (Wallac 1470 Wizard Automatic Gamma Counter, Perkin Elmer Life Sciences, Waltham, MA). For assay validation, a pool of mixed sex (equal amounts of male and female) and age chicken plasma was used. Intra- and inter-assay CVs were calculated from 6 independent assay runs in which 4 different pools were each analyzed 6 different times. During assay validation it was determined that $T_3$ had a intra-assay CV of 3.7%, an inter-assay CV of 5.6% and a cross reactivity with $T_4$ of 1.4%. During assay validation for $T_4$ the intra-assay CV was 7.9%, the inter-assay CV was 13.5% and the cross reactivity with $T_3$ was 3.3%. Intra-assay coefficient of variation values from the experimental runs for experiment 1 were 16.7% and 8.2% for $T_3$ and $T_4$, respectively. Intra-assay coefficient of variation values for experiment 2 were 16.9% and 9.3% for $T_3$ and $T_4$, respectively.

Corticosterone (CORT) levels were determined using a commercially available ELISA kit (catalog number 501320; Cayman Chemical, Ann Arbor, MI; Appendix A). Samples for the CORT assay were ether extracted twice with 5X volume each time. Briefly, all samples were placed in a 65°C water bath for 1 hr to denature proteins that may interfere with extraction. After samples were allowed to cool, a 5x volume of diethyl ether was added to each sample and vortexed for 30 sec. Samples were then placed in a dry ice bath for ~5 min until the aqueous phase froze. The ether fraction was then decanted into a new tube. The ether extraction was

completed twice for each sample. All CORT samples were diluted 1:4 with ELISA buffer solution, incubated with tracer and antibody for 18 h at 4°C. The plate was then washed and developed and read with an absorbance of 405 nm. This CORT assay detects levels as low as 30 pg/mL. Intra-assay coefficient of variation values for the experimental runs for CORT were 11.3% and 20.3% for experiment 1 and 2, respectively. The inter-assay CV for the experimental runs was 17.7%. This assay was validated with an ether extracted mixed sex/mixed age pool. Intra- and inter-assay CVs were calculated from 6 independent assay runs in which 4 different pools were each analyzed 6 different times. Upon validation it was determined that the intra-assay CV was 8.29% and the inter-assay CV was 9.6%.

Glucagon was determined using a commercially available Chicken GCG/Glucagon ELISA kit according to manufacturers instructions (catalog number LS-F16677; LifeSpan BioSciences, Inc, Seattle, WA). Samples were added to the plate and incubated overnight at 4°C, after incubation was complete the plate was washed. All glucagon samples were then incubated twice for 60 min at 37°C with detection agent. The plate was reading using an absorbance of 450 nm. The manufacturer determined that the glucagon assay could detect levels as low as 31.2 pg/mL. Intra-assay coefficient of variation values for glucagon were 24.5% and 23.1% for experiment 1 and 2, respectively. The inter-assay for glucagon was 22.9%. GH, IGF-1 and insulin levels were determined using commercially available ELISA kits (CSB-E09866Ch, CSB-E09867Ch, CSB-E13293C; Cusabio, Houston, TX) with modifications to the procedure (Appendix A). For each assay 3 plates were ran per experiment. During the analysis of IGF-1, GH and insulin, the samples were incubated with conjugate overnight at 4°C instead of being incubated for an hour at 37°C. The plate was then washed, HRP was added and the plate was incubated at 37°C for 30 min. The plate was washed again and then substrate buffer was added with another round of

incubation at 37°C for 15 min. An absorbance of 450 nm was used to read the plates. Samples used to analyze IGF-1 were diluted 1:3 with buffer solution. As per the manufacturer, GH, IGF-1 and insulin assays could detect levels as low as 625 pg/mL, 125 pg/mL, and 0.59μIU/mL, respectively. No significant cross reactivity or interference has been observed in the GH, IGF-1, or insulin assays from the manufacturer (CSB-E09866Ch, CSB-E09867Ch, CSB-E13293C; Cusabio, Houston, TX). Intra- and inter-assay CV values for GH were 4.4% and 5.5%, respectively, for experiment 1. Intra- and inter assay CV values for IGF-1 were both 5.8% for experiment 1. Intra- assay CV values for insulin were 3.3% and 2.9% for experiment 1 and 2, respectively. Inter-assay CV for insulin was 9.6%. Assays were validated using mixed sex/age pools. During assay validation for IGF-1 it was determined that intra-assay CV was 5.4% and inter-assay CV was 12.9%. During the assay validation of GH it was determined that intra-assay CV was 2.6% and inter-assay CV was 4.7%. It was determined during assay validation for insulin that the intra-assay CV was 5.0% and the inter-assay CV was 12.7%. Lastly, during the assay validation for glucagon it was determined that the intra-assay CV was 7.1% and the inter-assay CV was 10.0%.

#### *4.3.3.2 Dissection and Tissue Sampling*

On d 28, 35 and 42, dissections were carried out to obtain liver and breast tissue samples from all birds to be analyzed for glucidic content. Equal numbers of birds from each treatment were randomly selected for dissection on d 28, 35, and 42. All birds randomly selected for dissection were euthanized via cervical dislocation. Within 10 minutes of cervical dislocation, 20 g samples were taken from the left Pectoralis major and right lobe of the liver. Samples were snap frozen in

liquid nitrogen and immediately stored on dry ice. After dissections were complete, samples were transported and stored in a -80°C freezer until analysis was completed.

#### *4.3.3.3 Determination of Muscle and Hepatic Glucidic Potential.*

Glucidic metabolites were extracted from frozen muscle and liver samples as described by Dalrymple and Hamm (1973) and Yambayamba et al. (1996) with the exception that free glucose, glycogen as glucose units and lactate content of samples were analyzed on the YSI 2300 StatPlus glucose/lactate analyzer (YSI Incorporated, Dayton, OH). Prior to analysis, samples were crushed in a mortar cooled with liquid nitrogen. One gram of sample was removed and placed into 5.0 mL of perchloric acid (0.6N). Samples were then homogenized using a PT-MR 3100C homogenizer (Kinematica GmbH, Switzerland). Two hundred µL of homogenate was added to a centrifuge tube to be used for glycogen analysis using the amyloglucosidase procedure as described by Dalrymple and Hamm (1973). The original tube was used for glucose/lactate samples. The original tube was centrifuged (9000 rpm) for 10 minutes at 4℃. Two hundred µL of potassium carbonate (3M) was added to 2.3 mL of supernatant, this solution was vortexed for 30 sec. This solution was then centrifuged (3000 rpm) for 10 minutes at 4°C. This tube was used to assay lactate only in the liver samples and both glucose and lactate in breast tissue samples. Analysis of liver glucose required 383 µL of supernatant from the previous tube mixed with 1.917 ml of perchloric acid (0.6N) and 200 µL of potassium carbonate (3M) in a glass tube. This mixture was vortexed for 30 sec and centrifuged (3000 rpm) for 10 minutes at 4°C. Samples were placed in a refrigerator overnight and analysed the following day using a YSI 2300 StatPlus glucose/lactate analyzer (YSI Incorporated, Dayton, OH).

### *4.3.4 Statistical Analysis.*

#### *4.3.4.1 Statistical Analysis – Experiment 1 and Experiment 2*

All ANOVA were conducted using the HPMIXED and MIXED procedures of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). In experiment 1, individual bird was used as the experimental unit for dissection data, blood plasma data and glucidic analysis. The pen was used as the experimental unit for feed intake analysis. In experiment 2, individual bird was used as the experimental unit. MT, FT, and sex were used as sources of variation. Age was not included as a source of variation due to model convergence issues. Means were reported as different where $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$. Tukey's range test was used for multiple mean comparisons to reduce type II errors (false positives).

## 4.4 RESULTS AND DISSCUSION

### *4.4.1 Experiment 1 (Maternal Body Weight Experiment)*

Of all the plasma hormones measured in the offspring, only $T_4$ levels were significantly affected by MT. $T_4$ levels were 11.7% higher in HBW offspring when compared to SBW offspring (Table 4.2). $T_4$ is released by the thyroid gland and is de-iodinated to form $T_3$, which is thought to cause an increase in growth and metabolism (Stojević et al., 2000; Győrffy et al., 2009;). The previously mentioned results are consistent with the finding that offspring of HBW hens are 3.8% heavier at wk 6 then offspring of SBW hens (Chapter 3). However, MT did not have a significant effect on the plasma level of $T_3$, which is the more active thyroid hormone in the chicken and known to be more involved in increasing growth rate than $T_4$ (Stojević et al., 2000). It is possible that $T_4$ was being converted into $T_3$ in the target tissues which would not be detectable in the analysis of plasma.

The effect of MT and sex on the circulating concentration of $T_4$, shows a higher effect of MT in males but not females. HBW males had 2.4% higher $T_4$ levels than SBW males, while HBW females had 2.0% higher $T_4$ levels than SBW females. As previously mentioned, $T_4$ is de-iodinated to form $T_3$ and has been related to increased growth in broilers (Ellestad et al., 2019; Darras et al., 2000; Stojević et al., 2000). The trend described above was not observed for $T_3$. HBW offspring within the 60% of AL treatment group with 8.8% higher $T_3$ levels than their SBW counterparts. On the other hand, within the AL group, SBW offspring showed 13.3% higher $T_3$ levels than the HBW offspring. The first hypothesis stated that relaxing maternal feed restriction will increase circulating concentrations of GH, IGF-1, $T_3$ and $T_4$ which will increase final BW in offspring, therefore if this hypothesis was correct $T_3$ levels would have been the highest in the HBW offspring. However, that was not what was observed therefore, this study does not support the original hypothesis. Future studies should examine the level of hormones associated with increased growth and metabolism in the target tissues as well as the receptor quantities for these hormones.

The level of offspring feed restriction had a more profound effect on the circulating concentration of hormones than MT. CORT level was not significantly different between MT but was significantly higher in the 60% of AL group than the AL group (Table 4.2). The results of the current study are in agreement with the findings of de Jong et al. (2002) who concluded that broiler breeders restricted based on primary breeder recommendations showed higher CORT levels than their ad libitum fed counterparts due to stress caused by being continuously hungry. $T_3$ levels were also not significantly affected by MT but were significantly higher in the AL treatment than in the 60% of AL treatment. Conversely, $T_4$ levels were higher in the 60% of AL

group than in the AL group. The endocrine system of the birds exposed to the AL treatment may be prepared to express growth potential that cannot be expressed at the allowed level of intake in the restricted group (Győrffy et al., 2009). The AL fed birds were significantly heavier than the 60% of AL birds; therefore, finding increased circulating $T_3$ in these birds was expected, due to the involvement of $T_3$ in increasing growth rate (Chapter 3; Győrffy et al., 2009). $T_3$ is the more active of the three thyroid hormones in the chicken and is more involved in growth and metabolism than $T_4$ (Singh et al., 1967a; Singh et al., 1967b). Low levels of $T_3$ and higher levels of $T_4$ in the 60% of AL treatment may be due to the need for a slow metabolism to preserve the nutrients that are available (McMurtry et al., 1988). Increased $T_4$ levels are not associated with a decrease in metabolic rate. Metabolism is not increased when $T_4$ is not de-iodinated into $T_3$.

Previous research suggests that high IGF-1 levels are seen with increased growth (Goddard et al., 1987; McMurtry et al., 1997). However, in experiment 1 IGF-1 was 27.9% higher in the 60% of AL treatment than the AL treatment. The AL treatment had an increased opportunity to reach their growth potential than the 60% of AL treatment which would lead us to believe that their plasma IGF-1 levels would be higher than that of the 60% of AL treatment. However, it may be the case that the 60% of AL treatment birds were trying to reach their genetic potential for growth by secreting increasing amounts of a hormone associated with growth but due to nutrient deficiency they were not able to reach their growth potential.

Similar to the concentrations of plasma hormones, GP was most affected by FT. In the current study, AL fed birds had a higher liver GP and plasma insulin level than the 60% of AL birds (Table 4.3). Birds in the AL treatment consumed more feed (Chapter 3), therefore they were consuming more glucose than the 60% of AL treatment, which would cause the 0.5 μIU/mL

increase in insulin. However, there was no difference in the glucagon to insulin ratio (**G:I**) between AL and 60% of AL birds (Table 4.2). Feeding treatment had no effect on the level of breast GP. All birds were full fed at wk 4, which could be the cause for the observed age effect. GP of the breast decreased with age but was unaffected by other treatments (Table 4.4). This was expected because the liver is the major glycogen storage center in the chicken (Bennett et al., 2007). Glycogen storage in the breast happens in such minute amounts that none of the FT that birds were exposed to had an effect on breast GP (Klasing, 1998). No evidence of a maternal effect on liver GP was found in the current study, further research needs to be done to determine the effect of maternal nutritional availability on the offspring's ability to mobilize and store glucose.

The effect of FT differed in males and females; plasma CORT levels of the females in the 60% of AL treatment were 138% higher than those in the AL treatment, while the males in the 60% of AL treatment were only 7.4% higher than males in the AL treatment. CORT is known to have a positive effect on the release of glucose into the bloodstream (Kafri et al., 1988). Due to the level of restriction, the metabolism of the restricted group may have been attempting to mobilize their glycogen stores to compensate for the lack of nutrients as a result of feed restriction. However, significantly higher levels of glucagon were not seen in the 60% of AL treatment (Table 4.2). As stated previously, secretion of CORT was also increased in response to increased levels of stress, increased CORT levels in females of the 60% of AL treatment could also be due to stressed caused by feed restriction (Kafri et al., 1988). This result would suggest that females may be more susceptible to nutritional related stress than males. IGF-1 showed a similar trend. Females of the 60% of AL treatment showed 36% higher IGF-1 levels than their AL counterparts, while the 60% of AL males had only 20% higher IGF-1 levels than the AL treatment. It has been

proposed that IGF-1 also has a role in growth and metabolism in the chicken (Goddard et al., 1987). The metabolism of the birds in the 60% of AL treatment may be trying to overcome the restriction and reach their biological growth potential by secreting hormones that would aid in growth or preparing itself to grow to its full potential should the restriction be relaxed. More research needs to be done to solidify the function of IGF-1 in relation to growth and metabolism in the chicken.

### *4.4.2 Experiment 2 (Maternal Feeding System and Precision Feeding)*

The effect of MT on plasma glucagon level was dependent on age; PF offspring had 157% higher glucagon levels than CON offspring at wk 5 (Table 4.5). The opposite pattern was seen in wk 6, CON offspring had 28.8% higher levels of glucagon than PF offspring (Table 4.5). Even though there was observed differences in the plasma glucagon level, there was no difference detected in the amount of stored liver or muscle glycogen. We did not expect to see a difference in the glucagon level because, for homeostasis to be achieved the body must keep tight control on the circulating blood glucagon levels, which would in turn keep the circulating blood glucose level at a steady state, regardless of treatment. There was a trend towards PF offspring having a higher G:I than CON offspring at wk 5 and CON offspring having a higher G:I at wk 6 ($P = 0.030$; Table 4.5). CON fed hens ate meals less frequently than PF hens so a higher G:I ratio would be expected because CON fed hens would have to mobilize their stored glycogen using glucagon to keep up with their energy demand. If this is genetically heritable, CON offspring exhibit an increased G:I ratio better preparing them for a environment where meals occurred less frequently.

Similar to Experiment 1, the AL group had significantly higher levels of $T_3$ and lower $T_4$ levels than their 60% of AL counterparts (Table 4.5); which, is likely due to the difference in metabolic

needs of the two groups. According to Győrffy et al., (2009), feed restriction causes $T_3$ levels to decrease due to a reduction in $T_4$ activation and an increase in $T_3$ inactivation. It is in the interest of a restricted bird to conserve energy and protein stores, which can be done by reducing the activity of $T_3$. GP was not significantly different in breast or liver tissue between MT (Table 4.6; Table 4.7), which allows us to conclude that maternal feeding system does not affect how offspring store energy as glycogen in their liver or breast tissue. The effect of MT on $T_3$ levels was dependent on FT; within the AL group there was a trend towards CON offspring having 14.1% higher $T_3$ levels than PF offspring, while within the 60% of AL group, PF offspring had 5.9% higher $T_3$ levels than CON offspring ($P = 0.069$). This result is consistent with the hypothesis of van der Waaij et al., 2011 that matching the maternal and offspring nutritional environment will be beneficial to the offspring. Offspring from conventionally fed mothers that were exposed to the AL treatment had an environment that more closely matched their mothers than the CON offspring exposed to the 60% of AL treatment. CON offspring exposed to the AL feeding treatment had on average 14.1% higher $T_3$ level throughout the experiment than CON offspring on the 60% of AL treatment and this would suggest that these offspring would have an increased ability to reach their growth potential due to $T_3$ being associated with growth. However, as reported in chapter 3 no interactions between MT and FT influenced offspring performance.

No FT effect on plasma CORT was observed. We hypothesize that this is due to the use of the precision feeding system and the method used to house these birds. The precision feeding system gave the birds the ability to engage in feed-seeking behavior at all times, even if the birds were not successful in being given a feeding bout because they had already consumed their allotment for that particular day, they could still enter the feeding system. CORT levels were 46.9% higher

in experiment 1 as opposed to experiment 2. The process of entering the feeding system and attempting to feed may have lowered their stress level by satisfying a need to forage. It is also possible that the difference in environment reduced the stress level of the birds in the maternal feeding system trial as compared to those in the maternal BW trial. The cages used to house birds in experiment 1 did not provide them the same opportunity to engage in feed seeking behavior that the floor pens did in experiment 2.

### 4.5 Conclusion

$T_4$ was the only hormone that was found to be significantly higher in HBW offspring. Target tissue levels of $T_3$ were not measured but may have been altered. It may be possible that levels of $T_3$ were higher in the target tissues but not in the blood. Future experiments should be conducted that examine the difference in the amount of hormone receptors present or the change in hormone levels over time, which may result in significant results that were not seen in this study. It was also determined that altering maternal feeding system did not significantly decrease the amount of glycogen stored in liver and muscle tissue, therefore our 2$^{nd}$ hypothesis was rejected. However, we did observe that BW was significantly higher in the offspring of HBW hens than in offspring of SBW hens raised in a precision feeding system (Chapter 3). Altering MT does have an effect on the growth of the broiler; however, the mechanisms that make this possible are unclear. It can be concluded that maternal feed restriction in a precision feeding system does have an influence on the plasma concentration of $T_4$ in the chicken. The effects that the changes in hormone concentration have on offspring performance is still unknown. This research is only the beginning of what needs to be explored when it comes to maternal BW and maternal feeding system and their effect on offspring performance. Further studies should focus on increasing the level of feed restriction that offspring are subjected to, to more closely match maternal feed

restriction levels which may expose epigenetic effects that were passed from mother to offspring. Closer examination of changes in DNA methylation and histone modification in the offspring may also give further insight into changes in gene expression that are caused by the nutritional environment. There may also be significant value in exploring the effect that maternal BW and feeding system has on the concentrations of embryonic metabolic and growth hormones. To increase the final BW of broilers it is recommended that the BW target of broiler breeders in a precision feeding system be raised by 21% based on the results of this project and the results posted in Chapter 3.

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## 4.7 TABLES

**Table 4.1.** Composition and calculated analysis of broiler diets.

| | Starter | Grower | Finisher |
|---|---|---|---|
| Ingredient composition, g/kg | | | |
| Yellow Corn | 180 | 180 | 150 |
| Canola Oil | 37.73 | 33.60 | 41.30 |
| Fish Meal | 30.00 | 50.00 | 35.10 |
| Soybean Meal | 268.66 | 162.30 | 151.00 |
| Wheat | 429.31 | 532.20 | 580.30 |
| Calcium Carbonate | 14.99 | 10.50 | 10.70 |
| Dicalcium Phosphate | 15.45 | 10.00 | 10.80 |
| Salt | 4.25 | 3.40 | 3.60 |
| L-Lysine | 2.32 | 1.50 | 1.54 |
| DL-Methionine | 2.29 | 1.00 | 0.90 |
| L-Threonine | 0.48 | 1.00 | 0.26 |
| Broiler Vitamin Premix[1] (0.5% inclusion) | 5.00 | 5.00 | 5.00 |
| Choline Premix[2] (0.5% inclusion) | 5.00 | 5.00 | 5.00 |
| Vitamin E 5000 IU/kg | 3.00 | 3.00 | 3.00 |
| Avizyme | 0.50 | 0.50 | 0.50 |
| Coban | 0.50 | 0.50 | 0.50 |
| | | | |
| Calculated nutrient analysis, as-fed basis | | | |
| CP (%) | 23 | 20.2 | 19 |
| ME (kcal/kg) | 3,067.51 | 3,152.00 | 3,196.00 |
| Calcium (%) | 1.10 | 0.9 | 0.85 |
| Nonphytate phosphorous (%) | 0.50 | 0.45 | 0.42 |
| Lysine (%) | 1.35 | 1.10 | 1.01 |
| Methionine (%) | 0.60 | 0.46 | 0.42 |

[1]Broiler Vitamin Premix: 24,000 mg/kg of Manganese, 20,000 mg/kg of Zinc, 16,000 mg/kg of Iron, 4,000 mg/kg of Copper, 330 mg/kg of Iodine, 2,000,000 IU/kg of Vitamin A, 800,000 IU/kg of Vitamin D, 10,000 IU/kg of Vitamin E, 800 mg/kg of Vitamin K, 800 mg/kg of Thiamin, 2,000 mg/kg of Riboflavin, 13,000 mg/kg of Niacin, 1000 mg/kg of Pyridoxine, 3,000 mg/kg of d-Pantothenic Acid, 4,000 mg/kg of Vitamin B12, 400 mg/kg of Folic Acid, 40,000 mcg/kg of Biotin.
[2]Choline Premix: 80,000 mg/kg of Choline.

**Table 4.2.** Corticosterone, glucagon, growth hormone (GH), insulin like growth factor-1 (IGF-1), 3,5,3'-triiodothyronine ($T_3$), thyroxine ($T_4$), insulin and the glucagon to insulin (G:I) ratio of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]) hens at 5 and 6 wk of age exposed to ad libitum (100) and 60% of AL (60) feeding levels (Experiment 1).

| MT | FT | Age (wk) | Sex | CORT | SEM | Glucagon | SEM | GH | SEM | IGF-1 | SEM | $T_3$ | SEM | $T_4$ | SEM | Insulin | SEM | G:I | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | pg/mL | | | | | | | | ng/dL | | | | uIU/mL | | pg/mL | |
| HBW | | | | 1,065 | 107 | 136 | 29 | 1,540 | 120 | 2,102 | 48.1 | 2.1 | 0.11 | 15.2[a] | 0.6 | 5.3 | 0.14 | 0.64 | 0.13 |
| SBW | | | | 1,109 | 181 | 202 | 40 | 1,537 | 135 | 2,102 | 47.5 | 2.2 | 0.09 | 13.6[b] | 0.4 | 5.4 | 0.12 | 0.97 | 0.20 |
| | 100 | | | 842[b] | 93 | 143 | 30 | 1,636 | 147 | 1,844[b] | 36.7 | 2.4[a] | 0.12 | 12.0[b] | 0.4 | 5.6[a] | 0.16 | 0.62 | 0.12 |
| | 60 | | | 1,332[a] | 188 | 194 | 40 | 1,441 | 105 | 2,359[a] | 56.7 | 1.9[b] | 0.08 | 16.8[a] | 0.5 | 5.1[b] | 0.11 | 0.98 | 0.21 |
| | | 5 | | 1,302 | 189 | 203 | 42 | 1,653 | 144 | 2,171 | 49.8 | 2.2 | 0.11 | 14.2 | 0.5 | 5.4 | 0.12 | 0.96 | 0.20 |
| | | 6 | | 872 | 91 | 134 | 26 | 1,424 | 109 | 2,032 | 45.6 | 2.1 | 0.09 | 14.6 | 0.4 | 5.2 | 0.14 | 0.65 | 0.13 |
| | | | F | 1,105 | 147 | 205 | 41 | 1,438 | 107 | 2,169 | 55.1 | 2.0 | 0.08 | 15.4[a] | 0.5 | 5.2[b] | 0.13 | 1.03 | 0.21 |
| | | | M | 1,069 | 150 | 132 | 28 | 1,640 | 145 | 2,034 | 39.1 | 2.3 | 0.11 | 13.4[b] | 0.5 | 5.6[a] | 0.14 | 0.57 | 0.12 |
| Inter-assay CV (%) | | | | 11.3 | | 24.5 | | 4.4 | | 5.8 | | 16.7 | | 8.2 | | 3.3 | | - | |
| Source of Variation | | | | Probability | | | | | | | | | | | | | | | |
| MT | | | | 0.83 | | 0.20 | | 0.99 | | 0.99 | | 0.40 | | 0.027 | | 0.67 | | 0.19 | |
| FT | | | | 0.031 | | 0.32 | | 0.29 | | < 0.001 | | 0.002 | | < 0.001 | | 0.007 | | 0.15 | |
| Age | | | | 0.055 | | 0.18 | | 0.21 | | 0.053 | | 0.42 | | 0.58 | | 0.15 | | 0.22 | |
| Sex | | | | 0.86 | | 0.16 | | 0.27 | | 0.060 | | 0.053 | | 0.008 | | 0.040 | | 0.072 | |
| MT x FT | | | | 0.92 | | 0.29 | | 0.53 | | 0.99 | | 0.057 | | 0.95 | | 0.35 | | 0.20 | |
| MT x Age | | | | 0.54 | | 0.75 | | 0.59 | | 0.51 | | 0.77 | | 0.33 | | 0.95 | | 0.80 | |
| MT x Sex | | | | 0.33 | | 0.18 | | 0.81 | | 0.70 | | 0.17 | | 0.072 | | 0.89 | | 0.12 | |
| FT x Age | | | | 0.19 | | 0.94 | | 0.48 | | 0.99 | | 0.86 | | 0.57 | | 0.88 | | 0.84 | |
| FT x Sex | | | | 0.064 | | 0.49 | | 0.64 | | 0.052 | | 0.25 | | 0.17 | | 0.45 | | 0.38 | |
| Age x Sex | | | | 0.77 | | 0.87 | | 0.88 | | 0.73 | | 0.73 | | 0.45 | | 0.83 | | 0.94 | |
| MT x FT x Age | | | | 0.10 | | 0.87 | | 0.40 | | 0.90 | | 0.59 | | 0.90 | | 0.079 | | 0.77 | |
| MT x FT x Sex | | | | 0.67 | | 0.079 | | 0.60 | | 0.78 | | 0.41 | | 0.56 | | 0.74 | | 0.082 | |
| MT x Age x Sex | | | | 0.86 | | 0.70 | | 0.44 | | 0.70 | | 0.40 | | 0.34 | | 0.42 | | 0.92 | |
| FT x Age x Sex | | | | 0.30 | | 0.77 | | 0.36 | | 0.84 | | 0.82 | | 0.34 | | 0.68 | | 0.87 | |
| MT x FT x Age x Sex | | | | 0.74 | | 0.44 | | 0.99 | | 0.55 | | 0.94 | | 0.91 | | 0.97 | | 0.56 | |

[1] Standard maternal BW (SBW); 121% of SBW at 18 wk (HBW)

[a-c] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 4.3.** Glucose, glycogen and lactate levels, and glucidic potential (GP) in the liver of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]), fed on 3 different feeding treatments (FT: ad libitum (100), 60% of ad libitum) from 4 to 6 wk of age (Experiment 1).

| Age | MT[1] | FT | Sex | Glucose | SEM | Glycogen | SEM | Lactate | SEM | GP | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | μmol.g$^{-1}$ | | | | | | | |
| 4 | | | | 55.6$^{b}$ | 1.5 | 227.9$^{a}$ | 12.6 | 10.7 | 0.5 | 288.8$^{a}$ | 12.4 |
| 5 | | | | 58.2$^{ab}$ | 1.1 | 215.2$^{ab}$ | 11.1 | 9.4 | 0.3 | 278.1$^{ab}$ | 11.4 |
| 6 | | | | 60.9$^{a}$ | 1.2 | 183.5$^{b}$ | 8.9 | 9.2 | 0.3 | 249.1$^{b}$ | 9.4 |
| | HBW | | | 59.2 | 0.8 | 211.1 | 8.3 | 9.8 | 0.2 | 275.2 | 8.5 |
| | SBW | | | 57.3 | 1.2 | 206.7 | 9.5 | 9.8 | 0.4 | 268.9 | 9.7 |
| | | 100 | | 56.5$^{b}$ | 1.1 | 235.6$^{a}$ | 7.7 | 10.3$^{a}$ | 0.3 | 297.3$^{a}$ | 7.7 |
| | | 60 | | 60.0$^{a}$ | 0.9 | 182.2$^{b}$ | 10.1 | 9.2$^{b}$ | 0.3 | 246.8$^{b}$ | 10.3 |
| | | | F | 55.5$^{b}$ | 1.1 | 207.9 | 8.8 | 9.9 | 0.3 | 268.5 | 8.8 |
| | | | M | 61.0$^{a}$ | 1.0 | 209.8 | 9.1 | 9.6 | 0.3 | 275.6 | 9.3 |

| Source of Variation | Probability | | | |
|---|---|---|---|---|
| MT[1] | 0.20 | 0.73 | 0.96 | 0.63 |
| FT | 0.024 | < 0.001 | 0.024 | < 0.001 |
| Age | 0.031 | 0.012 | 0.057 | 0.031 |
| Sex | < 0.001 | 0.88 | 0.42 | 0.58 |
| MT*FT | 0.46 | 0.27 | 0.18 | 0.23 |
| MT*Age | 0.30 | 0.55 | 0.74 | 0.62 |
| MT*Sex | 0.51 | 0.47 | 0.15 | 0.45 |
| FT*Age | 0.013 | 0.12 | 0.68 | 0.13 |
| FT*Sex | 0.31 | 0.95 | 0.33 | 0.87 |
| Age*Sex | 0.088 | 0.15 | 0.74 | 0.15 |
| MT*FT*Age | 0.84 | 0.17 | 0.66 | 0.18 |
| MT*FT*Sex | 0.66 | 0.46 | 0.73 | 0.44 |
| MT*Age*Sex | 0.77 | 0.92 | 0.61 | 0.92 |
| FT*Age*Sex | 0.85 | 0.69 | 0.33 | 0.66 |
| MT*FT*Age*Sex | 0.34 | 0.071 | 0.85 | 0.088 |

[1] Standard maternal BW (SBW); 121% of SBW at 18 wk (HBW)
[a-b] Means within column within effect with no common superscript differ (P < 0.05)

**Table 4.4.** Glucose, glycogen and lactate levels, and glucidic potential (GP) in the breast muscle of female and male Ross 708 broilers from high and low BW maternal treatments (MT[1]), fed on 3 different feeding treatments (FT: ad libitum (100), 60% of ad libitum) from 4 to 6 wk of age (Experiment 1).

| Age | MT | FT | Sex | Glucose | SEM | Glycogen | SEM | Lactate | SEM | GP | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | µmol.g$^{-1}$ | | | | | | | |
| 4 | | | | 4.3$^{a}$ | 0.2 | 22.1 | 1.4 | 94.4$^{a}$ | 3.0 | 73.7$^{a}$ | 1.7 |
| 5 | | | | 3.4$^{b}$ | 0.1 | 25.4 | 1.1 | 79.1$^{b}$ | 2.2 | 68.3$^{ab}$ | 1.5 |
| 6 | | | | 3.4$^{b}$ | 0.1 | 24.4 | 1.0 | 78.8$^{b}$ | 2.2 | 67.2$^{b}$ | 1.4 |
| | HBW | | | 3.8 | 0.1 | 24.2 | 0.8 | 85.2 | 1.9 | 70.6 | 1.1 |
| | SBW | | | 3.6 | 0.1 | 23.7 | 1.1 | 83.0 | 2.2 | 68.9 | 1.4 |
| | | 100 | | 3.7 | 0.1 | 24.5 | 0.9 | 81.3 | 1.8 | 68.9 | 1.3 |
| | | 60 | | 3.7 | 0.1 | 23.4 | 1.1 | 86.9 | 2.2 | 70.5 | 1.3 |
| | | | F | 3.7 | 0.1 | 24.5 | 1.1 | 84.4 | 2.3 | 70.4 | 1.3 |
| | | | M | 3.7 | 0.1 | 23.4 | 0.9 | 83.8 | 1.8 | 69.0 | 1.2 |

| Source of Variation | Probability | | | |
|---|---|---|---|---|
| MT[1] | 0.34 | 0.72 | 0.47 | 0.34 |
| FT | 0.88 | 0.41 | 0.060 | 0.35 |
| Age | < 0.001 | 0.23 | < 0.001 | 0.011 |
| Sex | 0.85 | 0.41 | 0.85 | 0.44 |
| MT*FT | 0.59 | 0.45 | 0.50 | 0.99 |
| MT*Age | 0.86 | 0.13 | 0.31 | 0.86 |
| MT*Sex | 0.80 | 0.064 | 0.28 | 0.019 |
| FT*Age | 0.76 | 0.96 | 0.67 | 0.86 |
| FT*Sex | 0.94 | 0.77 | 0.86 | 0.93 |
| Age*Sex | 0.98 | 0.10 | 0.76 | 0.50 |
| MT*FT*Age | 0.82 | 0.16 | 0.53 | 0.36 |
| MT*FT*Sex | 0.47 | 0.66 | 0.78 | 0.96 |
| MT*Age*Sex | 0.96 | 0.70 | 0.77 | 0.46 |
| FT*Age*Sex | 0.95 | 0.044 | 0.31 | 0.63 |
| MT*FT*Age*Sex | 0.26 | 0.76 | 0.51 | 0.96 |

[1] Standard maternal BW (SBW); 121% of SBW at 18 wk (HBW)

[a-b] Means within column within effect with no common superscript differ (P < 0.05)

**Table 4.5.** Corticosterone, glucagon, $T_3$, $T_4$, insulin levels, as well as the glucagon to insulin ratio (G:I) of female and male Cobb broilers from hens fed using conventional systems (CON) or precision feeding systems (PF) at 5 and 6 wk of age exposed to ad libitum (100) and 60% of ad libitum (60) feeding levels (Experiment 2).

| MT[1] | FT | Age | Sex | CORT | SEM | Glucagon | SEM | $T_3$ | SEM | $T_4$ | SEM | Insulin | SEM | G:I | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | pg/mL | | | | ng/dL | | | | uIU/mL | | pg/mL | |
| CON | | | | 430 | 122 | 123 | 17 | 2.1 | 0.077 | 11.1 | 0.31 | 6.0 | 0.139 | 0.56 | 0.066 |
| PF | | | | 723 | 116 | 187 | 39 | 2.0 | 0.076 | 11.1 | 0.31 | 6.0 | 0.137 | 0.64 | 0.134 |
| | 100 | | | 524 | 121 | 154 | 36 | 2.2[a] | 0.079 | 10.2[b] | 0.31 | 6.1 | 0.137 | 0.52 | 0.108 |
| | 60 | | | 629 | 116 | 155 | 23 | 1.9[b] | 0.074 | 12.0[a] | 0.33 | 5.8 | 0.139 | 0.69 | 0.103 |
| | | 5 | | 552 | 116 | 182 | 39 | 2.0 | 0.079 | 11.3 | 0.33 | 5.8 | 0.135 | 0.67 | 0.132 |
| | | 6 | | 602 | 121 | 127 | 19 | 2.1 | 0.074 | 10.9 | 0.32 | 6.1 | 0.142 | 0.54 | 0.070 |
| | | | F | 570 | 120 | 169 | 29 | 2.1 | 0.076 | 11.5 | 0.33 | 6.0 | 0.135 | 0.73 | 0.131 |
| | | | M | 584 | 118 | 140 | 32 | 2.0 | 0.077 | 10.6 | 0.32 | 6.0 | 0.142 | 0.47 | 0.072 |
| Inter-assay CV (%) | | | | 20.3 | | 23.1 | | 16.9 | | 9.3 | | 2.9 | | - | |
| Sources of Variation | | | | Probability | | | | | | | | | | | |
| MT[1] | | | | 0.085 | | 0.17 | | 0.40 | | 0.91 | | 0.99 | | 0.61 | |
| FT | | | | 0.53 | | 0.99 | | 0.007 | | < 0.001 | | 0.15 | | 0.27 | |
| Age | | | | 0.77 | | 0.23 | | 0.24 | | 0.38 | | 0.12 | | 0.42 | |
| Sex | | | | 0.94 | | 0.51 | | 0.31 | | 0.059 | | 0.73 | | 0.10 | |
| MT x FT | | | | 0.18 | | 0.93 | | 0.069 | | 0.49 | | 0.28 | | 0.80 | |
| MT x Age | | | | 0.99 | | 0.048 | | 0.38 | | 0.57 | | 0.77 | | 0.030 | |
| MT x Sex | | | | 0.25 | | 0.95 | | 0.93 | | 0.70 | | 0.17 | | 0.44 | |
| FT x Age | | | | 0.58 | | 0.77 | | 0.61 | | 0.91 | | 0.91 | | 0.69 | |
| FT x Sex | | | | 0.81 | | 0.18 | | 0.48 | | 0.71 | | 0.33 | | 0.40 | |
| Age x Sex | | | | 0.031 | | 0.49 | | 0.064 | | 0.89 | | 0.61 | | 0.79 | |
| MT x FT x Age | | | | 0.67 | | 0.49 | | 0.97 | | 0.94 | | 0.89 | | 0.74 | |
| MT x FT x Sex | | | | 0.76 | | 0.32 | | 0.90 | | 0.15 | | 0.26 | | 0.78 | |
| MT x Age x Sex | | | | 0.94 | | 0.67 | | 0.44 | | 0.83 | | 0.36 | | 0.088 | |
| FT x Age x Sex | | | | 0.34 | | 0.40 | | 0.63 | | 0.77 | | 0.26 | | 0.93 | |
| MT x FT x Age x Sex | | | | 0.83 | | 0.21 | | 0.42 | | 0.50 | | 0.45 | | 0.38 | |

[1] Conventionally Fed Hens (CON); Precision Fed Hens (PF)

[a-c] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 4.6.** Glucose, glycogen and lactate levels, and glucidic potential (GP) in the breast muscle of female and male Cobb broilers from conventionally and precision fed maternal treatments, fed on 3 different feeding treatments (FT: ad libitum (100), 60% of ad libitum) from 4 to 6 wk (Experiment 2).

| Age | MT[1] | FT | Sex | Glucose | SEM | Glycogen | SEM | Lactate | SEM | GP | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | µmol.g$^{-1}$ | | | | | | | |
| 4 | | | | 3.3 | 0.2 | 39.6 | 3.3 | 74.7 | 4.7 | 80.2 | 3.3 |
| 5 | | | | 3.5 | 0.1 | 36.0 | 1.5 | 84.9 | 2.2 | 81.9 | 1.5 |
| 6 | | | | 3.2 | 0.1 | 38.7 | 1.5 | 85.2 | 2.2 | 84.5 | 1.6 |
| | CON | | | 3.1[b] | 0.1 | 40.2 | 1.8 | 81.0 | 2.6 | 83.8 | 1.8 |
| | PF | | | 3.5[a] | 0.1 | 36.0 | 1.9 | 82.2 | 2.7 | 80.7 | 1.9 |
| | | 100 | | 3.1[b] | 0.1 | 37.0 | 1.9 | 79.4 | 2.8 | 79.8 | 1.9 |
| | | 60 | | 3.5[a] | 0.1 | 39.2 | 1.8 | 83.9 | 2.5 | 84.7 | 1.8 |
| | | | F | 3.4 | 0.1 | 36.5 | 1.5 | 83.1 | 2.2 | 81.4 | 1.5 |
| | | | M | 3.3 | 0.1 | 39.7 | 2.1 | 80.1 | 3.0 | 83.1 | 2.1 |

| Source of Variation | Probability | | | |
|---|---|---|---|---|
| MT[1] | 0.018 | 0.12 | 0.76 | 0.23 |
| FT | 0.012 | 0.41 | 0.23 | 0.067 |
| Age | 0.15 | 0.38 | 0.12 | 0.34 |
| Sex | 0.41 | 0.21 | 0.43 | 0.53 |
| MT*FT | 0.19 | 0.18 | 0.88 | 0.19 |
| MT*Age | 0.31 | 0.54 | 0.039 | 0.37 |
| MT*Sex | 0.036 | 0.27 | 0.79 | 0.30 |
| FT*Age | < 0.001 | 0.093 | 0.091 | 0.64 |
| FT*Sex | 0.36 | 0.10 | 0.88 | 0.15 |
| Age*Sex | 0.35 | 0.75 | 0.32 | 0.45 |
| MT*FT*Age | 0.085 | 0.12 | 0.39 | 0.28 |
| MT*FT*Sex | 0.70 | 0.14 | 0.58 | 0.30 |
| MT*Age*Sex | 0.003 | 0.71 | 0.33 | 0.11 |
| FT*Age*Sex | 0.067 | 0.12 | 0.020 | 0.093 |
| MT*FT*Age*Sex | 0.13 | 0.022 | 0.25 | 0.31 |

[1] Conventionally Fed Hens (CON); Precision Fed Hens (PF)

[a-b] Means within column within effect with no common superscript differ ($P < 0.05$)

**Table 4.7.** Glucose, glycogen and lactate levels, and glucidic potential (GP) in the liver of female and male Cobb broilers from conventionally and precision fed maternal treatments, fed on 3 different feeding treatments (FT: ad libitum (100), 60% of ad libitum) from 4 to 6 wk (Experiment 2).

| Age | MT[1] | FT | Sex | Glucose | SEM | Glycogen | SEM | Lactate | SEM | GP | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | μmol.g$^{-1}$ | | | | | | | |
| 4 | | | | 63.2 | 2.6 | 260.6$^{ab}$ | 21.9 | 10.7 | 0.6 | 329.1$^{ab}$ | 21.5 |
| 5 | | | | 64.9 | 1.2 | 208.6$^{b}$ | 10.1 | 9.7 | 0.3 | 278.3$^{b}$ | 10.0 |
| 6 | | | | 62.3 | 1.2 | 247.1$^{a}$ | 10.4 | 10.4 | 0.3 | 314.6$^{a}$ | 10.2 |
| | CON | | | 63.7 | 1.4 | 245.2 | 12.2 | 10.4 | 0.3 | 314.1 | 12.0 |
| | PF | | | 63.2 | 1.5 | 232.3 | 12.6 | 10.2 | 0.3 | 300.6 | 12.4 |
| | | 100 | | 60.2$^{b}$ | 1.5 | 233.8 | 12.9 | 10.2 | 0.3 | 299.1 | 12.7 |
| | | 60 | | 66.8$^{a}$ | 1.4 | 243.7 | 11.9 | 10.4 | 0.3 | 315.7 | 11.7 |
| | | | F | 63.2 | 1.2 | 245.1 | 10.2 | 10.1 | 0.3 | 313.4 | 10.1 |
| | | | M | 63.7 | 1.7 | 232.4 | 14.2 | 10.5 | 0.4 | 301.3 | 14.0 |

| Source of Variation | Probability | | | |
|---|---|---|---|---|
| MT | 0.81 | 0.46 | 0.63 | 0.43 |
| FT | 0.002 | 0.57 | 0.68 | 0.34 |
| Age | 0.33 | 0.012 | 0.12 | 0.016 |
| Sex | 0.80 | 0.48 | 0.43 | 0.48 |
| MT*FT | 0.94 | 0.24 | 0.64 | 0.23 |
| MT*Age | 0.87 | 0.41 | 0.65 | 0.43 |
| MT*Sex | 0.13 | 0.62 | 0.037 | 0.77 |
| FT*Age | 0.12 | 0.45 | 0.91 | 0.58 |
| FT*Sex | 0.38 | 0.94 | 0.43 | 0.85 |
| Age*Sex | 0.36 | 0.76 | 0.62 | 0.82 |
| MT*FT*Age | 0.95 | 0.18 | 0.22 | 0.16 |
| MT*FT*Sex | 0.12 | 0.96 | 0.95 | 0.88 |
| MT*Age*Sex | 0.027 | 0.91 | 0.19 | 0.85 |
| FT*Age*Sex | 0.28 | 0.49 | 0.059 | 0.54 |
| MT*FT*Age*Sex | 0.71 | 0.88 | 0.84 | 0.83 |

[1] Conventionally Fed Hens (CON); Precision Fed Hens (PF)
[a-b] Means within column within effect with no common superscript differ ($P < 0.05$)

# 5.0 Synthesis

## 5.1 Introduction

The experiments that were a part of this thesis were designed to examine the effect of maternal feed availability and feeding system on offspring performance. The results of these experiments showed that offspring performance was affected by maternal feed availability in a precision feeding system but not maternal feeding method. Increasing the broiler breeder target BW in a precision feeding system by 21% increased broiler ADG and final BW. Commercially adopting an increase in broiler breeder BW may be advantageous for both breeder and broiler producers. Completing both projects would not have been possible without the innovative technologies used by the Zuidhof research group. The precision feeding system designed at the University of Alberta allowed for the collection of accurate feed intake and BW data in real time, increasing the accuracy of data analysis. The HPMIXED model used for statistical analysis allowed for the analysis of complex interactions with large numbers of variables in the model, which made the analysis definitive and accurate. Along with technology, collaborations with Dr. Jennifer Aalhus and Dr. Laura Ellestad allowed for the success of the experiments. Using their expertise made plasma and tissue analysis efficient and accurate. In the future, technologies and collaborations used as part of this thesis will continue to be used to further examine the effect of broiler breeder environment on broiler performance.

## 5.2 Optimizing Broiler Production

Optimizing broiler performance has always been on the forefront for both research and industry. The data presented in Chapter 3 provides evidence for the hypothesis that broiler breeder nutrition in a precision feeding system has a significant impact on offspring performance. The conclusions of this project and van der Waaij et al. (2011) suggest that the method being used to

feed broiler breeders in a precision feeding system is having a detrimental effect on offspring performance, by decreasing broiler ADG and final body weight. Increasing broiler breeder target BW curve by 21% using a precision feeding system will improve broiler performance by increasing final BW. However, more research needs to be done to determine the effect of increasing the target BW using conventional systems. A partial budget approach was used to analyse the economic value of the proposed changes. The conclusions of this project and the van der Klein et al. (2018) project could result in management changes at the broiler breeder level which will help to optimize broiler production and increase the welfare of broiler breeders if the proposed changes are adopted. The data suggests that increasing the target BW of broiler breeders will increase the final BW of broilers, therefore allowing broiler producers to produce the same amount of weight by placing fewer chicks. The partial budgets shown in Table 5.1 and Table 5.2 only include the changes in feed and chick costs that would result by using HBW offspring over SBW offspring. HBW offspring produced an average final BW of 2.486 kg at d 42, while SBW offspring were on average 2.389 kg at d 42. To produce 1000 kg of broilers, a producer would need to place 401 HBW offspring or 417 SBW offspring (Table 5.2). Placing less birds will reduce costs for the producer because they will buy less chicks and feed less birds. At the current broiler chick price, placing HBW offspring instead of SBW offspring would save the producer $12.54 per 1000 kg of broilers produced (Alberta Chicken Producers, 2018), it would also save the producer $25.33 in feeding costs per 1000 kg of broilers produced (Table 5.2). Increasing the target BW curve will not only benefit broiler producers by increasing the final BW of broilers but will also increase the number of hatching eggs produced by the broiler breeders in a precision feeding system (van der Klein et al., 2018), increasing hatching egg producer profit (Table 5.1). High BW hens produced on average 37 more eggs from wk 23 to 55

than the standard BW hens (van der Klein et al., 2018). This would result in an average increase in profit of $18.43/hen at the current market price of $0.5725/saleable chicks produced in a precision feeding system (Table 5.1). Some of the hesitancy to increasing the broiler breeder BW curve may come from the increase in feeding costs for broiler breeder producers. However, these feeding costs will be offset by an increased number of hatching eggs produced by the broiler breeders (Table 5.1; van der Klein et al., 2018). Based on the results of this project and the results of van der Klein et al. (2018) it is reasonable to predict that increasing the BW curve of broiler breeders in a precision feeding system will be beneficial for both the broiler breeder and broiler producer. Economic profits aside, increasing the amount of feed available to broiler breeders will also increase their quality of life by decreasing the severity of feed restriction (Renema and Robinson, 2004). Increasing the BW curve of broiler breeders may also improve the social perception of the poultry industry by improving the welfare of the birds. However, research is needed to determine whether relaxing feed restriction influences broiler breeder welfare.

### 5.3 Benefits and Drawbacks of Precision and Conventional Feeding to Feed Broilers

The two experiments completed as part of this thesis used two different feeding systems, conventional feeding and precision feeding. Conventional feeding has been the choice feeding method among poultry producers and researchers since the dawn of modern poultry production. However, precision feeding may be the future of efficiently and accurately feeding poultry in research and possibly in industry. Both methods have characteristics that make them successful in different situations, which I will elaborate on below. Conventional feeding of broilers is done

by filling feeders and giving birds ad libitum access to feed while the lights are on. This method has been producing growing broilers and providing a profit for producers; however, problems can arise when using a conventional feeding method. The larger, most dominant broilers may command the majority of the feeder space and the smaller less dominant birds have access to less feed, a problem that is commonly reported in broiler breeders (Zuidhof et al., 2015; Zuidhof et al., 2017; Zuidhof 2018). This issue, although uncommon in broiler production systems was observed in Experiment 1, as feeder design was not optimal. Feeders were placed in the corner of the pen and birds could block other birds from having access to the feeder. There was adequate feeder space as recommended by the National Farm Animal Care Councils guidelines for chickens, turkeys and breeders, however the feeder design could be changed to give optimal feeding opportunity for all birds. The feeder design may have caused a large gap in the weight of the heaviest and the smallest broilers however, this issue may not be prevalent in industry due to the significant amount of available feeder space. Feeder space may have not been the lone cause for problem: unconventional social group size, temperature, floor space and cage conditions could also have been contributing factors.

Precision feeding is a new technology designed at the University of Alberta that addresses the many problems that poultry researchers face when using conventional systems. One of those many problems is inaccurate feeding data caused by human error, as well as animal behaviour. The precision feeding system also allows for the accurate collection of feeding data in real time, an attribute that conventional systems do not possess (Zuidhof et al., 2017). Conventional systems have been the choice of researchers for much of the poultry related research in the past but recording feeding data by hand increases the risk of human error. Conventional feeding systems are also prone to error because of birds wasting and dust bathing in their feed, which

was a problem that was observed in Experiment 1, however these problems may not be seen in industrial production due to the difference in feeder design. The precision feeding system prevents the spilling and wasting of feed, an issue that is very common and difficult to control when using trough feeders like those used in Experiment 1. The precision feeding system has made aspects of poultry related research increasingly efficient, but it has not come without its challenges. In the first few weeks of life, birds need to be trained how to use the precision feeding system, which can be very labour intensive. Most birds catch on to the system quickly, however there are always a few birds that need more human intervention to be successful. During Experiment 2, 5% of the birds required additional training to successfully use the precision feeding system. The current implementation cost of the precision feeding systems many deter research and industry professionals from attempting to implement the technology. The unit cost along with the cost of fitting each bird with an RFID tag would put it out of most researchers and industries budget. However, continued innovation will make the cost of implementing this technology attainable to the poultry industry. The precision feeding system is proving itself to be an innovative technology that will help both producers and researchers reach their goals and with future innovation will be a staple in poultry related research.

The precision feeding system may also be used to implement feed restriction into broiler production systems. Research suggests that broilers subjected to feed restriction have reduced rates of sudden death syndrome and ascites, improved feed efficiency and reduced abdominal fat pad size (Yu and Robinson, 1992; Buys et al., 1998; van der Klein et al., 2016). When using conventional feed systems, it is impossible to effectively implement feed restriction strategies for every bird in the flock. The precision feeding system allows for the accurate implementation of feed restriction at anytime during the broiler production cycle. If the benefits of feed restriction

can be reproduced in an industrial setting, producers will most likely see increased feed efficiency and lower incidence of metabolic disease which had the potential to increase their profits by reducing dead loss and reducing feeding costs.

### 5.4 Innovations in Statistical Analysis

At the start of this program, I quickly learned that SAS is a useful and powerful tool for research. The models in SAS allow for complex statistical calculations to be done that take seconds or minutes to complete, if these calculations had to be done by hand, they would take a considerable amount of time to calculate. At the start of the program, I was using a mixed model in SAS to analyze my data. This model worked well when there were few variables in the random statement of the model. If there were too many variables in the random statement, the model would not converge, it also makes it difficult for the model to analyze the data if the data is not normally distributed. It is important to have all variables that are relevant and appropriate for analysis included in the model because this makes the statistical model robust and allows for more accurate reporting of results by accounting for the sources of variation. Having more variables included in the model gives a better estimate of the true probability of the outcome, this gives more reliable results as to whether means differ significantly or not by more accurately estimating variance for both fixed and random variables. The HPMixed model has not yet been fully adopted within the poultry science community. Hopefully with the information contained in this thesis the HPMixed model will be adopted poultry science community, which will lead to the community to a better statistical model. The HPMixed model allows for the convergence of more complicated models, as well as the inclusion of more variable in the random statement without the need for normal distribution. The HPMixed model also helps combat the problem of partial data, data that contains a lot of zero and missing values known as a sparse matrix.

Analyzing data with a high amount of zero and missing values, (common in covariance matrices) is computationally expensive (Pissanetzky, 1984), and is why the MIXED model took a large amount of time to converge on a solution or did not complete it at all. The MIXED model would not complete the feeding analysis, which was the largest set of data because of infinite likelihood but HPMIXED was able to complete the analysis. HPMIXED was designed specifically to handle partial data and can complete the analysis much faster and with more variables than the MIXED model. As explained above, this is of importance because including all the appropriate variables in the random statement causes the analysis to be more robust. Robust analysis allows science to improve the estimate of probability, increasing the accuracy of our conclusions benefits both industry and research community by ensuring what is reported is true and supported by the data.

### 5.5 Collaborations that made this Project Possible

I was very fortunate to be able to make excellent use of the numerous connections that Dr. Zuidhof has outside the University of Alberta. The metabolic analysis was aided tremendously by the help of both Dr. Jennifer Aalhus and Dr. Laura Ellestad. Dr. Jennifer Aalhus at the Government of Canada Research Centre in Lacombe, Alberta and her team taught me the techniques used to complete glucidic analysis on breast and liver tissue in her lab. The pipetting techniques learned in Dr. Aalhus' lab also became useful in Dr. Laura Ellestad's lab at the University of Georgia. Dr. Ellestad welcomed me into her lab and taught me the techniques needed to complete hormone analysis using ELISA kits and RIA for blood plasma. Collaborating with both researchers greatly increased the speed of which the analysis was completed. Expertise from many different areas can be brought together to effectively complete a project through

collaboration; one person gaining the knowledge to complete a project on their own would take an astronomical amount of time but bringing together the knowledge of many people increases the efficiency of research projects. Collaborations between researchers is of the utmost importance for continued advancement in science and research. Bringing together experts from all areas to work on a single project allows for integrative, intuitive and effective research.

### 5.6 Study Limitations

I believe that the experiments conducted were a success, however, they were not without their limitations. Firstly, it was originally planned to complete two experiments that were identical to Experiment 1 described in the above chapters. Due to egg laying issues with the broiler breeders, we were not able to collect enough eggs to run the second experiment. This was unfortunate because replicating the first study would have allowed for more robust conclusions. However, in place of that study we did discover that breeder feeding method did not affect offspring performance, which is an interesting discovery that would not have happened if all the experiments went as planned. Secondly, there were limitations with the cage system and feeding method used in Experiment 1. Grates were placed over the feeders that allowed the birds to feed but deterred birds from wasting feed, however for some birds these were ineffective. Feed would be flicked out of the container and would fall through the wire floor of the pen. This made recording accurate cage level feed intake difficult for a small number of cages. Thirdly, in my opinion, the small cage size contributed to increased stress levels in the birds. The CORT levels of Experiment 1 were almost double the CORT levels of Experiment 2 in both the 60% of AL and ad libitum feeding treatments. This would lead us to believe that the birds in Experiment 1 were experiencing more stress than birds in Experiment 2 (Table 4.2; Table 4.6). These

differences could be due at least in part to the housing system; birds that were housed in cages did not have the opportunity to interact with other birds and the environment in the same way that birds in Experiment 2 did. In both experiments, the birds were restricted to the same level but in Experiment 2 the birds had more room, more opportunities to interact with their counterparts, and the ability to forage successfully throughout the day. This may have reduced the stress level in the birds because they had more opportunities to engage in feed seeking or foraging behaviour with successful attempts throughout the day instead of being limited to a single feeding event. Another cause of the difference in CORT levels between the two experiments could have been the use of different bird strains, in Experiment 1 Ross 708 broilers were used and in Experiment 2 Cobb broilers were used. In further studies, I would avoid the use of the cages for the more favorable floor pens and precision feeding system.

Another limitation to both experiments was the level of feed restriction imposed on the broilers. The most severe level of feed restriction was set at 60% of ad libitum. This may not have been severe enough to match the maternal level of restriction and because the offspring environment did not match the maternal environment the offspring may not have performed as expected. Broiler breeders are restricted to 25 to 35% of what they would consume ad libitum (de Jong et al., 2002); only restricting the broilers to 60% of ad libitum is far from matching the 25 to 35% of ad libitum that the broiler breeders would get. It is conceivable that we would have seen more significant results if the broilers were exposed to more severe levels of feed restriction because it would have more closely matched their parents' level of restriction. If we did see increased growth with maternal and offspring environment matching it would be conceivable that broilers could be fed less to achieve the same level or a greater level of gain than they previously achieved. However, feed restricting broilers at a level lower than 60% would cause many welfare

issues due to broilers having a high drive to feed. The level of feed restriction in broiler breeders is much more severe than 60% but broiler breeder feed restriction has become acceptable because it is believed that feed restriction produces reproductively efficient broiler breeders. The biological limit of feed restriction of broiler breeders in a precision feeding system may have been passed as van der Klein et al. (2018) found that increasing the target BW of breeders increased the number of eggs from breeders in 52 wk when compared to breeders raised on an industry standard BW target. In the future more work will be needed to determine the optimal level of feed restriction and egg production.

**5.7 Future Considerations**

To build upon the work done as part of this project, I would suggest that a more intensive look into epigenetic mechanisms be done. The current thesis provided clear evidence that increasing broiler breeder BW target in a precision feeding system increased the BW and ADG of their offspring. The mechanisms responsible for this change in performance are not yet known. The mechanisms responsible for the observed differences are most likely genetic but could also be due to difference in hormone concentrations in the eggs, as well as egg nutrient composition. Bowling et al. (2018) found that hens exposed to medium levels of feed restriction had lower yolk corticosterone level when compared to the eggs of hens exposed to high levels of feed restriction, they also found similar effects on broiler BW with increased maternal BW as presented in the above chapters. To fully understand how broiler breeder feeding is affecting broiler performance, we must start to understand the mechanisms that are driving these changes to happen. Analysing the DNA methylation and histone modification patterns of offspring from restricted broiler breeders and ad libitum fed broiler breeders would provide valuable insight. Also completing nutrient analysis and analysing yolk corticosterone levels (Bowling et al.,

2018), on eggs from breeders on different BW curves would provide valuable information to create a more conclusive story.

It would also be of great value to further explore how matching and mismatching the maternal and offspring environments affects offspring performance. This would be a great opportunity for further collaborations with researchers that have done DNA methylation and histone modification analysis in the past. It may also be beneficial to speak with Mandy Bowling who published Bowling et al. (2018); she has completed yolk corticosterone analysis with the goal of discovering how maternal restriction affects the offspring. As described above, one of the limitations of both studies was the level of restriction imposed on the broilers. Future studies could be done where the broilers are exposed to greater levels of restriction, 40% or 30% of ad libitum. However, this level of restriction would cause concern for the welfare of the birds because they have a high drive to feed and would most likely be very hungry.

### 5.8 Conclusion

Even though it has been long believed that broiler breeders require intensive feed restriction in order to efficiently produce offspring, this project along with other research has shown that reducing the severity of restriction may increase the productivity of broilers in a precision feeding system. The current study suggests that raising the BW of broiler breeders in a precision feeding system will increase the final BW of broilers and hopefully increase producer profits and improving welfare of broiler breeders, as well as the social perception of the chicken industry. Due to the findings of this project, we are recommending that the target BW curve for broiler breeders in a precision feeding system be increased by 21% above the recommended Ross 708 target in precision feeding systems to increase the final BW of broilers. The broiler breeder study

was not within the scope of this project however van der Klein et al. (2018) showed that increasing the BW target of broiler breeders increased their productivity. To determine the optimal growth curve in precision feeding systems more research needs to be completed, which will help us to better understand how precision feeding affects changes to the maternal DNA structure and how those changes are passed to the offspring. Increasing the target BW of broiler breeders will not only benefit broiler producers but will also increase the number of hatching eggs produced by the breeders (van der Klein et al., 2018). Even though this research has increased our understanding of how to optimize broiler production there is still much to be discovered. Further studies should focus on determining if there are any hormone or nutrient composition differences in the egg. This is an excellent opportunity for collaborations within the poultry research industry. Increasing the severity of feed restriction experienced by the broilers may also provide valuable insight into the hypotheses of van der Waaij et al., (2011) by more closely matching the maternal and offspring nutritional environment. Our knowledge of how environmental conditions affect the DNA structure and cause epigenetic changes is limited but with the information published in this thesis and continuing work on the subject we will one day be able to understand and harness changes in the DNA structure to produce more efficient broilers.

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**Table 5.1.** Partial budget with cost and benefit analysis of raising high BW (HBW) and standard BW (SBW) broiler breeders. Only direct costs and benefits associated with implementing an increased target BW for broiler breeders. Costs are presented as per hen.

| | | | HBW[4] Hens | | SBW[5] Hens | | |
|---|---|---|---|---|---|---|---|
| **Description** | **Units** | **$/unit** | **Quantity** | **Total** | **Quantity** | **Total** | **Difference (HBW – SBW)** |
| Costs | | | | | | | |
| Feed cost[1] d 16 to wk 18 | tonne | $408.13 | 0.0080 | $3.25 | 0.0065 | $2.66 | $0.59 |
| Feed cost[1], wk 18 to wk 55 | tonne | $395.35 | 0.0343 | $13.56 | 0.0280 | $11.07 | $2.49 |
| Total feed cost | tonne | Variable[1] | 0.0423 | $16.81 | 0.0345 | $13.73 | $3.08 |
| Benefits | | | | | | | |
| Hatching Egg Revenue[2,3] | ea | 0.5725 | 129.4 | $74.08 | 92.8 | $53.13 | $20.95 |
| Net benefit of HBW | | | | | | | $17.83 |

[1]Price per tonne of feed was obtained from a broiler breeder feeding program from Trouw Nutrition used in Sherwood Park, Alberta.

[2]Feed intake and egg data of SBW and HBW hens was published in van der Klein et al. (2018).

[3]Hatching egg price of 57.25 cents per live chick was obtained from the Alberta Hatching Egg Producers (N. Robinson, personal communication, February 7, 2019).

[4]HBW hens were raised at 21% above the Ross 708 broiler breeder target, using a precision feeding system.

[5]SBW hens were raised in accordance to the Ross 708 broiler breeder target, using a precision feeding system.

**Table 5.2.** Partial Budget for Raising Broilers from High Body Weight (HBW) and Standard Body Weight (SBW) Hens. Calculations were done on the basis of growing 1000 kg (broilers, live weight). To grow 1000 kg of HBW offspring the producer would need to place 401 chicks, to grow 1000 kg of SBW offspring the producer would need to place 417 chicks.

| | | | HBW Offspring[4] | | SBW Offspring[5] | | |
|---|---|---|---|---|---|---|---|
| **Description** | **Unit** | **$/unit** | **Quantity** | **Total** | **Quantity** | **Total** | **Difference (HBW – SBW)** |
| Costs | | | | | | | |
| Chicks | ea | $0.7836 | 401 | $314.22 | 417 | $326.76 | -12.54 |
| Feed[1], d 1-7 | tonne | $475.00 | 0.0646 | $30.67 | 0.0671 | $31.87 | -$1.20 |
| Feed[1], d 8-14 | tonne | $446.50 | 0.1740 | $77.70 | 0.1808 | $80.75 | -$3.05 |
| Feed[1], d 15-25 | tonne | $400.50 | 0.4780 | $191.44 | 0.4967 | $198.93 | -$7.49 |
| Feed[1], d 26-33 | tonne | $401.80 | 0.3822 | $153.55 | 0.3971 | $159.56 | -$6.01 |
| Feed[1], d 34-mkt | tonne | $384.30 | 0.5041 | $193.71 | 0.5238 | $201.29 | $-7.58 |
| Total feed cost | Tonne | Variable[1] | 1.6028 | $647.07 | 1.6656 | $672.40 | $-25.33 |
| Benefits | | | | | | | |
| Sale of Broilers[3] | kg | 1.593[2] | 1000 | 1,593 | 1000 | 1,593 | 0 |
| Net benefit of HBW | | | | | | | $37.87 |

[1]Feeding costs were calculated using feed intake data presented in Chapter 3 and feeding costs obtained from the Poultry Research Center at the University of Alberta (K. Nadeau, personal communication, May 10, 2019).

[2]Live price of broilers = 1.593 (Alberta Chicken Producers, 2018).

[3]This table was calculated based on the growth of 1000 kg of broilers, HBW offspring grew to an average weight of 2.486 kg at 42 d of age and SBW offspring grew to an average of 2.389 kg at 42 d of age (Table 3.2).

[4]HBW offspring were hatched from hens were raised at 21% above the Ross 708 broiler breeder target.

[5]SBW offspring were hatched from hens were raised in accordance to the Ross 708 broiler breeder target.

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## Appendix A. Plasma Analysis Protocols

### Corticosterone ELISA Protocol (Cayman Chemicals Cat #501320)

Procedure:

1. Note the lot numbers and expiration dates for the entire kit and all components.
2. According to plate map (Appendix B), load the standard, sample, and control wells. Add NOTHING to the blank wells.
3. Add ELISA buffer to NSB (100 µL) and B0 (50 µL) wells.
4. Add 50 µL of each standard to appropriate wells. Equilibrate pipette tip in each standard prior to pipetting.
5. Add 50 µL of each sample or control pool to appropriate wells. Equilibrate pipette tip in each sample prior to pipetting.
6. Using the 8-channel manual pipette to add 50 µL corticosterone tracer to each well EXCEPT total activity (TA) and blank wells.
7. Working in the same direction, use the 8-channel manual pipette to add 50 µL corticosterone antiserum to each well EXCEPT total activity (TA), non-specific binding (NSB), and blank wells.
8. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate overnight at 4oC (15 – 18 h).
9. The next day, carefully unseal the plate and empty wells into a waste container by shaking one or two times and tapping firmly on absorbent paper towels.
10. Using the electronic 12-channel, wash each well 5 times with 200 µl – 300 µl wash buffer. Between washes, fully empty the wells by shaking the contents into a waste container and tapping firmly on absorbent paper towels, as in step 10. If you notice a lot of bubbles in the pipette tips or the wash buffer is getting dangerously close to aspirating into the pipette, change tips.
11. Prior to emptying wells after the final wash, reconstitute Ellman's reagent with DI water. See preparation section above.
12. Using the 8-channel pipette, add 200 µl Ellman's reagent to each well.
13. Add 5 µl tracer to TA well.
14. Cover plate with plastic film and foil and develop in the dark on an orbital shaker at ~500 rpm for ~90-120 min (see below).
15. Carefully remove the plate seal and read the plate at a wavelength between 405 – 420 nm.
    - Be sure Ellman's reagent is not present on plate seal. If so, carefully pipet it back into the well from which it came.
    - Allow the plate to develop until absorbance of the blank subtracted B0 wells is between 0.3 – 1.5.
    - If absorbance exceeds 2.0, wash plate 5X as in steps 9-11 and re-develop with fresh Ellman's reagent.

**Generic Protocol for Cusabio ELISA Kits [IGF-1 (Cat #CSB-E13293C), Insulin (Cat #CSB-E13293C), & Growth Hormone (Cat #CSB-E09866Ch)**

Procedure:

1. Note the lot numbers and expiration dates for the entire kit and all components.
2. According to plate map (Appendix B), load the standard, sample, and control wells. Add NOTHING to the blank wells. If running multiple plates at once, be sure to label them appropriately (ELISA type, plate #, date, initials).
3. Add 50 µL of each standard to appropriate wells. Equilibrate pipette tip in each standard prior to pipetting.
4. Add 50 µL of each sample, control pool, and PBS to appropriate wells. Equilibrate pipette tip in each sample prior to pipetting.
5. Gently shake the plate to distribute the standards or samples in the wells.
6. Add 50 µL conjugate to all wells EXCEPT BLANKS using the 8-channel pipette.
7. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate overnight at 4°C (15 – 18 h). If running multiple plates at once, be sure to label the seals appropriately (ELISA type, plate #, date, initials).
8. The next day, carefully remove plate seal and use the 8-channel pipette to pull off all liquid from each well. MINIMIZE CONTACT WITH THE PLATE BOTTOM. It works best to put the multichannel along one sidewall of each well, and guide the pipette tips along the side towards the bottom to access as much liquid as possible. BE CONSISTENT WITH THE SIDEWALL THAT YOU CHOOSE. Try to avoid touching the bottom altogether, if possible. Aim for pulling off ~90% of the liquid, as trying to get more will likely result in greater chances of scraping the bottom of the well with the pipette tips.
9. Using the electronic 12-channel pipette, wash each well 5 times with 200 µl – 300 µl wash buffer. Between washes, fully empty the wells by shaking the contents into a waste container and tapping firmly on absorbent paper towels. If you notice a lot of bubbles in the pipette tips or the wash buffer is getting dangerously close to aspirating into the pipette, change tips.
10. Add 50 µL HRP-avidin to all wells EXCEPT BLANKS using the 8-channel pipette.
11. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate at 37oC for 30 min.
12. Carefully remove plate seal and pull off the liquid using the 8-channel pipette as in step 8.
13. Wash each well 5 times using the electronic 12-channel pipette as in step 9.
14. Add 50 µL Substrate A to all wells.
15. Add 50 µL Substrate B to all wells.
16. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate in dark at 37°C for 10 – 15 min.
17. Add 50 µL Stop solution to each well. The color should change from blue to yellow immediately. You may need to gently shake the plate to get a uniform yellow color.
18. Read the plate at 450 nm WITHOUT THE PLATE SEAL IN PLACE.

**Protocol for Glucagon ELISA (LifeSpan Biosciences Cat#LS-F16677)**

Procedure:

1. Note the lot numbers and expiration dates for the entire kit and all components.
2. According to plate map (Appendix B), load the standard, sample, and control wells. If running multiple plates at once, be sure to label them appropriately (ELISA type, plate #, date, initials).
3. Add 100 µl sample diluent to the blank wells and negative control wells.
4. Add 100 µl each standard to appropriate wells. Equilibrate pipette tip in each standard prior to pipetting.
5. Add 100 µl each sample or control pool to appropriate wells. Equilibrate pipette tip in each sample prior to pipetting
6. Gently shake the plate to distribute the standards or samples in the wells.
7. Cover with plate seal and incubate overnight at 4°C (15 – 18 h). If running multiple plates at once, be sure to label the seals appropriately (ELISA type, plate #, date, initials).
8. The next day, carefully remove plate seal and use the 8-channel pipette to pull off all liquid from each well. MINIMIZE CONTACT WITH THE PLATE BOTTOM. It works best to put the multichannel along one sidewall of each well, and guide the pipette tips along the side towards the bottom to access as much liquid as possible. BE CONSISTENT WITH THE SIDEWALL THAT YOU CHOOSE. Try to avoid touching the bottom altogether, if possible. Aim for pulling off ~90% of the liquid, as trying to get more will likely result in greater chances of scraping the bottom of the well with the pipette tips.
9. Using the electronic 12-channel pipette, wash each well 3 times quickly with 200 µl – 300 µl wash buffer. Between washes, fully empty the wells by shaking the contents into a waste container and tapping firmly on absorbent paper towels. If you notice a lot of bubbles in the pipette tips or the wash buffer is getting dangerously close to aspirating into the pipette, change tips.
10. Add 100 µL 1X Detection Reagent A to all wells using the 8-channel pipette.
11. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate at 37°C for 60 min.
12. Carefully remove plate seal and aspirate liquid using the 8-channel pipette as in step 5.
13. Wash each well 5 times with 200 µl – 300 µl wash buffer using the electronic 12-channel pipette. Incubate plate for 1 min on plate shaker before emptying the wash buffer each time. Between washes, fully empty the wells by shaking the contents into a waste container and tapping firmly on absorbent paper towels. If you notice a lot of bubbles in the pipette tips or the wash buffer is getting dangerously close to aspirating into the pipette, change tips.
14. Add 100 µL 1X Detection Reagent A to all wells using the 8-channel pipette.
15. Cover with plate seal, mix on orbital shaker at ~500 rpm for 1 min, and incubate at 37°C for 60 min.
16. Aspirate wells and wash 5 times as described in step 10.

17. Add 90 µl TMB Substrate to each well, cover with a new seal, and incubate at 37°C for 10-20 min. Protect from light and monitor for optimal color development. Wells should turn blue.
18. Add 50 µL Stop Solution to each well. The color should change from blue to yellow immediately. You may need to gently shake or tap the plate to get a uniform yellow color.
19. Read the plate at 450 nm WITHOUT THE PLATE SEAL IN PLACE.

**T3 Solid Phase Radioimmunoassay (MPBiomedicals)**

Procedure:

1. Note the lot numbers and expiration dates for the entire kit, the highest standard (F), the antibody coated tubes, and the 125I-T3 tracer.
2. Determine the number of tubes you will need for the assay and number them appropriately. For each assay run, 6 plain 12x75 mm polypropylene tubes are needed to measure total activity (TA) and nonspecific binding (NSB), and 27 antibody-coated tubes (from the kit) are needed to determine maximum binding (B0) and for the standard curve (Std A-Std H). Standard tubes are run in triplicate, and sample tubes are run in duplicate. See Table 1 at the end of the protocol for an example assay set-up.
3. Prepare the standard curve. Dilute the highest standard that comes with the kit (Standard F; 800 ng/dL) 2-fold in steroid-free serum, so that the concentration of the highest standard in the assay is 400 ng/dL (Standard H). Perform seven two-fold serial dilutions using steroid-free serum, starting with Std H, to create the standard curve.
4. Dilute samples with steroid-free serum as necessary so that they fall within the range of the standard curve and exhibit parallelism. For chickens, the following dilutions should fall within the range of standards.
5. Adult plasma – undiluted to up to 5-fold dilution
6. Hatchling plasma – undiluted to up to 5-fold dilution
7. Thyroid glands – must be diluted at least 4-fold to exhibit parallelism; up to 8-fold should be okay
8. Add 100 µL of the steroid-free serum (tubes #4-6 for NSB and tubes #7-9 for B0), standard, or sample to appropriate tubes.
9. Add 1 mL 125I-Total T3 tracer to each tube using a repeat pipettor.
10. Once the tracer has been added to all tubes, vortex each tube for 5 sec. Be sure to keep tubes in the appropriate order.
11. Place samples in a tupperware container labeled with radioactive tape, and incubate overnight (15 – 18 h) at 4°C in a radiation-certified refrigerator.
12. Set aside the total activity tubes (#1-3). Thoroughly decant liquid from the remaining tubes. Leave tubes inverted on a peg rack with kimwipes folded into the bottom and allow them to drain for several hours. Place a second peg rack on top of the tubes to stabilize them, and strike the rack sharply on the bench to shake off residual tracer droplets. Allow tubes to dry at least overnight, and preferably for 2-3 days.
13. Count the tubes for 1 minute in a gamma counter.

**T4 MAb Solid Phase Radioimmunoassay (MPBiomedicals)**

Procedure:

1. Note the lot numbers and expiration dates for the entire kit, the highest standard (F), the antibody coated tubes, and the 125I-T4 tracer.
2. Determine the number of tubes you will need for the assay and number them appropriately. For each assay run, 6 plain 12x75 mm polypropylene tubes are needed for total activity (TA) and nonspecific binding (NSB), and 24 antibody-coated tubes (from the kit) are needed to determine maximum binding (B0) and for the standard curve (Std A-Std G). Standard tubes are run in triplicate, and sample tubes are run in duplicate. See Table 1 at the end of the protocol for an example assay set-up.
3. Prepare the standard curve. Dilute the highest standard that comes with the kit (Standard F; 20 µg/dL) 2-fold in steroid-free serum, so that the concentration of the highest standard in the assay is 10 µg/dL (Standard G). Perform six two-fold serial dilutions using steroid-free serum, starting with Std G, to create the standard curve. Refer to Table 2 at the end of the protocol for how to set up the dilutions and suggested volumes.
4. Dilute samples with steroid-free serum as necessary so that they fall within the range of the standard curve and exhibit parallelism. For chickens, the following dilutions should fall within the range of standards.
5. Adult plasma – undiluted (will be at the low end of the curve)
6. Hatchling plasma – undiluted (will be at the low end of the curve)
7. Thyroid glands – must be diluted at least 2-fold to exhibit parallelism; up to 16-fold and possibly higher (20-fold) should be okay.
8. Add 25 µL of steroid-free serum (tubes #4-6 for NSB and tubes #7-9 for B0), standard, or sample to appropriate tubes. Refer to Table 1.
9. Add 1 mL 125I-Total T4 tracer to each tube using a repeat pipettor.
10. Once the tracer has been added to all tubes, vortex for 5 sec.
11. Place samples in a tupperware container labeled with radioactive tape, and incubate overnight (15 – 18 h) at 4°C in a radiation-certified refrigerator.
12. Set aside the total activity tubes (#1-3). Thoroughly decant liquid from the remaining tubes. Leave tubes inverted on a peg rack with kimwipes folded into the bottom and allow them to drain for several hours. Place a second peg rack on top of the tubes to stabilize them, and strike the rack sharply on the bench to shake off residual tracer droplets. Allow tubes to dry at least overnight, and preferably for 2-3 days.
13. Count the tubes for 1 minute in a gamma counter.

**Ether Extraction for Steroid Hormone Measurement**

1. Label two sets of 12x75 mm (for ≤200 µl plasma) or 18x150 mm (for >200 µl plasma) glass culture tubes for each sample.
2. Pipet 50 – 1000 µl plasma into one of the appropriately-sized labeled tubes and cover with foil.
3. Heat plasma in 65ºC water bath for 1 hr to denature proteins that may interfere with extraction. Allow samples to cool to room temperature.
4. In the fume hood, add 5x volumes diethyl ether to each sample and vortex vigorously for 30 sec. For example, add 1 ml diethyl ether to extract 200 µl plasma.
5. Cover with foil and allow samples to stand for ~2 min until fractions separate.
6. Wearing orange freezer gloves to prevent freezer burn, place samples in -80ºC freezer for ~5 min until the lower aqueous phase freezes. Alternatively, samples can be placed in a methanol/dry ice bath for ~5 min until the lower aqueous phase freezes. Retrieve samples using orange freezer gloves.
7. In the fume hood, decant ether fraction into the second labeled tube for that sample. Work quickly and be sure not to allow the frozen phase to thaw and decant. It is best to remove samples from the -80ºC freezer or methanol/dry ice bath a few tubes at a time to prevent thawing, particularly when extracting small volumes (<100 µl).
8. Allow the extracted plasma to thaw.
9. In the fume hood, add 5x volume diethyl ether to each sample and vortex vigorously for 30 sec.
10. Repeat steps 5 through 7, so that samples are extracted twice and both ether fractions are combined into the same tube.
11. Allow the tubes containing the extracted plasma to dry thoroughly in the fume hood and dispose of in broken glassware container.
12. Allow tubes with the ether fraction containing the steroids to stand at least overnight in fume hood to allow ether to fully evaporate. As they are drying down, vortex periodically to rinse down any steroid that may stick to the side of tube.
13. Add 1X EIA buffer to reconstitute the samples, the volume of which will depend on the dilution appropriate for the assay and starting volume of plasma. If volume is adequate, it is suggested to reconstitute with 1X volume EIA buffer to reduce the risk of undetectable levels of hormones in the extract. In some cases, you may want to concentrate the level of steroids in the extracted sample by reconstituting with less volume than was originally extracted. Be sure to account for any dilution in final calculations of hormone levels. For example, if you extracted 50 µl plasma and reconstituted with 200 µl, that is a 4-fold dilution.
14. Vortex for 30 sec, cover with foil, and place in 65ºC water bath for 1 hr. Vortex for 15-20 sec every 15 min to rinse any steroids down from the sides of the tube.
15. Allow samples to stand for ~5 min to drain to the bottom of the tube and transfer to a clearly and legibly labeled 1.7-mL polypropylene microcentrifuge tube.
16. Store extracted plasma samples at -20ºC or -80ºC.

## Appendix B – Sample Plate Map for ELISA Kits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TA | S1 | S1 | S1 | Sample 1 | Sample 1 | Sample 9 | Sample 9 | Sample 17 | Sample 17 | Sample 25 | Sample 25 |
| B | Blank | S2 | S2 | S2 | Sample 2 | Sample 2 | Sample 10 | Sample 10 | Sample 18 | Sample 18 | Sample 26 | Sample 26 |
| C | Blank | S3 | S3 | S3 | Sample 3 | Sample 3 | Sample 11 | Sample 11 | Sample 19 | Sample 19 | Sample 27 | Sample 27 |
| D | NSB | S4 | S4 | S4 | Sample 4 | Sample 4 | Sample 12 | Sample 12 | Sample 20 | Sample 20 | Sample 28 | Sample 28 |
| E | NSB | S5 | S5 | S5 | Sample 5 | Sample 5 | Sample 13 | Sample 13 | Sample 21 | Sample 21 | Sample 29 | Sample 29 |
| F | $B_0$ | S6 | S6 | S6 | Sample 6 | Sample 6 | Sample 14 | Sample 14 | Sample 22 | Sample 22 | Sample 30 | Sample 30 |
| G | $B_0$ | S7 | S7 | S7 | Sample 7 | Sample 7 | Sample 15 | Sample 15 | Sample 23 | Sample 23 | Pos Ctrl | Pos Ctrl |
| H | $B_0$ | S8 | S8 | S8 | Sample 8 | Sample 8 | Sample 16 | Sample 16 | Sample 24 | Sample 24 | Neg Ctrl | Neg Ctrl |